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Current status and future prospects of bioactive molecules delivered through sustainable encapsulation techniques for food fortification

Divakar Dahiya,[a] Antonia Terpou,[b] Marilena Dasenaki [c] and Poonam S. Nigam *[d]

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In a world of growing population and climate changing, health and sustainable food production are nowadays considered the most pressing challenges. Foods enriched with bioactive compounds provide numerous health benefits to consumers. For instance, essential oils and extracts prepared from several natural edible resources including herbs, spices, fruits, seeds, flowers and medicinal plants are selected to obtain bioactive compounds. These ingredients have been selected based on their active components providing antioxidant activity, antiinflammation activity, aroma, and various other health-promoting attributes. Although having applications in foods and nutraceuticals, certain bioactive compounds are found susceptible to oxidative degradation, while some are known to be chemically unstable, which results in minimizing their health-promoting effects. Similarly, the instability of bioactive compounds during several stages of food manufacturing and storage, in conjunction with poor bioavailability, fast release, low solubility, and chemical instability when exposed to different conditions in the gastrointestinal tract, significantly compromise their anticipated benefits. Therefore, effective vehicles and sustainable techniques are essential for the delivery of bioactive compounds in foods to retain requisite benefits. The encapsulation process is one of the most popular techniques applied for the protection and controlled release of bioactive compounds, as well as for masking undesirable odours and bitter tastes of certain food ingredients. This review gives insight into studies published on bioactive preparations and the techniques of encapsulation for their integration to obtain enhanced and sustainable health-promoting food products. The benefits achieved include the protection of bioactive compounds against adverse environmental conditions, improvement of their physicochemical functionalities, stability during processing and storage, and enhanced bioavailability, providing health-promoting and anti-disease activities to the consumers.

1. Introduction

Food affects human health in many ways besides providing the human body with energy mainly provided by macronutrients (carbohydrates: 4 kcal per gram, proteins: 4 kcal per gram, and fat: 9 kcal per gram) as food contains other ingredients such as micronutrients essential for healthy functioning and development.[1] In addition to ingredients essential to the human body, providing a stable supply of nutritious food, there are functional ingredients that can enhance human well-being. Specifically, functional foods containing bioactive compounds and other bio-sourced ingredients have attracted the attention of researchers and the food industry in recent years.[2]

The need for research and formulations for functional healthy foods is mainly driven by consumers' demand for well-being, health improvement and disease prevention through the intake of a diet enriched with bioactive ingredients.[3,4] These biologically active compounds can include dietary compounds and metabolites, living probiotic cells, dietary fibers, and active ingredients from different plant resources such as phenolic compounds, antioxidants, bioactive peptides, polyunsaturated fatty acids (*e.g.*, omega-3), and vitamins. For instance, fruits and vegetables being rich in phenolic compounds, terpenes, terpenoids, alkaloids, *etc.* also include dietary fibers providing unique beneficial effects to the consumer. These biologically active compounds can be isolated and are widely used for food fortification.[5–7]

Functional foods *i.e.*, foods that have a potentially positive effect on health beyond basic nutrition, as well as bioactive supplements have become popular because of their nutraceutical benefits.[6,8,9] Different preparations of functional foods and

[a]*Wexham Park Hospital, Wexham Street, Slough SL2 4HL, UK*
[b]*Department of Agricultural Development, Agrofood and Management of Natural Resources, School of Agricultural Development, Nutrition & Sustainability, National and Kapodistrian University of Athens, 34400 Psachna, Greece*
[c]*Laboratory of Food Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis Zographou, 15771 Athens, Greece*
[d]*Biomedical Sciences Research Institute, Ulster University, Coleraine BT52 1SA, UK. E-mail: p.singh@ulster.ac.uk*

beverages enhanced with bioactive compounds have been studied and designed to meet the requirement of a large group of consumers, including vegans, vegetarians, and people with a compromised digestibility for dairy-based products.[10,11] The presence of bioactive compounds in the diet is linked with beneficial effects on specific human body functions, beyond the adequate nutritional effects, which mainly depend on the biochemical state of bioactive compounds in a product at the time of consumption, reaching the consumer's bloodstream.[9,12] Specifically, the health effects from the intake of functional foods have been established to confer anti-tumour, anti-inflammatory, anti-oxidative, anti-hypertensive, and anti-hyperlipidemic effects, in addition to their basic nutritional functions.[10,13]

The constituents of food intake especially bioactive constitutes positively affect the function of the gastro-intestinal tract (GIT) as well as human metabolism; however, bioactives are known to be sensitive to a variety of environmental factors resulting in low bioavailability.[13–15] As a result, encapsulation is presented as a viable vehicle for ensuring bioactive stability and viability through food storage as well as though food passage in the GIT.[16] Moreover, bioactive encapsulation can also be applied for masking unwilling aromas derived from bioactive ingredients. For instance, functional chemicals, such as polyphenols when administrated in foods at high content may confer bitter taste and astringency while encapsulated polyphenols mask any unpleasant bitterness from the consumer.[17] Likewise, microalgae when administrated in foods they may provide fishy and/or marine odors while the density is mainly attributed to the food matrix, highlighting the need for their encapsulation beyond the stability and control release needs.[18,19]

The reasons to use encapsulation applications span across diverse sectors, including food, pharmaceutical, cosmetic, metrology, and analytical chemistry industries as well as research. Central to most dietary and health applications is the protection of bioactive components in food systems and their effective delivery to the consumer. Thus, this review will discuss reports on the sources of bioactive molecules, which have been isolated, extracted, and studied for their bioactivities (antioxidants, proteins, lipids, polysaccharides, flavoring, and colour), targeting their sustainable integration into food products through the application of encapsulation techniques.

