Advances in the encapsulation of bioactive compounds present in Brazilian fruits.

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ABSTRACT

Recent advances in the encapsulation of bioactive compounds that are found in some important Brazilian fruits are discussed in this paper. Bacuri, Tucumã, Ticazo, Taperebá, Pineapple, and Jabuticaba are examples of typical fruits from the Brazilian Cerrado region, where bioactive compounds known for their sensory attributes such as color, flavor, and aroma, in addition to, their high nutritional values grow increasingly. However, many of the bioactive compounds in the fruits degrade naturally quickly, with functional loss due to several factors. Thus, in the food industry, the immediate need for the development of systems to protect and release the bioactive compounds present in the various fruits is evident. Microencapsulation and nanoencapsulation of bioactive fruit compounds are technologies that prevent degradation and possible functional loss of the bioactive compounds. Different techniques used in encapsulating will be presented, as well as, encapsulation materials and the principal results achieved by various studies of micro and nanoencapsulation.

KEY WORDS: Microencapsulation, nanoencapsulation, spray-drying, Brazilian fruits, bioactives, capsule materials

INTRODUCTION

Native Brazilian plants include a great variety of fruits growing throughout its regions. However, only a fraction of the edible Brazilian fruits is known and properly made use of. Some of these fruits are becoming, or are already, popular worldwide, such as açaí (*Euterpe oleracea*) and passion fruit (*Passiflora edulis*). Others, such as pequi (*Caryocar coriaceum Wittm.*) and Camu-camu (*Myrciaria dubia [HBK] McVaugh*) (1), are better known locally.

Most Brazilian fruits are sources of several bioactive compounds, that is, primary and secondary plant metabolites such as fatty acids, dietary fiber, heteropolysaccharides, carbohydrates, flavonoids, polyphenols, terpenoids, alkaloids, tocopherols, coumarins, and phenolic acids (2). These bioactive compounds can be defined as substances that interact with one or more components of living tissues or organs and result in potentially beneficial physiological effects (3). Thus, these compounds are widely known for their anti-inflammatory and antioxidant activities, in addition to their potential for preventing and treating non-communicable diseases, including cancer (1).

However, despite the positive characteristics cited,

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most isolated compounds are highly instable which limits their application in food processing and drug formulations because of factors, such as rapid chemical degradation, high volatility, thermal instability, low solubility, bio accessibility, and bioavailability (4). In view of this, encapsulation can be used as a strategy to preserve the bioactive components or to improve their use in food, nutraceutical and supplement processing. Encapsulation is defined as a method for temporarily trapping an active substance within a shell-forming material. Encapsulation provides high protection efficiency, better bioavailability, higher stability, controlled release and allows masking of undesirable flavors (3-5).

BIOACTIVE COMPOUNDS

Bioactive compounds are mainly derived from plant sources that exhibit specific metabolic or physiological activities in humans and provide a wide range of health benefits, such as antioxidant, anticancer, anti-inflammatory, and anti-aging activities. These effects bring these compounds to the forefront for researchers, producers, and consumers (5-6).

In addition, bioactive compounds have a significant relevance in the combat and prevention of chronic diseases, such as cardiovascular diseases (7). Furthermore, extracts of bioactive compounds present important biological effects that indicate their use as food preservatives and their application in cosmetics or medicines (8).

Among the bioactive compounds, fatty acids, essential oils, vitamins, polyphenols, flavonoids, carotenoids, terpenes, alkaloids, and bioactive peptides stand out. Plants are the main sources of these compounds because all plant parts, including bark, roots, fruits, leaves, tubers, gums, flowers, and rhizomes, produce bioactive compounds in varying concentrations (5).

