Enhancing the Availability, Assimilation, and Accessibility of Bioactives through Nanoencapsulation

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ABSTRACT: Bioactive compounds are secondary metabolites secreted by plants and plant derived products which are essential for human health. Because bioactive chemicals have the potential to maximize therapeutic efficacy and minimize side effects, there has been a lot of attention focused on developing effective delivery systems for these compounds in recent years. One method that has shown promise for overcoming the difficulties in delivering bioactive substances is nanoencapsulation. The state-of-the-art in bioactive compound delivery systems using nanoencapsulation is thoroughly reviewed in this paper. Present review deals with the brief study of bioactive delivery and their improvement for better biostability via nanoencapsulation. Nanotechnology holds potential perspectives for food processing, including superior antimicrobial effects, improved sensory quality and safety, enhanced nutrient bioavailability in food preparations, and targeted delivery and controlled release of bioactive ingredient along the gastrointestinal tract. The review commences with an explanation of the principles of nanoencapsulation, covering different methods for encapsulating bioactive substances in nano-sized carriers, including coacervation, emulsification, and nanoprecipitation. The benefits of nanoencapsulation are also emphasized, including enhanced solubility, stability, bioavailability, and targeted distribution.

Keywords: Nanoencapsulation, bioactive compounds

I. Introduction

Bioactive compounds are abundantly present in the various kind of foods containing beneficial compounds for human health in addition to their basic nutritional value. Numerous biological activities, including anti-inflammatory, antibacterial, antioxidant and anti-cancer effects are possible for these molecules. Some of the recently reported compounds are Gallic acid, Ellagic acid, Protocatechuic acid, Vanillic acid, Omega-3 fatty acids, polyphenols, carotenoids, and flavonoids are a few examples of bioactive substances. They have been connected to a lower risk of chronic illnesses like diabetes, cancer, and heart disease and are frequently present in fruits, vegetables, whole grains, nuts, and seeds. To enhance health outcomes, bioactive substances can be isolated and utilized as functional ingredients in food products, dietary supplements, and nutraceuticals. Food proteins yield bioactive peptides (BPs) that have potential therapeutic applications against degenerative and cardiovascular disorders, including cancer, diabetes, and inflammation. There is a dearth of knowledge regarding the stability and bioactivity of these peptides when they are integrated into dietary matrices, despite the abundance of reports on the in vitro, animal, and human research of BPs (Ashaolu et al. 2023). There are two well established approaches for the production of nanostructured particles 'top-down' or 'bottom-up' method. The top-down method involves physical forces like grinding, milling, homogenization and ultrasonication whereas, 'bottom-up' include heat-induced aggregation, nano-complexation with other polymers (e.g., polysaccharides), covalent crosslinking, self-assembly, and emulsification-evaporation (Tang, 2019). Biologically active constituents which are responsible for functionality and inducing probiotic effects on metabolism are generally termed "bioactive compounds".

Bioactive compounds have the ability to interact with one or more components of living tissue by providing a wide range of potential effects and are derived from plant, animal, or other sources such as microorganisms, which are "generally regarded as safe (GRAS). BACs are of great interest due to their various biological and functional activities such as antioxidant, anti-inflammatory, antidiabetic, anticancer, antiviral, and antitumor activities, thereby protecting the human body from high levels of free radicals and reactive oxygen species (ROS) that may easily react with other molecules, resulting in damages of the cell. BACs are major elements such as vitamins and phytochemicals that originate from nature during the processing of foods or plants with the capacity to give beneficial purposes. Consumption of BACs in functional foods has received enormous attention from many researchers because of the beneficial roles played in human health. Biopolymers such as alginate, gelatin, whey protein, starch, chitosan, and pectin have been used to develop biodegradable films that can be used as vehicles for transporting BACs. Aside from that, encapsulation has become one of the most widely used methods for incorporating BACs into food matrices in recent years (Bazana et al., 2019).