2. Sources of bioactive compounds

Nowadays, various chronic diseases tend to rise including obesity, diabetes, cancer, hypertension, and cardiovascular diseases most likely as a result of modern lifestyle and bad human dietetic habits.[13] As a result of the increased interest from consumers globally, novel food products and nutraceuticals are being developed containing bioactive compounds obtained from several sources. These bioactives can be incorporated into food matrices in order to improve their nutritional value and provide health benefits.[13] Researchers have investigated different natural resources, and optimized chemical and physical bioprocesses to yield bioactive compounds possessing different characteristics. The relevant references on the isolation and extraction of bioactive compounds originating from several natural edible resources including herbs, spices, fruits, seeds, flowers, and medicinal plants are summarised below:

1. Essential oils have been recovered from culinary and medicinal herbs, like basil. The plants belonging to the *Ocimum* genus of the Lamiaceae family are considered to be a rich source of essential oils, and have expressed biological activity and use in different areas of human activity.[20] The biologically active compounds present in volatile oils of different species of *Ocimum* are studied as a source of natural antibacterial ingredients while the plant has been traditionally consumed for the treatment of digestive, respiratory, and sedative disorders.[21,22] A representative example of the species is *Ocimum americanum* which contains several phytochemical components, such as phenolic acids, flavonoids, tannins, terpenoids, alkaloids, saponin, steroids and glycosides.[22] The abundance of these bioactive compounds in *Ocimum* essential oils can provide antimicrobial, antioxidant and anti-inflammatory effects to the consumer.[22,23]

2. Essential oils were isolated from several *Mentha* species, such as spearmint, peppermint, and garden mint, which are used as an aroma additive in several commercial products.[24] These oils have been characterized for several bioactivities.[24] Particularly, seasonal and climatic variations have an effect on the content and chemical composition of bioactive compounds in *Mentha* plants. Therefore, the activities of essential oils recovered from different *Mentha* species could be different, as this criterion has been studied by Hussain *et al.* analysing the oils obtained from four species of *Mentha*.[25]

3. Rosemary leaves are reported to be a natural source of bioactive compounds. Essential oils have been extracted from the aromatic evergreen leaves of this culinary herb (*Rosmarinus officinalis*). Rosemary is a potent antioxidant herb rich in polyphenols belonging to the family Lamiaceae, and is popularly used as a spice and medicine.[26] Sharma *et al.* reported an efficient method of extraction yielding a high concentration of rosmarinic acid up to 33.49 mg g^{-1}, which was found to contribute substantially to the high antioxidant potential of the extracts.[27] Though this plant is grown for its aromatic and unusual flowers, researchers have reported its antiproliferative, antioxidant, and antibacterial activities.[28]

4. Pomegranate (*Punica granatum* L.) possesses different bioactive compounds, and belongs to the family Punicaceae. Pomegranate juice is known for its high levels of bioactive compounds such as flavonoids and other phenolics, exhibiting antioxidant, antimicrobial, and antimutagenic properties.[6] Significantly, all parts of the pomegranate plant; the seed, the peel, the juice, and leaves are known to be rich in bioactive compounds including anthocyanin, gallic acid, catechin, quercetin, alkaloids, flavonoids, *etc.*[6,29] Many methods target the extraction of polyphenols from red-coloured natural fruit juices like pomegranate (*Punica granatum*) or juice by-products targeting application in fortified novel products.[30,31] Agro-industrial by-products are of significant importance as many studies reveal their valorization potential which in many cases

exceeds the original product value. For instance, recent studies reveal that the extract of pomegranate peels show significantly higher antioxidant capacity than pomegranate seeds or juice[31]

5. Bioactive metabolites have been found in endophytes, like Endolichenic fungi.[32] Endolichenic microorganisms are an intriguing source of bioactive compounds with pharmacological potential. For instance, polysaccharides, sterols, and alkaloids have been reported for their biosynthesis employing a bioagent sourced from a medicinal plant *Swertia chirayita*.[33]

6. Extracts from several Centaurea species (family Asteraceae, tribe Centaureinae) such as Polyclada Dc. belonging to family Asteraceae, are a source of novel bioactive compounds. The Centaurea genus includes approximately 500 species mainly distributed in the Mediterranean region.[34] Phytochemical investigations on this genus generally revealed the isolation of sesquiterpene lactones, flavonoids, phenyl propanoids, lignans and phenolic compounds. These compounds are reported for their anti-inflammatory, antimicrobial, antidiabetic and anticancer activities.[34,35] Associated with this exuberant chemodiversity, a plethora of herbal remedies have been valorized through the years to treat abscesses, asthma, haemorrhoids, peptic ulcers, malaria, common cold, stomach upset and abdominal pain.

