Application of inulin for the formulation and delivery of bioactive molecules and live cells

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ABSTRACT Inulin is a fructan biosynthesized mainly in plants of the *Asteraceae* family. It is also found in edible vegetables and fruits such as onion, garlic, leek, and banana. For the industrial production of inulin, chicory and Jerusalem artichoke are the main raw material. Inulin is used in the food, pharmaceutical, cosmetic as well biotechnological industries. It has a GRAS status and exhibits prebiotic properties. Inulin can be used as a wall material in the encapsulation process of drugs and other bioactive compounds and the development of their delivery systems. In the review, the use of inulin for the encapsulation of probiotics, essential and fatty oils, antioxidant compounds, natural colorant and other bioactive compounds is presented. The encapsulation techniques, materials and the properties of final products suitable for the delivery into food are discussed. Research limitations are also highlighted.

1. Introduction

Inulin is a linear polydisperse fructan composed of β-(2 → 1) linked fructofuranosyl units normally, but not necessarily terminated by a glucopyranose residue through a sucrose-type linkage (Fig. 1) (Mensink et al., 2015a). Inulin is found mainly in plants of the *Asteraceae* family, such as globe artichoke, Jerusalem artichoke, chicory, and dahlia. Edible vegetables and fruits such as onion, garlic, leek, and banana are also known as a source of inulin. For the industrial production of inulin, chicory and Jerusalem artichoke are mainly used (Bhanja et al., 2022; Kaur & Gupta, 2002). The degree of inulin polymerization (DP) varies from 2 to 60. Inulin with a DP not higher than 10 is referred to as oligofructose (Niness, 1999). However, DP depends on the source of inulin, the time of plant harvest, storage conditions, and inulin processing conditions (Krivorotova & Sereikaite, 2014; Krivorotova & Sereikaite, 2018; Mensink et al., 2015a). Inulin is generally recognized as safe (GRAS status) by the United States Food and Drug Administration, and a daily intake of up to 10 g is well tolerated by healthy people (Bonnema et al., 2010; Coussement, 1999). It serves as a prebiotic, *i.e.*, it is a non-digestible polysaccharide by human gastrointestinal enzymes, and is fermented by colon bacteria. Inulin stimulates the growth of colon bacteria like bifidobacteria associated with human well-being (Holscher, 2017; Roberfroid, 2007). In addition, all other health benefits attributed to inulin consumption are summarized in Fig. 2 (Gupta et al., 2019; Shoaib et al., 2016; Tawfick et al., 2022). Inulin finds its application in various fields, primarily in the food industry (Fig. 3) (Bhanja et al., 2022; Chi et al., 2011; Jackson et al., 2022; Mensink et al., 2015b). For cosmetic formulations, chemically modified inulin is being investigated. Carboxymethyl or quaternized inulin can serve as humectants and substitute hyaluronic acid (Bhanja et al., 2022). Hydrophobically modified inulin is a suitable emulsifier for anti-aging ingredients formulation and other applications in cosmetics and personal care (Han et al., 2020; Kokubun et al., 2018; Yang et al., 2022). In industrial biotechnology, for biofuel production, inulin-rich feedstocks are usually used (Sing et al., 2022). Over the past ten years, inulin has found its application in micro/ nanotechnology. Natural or hydrophobically modified inulin particles can be produced by the self-assemblage and applied in various biological processes as nanostructured prebiotics, as an example, to stimulate the production of pediocin in *Pediococcus acidilactici* (Jimenez-Sanchez et al., 2019; Kim et al., 2018). On the other hand, inulin alone or together with other biopolymers can be used as a wall material for the encapsulation of bioactive compounds and the development of their delivery systems. In addition to the term “wall material”, others such as the matrix, carrier, shell, or encapsulant are also used. The encapsulation process allows the protection of the bioactive substance from adverse environmental conditions. Moreover, encapsulation can improve the physicochemical and biological properties of bioactive molecules and ensure their controlled release. The micro/nanoencapsulated bioactive substances are used in various fields such as medicine, the pharmaceutical and food industry, and agrochemistry (An et al., 2022; Katouzian & Jafari, 2016; McClements & Ozturk, 2022; Pisoschi et al., 2018). The application of inulin for drug delivery was largely reviewed in recent publications (Afinjuomo et al., 2021; Giri et al., 2021). In this paper, the use of inulin for the encapsulation of bioactive compounds and probiotics for their delivery into food products is reviewed. Functional foods being consumed as part of the normal diet improve the health and well-being and reduce the risk of diseases. To increase the functionality of food, the addition of bioactive ingredients attracts the growing interest and promotes the development of new formulations.