Foods that contain bioactive chemicals have health benefits for humans in addition to their basic nutritional value. Numerous biological activities, including anti-inflammatory, antibacterial, antioxidant, and anti-cancer effects, are possible for these molecules. Omega-3 fatty acids, polyphenols, carotenoids, and flavonoids are a few examples of bioactive substances. They have been connected to a lower risk of chronic illnesses like diabetes, cancer, and heart disease and are frequently present in fruits, vegetables, whole grains, nuts, and seeds. To enhance health outcomes, bioactive substances can be isolated and utilized as functional ingredients in food products, dietary supplements, and nutraceuticals (Kour et al. 2022).

By-products of fruit processing, such as pomace, peels, rinds, and seeds, are largely wasted and underutilized. These biowastes are abundant in health-promoting bioactive substances, including vitamins, oils, polyphenols, carotenoids, and other biomolecules. Consequently, it is possible to extract and recycle these bioactive materials from agro-waste to make functional food products that can become more well-liked (Shukla *et al.* 2023).

The interaction stability of whey protein concentrates and pectin with D-limonene, and stated that hydrocolloid used as a carrier agent for D-limonene in nanocomplex formation was widely affected by the pH of the suspension formed. The negative charge of pectin due to carboxylic group at its surface and positive charge of WPC subunits created much stronger force then electrostatic interaction. pH of the final nanocomplex formed also determines the stability, viscosity and colour of the nanocomplex formed for example, at higher pH (9) the maximum stability (98.3%) was noted for the nanocomplex formed whereas, minimum stability (83%) was observed for the low pH (3) (Ghasemi et al. 2018). It is evident that pH widely interferes with the network structure of the nanocomplex. Whereby increment in pH above its isoelectric point accumulates higher number of OH^- groups on the surface of amino acids in comparison to H^+ groups thereby, the negative charges on the protein are dominated. At pH near its isoelectric point, where the overall charge is close to zero formation of spherical aggregates are observed, while for pH far beyond its isoelectric point the formation of linear aggregates are seen. Temperature also plays noteworthy role in texture defining of the final suspension formed for example, the morphology of heat-induced aggregates formed in proteins containing suspensions may vary at pH values, in that case the role of pH is recessive over the temperature (Tang, 2019).

The interdependency between pH and temperature have influences the whole rheological frame of the suspension formed for example, Qiao *et al.* (2023) examined the effect of pH-shifting temperature on structure and property of PNPs formed. The nature of PNPs, and the ability to fabricate and stabilize Pickering emulsion gels was investigated in the Heat-assisted pH-shifting method which is easy to accomplish and relatively safe to fabricate the protein nanoparticle its Improved solubility, interfacial and emulsifying properties by pH-shift. Moreover, proteins are temperature-sensitive at extreme pH conditions, thus thermal treatment could lead to the enhancement of the effect of pH-shifting treatment, such as improved functional characteristics for example, emulsifying, foaming and gelling properties. Heat induced changes in SPI (soy protein isolate) in salt free aqueous solution at neutral pH had varied influence on its rheology as flexible self-similar aggregates were observed during temperature driven gelation of the soy protein at different concentrations (0.3–90 g/L) and temperatures (50–95° C). It was found that at higher temperatures the aggregation rate of Soy globulin increased and at 70°C after 4 days the complete gelation was acquired, also the concentration of the protein strongly influenced the aggregation and gelation rate, with higher concentrations leading to quicker gelation (Chen *et al*. 2016). Enhanced nutrient bioavailability in food preparations, improved antimicrobial effects, targeted delivery and controlled release of bioactive ingredients along the gastrointestinal tract are just a few of the many potential perspectives that nanotechnology offers for food processing.

Encapsulating in a suitable matrix can be a possible solution and inexpensive process to solve these difficulties and limitations. In addition, encapsulating essential oils has many benefits over conventional methods: longer shelf life, enhanced water solubility, bioavailability, reduced strong odour, targeted and sustained release of the EOs, enhanced stability, long-lasting protection, improved water-solubility, and palatability of the encapsulated materials. The scientific community no longer views encapsulation as a revolutionary technology, but there is ongoing discussion regarding the underlying mechanisms of action that control or reduce the infection of food products by microbes (Yousef *et al.* 2024).