7. Pumpkin (*Cucurbita pepo*), watermelon (*Citrullus lanatus*), and melon (*Cucumis melo*) belong to the Cucurbitaceae family and they can be also mentioned as cucurbits growing extensively in tropical and subtropical regions. The bioactive composition and health benefits have also been studied in the fruits of the Cucurbitaceae family (*Momordica charantia* L.).[36] In addition, bioactive compounds possessing antioxidant activity have been extracted from the edible seeds of the Cucurbitaceae family. Cucurbitaceae seeds are an alternative source of plant oil, which is used as a raw material for certain food applications. Pumpkin (*Cucurbita pepo*) seeds are reported as nutraceutics for their bioactive properties mainly retrieved from their phenolic content.[36,37]

8. Endophytes are microbes that asymptomatically colonize the biotopes reported to survive in a symbiosis relationship with host plants having medicinal properties. Endophytes promote plant growth, development and defence by the production of metabolites.[38] Endophytes have been studied as a promising source of novel bioactive compounds, highlighting the potential to obtain targeted bioactive compounds faster through laboratory production than by bioactive producing plants.[39] Significantly, endophytes are emerging as an eco-friendly candidate producing bioactive compounds with therapeutic use while promoting crop yield productivity as biostimulants.[39] This approach can address environmental and agricultural concerns, producing high-value metabolites within a sustainable agriculture perspective.

9. Compounds rich in potent anti-inflammatory and antioxidant properties, such as curcumin have been isolated from different spices such as turmeric. The rhizomes of turmeric (*Curcuma longa* L.) are rich in essential oils (5–10%, v/w) and curcuminoids (1–2%, w/w).[40] Turmeric is a widely used spice in the Indian subcontinent and its extracts have been widely used in traditional medicine and Ayurveda.[40] A combination of curcumin isolated from turmeric and alpha-linolenic acid have been studied for its beneficial properties against cancer cells.[41] Likewise, saffron (*Crocus sativus* L.), which is a perennial herb belonging to the family Iridaceae, is a widely popular product of Greece (Kozanis Crocus; "red gold") providing a significant proportion of antioxidants, with more than 300 volatile and non-volatile compounds. The main constituents of saffron are carotenoids, glucosides, flavonoids and monoterpenes while its most significant bioactive components are apocarotenoids such as safranal, picrocrocin, and crocins known for their beneficial effects.[42,43]

10. Vegetative sources rich in antioxidant properties have been investigated, such as the leaves of the plant *Camellia sinensis* (popular as tea). Antioxidant properties were assayed as the free radical quenching capacity of several types of commonly used tea leaves (Earl grey, black tea, Ceylon tea, & green tea). The infusions of tea leaves were investigated for antioxidant activity using ascorbic acid, trolox or gallic acid as reference antioxidants. The radical quenching capacity of tea infusions were expressed as trolox equivalent antioxidant capacity (TEAC) or ascorbic acid equivalent antioxidant capacity (AAEAC).[44]

11. Plants of *Origanum* species like common oregano and wild marjoram were used to isolate essential oils. The essential oil of oregano (*Origanum vulgare*) presents antioxidant and antimicrobial activities, mainly due to the presence of carvacrol and thymol. The chemical composition of the essential oils distilled from the extract of *Origanum* species may provide antiproliferative, antioxidant, anti-inflammatory, and antidiabetic activities, and more recently, cancer suppressive activity has been reported.[45]

12. *Rubia cordifolia* L. (commonly known as madder) is a species of a flowering herbal plant of the coffee family, Rubiaceae, utilized for ages in China as a medicinal plant.[46] *R. cordifolia* is usually cultivated for a red pigment derived from its roots employed to treat hematemesis, haemorrhage, rheumatism, metrorrhagia, contusion, and chronic bronchitis. Various studies have also demonstrated the anti-inflammatory, antioxidant, anticancer, and antimicrobial effects of the aqueous root extract of *R. cordifolia*.[47,48]

13. Ashwagandha or the "Indian Ginseng" (*Withania somnifera*) is a medicinal herb, and the bioactivity of its root extracts is being used since ancient times for health restoration in therapeutic Ayurveda.[49] Recent scientific reports show that the aqueous extracts of its roots possess antioxidant, anti-osteoporotic, anti-arthritic, anti-epilepsy, anti-Alzheimer, anticancer, and antimicrobial activities mainly attributed to the bioactive compound triterpenoid steroidal lactone.[49] Unfortunately, the yield of secondary metabolites retrieved from cultivated plants is not always produced in adequate amounts.

14. Extracts in different solvents, polar and non-polar, were prepared from the dark orange-coloured petals of *Calendula officinalis*, a flowering plant in the daisy family Asteraceae. Efstratiou *et al.* studied different extracts of Calendula and reported its bioactivity against broad spectrum pathogens.[50]

15. Extracts were obtained from *Stachys schtschegleevii*, an endemic species of Iran. Stachys is a genus of plants, one of the

largest in the mint family Lamiaceae; it is considered a valuable medicinal plant that is widely used in herbal medicines. Its different preparations have been studied for the presence of free-radical-scavenging and antibacterial properties.[51]

3. Protection of bioactivities in ingredients and products

Food functionality can be claimed when health beneficial effects are provided to the consumer through food intake. Likewise, food fortification with bioactive compounds targets the production of novel foods with improved beneficial characteristics.[8,13] The direct fortification of food products with bioactive compounds is a complex procedure as in many cases the bioactives may become unstable depending on the food matrix and negative effects on the quality of the final product (*e.g.*, sensory properties such as weird taste, appearance, and bad odor) may also occur.[52]