2. Inulin as a wall material for the encapsulation of bioactive ingredients

2.1. Probiotics The interest of consumers in food products functionalized by probiotics is increasing. Nowadays, the role of the gut microbiota in human well-being and the prevention of some diseases is well known and is being extensively investigated (Fan & Pedersen, 2021; Markowiak & Slizewska, 2017). The beneficial effect on human health is provided if there is at least 106 CFU per g or mL of viable cells at the time of product consumption. However, the viability of probiotics is reduced during their passage through the human gastrointestinal tract. Probiotics exhibit high susceptibility to the acid environment and bile salts. In addition, there is a need for their protection during food processing, storage, and handling. Microencapsulation is a modern method for improving probiotic viability and facilitation of their application in functional foods (Terpou et al., 2019). Spray-drying is the most common method for encapsulation of probiotics using inulin or its mixture with other biomaterials (Table 1). In general, spray-drying is popular for probiotics encapsulation because it is a low-cost, rapid processing, and easy to scale up method. Spray-drying results in a product that is simple for storage, transportation, and application in functional foods (Sharma et al., 2022). However, spray-drying has a disadvantage as a result of the high temperature used in the process. To protect cells from damage, the choice of carrier is of primary importance. Inulin is used in the combination with proteins, carbohydrates, and natural gums (Table 1). The encapsulation efficiency generally varies from 84.4 to 95.5 %. Since the authors use different processing conditions, *i.e.*, inlet and outlet air temperature it is difficult to compare the protective effects of wall materials (Dias et al., 2018; Nunes et al., 2018; Pinto et al., 2015a; Rosolen et al., 2019). Moreover, different species and strains of probiotic bacteria used in the experiments make the comparison impossible. Bustamante et al. (2020) tested the mixture of inulin and maltodextrins for the encapsulation of *Lacticaseibacillus rhamnosus* at the three different inlet temperatures of 90, 110, and 130 ◦C. The encapsulation efficiency decreased from 78.47 % at 90 ◦C to 58.14 % at 130 ◦C (Bustamante et al., 2020). On the other hand, even at the higher inlet temperature of 160 ◦C using the inulin and gum Arabic mixture for *Lactobacillus rhamnosus* HN001, and the inulin, gum Arabic, and whey protein isolate mixture for *Limosilactobacillus reuteri* DPC16, the encapsulation efficiency was higher and equal to 85 and 93.97 %, respectively (Barajas-Alvarez et al., 2022; Wang & Mutukumira, 2022). For *Bifidobacterium-BB-12*, the use of the mixture of whey and inulin resulted in a higher encapsulation efficiency of 93.55 % compared to the mixture of inulin and polydextrose when encapsulation efficiency of 88.30 % was found (Pinto et al., 2015b). Water activity (aw) is another very important characteristic of spray-dried powders. It is assumed that aw has to be lower than 0.25 to ensure the longest shelf life of encapsulated probiotics (Terpou et al., 2019). In most cases, including inulin as an alone wall material, this parameter is achieved (Avila-Reyes et al., 2014; Pinto et al., 2015a; Wang & Mutukumira, 2022). However, the blend of inulin with gum Arabic resulted in almost two times higher aw (Barajas-Alvarez et al., 2022). Inulin, as an encapsulant of probiotics, is also used in electrospinning. The modern encapsulation technique has some advantages since it does not involve organic solvents, the process is simple and performed at room temperature, and food-grade biopolymers can be used for nanofibers formation (Ghorani & Tucker, 2015; Wen et al., 2017). However, the number of publications related to probiotic encapsulation in nanofibers using inulin is limited. Duman and Karadag (2021) encapsulated *Lactobacillus fermentum* in nanofibers composed of poly (vinyl alcohol) (PVA), sodium alginate, and inulin of different degrees of polymerization. The encapsulation efficiency ranged from 74.26 to 80.63 %. The average diameter of bacteria-loaded nanofibers was about 400 nm. Nanofibers without bacteria were uniform in morphology with a size of approximately 200 nm. Mojaveri et al. (2020) fabricated

Bifidobacterium animalis Bb12 loaded nanofibers based on chitosan, PVA, and inulin. They were nonuniform and noncontinuous with an average diameter of 117 nm. In another study, electrospraying was used. Double–layered Ca - alginate/chitosan microcapsules containing inulin inside were prepared with an encapsulation efficiency of 79 % for *Lactobacillus* and *Bifidobacterium*. However, the size of the microcapsules was large and equal to 830 μ m (Zaeim et al., 2019). Besides, the addition of inulin was evaluated and the microcapsules were fabricated by extrusion - external gelation techniques. Inulin was used in combination with alginate (Poletto et al., 2019) or with the mixture of alginate and Persian gum (Nami et al., 2020) or alginate and goat milk (Prasanna & Charalampopoulos, 2019). In all studies, the encapsulation efficiency was higher than 90 %, the size of particles ranged from 91.58 μ m using only IN-alginate (Poletto et al., 2019) to 460–560 nm using the mixture of IN, alginate and Persian gum (Nami et al., 2020). *L. lactis* encapsulated in the last mixture was successfully applied in the preparation of probiotic-carrier orange juice. Encapsulated L. *lactis* showed high storage viability depending on the concentration of IN under the storage at 4 ◦C for 180 days while the viability of free cells decreased from 9.84 to 2.98 log CFUg⍰ 1. Higher concentration of inulin (up to 2 %) ensures the denser and stronger structure of matrix and the better protection of the probiotic. On the other hand, inulin, being a prebiotic, stimulates the viability of cells (Nami et al., 2020). The encapsulation of *Bifidobacterium animalis* subsp. *lactis* using the mixture of IN, alginate and goat milk resulted in the large particles up to 3.41 mm. Moreover, the size of capsules depended on the concentration of inulin (Prasanna & Charalampopoulos, 2019). The prepared capsules were used for the functionalization of yogurt. During the storage at 4 ◦C for 28 days, the viable count of encapsulated cells did not decrease below 6 log CFUg⍰ 