Major groups of bio actives found in Brazilian fruits

Phenolic compounds

Phenolic compounds are bioactive substances with antioxidant and antibacterial properties that are found in vegetables, fruits, and herbal medicinal plants (9). They are characterized by an aromatic ring with at least one hydroxyl group, and they can be classified into two subgroups: flavonoid and non-flavonoid compounds. The flavonoids are anthocyanins, flavonols, flavanones, flavonols, flavanones, and isoflavones; whereas the non-flavonoids are phenolic acids, coumarinoids, tannins, stilbenes, xanthones, and lignins (10). Brazilian fruits that contain phenolic compounds are Camu-camu (*Myrciaria dubia [HBK] McVaugh*), guaraná (*Paullinia cupana*), açaí (*Euterpe oleracea*), jabuticaba sabará (*Plinia cauliflora*), passion fruit (*Passiflora edulis*), pinhão (*Araucaria angustifolia*), and taperebá (*Spondias mombin*) (11, 12, 13, 14, 15, 16).

Anthocyanins

Anthocyanins are bioactive compounds found in fruits and vegetables. They are natural food dyes with purple, red, or blue colors that have antioxidant, antiallergenic, antiviral, anti-inflammatory, and vasodilatory properties. Moreover, they are cytotoxic against cancer cells and inhibit oxidative and inflammatory enzymes (17). They can be found in Brazilian fruits such as jabuticaba (*Plinia cauliflora (Mart.) Kausel*), açaí (*Euterpe oleracea*), pitanga *(Eugenia uniflora*), and juçara (*Euterpe edulis*) (17, 12, 18, 19).

Carotenoids

Carotenoids are natural hydrophobic pigments (varying from yellow to red tones) that can be found in various vegetables. They are beneficial to health because of their antioxidant and pro-vitamin activities (20). Carotenoids are terpenoids with 2-methyl-1,3-butadiene as a fundamental unit. They can be categorized as carotenes and xanthophylls (21). These compounds can be found in Brazilian fruits such as tucumã (*Astrocaryum vulgare Mart.*), bacuri (*Platonia insignis*), pitanga (*Eugenia uniflora L.*), pequi (*Caryocar coriaceum Wittm.*), and cajá (*Spondias mombin L.*) (20, 22, 23).

Vitamin C

Vitamin C, an organic substance composed of ascorbic acid and dehydroascorbic acid, is configured as an efficient polar antioxidant of which fruits are its main source. Vitamin C can provide antioxidant activity *in vitro*, as well as actively restore vitamin E *in vivo*. This vitamin is important for human nutrition and is used widely in the food industry as an additive in processed foods. It is important for several vital processes, such as wound healing and iron absorption (24). It can be found in Brazilian fruits such as cajuí (*Anacardium spp*), jenipapo (*Genipa americana L.*), mangaba (*Hancornia speciosa Gomes*), bacuri (*Platonia insignis Mart.*), and camu-camu (*Myrciaria dubia [HBK] McVaugh*) (23-11).

Tocopherols

Tocopherols (vitamin E) are organic compounds and act as lipophilic antioxidants that neutralize imminent membrane damage caused by chain reactions involving free radicals. In addition, tocopherols can be restored by vitamin C, carotenoids, and phenolic compounds *in vitro* and *in vivo* (24). They can be found in Brazilian fruits such as: bacaba (*Oenocarpus distichus*), bacupari (*Rheedia brasiliensis*), marmalade (*Alibertia verrucosa*), mirindiba (*Buchenavia tomentosa*), and ticazo (*Plukenetia volubilis*) (23-25).

ENCAPSULATION AND CHARACTERIZATION

Encapsulation is the process that incorporates a material (active compound) in the structural component of another molecule, resulting in nanoscale (nanoencapsulation), micrometer (microencapsulation), or millimeter-sized particles (26). Particles can be categorized according to size or morphology (27). Classification by size corresponds to nanoparticles (1-100 nm), microparticles (1-1000 μm), and macroparticles (>1000 μm). The morphology can be characterized by the matrix or reservoir model (Figure 1). The shape can be presented as spherical or non-spherical, and it can also have single or multiple cores (28-29).