One of the most imperative challenges of foods enhanced with bioactive compounds is the controlled release of the compound of interest from the food matrix to the gastrointestinal tract while retaining its bioactivities.[13] Bioactive compounds can be present in food matrices but do not always reach the bloodstream as in many cases they are decomposed, losing their beneficial value. Moreover, the application of bioactive compounds in the food industry remains limited up until nowadays. The loss/reduction of functionality of these health promoting bioactives remains a serious hazard in food industrial production as the environmental conditions (*e.g.*, oxygen, heat, and light) and food manufacturing conditions (*e.g.*, high temperatures and high pressure) can cause serious effects on the activity of bioactives.[52] To overcome these shortcomings, different food grade matrices have been proposed by researchers to encapsulate bioactive compounds in order to enhance their stability, bioactivity and bioavailability while increasing their concentration in the produced novel foods. The desired characteristics of different biocatalysts applied as encapsulation matrices are presented, highlighting sustainable production and high bioavailability.[53]

Encapsulation is a technique that involves the introduction of bioactive compounds in a secure way into the matrix of products. More specifically, using an effective vehicle, the ingredients and additives with the required bioactivity can be entrapped within or coated with another material, or system (encapsulating agent).[16] This strategy targets the protection of the activity of additive compounds from external conditions, restraining their direct contact with the conditions of the surrounding environment, and/or controlling their release (Fig. 1).

The coated material is called the core material while the material used for coating is called the shell, carrier, or encapsulant. Moreover, encapsulation can also be used to mask any undesirable strong aroma and bitter taste of additives and food ingredients.[54] The entrapment of bioactive molecules protects them against adverse environmental conditions, improves stability during processing and storage, and allows the controlled release of bioactive compounds. Encapsulation technology is applied in the pharmaceutical, chemical, cosmetic, and food industries. Several encapsulation technologies have been studied that include emulsification, entrapment, spray chilling and cooling, spray drying, fluidized bed coating, extrusion, inclusion complexation, and coacervation.[54–56]

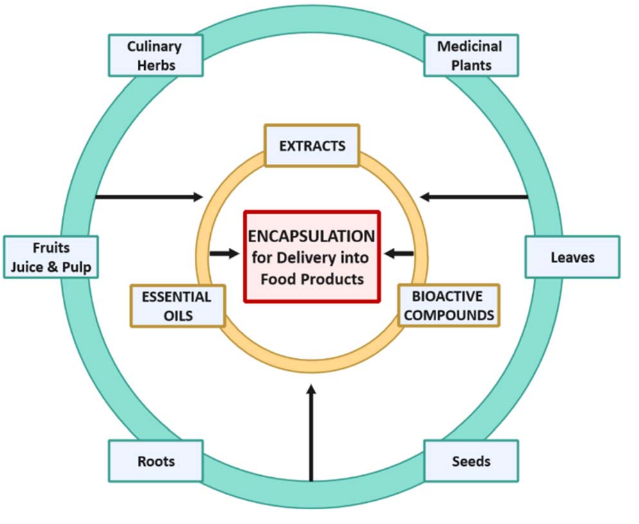

Fig. 1 Sources of bioactive molecules for application in food products.

4. Encapsulation techniques

Encapsulation can be defined as a process of holding active agents (bioactive compounds) within a food-grade carrier/matrix to improve the delivery of these active compounds into food products.

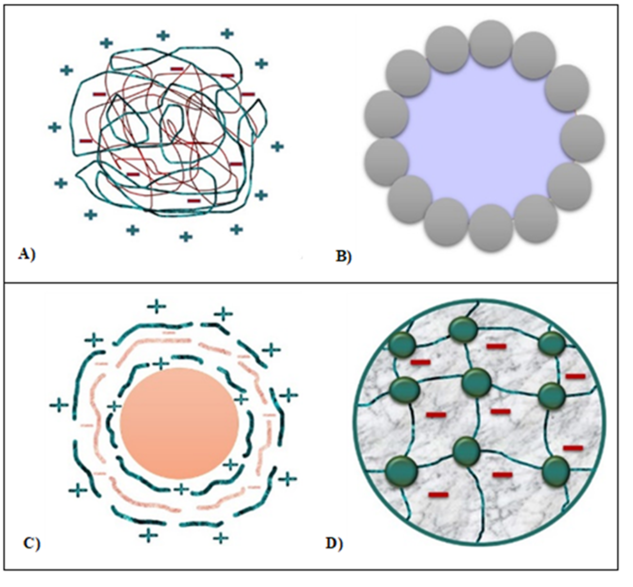

Fig. 2 Encapsulation systems of bioactive compounds classified by their building structures including (A) complex coacervates, (B) layer-by-layer microcapsules, (C) Pickering emulsion delivery systems, and (D) cross-linked biopolymers.