1. The authors revealed that 1 % of inulin could be used to increase the survival rate of encapsulated probiotics. To improve the survival of probiotics, alginate-inulin beads obtained by ionic gelation can be additionally coated with chitosan (Darjani et al., 2016; Krasaekoopt & Watcharapoka, 2014) or skim milk (Wang et al., 2016). To be suitable for application, microencapsulated probiotic bacteria must exhibit long-term stability. Usually, viability is analysed at the ambient temperature of 20 or 25 ◦C, under refrigeration at 4–7 ◦C and below freezing at ⍰ 18 or - 20 ◦C (Table 2). To reduce the cost of storage, the viability of encapsulated probiotics at the ambient temperature is very desirable. The storage period of encapsulated probiotics at 20 or 25 ◦C is different in various studies. In addition, encapsulation techniques, bacteria, and encapsulants are different (Table 2). *L. lactis* encapsulated by spray drying using IN in the combination with whey was viable (>8 log CFUg⍰ 1) for 6 months at 25 ◦C (Rosolen et al., 2019). *Lb*. *acidophilus* encapsulated by the emulsification-internal gelation method using the mixture of IN and pectin was viable for 4 months (>6 log CFUg⍰ 1) at the same temperature (Raddatz et al., 2020). On the contrary, the viable count of bifidobacteria in nanofibers composed of poly (vinyl alcohol), alginate and inulin and produced by electrospinning was below the recommended level after 35 days at 25 ◦C (Duman & Karadag, 2021). Spray-dried bifidobacteria using the mixture of inulin, maltodextrin and passion fruit juice was not suitable for application after 15 days (< 6 log CFUg⍰ 1) (Dias et al., 2018). Pinto et al. (2015a) spray-dried bifidobacteria using whey in the presence and absence of inulin and found that inulin had no effect on cell viability under the storage at 4 and - 20 ◦C. Microencapsulation improves the functionality of probiotics in the gastrointestinal tract (Champagne et al., 2018). The higher survival of encapsulated cells during the passage through the gastrointestinal tract compared to free cells demonstrates the need for that approach. Some studies report that the use of inulin in combination with other wall materials strengthens the effect of microencapsulation. The addition of inulin improved the survival of *Lactococcus*. *lactis* in alginate-Persian gum beads under gastrointestinal stress by 44–52 % depending on its concentration (Nami et al., 2020). The same effect was observed for *Bifidobacterium animalis* subsp. *lactis*. The enrichment of the alginate-goat milk matrix with inulin increased cell count under simulated gastric conditions (Prasanna & Charalampopoulos, 2019). The viability of *Lactobacillus plantarum* encapsulated in skim milk coated alginate-inulin beads did not change during 2 h of incubation under acidic gastric conditions (Wang et al., 2016). Biopolymer microgels are highly porous, and probiotics are ineffectively protected from gastrointestinal juice. Inulin is supposed to ensure higher cell protection by increasing the density of beads (Nami et al., 2020; Yao et al., 2020). The effect of inulin depends on the encapsulation method. Its addition to electrospun PVA-sodium alginate fibers did not significantly increase the survival of *Lactobacillus fermentum* and *Bifidobacterium animalis* subsp. *lactis* compared to fibers without inulin (Duman & Karadag, 2021; Mojaveri et al., 2020). Zaeim et al. (2019) compared the viability of *Bifidobacterium lactis* encapsulated in PVA-calcium alginate microcapsules having inulin or resistant starch by electrospraying and found that under simulated gastrointestinal conditions the addition of resistant starch results in more effective protection. *2.2. Essential and fatty oils* Essential oils find application in the food industry as aromatic ingredients and substitute chemical additives. Their use increases due to the customer demands for natural, healthy, and safe food. In addition, essential oils exhibit antimicrobial and antioxidant properties. They are extracted from plants, *i.e.*, leaves, seeds, fruits, flowers, and roots. The composition of essential oils is complex. They are a mixture of various volatile compounds that are degradable under the influence of oxygen, temperature, and light (Kant & Kumar, 2022). To preserve the efficiency and biological potential of essential oils and to prevent changes in their chemical composition due to oxidation or volatilization during their application, delivery systems based on encapsulation are developed (Bakry et al., 2016). Emulsification and subsequent spray-drying is the main technique for essential oil encapsulation using inulin as a wall material (Table 3). Since inulin has poor emulsification properties, it is used with other compounds such as gum Arabic, whey protein isolate, chitosan, or modified starch (Table 3). The application of inulin as a wall material allows partial replacement of expensive and limited-supply materials such as gum Arabic (Prasad et al., 2022). Wall materials are an important factor in determining the encapsulation efficiency and physicochemical properties of final powdered products (de Barros Fernandes et al., 2016b). As seen, the encapsulation efficiency varies from 29.5 % to 91.0 % (Table 3). Noghabi and Molaveisi (2019) demonstrated that for cinnamon essential oil the encapsulation efficiency depended on wall material constituents and the composition ratio. For rosemary essential oil, the modified starch and inulin blend was more effective than the gum Arabic and inulin blend (de Barros Fernandes et al., 2014). Inulin serves as a matrix former and filling agent, but the encapsulation efficiency of essential oil is not always higher compared to other carbohydrates as wall materials. As an example, the encapsulation efficiency of ginger essential oil is 48.14 % using the blend of whey protein isolate and inulin as a wall material and 61.64 % using whey protein isolate and maltodextrin (de Barros Fernandes et al., 2017). The encapsulation efficiency of the essential oil of ginger was almost two times higher using the blendof gum Arabic and maltodextrin compared to the one of gum Arabic and inulin (de Barros Fernandes et al., 2016b). Silva and Meireles (2015) encapsulated annatto oil by emulsification ‑ freeze-drying using inulinof different DP (≥10 and ≥ 23) as the only wall material. The authors found that the higher DP of inulin resulted