Nanoencapsulation holds the potential to improve bioavailability and delivery of bioactive compounds in food products. Nanoencapsulation has been singled out as an effective technique to optimize the stability and maintain the multiple bioactivities of PAs. Moreover, the remarkable sustained release obtained through nanoencapsulation could improve the bio accessibility and bioavailability of PAs. Encapsulation can be accomplished through a variety of methods, including spray drying, freeze-drying, spray cooling and chilling, fluidized bed coating, coacervation, liposome entrapment, co-crystallization, nano emulsion, interfacial polymerization, and molecular inclusion (Banwo *et al*. 2021).

II. Techniques in nanoencapsulation

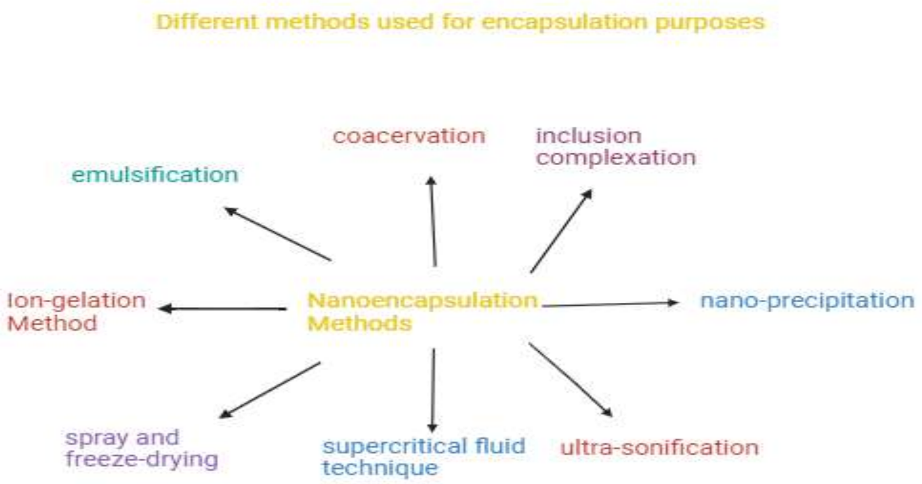

Fig.2. Different methods used for encapsulation purpose

The layer-by-layer method, alternating layers of polymers with opposite charges are sequentially deposited onto a substrate or template. Through covalent attachment or adsorption, bioactive compounds can be integrated into the multilayered structure to generate nonencapsulated particles that allow for fine control over the kinetics of the coating material and bioactive ingredient release into the suspension. Among the most popular polysaccharide-based biopolymers for edible fruit coatings, chitosan is receiving a lot of attention due to its abundance, capacity to form films, biocompatibility, non-toxicity, biodegradability, and potent barrier qualities against the penetration of carbon dioxide and oxygen (Coatings made of gelatine and chitosan can be beneficial in L-b-L coating method for nanoencapsulation for example, in L-b-L coating of the guava fruits following treatments were designed for optimization of wall material for layer by layer coating of the guava fruits with immersed method the following treatment design were used as T_1 (Control treatment); T_2 (chitosan (1.5 % w/v) and glycerol (1.0 % w/v); T_3 (gelatin (5 % w/v) and glycerol (1 % w/v); and T_3 (chitosan (1.5 % w/v) and glycerol (1 % w/v) under gelatin (5 % w/v) and glycerol (1 % w/v), with treatment time 30 s for all treatment, followed by drying under forced ventilation at room temperature (Pereira et al. 2022).