Table 1 Bioactive compounds encapsulated *via* different encapsulation techniques within the core material

Technique of encapsulation	Bioactive compound	Material of encapsulation	Ref.
Spray drying	*Spirulina* sp. LEB-18	Maltodextrin and soy lecithin	19
Spray drying	Pomegranate seed oil	Succinylated taro starch and β-cyclodextrin	57
Spray drying	Anthocyanin	Gum arabic, maltodextrin, and gelatin	58
Freeze drying	Probiotic cells	*Pistacia lentiscus* resin	5
Freeze drying	Sour cherry pomace extract	Whey and soy proteins	59
Freeze-drying	*L. casei* ATCC393	Wheat bran	60
Cross-linked biopolymer	Lactoferrin (glycoprotein)	Calcium alginate	61
Spray-drying and ionic gelation	Anthocyanins	Maltodextrin, whey protein, and gum arabic	62
Complex coacervation	Apple polyphenols	Cyclodextrin	63
Complex coacervation	Probiotic cells	Whey protein isolate and gum arabic	64
Complex coacervation	Flaxseed oil	Gelatin-gum Arabic	65
Complex coacervation	β-Carotene	Palm oil with chitosan/carboxymethylcellulose	66
Complex coacervation and spray drying	Peppermint oil	Albumin, gum acacia and an oxidized starch crosslinker	67
Cross-linking and microemulsification	Gallic acid	Whey protein hydrolysates	68
Microemulsification	Caffeine	Whey protein isolate	69
Self-assembled nanoparticle microcapsules	Vitamin D3	Whey protein isolate	70
Core-shell structure microcapsules/freeze-drying	Yeast	Pine sawdust	71
Pickering emulsion	Thymol	Zein/gum Arabic nanoparticle	72
Pickering emulsion	β-Carotene	Hydrolyzed soya protein isolate	73
Pickering emulsion	Chlorophyll	Gelatin, agar, oil phase, and water	74

Among encapsulation techniques, Pickering emulsions (Fig. 2C), cross-linked polymer gels (Fig. 2D), complex coacervates (Fig. 2A), core-shell structure microcapsules and self-assembled structures (Fig. 2B) are the most popular delivery systems to enhance the bioavailability and stability of bioactive compounds (Fig. 2).

Carrier agents, biopolymer microparticles or nanoparticles are applied to sustain the functional characteristics of bioactive compounds within the food matrix.[75] Likewise, microencapsulation can be defined as a process where bioactive compounds are contained within carrier agents acting as droplets surrounded by a coating or embedded in a homogeneous or heterogeneous matrix to produce effective capsules protecting the bioactive compounds. In general, core materials (essential oils, vitamins, flavonoids, polyunsaturated fatty acids, probiotics, *etc.*) selected as bioactive compounds are blended within the matrix and encapsulated by using a wall material (Table 1).

Selection of the wall material is of paramount importance and mainly depends on the chemical characteristics of the produced food as well as the inserted bioactives (Table 1). The wall material must have low production costs and provide sustainability and high encapsulation efficiency, retaining its characteristics throughout food production and storage.[5,71,75]

5. Bioactive compounds encapsulated for enhanced food quality

Encapsulation is considered a significant method for improving bioactive distribution in food as well as helping in the delivery of bioactives to the gastrointestinal tract. Encapsulation or microencapsulation is actually a method that encloses bioactive molecules either by coating or insertion in a complex matrix targeting the creation of capsules which contain molecules storing beneficial features.[8] The most frequently encapsulated bioactive molecules are lipids, antioxidants, proteins, carbohydrates, food colours and various flavoring compounds (Fig. 3).

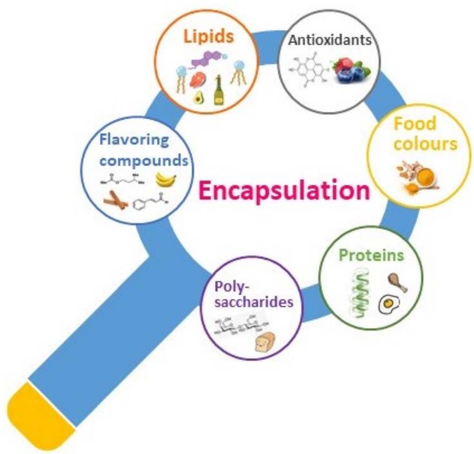

Fig. 3 Encapsulated bioactive compounds targeting enhanced food quality.

Overall, encapsulation techniques increase the stability of bioactive compounds, enable them to resist low pH environments and enzymatic activity of the gastrointestinal tract, promote targeted delivery of active compounds, improve viscosity, promote low water solubility, and facilitate their incorporation in many food products.[52,76]

5.1 Flavoring compound encapsulation

Flavors are known as the essence of foods and play important roles in consumer acceptance and consumption of foods. Most flavoring compounds that exist in foods are volatile and chemically unstable in the presence of air, light, moisture, and high temperatures; therefore, encapsulation provides the means for stabilization when exposed to air, light, heat, and moisture.[75] Microencapsulation can also be used to mask unpleasant flavors and odors as well as to provide barriers between sensitive bioactive materials and the environment. Moreover, the controlled release of flavour ingredients according to their threshold values can cover the effects of other unpleasant odours in food products. For instance, below threshold levels, organic acids contribute to the complexity of wine aroma, but very high concentrations may pose a negative effect on the final products.[12,77,78] For example, Shinohara (1985) reported that the presence of organic acids (C6–C10) in wine at regular concentrations (4–10 mg L^{-1}) contributes to a pleasant aromatic profile while a higher than 20 mg L^{-1} organic acid concentration can negatively affect the final aromatic profile of wine.[77] In the wine industry it has been well documented that esters are characterized by low flavour thresholds, and are known to pose a major impact on the flavour of alcoholic beverages.[12] Specifically, ethyl esters of fatty acids are associated with flowery or fruity aromatic profiles and are highly desirable in wine and other alcoholic products. Likewise, the acetates of higher alcohols and terpenes (*e.g.*, D-limonene, β-myrcene, linalool, α-terpineol, and β-citronellol) are significant volatiles that contribute to wine aroma, imparting flowery notes. Thus, encapsulation and controlled release of flowery or fruity aromatic volatiles can mask unpleasant effects posed by a high organic acid concentration of wine products.[12] Volatile compound encapsulation prior to use in foods or beverages may limit aroma degradation or loss during processing and storage.[79]