in the formation of microparticles with the higher encapsulation efficiency of ~42 % compared to ~27 % in the presence of inulin with DP ≥10. The use of inulin in essential oil formulations improves the wettability of particles. That physical property is related to the reconstitution time of powders and is desirable to be quick (Campelo-Felix et al., 2017). Inulin having a large number of hydrophilic groups improves the adsorption of water. As an example, the wettability time of spray-dried chitosan powders carrying coriander essential oil is 332 s while its blending with inulin results in a shorter wettability time of 112 s (Dima et al., 2016). In the case of rosemary essential oil, the replacement of inulin by maltodextrin in the mixture with gum Arabic increased the wettability time three times, although the encapsulation efficiency of the oil was significantly higher in the presence of maltodextrin (de Barros Fernandes et al., 2014). For ginger essential oil encapsulation, the use of cashew gum and inulin at the ratio of 1:3 resulted in the short powder wettability time of 91 s, but the encapsulation efficiency was very low (de Barros Fernandes et al., 2016a). Essential oils, encapsulated in the matrix containing inulin, maintain their antioxidant activity as exemplified by lime, cinnamon, or oregano oils (Beirao da Costa et al., 2012; Campelo-Felix et al., 2017; Noghabi & Molaveisi, 2019). During the storage, the loss of antioxidant activity is lower compared to the pure oil. The antioxidant activity of cinnamon essential oil encapsulated in gum Arabic-maltodextrin-inulin matrix decreased approximately by 55 % under the storage for 30 days at 25 ◦C while its reduction for the pure oil was approximately 80 % (Noghabi & Molaveisi, 2019). Campelo-Felix et al. (2017) found that the maintenance of antioxidant activity depended on the DP. After 42 days of storage, the antioxidant activity of lime essential oil was higher in the matrix containing inulin with higher DP. Encapsulated essential oils as natural antimicrobials could be an alternative to chemicals. Oregano essential oil encapsulated in inulin by emulsification and following spray-drying exhibited the antimicrobial activity against food pathogenic bacteria such as E. coli, S. aureus and L. monocytogenes (Beirao da Costa et al., 2012). To enhance antimicrobial activity, oregano oil can be co-encapsulated with phenolic compounds such as resveratrol. The emulsion was prepared using sodium caseinate and inulin and ensured lower loss of essential oil and resveratrol during the storage compared to the system stabilized only by sodium caseinate. As suggested, inulin additionally stabilizes the emulsion by interaction with protein and forms a barrier to physically prevent the oxidation of antimicrobials. The higher retention of bioactive compounds resulted in the higher antimicrobial activity (Ai et al., 2022). For the application of encapsulated essential oils in the food industry, the burst release of aroma compounds is a very important factor. This effect was observed for oregano essential oil entrapped in inulin (Beirao da Costa et al., 2013) or for cinnamon essential oil encapsulated in gum Arabic, maltodextrin and inulin blend (Noghabi & Molaveisi, 2019). Noghabi and Molaveisi (2019) found that the release of cinnamon essential oil was driven by the Fickian diffusion mechanism. The release of lime essential oil in the WPI matrix followed the same mechanism; however, the addition of the prebiotic to the encapsulation matrix resulted in non- Fickian or anomalous transport (diffusion-swelling controlled process) (Campelo-Felix et al., 2017). The same mechanism is characteristic for the release of coriander essential oil from chitosan-inulin microcapsules (Dima et al., 2016). To improve organoleptic properties and prevent the deterioration of fatty oils with nutritional values, encapsulation is also the first choice.Inulin was used for the formulation of black cumin (Santiworakun et al., 2022), chia (Razavizadeh & Tabrizi, 2021; Razavizadeh et al., 2022), pequi (Oliveira et al., 2018), buriti (de Oliveira et al., 2022), Camelina sativa (Kanclerz et al., 2019), flaxseed (Koume et al., 2023), algal (Wang et al., 2020), canola (Ortiz-Deleon et al., 2023), olive-based diacylglycerol (Guo et al., 2023) and fish oil, which has been known for a long time and is widely used (Botrel et al., 2014b; Botrel et al., 2014a; Bakry et al., 2017; Nawas et al., 2019). Usually, fatty oils are encapsulated using the blend of inulin with other wall materials such as gum Arabic, whey protein isolate, maltodextrin or with the mixture of soy protein isolate and maltodextrin. The possibility of replacing Arabic gum by Brea gum in the mixture with inulin was demonstrated (Castel et al., 2018). Brea gum has physicochemical and functional properties similar to those of gum Arabic (Castel et al., 2017). Instead of soy protein isolate, other plant-derived proteins such as pea protein isolate can be used in combination with inulin (Le Priol et al., 2022). Hydroxypropyl-inulin was also developed as a novel encapsulation agent (Encina et al., 2021). Emulsification - freeze-drying and emulsification – spray-drying are the most often used techniques. Encapsulated oil can be used in aqueous media. The addition of inulin improves the wettability of the microcapsules. As an example, the wettability time of black cumin oil microcapsules using gum Arabic as a wall material is 52.88 min, while using the gum Arabic-inulin blend, the time is 10.52 min (Santiworakun et al., 2022). The highest solubility (88.26 %) and the lowest wettability time (130 s) of microencapsulated pequi oil powders was found using whey protein isolate-inulin mixture in comparison with whey protein isolate (81.76 % and 424 s, respectively) or its mixture with maltodextrin (82.4 % and 193 s, respectively) (Oliveira et al., 2018). For algal oil encapsulation, the blend of inulin with octenyl-succinic anhydride starch (IN/OSA) or ternary systems composed of inulin - OSA starch - chitosan (IN/OSA/CS) and inulin - OSA starch – maltodextrin (IN/OSA/ MD) was used as wall materials. The lowest wettability time (66 s) was found for IN/OSA and the highest one (287 s) – for IN/OSA/CS. However, the last ensured the highest solubility (96.46 %) and encapsulation efficiency (98.57 %) (Wang et al., 2020). IN/OSA and the ternary system IN/OSA/WPI were also used for