There are various other techniques used in nanoencapsulation such as Supercritical fluid techniques, Coacervation, Emulsification, nanoprecipitation etc., The mentioned techniques depend on feasibility and microchemical behaviour of the coating material and its compatibility with the products on which it is supposed to be coated. In recent years inclination towards the nanoencapsulation of various bioactive compounds are growing with their extraction with different techniques such as ultrasonic assisted bioactive extraction for flavonoids (He et al. 2024), emulsions extraction using Supercritical Fluid Extraction of Emulsions (SFEE) process for the development of nutraceutical products and functional foods (Cerro et al. 2023). Particle size and distribution uniformity is a prerequisite for many nanoencapsulated and microencapsulated procedures to function well. In order to guarantee the ideal design of particle size in both microencapsulated and nanoencapsulated pharmaceuticals, as well as the capacity to regulate drug loading operations at different temperature, pressure, and flow ratio ranges, the use of supercritical carbon dioxide (CO2) has demonstrated encsouraging benefits. In the nanoencapsulation process known as "coacervation," polymers separate into a coacervate phase, which encapsulates the target bioactive molecule.

A few instances of coacervation in nanoencapsulation are as follows: A popular biopolymer for coacervation-based encapsulation is gelatin. For example, when encapsulating bioactive substances such as vitamins or tastes, gelatin can be dissolved in water and subsequently exposed to regulated phase separation via pH or temperature variations. The bioactive substances are captured in the ensuing coacervate phase, which can subsequently solidify to create gelatin nanoparticles. Another biopolymer that's frequently used for coacervation-based encapsulation is gum arabic. It is widely used in the encapsulation of medicines, colors, and flavors. Gum arabic is dissolved in water, and phase separation is induced by adding salts or adjusting the temperature. The bioactive compounds are encased in the coacervate droplets that form, and they can be spray-dried to produce powdered nanoencapsulated particles. The process of creating a coacervate phase in polyelectrolyte complex coacervation entails the interaction of oppositely charged polymers. For instance, to encapsulate bioactive substances like proteins or nucleic acids, alginate, a negatively charged polysaccharide, can form a combination with chitosan, a positively charged polymer. Coacervate droplets are created as a result of the electrostatic interactions between chitosan and alginate. These droplets encapsulate the bioactive ingredients and provide controlled release and protection. Additionally, proteins can coacervate to encapsulate bioactive substances. Whey protein isolate (WPI), for example, can be used to encapsulate hydrophobic or bioactive peptides by coacervation. The beneficial chemicals can be generated in coacervate droplets by varying parameters including pH, temperature, and protein concentration. To stabilize the coacervate droplets and improve encapsulation efficiency, crosslinking agents can be utilized. The process of creating micro- or nanoparticles through the electrostatic interaction of oppositely charged particles—at least one of which is subjected to mechanical stirring—is known as ionic gelation. Because of its favourable biocompatibility, biodegradability, and distinct bioactive release characteristics, chitosan is a biopolymer that has been widely employed to create nanoparticles via ionic gelation. This method of creating nano/microparticles is easy to utilize, economical, and requires less equipment and time than organic solvents. Reversible physical cross-linking, as opposed to permanent chemical cross-linking, prevents potential reagent toxicity and other negative effects (Culas, Popovich and Rashidinejad, 2023).