Another mechanism of action in the application of encapsulation techniques in food ingredients containing off-flavors is by targeting deodorization.[80] For instance, algae and microalgae contain off-flavors and odors (*e.g.*, fishy and/or marine odor caused by the production of dimethyl sulfide) which affect the quality of the final product and make it less desirable to the consumers.[18] Algae-fortified new food products can be implemented with other flavouring agents that mask these off-flavor compounds.[18,19,81] Microencapsulation of food ingredients containing off-flavors like microalgae may mask the characteristic flavour providing novel food with accepted organoleptic characteristics.[19]

Several methods have been reported for flavor microencapsulation. Usually, spray drying of flavor components dominates the techniques used for the production of flavor powders.[82] Spray drying is one of the most frequently used encapsulation techniques for thermosensitive bioactive components as it promotes faster drying and short-term exposure to heat, it is easy to apply, it is cost-effective, and it creates high-quality microcapsules.[19,83] Complex coacervation, however, is a new promising low cost technique for flavor compound encapsulation providing high encapsulation yields (up to 99%) and controlled release of flavoring compounds.[84] Even though it has been previously reported that coacervation showed limited application in flavour encapsulation such as evaporation of volatiles, dissolution of an active compound into the processing solvent and oxidation of the product, recent reports provide us with more promising results.[85]

The protein–polysaccharide combinations that have been reported for flavor encapsulation by complex coacervation include gelatin/gum Arabic, gum Arabic/albumin and xanthan gum/gelatin.[86] In cases where a flavor is being encapsulated by a process based on complex coacervation, water-miscible or partially water-soluble components present in the flavor can affect the coacervation process and nature of the formed coacervate.[75,79] Gelatin-based complex coacervation systems for flavor compounds introduce protocols depending mainly on the type of gelatin applied for coacervation. In many cases a cross-linking factor can be added to improve the stability of the microcapsules. Numerous variations of this process have been reported throughout the literature because of the different polyanions that can be applied to produce complex coacervates suitable for microcapsule formation. A representative example is the microencapsulation of garlic oil *via* complex coacervation using gelatin and gum acacia as e cell wall material in which the cross-linked garlic oil contained in coacervates allowed the controlled release of garlic oil in pepsin solution within 5 h. The studied systems remained stable and effective protecting garlic oil against primary and secondary oxidation throughout storage (45 °C) compared to free garlic oil which showed high oxidation under the same conditions.[87] Another study targeted the formation of heat-resistant jasmine oil applying complex coacervation microcapsules of gelatin and gum Arabic. Their heat-resistance test showed that the nano-capsules cross-linked under alkaline conditions showed sustainability while chromatographic analysis showed a destruction in the fractions of free jasmine essential oil.[88] In conclusion complex coacervation can be noted as a very promising low-cost microencapsulation technology showing optimum characteristics in flavor compouns encapsulation as it provides high encapsulation yields (up to 99%) verifying the controlled release and stability of flavor compounds.[75,79]

5.2 Lipid encapsulation

Lipids belong to a broad category of food compounds that include different types of molecules such as carotenoids, fatty acids, oil-soluble vitamins, antioxidants, phospholipids, and phytosterols. Lipids are known to have low water solubility and the majority of them are highly susceptible to oxidation; therefore, encapsulation offers an alternative to the food industry to facilitate their incorporation *via* sustained product quality, storage, and protection against oxidation in a large variety of food products.[83]

Several encapsulation methods and wall materials have been tested for lipid encapsulation depending on rheological properties, achievement of controlled release, dispersion and stabilization of the encapsulated oil, and the ability to hold the core within the capsule.[67,83] The most widely applied method to increase their stability is the use of microencapsulation. A combination of wall materials such as pectin, gum arabic, and protein isolate has been proved to improve lipid stability while utilization of sustainable and other renewable resources is also considered very promising according to recent data. The main strategies for lipid encapsulation include spray drying, freeze-drying, complex coacervation, extrusion, spray-chilling/cooling, and ionic gelation.[57,67]

As lipids are known to be susceptible to oxidation, encapsulation by spray-drying is proposed as a technique to protect them against harsh environmental factors.[83] Even though many studies have highlighted the successive effect of this technique on lipid encapsulation, more recent data provide a controversial input. For instance, according to Łozińska *et al.* (2020), the best wall materials for fish oil micro-encapsulation are protein + lipid + carbohydrate and protein + lipid, the result in this case highlighted that spray-drying methods were of lower expectations.[89] Another study by Linke *et al.* (2020) evaluated the rate of oxidation of encapsulated and non-encapsulated fish oil highlighting that the encapsulated oil could be oxidized even when incorporated within the protective matrix.[90] In addition, the oxidative stability of the encapsulated fish oil was determined by its oxidation behavior, and as a result, the solid protective matrix was not proved sufficient to obstruct the penetration of environmental oxygen. Therefore, more complex materials need to be studied targeting efficient protection of lipids from oxidation.