olive-based diacylglycerol oil encapsulation (Guo et al., 2023). As in the previous case, mentioned above, the ternary system significantly improved the solubility (86.11 %) compared to IN/OSA coated microcapsules (76.22 %). In the case of IN/ OSA, the microcapsule wettability time was 81.5 s and slightly increased with the IN/OSA/WPI system (84.5 s). For fish oil encapsulation, other encapsulation methods were also developed. Rios-Mera et al. (2019) optimized the encapsulation of fish oil by complex coacervation. Soy protein isolate and inulin were used as wall materials. To increase the pH and heat stability of the complex coacervate, additional treatment using transglutaminase was applied and ensured encapsulated oil retention of 81 %. Canola oil was encapsulated in the oil-in-water emulgel system stabilized with whey protein isolate-alginate-inulin ternary complex coacervate and used as a fat replacer in yogurt (Ortiz-Deleon et al., 2023). To increase nutritional value, fish oil can be encapsulated in combination with fish protein hydrolysate known for its anti-hypertension activity (Yathisha et al., 2019). Double emulsion of water-in-oil-in-water was developed using whey protein concentrate complex with inulin or both inulin and fucoidan and finally freeze-dried (Jamshidi et al., 2018, 2019). As demonstrated (Jamshidi et al., 2020), high-pressure homogenisation increased the stability and encapsulation efficiency of the double emulsion containing fish oil and fish protein hydrolysate encapsulated within a complex of whey protein concentrate and inulin compared to the sample without high-pressure homogenisation. Natural yogurt was fortified with microcapsules, and sensory analysis revealed that consumers noticed a lower fishy flavour using the whey protein concentrate-inulin microparticles compared to the whey protein concentrate-inulin-fucoidan or whey protein concentrate-fucoidan microparticles (Jamshidi et al., 2018). There are some examples when fatty oils are co-encapsulated with other lipophilic compounds. Soybean oil was enriched with antioxidants from Thai rice germs and encapsulated by complex coacervation using soy protein isolate and inulin as wall materials.

Oil-loaded nanoparticles were fabricated due to electrostatic interactions between soy proteins and inulin molecules (Wangsuntornpakdee et al., 2023). Tuna oil and mint essential oil was co-encapsulated by an emulsification - spray-drying process, using whey protein isolate and inulin as the encapsulants. During storage, the loss of eicosapentaenoic and docosahexaenoic acids was slightly less compared to microcapsules without mint oil. The protective effect could be related to the antioxidant activity of essential oil (Bakry et al., 2017). Prevention of fatty oil oxidation is one of the most important goals of encapsulation. Le Priol et al. (2022) demonstrated that pea protein/ inulin wall material provided more efficient protection of sunflower oil against oxidation compared to pea protein. The positive effect of inulin was also confirmed for *Camelina sativa* oil. After seven days of storage, the degree of oil oxidation in microcapsules with inulin was 5.92 eq/kg compared to 41.02 eq/kg for unencapsulated oil and 16.11 eq/kg for oil encapsulated with pectin. However, gum Arabic ensured slightly better oxidative protection (4.52 eq/kg) compared to inulin (Kanclerz et al., 2019). After 60 days storage, the peroxide value of fish oil encapsulated with the blend of modified starch and inulin was 78.18 % less compared to unencapsulated oil and was equal to 17.93 meq/kg. The use of the blend of gum Arabic or modified starch with maltodextrin resulted in a peroxide value of 34.37 meq/kg and 26.30 meq/kg, respectively (Nawas et al., 2019). *2.3. Antioxidant compounds* Table 4 summarizes the applications of inulin in the design of antioxidant delivery systems. As seen, for the encapsulation experiments, plant extracts or even juice are mainly used. Some papers have also considered the encapsulation of individual antioxidant compounds (Amjadi et al., 2022; Goelo et al., 2020). Plant extracts are generally used to enhance food taste, aroma and color (Liang et al., 2022; Rodriguez-Mena et al., 2023; Serrano et al., 2020; Zang et al., 2022). Moreover, plant extracts are a source of different bioactive molecules such as ascorbic acid, betalains, carotenoids and various phenolic compounds (Kuhn et al., 2020; Nguyen et al., 2022; Sakulnarmrat et al., 2022; Wyspianska et al., 2017) that exhibit health-improving properties. Therefore, they can be used for food fortification and the development of functional foods (Kandyliari et al., 2023). Consumption of foods rich in antioxidants can reduce the risk of chronic degenerative diseases (Araujo-Diaz, Leyva-Porras, Aguirre-Banuelos, Alvarez-Salas, & Saavedra-Leos, 217). Many plant extracts find the application in traditional folk medicine (Daza et al., 2016; Lahlou et al., 2022; Mahendran et al., 2021). Inulin as an encapsulant of antioxidants is mostly used for spray-drying (Table 4). Other methods of antioxidant encapsulation using inulin such as extrusion - gelation, freeze-drying, or entrapment into liposomes are rarely found in the scientific literature. They are used mainly for comparison of the effectiveness of the spray drying process and the properties of the final product (Mar et al., 2020; Nguyen et al., 2022; Sturm et al., 2019; Tkacz et al., 2020). Inulin is used alone or in combination with other encapsulants. For spray-drying, maltodextrin is the most common choice. (Table 4). Full or partial replacement of conventional coating materials with inulin, as dietary fibre, can add additional value to the final product (Pettinato et al., 2017). The application of free plant extracts in food matrices presents some limitations. Antioxidant compounds can have low solubility in water or an unpleasant and bitter taste. These drawbacks can be overcome by using encapsulation. Moreover, the most important problem that encapsulation helps solve is increasing the stability of antioxidants during food processing and storage. As exemplified, the antioxidant activity of the pineapple peel extract encapsulated by spray-drying using inulin as a wall material did not change significantly after six months of storage (Lourenco et al., 2020). The addition of hawthorn or onion husk extract encapsulated in inulin by spray-drying to the recipe