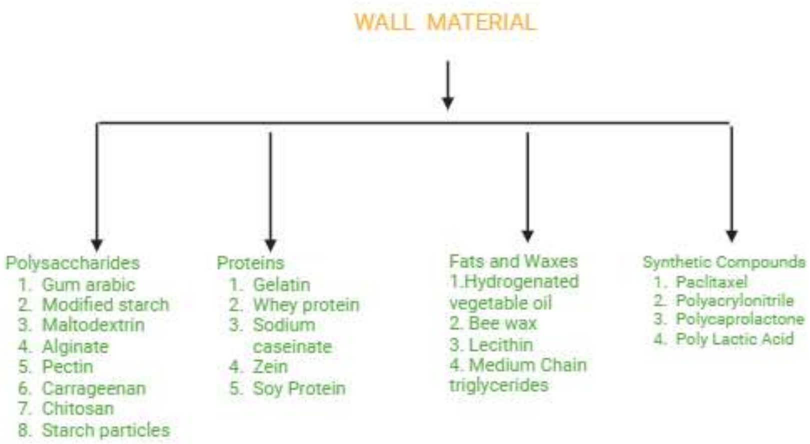

Fig.1.1 Different classes of wall materials used for nanoencapsulation

III. Applications of Nanoencapsulation in Food Products

The wider spectrum on nanoencapsulation has several applications in various fields such as food industry, pharmaceutical and industry cosmetic etc. Nanoencapsulation refers to encapsulation of coating material at the nanoscale range. Whereas Encapsulation is any technological process allowing enclosure of one or more active compounds within an inert material, it has various applications such as protection against harsh environments. Several studies have highlighted the fundamentals of encapsulation in shelf-life increment in several horticultural products i.e., guava (Pereira et al., 2022); (Formiga et al., 2022); and tomato (Ali et al., 2010). Nanostructured based biopolymer coating has been used in securing the safety against covid (Ghosh and Singh, 2022) and for its antimicrobial activity (Kyriakoudi and Tsimidou, 2018), nanoencapsulation has also proven advantages in keeping quality of meat and poultry products (Andrade et al. 2021). The reason nanoencapsulation is widely accepted as efficient and reliable technique in protecting the core material and ultimately releasing and easing the delivery of bioactive compound when required. Some of the most common applications of nanoencapsulation are targeted drug delivery systems the body (Chang et al. 2017) where author forwarded the in oral drug delivery of Leucine in the body with trehalose as the carrier agent for bioactive delivery. Several other applications are mentioned on the table presented below (Table 1.1).

Table. 1.1 Application and coating material used in nanoencapsulation.

Carrier material (wall material)	Product/ Bioactive compound used	Application	Method used	References
chitosan and gelatin	Korean red ginseng	Taste masking and stability improvement	ionic gelation method	Han et al. 2023
maltodextrin (MD), whey proteins isolate (WPI)	pomegranate peel	Shelf-life extension of edible oils		Rashid et al. 2022
-	grapefruit peel	improving oxidative stability of mustard oil	Response surface methodology	Nishad et al. 2021
Gum Arabic, Lecithin	Eugenol oil	Antimicrobial activity and shelf-life extension	Spray drying gas method	(Kyriakoudi and Tsimidou, 2018)
Pectin, gum arabic, alginate, carboxymethyl cellulose and carrageenan	Polysaccharide-coated solid lipid nanoparticles	Antimicrobial activity and shelf-life extension	Spray drying technology	Veneranda et al. 2018
Caseinate-zein-polysaccharide	curcumin	Improving bioactive delivery	Spray drying technology	Feng et al. 2011
Trehalose	Leucine	Improving oral delivery of drugs	Spray drying technology	Chang et al. 2017
Zein, sodium caseinate, Pectin	Eugenol	Antimicrobial properties and bioactive delivery	Spray drying technology	Wang et al. 2016
Maltodextrin	Saffron extracts	evaluation and implication of drying technologies	Spray drying technology	Hu et al. 2016