Flaxseed oil, for example, is a significant source of omega-3 fatty acids and has been acknowledged for its role in disease prevention and human health.[91] Due to its incompatibility with many food systems, encapsulation has been studied as an alternative option for food industries. Liu *et al.* (2010) encapsulated flaxseed oil within gelatin–gum arabic (GA) complex coacervates. The produced capsules showed high encapsulation efficiency (∼84%) providing in parallel a protective effect against oxidative products when compared to the original oil.[65] Consequently, coacervates can be proposed as successful encapsulation materials when applied as lipid protective capsules.

5.3 Encapsulation of antioxidants

Antioxidants as food preservatives have gained exquisite attention in recent years as they may prevent food deterioration in parallel with preserving their nutritional food value. Antioxidant therapy has been proved as a powerful tool against oxidative stress as bioactive antioxidant compounds can reduce or terminate chain reactions caused by free radicals. Specifically, natural antioxidants are mostly preferred as they possess enhanced thermal stability and antioxidation ability compared to synthetic antioxidants providing in parallel potential health benefits and reinsuring safety in food applications.[92]

Food antioxidants can be classified into different categories based on their properties including water soluble compounds like phenolic compounds, citrates, flavonoids, and anthocyanins, and lipid soluble compounds like terpenoids, carotenoids, tocopherols, and vitamins.[93] In general, for antioxidant compounds to be characterized as efficient they need to confer high reactivity, biological availability, ubiquity, versatility and the ability to cross physiological barriers. More importantly, bioactivity of antioxidants may be influenced by oxygen, light, temperature and moisture. Thus, encapsulation techniques have been extensively studied targeting their efficient stabilization thought-out food processing and storage.[94] More specifically, microencapsulation has been proved to promote the delivery of vitamins and minerals to foods mainly by preventing their interaction with other food components; for example, iron bioavailability can be severely affected by interaction with food ingredients (*e.g.* tannins, polyphenols & phytates).[7] Apart from oxygen, acids can also cause problems in conjunction with other food ingredients, such as a decrease in flavour, providing undesirable odors and providing undesirable changes in pH.[95]

Ascorbic acid, which is a water-soluble vitamin, is considered as one of the most important antioxidants. However, environmental factors, such as pH, temperature, oxygen, metal ions, UV light and X-rays can affect its stability.[96–99] Therefore, several microencapsulation techniques have recently been utilized to reduce these problems.[100] For example, complex coacervation of gelatin-A and sodium alginate as a new microencapsulating material has been applied using gelatin as the crosslinker and ascorbic acid as a model active agent.[101] Likewise, to enhance microencapsulation, the use of the double emulsion technique made it possible to obtain microcapsules with a hydrophilic core providing high encapsulation efficiency. To conclude, incorporation of novel technologies seems to be very promising for revealing the incentive quality of processed substances, and tailor-made microcapsules need to be considered for specific applications.

5.4 Food colour encapsulation

Plant pigments are unique chemical substances considered as secondary compounds involved in plant photosynthesis, metabolism, light-harvesting, and protection from photo-oxidation while being responsible for the colourful appearance of fruits and plants.[102] In particular, the most common plant pigments are carotenoids (yellow-orange), chlorophylls, anthocyanins (red-purple) and betalains (red-yellow). In most cases these colourants can also exceed antioxidant capacity when administrated in adequate amounts in foods. Natural colourants are being used as additives in foods targeting optimum organoleptic characteristics in addition to their medicinal effects.[74] Unfortunately, natural colourants show low bioavailability with regard to their beneficial effects and in many cases, a lack of stability, showing less attractive colours which are not attractive to the consumer. As a result, encapsulation of colour pigments has been applied to reinsure colour sustainability.[74,102] Targeting a more sustainable point of view, agricultural residues can become a significant source of natural pigments with low costs, reducing the environmental fingerprint during food supplement applications.

Carotenoids can be easily oxidized under processing and storage conditions. For instance, lycopene, which is a carotenoid sensitive to oxidation, is usually introduced into foods to provide color. However, lycopene's application in the food industry is limited due to its poor solubility and low bioaccessibility. Several delivery systems have been employed for the delivery of lycopene including protein-based nanoparticles, emulsions, liposomes, *etc.* Protein-based spray-dried microcapsules have been prepared economically and widely used.[103] Silva *et al.* (2012) encapsulated lycopene by complex coacervation using gelatin and pectin, and despite the high encapsulation efficiency, it did not provide sufficient protection of the lycopene during storage.[104]

Chlorophyll is also known as an unstable compound as its stability can be highly affected by heat, light, temperature, and pH. On the other hand, chlorophyll has been proven to be more thermolabile compared to carotenoids.[74]

Application of anthocyanins in the food and pharmaceutical industries is limited as they are prone to degradation, being extremely sensitive to oxygen, light, temperature, pH, and enzymes.[58]

5.5 Protein encapsulation

Bioactive proteins are also considered one of the most important families of bioactive molecules which are applied for encapsulation, as numerous food-derived peptides can act as antioxidants, growth factors, immune regulatory factors, or anti-hypertensive agents. In order to exert a beneficial health effect, some bioactive proteins have to reach the uptake site in the small bowel intact, but most proteins are highly susceptible to biodegradation during transit through the gastrointestinal tract. Other proteins may require hydrolysis in the stomach and small intestine in order to release specific bioactive peptides or amino acids.[105] Thus, the encapsulation of proteins depends on the type of protein, its health effect, and the product that will serve as a vehicle for the bioactive protein.[106]