of wheat bread resulted in the bread with the antioxidant activity of 120 and 125 μ mol of Trolox equivalent/DW, respectively (Czubaszek et al., 2021). The recovery of antioxidants and the properties of the final encapsulated product including the antioxidant activity depend on the plant extract used, the concentration of inulin or in combination with other biopolymers and the encapsulation method (Table 4). On the other hand, the choice of inulin as wall material is not always the best. Indeed, Daza et al. (2016) demonstrated that the encapsulation of Cagaita fruit extract by spray-drying using gum Arabic resulted in the longer stability of total phenolics compared to the use of inulin. The effect of encapsulation in inulin on antioxidant bioaccessibility was investigated for some compounds. To this aim, oral, gastric and small intestine conditions were simulated *in vitro*, as bioaccessibility is defined as the fraction of antioxidants released from the matrix in the gastrointestinal tract and available for absorption (Gonzalez et al., 2020). For anthocyanins of maqui juice, encapsulation in inulin resulted in 10 % increase of bioaccessibility compared to free anthocyanins, with bioaccessibility in the range 19.6–85.7 % depending on the specific compound (Fredes et al., 2018). This result was attributed to the protective effect of the wall material. Encapsulation of betacyanins from Bougainvillea bracts extract led to a complete release in the gastric environment where these compounds are stable, while degradation of these compounds was not completely prevented under small intestine conditions, where they undergo alkaline hydrolysis. Hence, at the end of the intestinal phase only 40 % of encapsulated betacyanins were bioaccessible (Kuhn et al., 2022). Free oleuropein formulated into a starchy matrix was completely released at the end of simulated digestion, while oleuropein encapsulated in inulin before formulation in the starchy matrix, showed 60 % bioaccessibility. However, the remaining fraction of oleuropein was not degraded, instead it was retained in the wall material. The presence of a residual amount of encapsulated oleuropein was considered advantageous, since it was assumed that encapsulated oleuropein can reach the colon and then be released and adsorbed (Pacheco et al., 2018). In a further study, the evolution of release of free oleuropein and oleuropein encapsulated either in inulin or in maltodextrin was studied by simulated *in vitro* digestion, followed by simulated colon fermentation (Gonzalez et al., 2020). Upon application of either maltodextrin or inulin as wall materials, the content of oleuropein at the end of simulated colon digestion was higher compared with free oleuropein, with bioaccessibility of 15, 12 and 1.5 %, for maltodextrin-oleuropein, inulin-oleuropein and free oleuropein, respectively. Moreover, oleuropein metabolites, *i.e*., oleoside 11 methyl ester and oleoside were found at the end of *in vitro* digestion of the encapsulated systems, but they were not found upon *in vitro* digestion of free oleuropein. This result was attributed to a protective role of the non-covalent polysaccharides-oleuropein complexes. The bioaccessibility of ß-carotene from mango peel was also increased by inulin used as wall material in combination with maltodextrin and gum Arabic. In fact, the bioaccessibility of ß-carotene was 47.7 % in presence of inulin and 28.2 % when only maltodextrin and gum Arabic were used (Cabezas-Teran et al., 2023). Inulin was also effective in improving the bioaccessibility of bioactive antioxidants from Amazonian berry when used in combination with alginate (Mar et al., 2021). Limited studies have investigated the effect of antioxidant encapsulation in inulin on their potential bioavailability. Potential bioavailability was defined as the fraction of antioxidant recovered upon simulated gastric digestion and colon fermentation by tangential filtration through a ceramic microfiltration membrane (Gonzalez et al., 2020). By this approach, after *in vitro* digestion of oleuropeinencapsulated in inulin, potential bioavailability was not detected for oleuropein, probably due to its polarity that limits diffusion, while its metabolites were found to be potentially bioavailable (Gonzalez et al., 2020). Following assessment of bioaccessibility of ß-carotene from mango peel, prediction of bioavailability was performed by studying the uptake of ß-carotene by the Caco-2 cells. Interestingly, inulin resulted in a higher ß-carotene uptake by Caco-2 cells (Cabezas-Teran et al., 2023). In some studies, antioxidant encapsulated in inulin have been formulated in food matrices and assessed for bioaccessibility. Extract from Saskatoon berry fruits rich in phenolic acids, flavanols, procyanidins, anthocyanins and flavonols was encapsulated in inulin or maltodextrin by spray-drying and then formulated in wheat bread. Encapsulation with maltodextrin and inulin provided average protection of 27 % and 28 % of thermolabile bioactive compounds during exposure to high temperature in the bread making process. Moreover, enriched breads were characterized by a high bioaccessibility of flavanols phenolic acids, and anthocyanins (Lachowicz et al., 2021). Similarly, plant extracts from hawthorn bark, rich in procyanidins, soybeans, rich in isoflavones and onion husks, rich in flavonols, were encapsulated by spray-drying either in maltodextrin or inulin carriers and then used to enrich wheat bread. Addition of all these extracts resulted in increased antioxidant activity of bread, with no difference between the two carriers. In general, during *in vitro* digestion of enriched breads, the maximum release of antioxidants occurred in the gastric stage, and their content decreased in the intestinal phase. At the end of the intestinal phase, only procyanidins and isoflavones were found to be bioaccessible, while flavanols were completely degraded (Czubaszek et al., 2021). Tomato pomace extract, encapsulated by spray-drying with both inulin and Arabic gum as wall materials was added to yogurt and lycopene bioaccessibility was studied. The feed composition with 20 % inulin showed the best protective ability and enabled release of the bioactive compounds preferentially in the intestine. At the end of the simulated intestinal stage, a final lycopene release of 26.1 % was observed for lycopene encapsulated in inulin, while only 15 % of lycopene was recovered at the end of digestion of the free extract (Corrˆea- Filho et al., 2022). There is a limited number of papers describing the application of inulin for the co-encapsulation of antioxidants with other bioactive ingredients. Nevertheless, using the blend of inulin and maltodextrin the microcapsules carrying quercetin and probiotic bacteria *Bacillus claussi* were prepared by spray-drying (Saavedra-Leos et al., 2022). These bacteria were also co-encapsulated with resveratrol using inulin for spray-drying (Vazquez-Maldonado et al., 2020). Both active ingredients preserved their activity and, as a result, a functional powder with antioxidant and probiotic activities was obtained. *2.4.*