III. Bioactive Compounds Encapsulated by Nanotechnology

Nano-encapsulation is a promising technology to protect bioactive compounds and could be suitable for delivering such protected compounds to target biological systems. Bioactives of various kinds having potential antioxidant property (Nishad et al. 2021), antimicrobials properties (Yousef et al., 2024), taste masking in Korean red ginseng (Han et al. 2023) are frequently employed in nanoencapsulation. In order to improve the nutritional content and health advantages of foods and nutraceuticals and improving the bioactive delivery and oral drug delivery these components are beings used extensively. In food and nutraceutical applications, nanoencapsulation materials are commonly based on carbohydrates, proteins, or lipids for example various formulations have been used for this purpose as indicated on table1.1. For food and nutraceutical applications, nanoencapsulation offers a number of potential advantages, including as increased transport to targeted body locations, better control over the release of bioactive chemicals, and higher stability and solubility of such compounds for example to Improving the solubility of silymarin the comparative studies were performed where nanoencapsulated and non-nanoencapsulated silymarin with water-soluble chitosan (WCS) and poly-gamma-glutamic acid (Gamma-PGA) were compared after lyophilization at concentration of 0.75 mg/mL containing 8.3% ethanol, the solubility for both were analysed in 8.3% ethanol the result signifies no significant difference in solubility of silymarin in 8.3% ethanol despite nanoencapsulation of silymarin, after redispersion in DW, the reconstituted silymarin nanoparticle suspension showed significantly higher (7.7-fold) solubility than the non-nanoencapsulated silymarin/DW suspension. Author propounded a through justification as with decrease in molecular mobility of silymarin the surface area exposed to dissolution media increased causing improved solubility of silymarin (Lee et al., 2017). The bioactive compounds composition and techno-functional properties of amaranth protein can induce its solubility, emulsification, gelation, foaming, and binding properties and holds potential for nanoparticle-based delivery systems for bioactive compounds (Hadidi et al., 2024). Thus, it can be said that encapsulation can improve bioavailability of such bioactive compounds as it provides outer shielding which protects core material from environmental factors and allow controlled and precise release of bioactive compounds in gastrointestinal tract allowing bioavailability and biostability of bioactive in the body. In addition, it may prolong the shelf life of food items and enhance the sensory qualities. From food processing to packaging, nanotechnology-assisted bioactive component encapsulation offers a number of clear benefits, including increased stability and bioavailability. Additionally, it promotes health by shielding and regulating the release of bioactive substances. To encourage the safe commercialization of novel nanotechnology goods that are health-beneficial, international laws must be drafted in order to better understand the safety and toxicity of these nanomaterials. Because they are hydrophobic, the majority of bioactive chemicals have limited solubility even though they are thought to be essential to human health. Phenolic chemicals, essential oils, essential fatty acids, and carotenoids. The above-discussed low stability and bioavailability of these compounds are the main barriers to their use in the food and pharmaceutical industries. Bioactive compounds encapsulated in pectin-based nanostructures may elude the microbiota's breakdown and remain protected because the gut bacteria progressively break down the pectin surrounding the nanoparticles. The encapsulation of essential oils, bioactive compounds, and functional components at the nanoscale has been shown to increase component uptake during digestion and the physical stability of the chemicals entering and exiting the stomach (Rashidinejad and Jafari, 2020).

Table.1.2. Bioactive compounds and its applications

S.No.	Bioactive Compound	Application
1	Folic acid	High encapsulation efficiency (83.9%). Improved folic acid stability under different storage conditions over 60 days.
2	Caffeine	a-Lactalbumin nanotubes synthesized in the presence of Mn2+ were highly stable during freeze-drying. a-LA nanotubes were very efficient at caffeine encapsulation (around 100%). Low temperature and high pH prevented caffeine release from a-LA nanotubes.
3	Tea tree oil	The nanofibers were plasma-treated to modify their surface; after plasma treatment, the release efficiency of nanofibers and the antibacterial activity of tea tree oil improved. The nanofibers increased the shelf life of beef.
4	Fish oil and ferulic acid	Zein nanofibers exhibited nanoscale size of 440 nm and showed high encapsulation efficiency (94%) and loading capacity of 20%. Loading ferulic acid into the nanofibers enhanced the oxidative stability of encapsulated fish oil. Fish oil loaded nanofibers showed an excellent release profile under gastrointestinal and enzymatic conditions
5	Vitamin E	Revealed mean particle size around 86 nm; high encapsulation efficiency of near 100%; improved antioxidant and antimicrobial activity
6	Curcumin	SLNs and NLCs both exhibited high loading capacity. SLNs revealed excellent stability under storage conditions and in simulated gastrointestinal conditions
7	a-Tocopherol	Niosomes were found on the nanometer scale (106.8–190 nm). The optimum condition for the encapsulation of a-tocopherol enhanced stability, encapsulation efficiency, and prolonged release.

Adapted from (Bazana et al., 2019).

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