5.6 Polysaccharide encapsulation

Another family of bioactive molecules that may benefit from encapsulation are carbohydrates, mainly referring to bioactive carbohydrates that are found in dietary fibers. These fibers are very heterogeneous and vary in the type and distribution of polysaccharides as well as in the quantity of monosaccharides. They are usually classified according to their molecular structure and physicochemical properties, their origin, or according to their physiological effects.[105] Polysaccharides are mainly applied as vehicle matrices for the delivery of other bioactive molecules or microorganisms.[8]

The fibers that benefit from encapsulation are the soluble non-digestible polysaccharides that have been used for cholesterol reduction, prevention of constipation, reduction of glycaemic fluctuations, prebiotic effects, and antioxidant effects.[107] For instance, polysaccharides isolated from seaweed have shown rich antioxidant activity, and their encapsulation would provide food supplements and products of high standards.[107] The main challenge for the encapsulation of these compounds is not the targeted release but the increment of the total fiber content in food to exert the aforementioned health benefits. Thus, most efforts are focusing on increasing the total fiber load in the food load by packing enough fibers in capsules without interfering with the product quality such as changes in texture, mouthfeel, or flavor.[108]

6. *In vitro* digestibility and release profile of encapsulated bioactive compounds

The successful application of encapsulation depends on the selection of food-grade carrier materials, which should possess the desired physicochemical and rheological properties. Such an encapsulation process is a necessary support to preserve the targeted bioactive compound within a specific food matrix. This strategy targets the protection of the activity of additive compounds from external conditions, restraining their direct contact with the conditions of the surrounding environment, and/or controlling their release. Studies have been designed to investigate the bioavailability and bioactivity of encapsulated phenolics and carotenoids isolated from red pepper waste during *in vitro* simulated gastrointestinal digestion. The *in vitro* digestion of freeze-dried encapsulates and spray-dried encapsulates was determined by simulation of digestion in gastric fluid and intestinal fluid. In another study, the effect of *in vitro* gastrointestinal digestion on encapsulated and nonencapsulated phenolic compounds of Carob (*Ceratonia siliqua* L.) pulp extracts and their antioxidant capacity were investigated. The effects of *in vitro* gastrointestinal digestion on the release and bioactivity of encapsulated bioactives have been studied after each digestion step. The results obtained in *in vitro* tests showed that the release of bioactive molecules from encapsulated entities, phenolics and carotenoids, as well as the antioxidant, anti-hyperglycemic, and anti-inflammatory activities were influenced by the pH and level of intestinal fluid.[109,110]

7. Conclusions

Bioactive compounds can be applied in food enrichment to produce functional foods, food preservative-antimicrobials, food colorants, anti-cancer agents, or anti-inflammatory agents. Beyond academic research and innovation, it is the industry that must focus their development on scaling up the production of foods fortified with bioactive compounds. The bioavailability of these compounds is the main obstacle that food producing companies will have to overcome in order to align with food authority regulations on health claims. The European Food Safety Authority (EFSA), for instance, only authorises health claims if they are based on sound scientific evidence and are easy to understand by consumers.[111] Thus, ascertaining bioavailability is the main target in functional food production.

The bioavailability of bioactive compounds depends on the matrix of food in which the bioactive delivery system is incorporated, and on other food ingredients consumed in the diet along with the enriched product. Encapsulation, a technique which involves the introduction of bioactive compounds into a matrix, has been proven as one of the most effective techniques for the delivery of health-promoting ingredients in adequate amounts to targeted regions after consumption. As the bioavailability varies between different individuals and within a single person, depending on the food composition, consumers' health condition, and other factors, a proper experimental design regarding the encapsulation of bioactive compounds is of paramount importance. Interestingly the main target to sustain the bioactivity, as well as the concentration of bioactive compounds in food products, has been proven successful in many cases. In conclusion, the incorporation of novel technologies seems to be very promising for revealing the incentive quality of processed substances, and tailor-made microcapsules need to be considered for their specific applications.

8. Future prospects

Future studies should emphasize on the limitations of encapsulation in meeting the commercial demands for industrial-scale production. From a more ambitious perspective in the future, the field of bioactive delivery may include smart selective targeted delivery systems designed to release bioactive compounds to particular regions or cells of the consumer, providing personalized delivery systems based on each individual's habits and health needs.

Bioavailability is a key step in ensuring the bio-efficacy of bioactive compounds after consumption. The bioavailability of bioactive compounds mainly refers to the fraction of the ingested compound when it reaches the blood.[112] As mentioned before, most of the bioactive compounds show low bioavailability which is the major concern. Numerous encapsulation techniques have been enlisted to enhance the bioavailability of bioactive compounds. The compounds which show instability during food processing and within the gastrointestinal tract should be encapsulated in food-grade carrier agents. This will allow their timely release after consumption, facilitating bioactive uptake. Controlled release and increased residence within the gastrointestinal tract can be achieved by encapsulation, but for a bioactive compound, a tailor-made bio-capsule is required. Finally, regarding the food sector, two main aspects for applying these insights need to be kept under consideration. In the first instance, the procedure is important, and the encapsulation material needs to be of low cost. Finally, and more importantly, the production industry must keep in mind the consumers' preference for natural ingredients used as additives.

Conflicts of interest

There are no conflicts to declare.

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