Natural colorants Natural colorants are an alternative to synthetic ones. Scientific research on natural colorants has expanded and their application in the food industry is growing and is promoted by consumer interest in safe food. Among natural antioxidants, different compounds beside providing health benefits can also act as food colorants (Rocha et al., 2023). Table 5 summarizes the applications of inulin as wall material for the encapsulation of antioxidants to be used as food colorants. Carotenoids found in all photosynthetic organisms and in some non-photosynthetic prokaryotes and fungi add color to food. They are isoprenoids compounds that maximally absorb light in the 400–500 nm range and present a range of colors from pale yellow to red (Melendez- Martinez et al., 2022). However, they are unstable and insoluble in water. Carotenoids degrade in the presence of light and oxygen and under the influence of heat. For their use as food colorants, efficient formulations have to be developed. In addition, carotenoids serve as a functional food ingredient since they exhibit antioxidant activity and other health-promoting properties (Melendez-Martinez et al., 2021). In the context of sustainability and circular economy, a lycopene-rich extract was obtained from tomato processing byproduct, *i.e.*, the pomace, and then encapsulated by spray-drying using inulin or gum Arabic as a carrier (Correa-Filho et al., 2019). The authors found that the concentration of inulin of 10 wt% and the inlet temperature of 160 ◦C were the best in terms of encapsulation efficiency, which resulted to be 23.5 %, slightly higher than that found for gum Arabic. Loading capacity, drying yield and the antioxidant activity of final product were also higher using inulin compared to Arabic gum. Lycopene was also extracted from tomato pomace and encapsulated by spray-drying using inulin in combination with maltodextrin (Li et al., 2022). The optimal ratio of inulin to maltodextrin was found to be 21.7:78.3, which resulted in encapsulation efficiency of 73 %. Carotenoids from corn by-products (Vulic et al., 2022) and carrot waste (Seregelj et al., 2021) were extracted, and then freeze- and spray-drying were compared for their encapsulation. The extract from corn by-products is rich in zeaxanthin, β -cryptoxanthin and lutein and the extract from carrots has a significant amount of β - and α -carotene. For corn by-product extract, freeze-drying using inulin resulted in higher encapsulation efficiency of 40.03 % and higher chroma (C^*) that is an important parameter considering the application of final product as a colorant. On the other hand, in the same study pea protein resulted to provide higher encapsulation efficiency for total carotenoids, equal to 92.74 % (Vulic et al., 2022). Carrot waste extract was encapsulated using inulin in combination with maltodextrin or whey protein, or with the mixture of maltodextrin and whey protein. For spray-drying, the optimal wall material was a mixture of whey protein and inulin with 71:29 ratio, while for freeze-drying, whey protein alone was the best. Moreover, freeze-drying resulted in lower lightness and greater yellowness compared to those parameters of the product obtained by spray-drying (Seregelj et al., 2021). Lutein, which is an important carotenoid for retarding the development of age-related eye diseases, was also encapsulated by spray drying and seven carbohydrates including inulin, were tested as a carrier (Ding et al., 2020a). The final product using inulin was of orange-red color and maintained its color during the storage at 25 ◦C for 20 days. The protection effect of inulin was the highest compared to trehalose, modified starch and maltodextrins of different degree of polymerization. After 20 days of storage at 25 ◦C, the retention of lutein was 61.99 %. However, the mixture of inulin and modified starch (1:1) resulted in the highest encapsulation efficiency of 81.0 % compared to 75.7 % using only inulin, as well as higher stability during storage (Ding et al., 2020b). The same spray-drying method was used for the encapsulation of carotenoid-rich goldenberry juice by testing various carbohydrates including inulin as wall material (Etzbach et al., 2020). Goldenberry comprises β -carotene (>50 %), lutein, and a considerable amount of carotenoid fatty acid esters. However, encapsulation efficiency of inulin for total carotenoids was 65 %, lower than that of cellobiose (77.2 %). Carotenoids retention after 6 weeks of the storage at 30 ◦C was the highest (32.43 %) using cellobiose as a carrier compared to 19.79 % in the presence of inulin. Beet extract is also a good alternative to synthetic colorants. It contains betalains that are classified into two groups of compounds, *i.e.*, betacyanins and betaxanthins. Betacyanins exhibit a red-violet color and betaxanthins exhibit a yellow-orange coloration. Betanin as one of the most abundant betacyanins is signed by the EU with the number E162 (Carocho et al., 2015; da Silva et al., 2019; Delgado-Vargas et al., 2000). Furthermore, betalains are strong antioxidants, but sensitive to temperature, light, alkaline pH, and enzymatic degradation (Slimen et al., 2017). Omae et al. (2017) encapsulated beet extract by spray-drying with inulin and incorporated it into sorbet. The encapsulation efficiency was 58.4 %. During 6 months at ⍰ 18 ◦C the color stability of sorbet fortified with the microcapsules was higher compared to the sorbet prepared with beet extract. do Carmo et al. (2018) analysed inulin and its blends with maltodextrin and whey protein isolate encapsulation of beet extract by freeze-drying. The best results in terms of powder stability were obtained using the inulin-whey protein isolate blend. The samples with inulin alone were more hygroscopic and showed lower thermal stability. For freeze-drying techniques, maltodextrin and inulin were compared. A higher total amount of colorants was found in the samples encapsulated with maltodextrin. Furthermore, the use of maltodextrin resulted in a higher glass transition temperature and ensured a lower hygroscopicity and greater stability of the samples (Flores-Mancha et al., 2020). Inulin and maltodextrin were also compared for the encapsulation by spray-drying of cactus pear fruit extract rich in betalains., but the encapsulation efficiency was not provided. The use of inulin resulted in the better color parameters. *i.e.*, lower lightness and higher redness. In general, the chroma (*C**) was 61.7 in the presence of inulin compared to 19.2 in the case of maltodextrin. Furthermore, the extract encapsulated with inulin exhibited three times higher antioxidant activity (Saenz et al., 2009). The extract of *Bougainvillea glabra* bracts is rich in betacyanins. The capsules were fabricated by external ionic gelation method using alginate and 20 % (*w*/w) inulin. The color parameters of capsules *L**, *a**, *b** and *C** were 14.60, 25.00, − 5.62 and 25.45, respectively. The capsules were used as a colorant in a gummy candies formulation. The rate constant of betacyanin degradation in gummy candies was 0.0942 days^{-1} (de Azevedo & Norena, 2021). The extract of strawberry by-products rich in anthocyanins can be exploited as a natural colorant. Anthocyanins are a group of natural water-soluble pigments that possess a large color spectrum, especially purple, dark blue and red colors, and are classified as a subgroup of flavonoid. Anthocyanins are signed by the EU with the number E 163. The interest into anthocyanins is also related to their broad range of health effects. However, their use is limited by their instability during processing and storage due to temperature, oxygen and light exposure (Neves et al., 2021). Encapsulation of strawberry byproduct extract was performed by freeze-drying using inulin as a carrier. A recovery of 55 % of anthocyanin was observed upon freeze-drying. Encapsulation ensured the light stability of extract. Indeed, for the encapsulated extract, tristimulus color parameters (*L**, *C**, *h*) did not change over one month while color changed for the free extract and the chroma (*C**) decreased approximately twice (Gomes et al., 2021). The extract of spinach (*Spinacia oleracea*) as a source of natural chlorophylls as well the extract or biomass of microalgae *Chlorella vulgaris* could be utilized as a natural colorant due to their vivid green color. In addition to chlorophylls, microalgae are a source of carotenoids and other various macro- and micronutrients. For the development of natural colorant delivery systems by spray-drying techniques, inulin was included in the composition of wall material (Agarry et al., 2023; Sansone et al., 2023; Tamturk et al., 2023). *2.5. Other bioactive compounds* Inulin finds the application in the formulation of different bioactive ingredients such as vitamins and even antimicrobial peptides. Vitamin D3 was encapsulated by spray drying together with chia seed oil rich in omega-3 fatty acids. The mixture of soy protein isolate, maltodextrin and inulin at the ratio of 5/5/2 was found optimal and resulted in the encapsulation efficiency of 88 %. The retention of core material after 21 days of storage at 25 °C and 75 % relative humidity was 86 % at the core/wall ratio of 1/5 (Razavizadeh et al., 2022). Vitamin E was encapsulated by spray-drying using various wall materials including inulin. The encapsulation efficiency varied from 70.1 to 99.4 % depending on the wall material. The highest encapsulation efficiency <of 99.4 % was obtained using inulin. The authors determined the release time of vitamin E as affected by the wall material using octanol, an organic solvent referred as a good mimic of phospholipids membrane characteristics due to its amphiphilic nature. In the presence of inulin, total release of vitamin E was detected after 413 min while in the presence of starch, modified chitosan or gum Arabic the period was longer and found in the range of 815–999 min (Ribeiro et al., 2021). The antimicrobial peptide nisin-loaded particles were prepared using pectin with a different degree of esterification in combination with inulin. The addition of inulin increased the efficiency of nisin loading compared to pectin as a coating material. The particles exhibited antimicrobial activity in a microorganism-dependent manner. The combination of two different biopolymers offers new perspectives for antimicrobials preparation especially due to higher loading efficiency (Krivorotova et al., 2016).

2. Conclusions and future perspectives

Inulin is applied for the development of the formulation of various bioactive compounds. It is used alone or in combination with other biopolymers as a coating material in the encapsulation process. In general, wall materials act as a barrier to ensure the protection of sensitive core compounds from light, oxygen and the isolation of other harmful external factors. Furthermore, the water solubility of encapsulated hydrophobic compounds is also improved. Inulin is a hydrophilic compound and due to the large number of hydroxyl groups can form the network of hydrogen bonds with core compounds or other wall materials. As a result, the encapsulating matrix is reinforced and prevents the penetration of oxygen and protects against other environmental factors. The effectiveness of the process and the functionality of the final product depend on the bioactive ingredient itself, the chosen encapsulation method, and other wall materials used in combination with inulin. Spray-drying is the most popular choice. However, inulin could be more extensively tested for a wider range of methods. For the optimization of target applications, the effect of inulin polymerization degree on the encapsulation efficiency and properties of encapsulated system should be investigated. In addition, the research could be focused on the co-encapsulation of different bioactive compounds using inulin. Currently, investigations in this regard are limited. Moreover, studies on the final product in real food matrices are not numerous and could be expanded.

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