Particle size is essential as it can influence several parameters such as quality, segregation, stability, uniformity, and others. Understanding particle morphology is also important, as it allows for characterizing the shape and differentiating particles with varying processabilities (30).

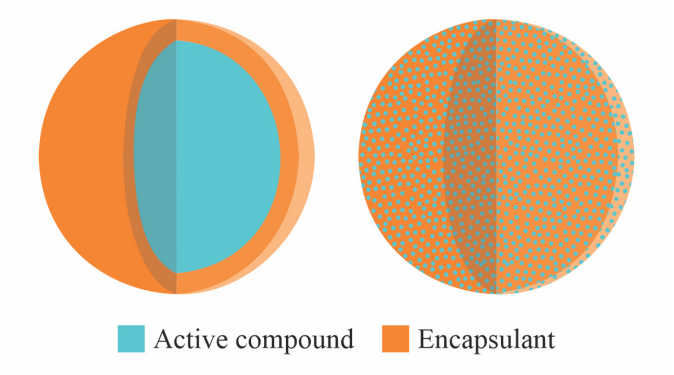

Figure 1 Matrix and reservoir microparticles.

Dynamic light scattering (DLS) is among the most popular methods for determining particle size. It measures the hydrodynamic diameter through Brownian motion. The interpretation of this method is defined by the amount of light scattered by backscattering; the larger the particle, the smaller the amount of scattered light (31).

Particle morphology is usually detected by microscopic imaging methods, such as the scanning electron microscope (SEM) (32). The SEM works through the emission of electron beams by inducing the potential difference because the principle of the technique is based on the electrical conductivity of the material to be analyzed (33).

TYPES OF ENCAPSULANTS

The encapsulant (or wall material) is the most important component in encapsulation because the selection of the appropriate encapsulant impacts the physical and chemical properties of the resulting micro/nano capsules. The encapsulant must have properties such as being inert and stabilizing the active compound, film-forming, malleable, tasteless, non-hygroscopic, of moderate viscosity, economical, soluble in an aqueous medium or solvent, and ensure controlled release at

the specific location under specific conditions (34). The combination of different encapsulants is of special commercial interest because it allows new functionalities, on the one hand, and reduces the level of inputs, on the other (35).

Organic encapsulants

Protein-based polymers, such as albumin and gelatin, can be used as encapsulants. Albumin is a biodegradable, water-soluble protein. It is stable at pHs between 4 and 9, and it can be heated at 60°C for up to 10 hours without any deleterious effects. However, it is subject to degradation by proteases, which aid in the release of the active compound in the small intestine. It also facilitates the release of nano capsules within endosomes. Gelatin is biodegradable, inexpensive, easily sterilized, non-pyrogenic, non-toxic, non-immunogenic, and easy to cross-link or chemically modify (34).

Monosaccharide polymers, such as vegetable polysaccharides, can also be used as encapsulants. They include starches, cellulose, and pectin. Animal polysaccharides, chitosan, polysaccharides obtained by fermentation; xanthan and enzymatic gum; and maltodextrin are other examples. Chitosan has a viscosity directly proportional to its concentration. The solubility can be improved when the amine group is protonated at low pH because these characteristics are manipulable, making this compound versatile for promoting encapsulation. (34, 36, 37).

Xanthan gum, a polysaccharide obtained by fermenting a carbohydrate, has been widely used as an encapsulating agent because it has useful sensory characteristics, such as being tasteless, odorless, colorless, with a smooth texture, in addition to having excellent thermal stability (38). Maltodextrin is a partially hydrolyzed starch, a material widely used in food and drug processing. It effectively protects nutrients and bioactive substances from oxidation and thermal degradation (39).

Hydrophobically modified starches, such as starches modified with octenyl succinic anhydride (OSA-starches), serve as surfactant food additives, and they are widely used in the microencapsulation of flavors, nutrients, fragrances, and oil-based pharmaceutical actives (40). Lipid encapsulants are also common, mainly in solid-lipid nanoparticles (SLN) and second-generation nanostructured lipid carriers (NLC), because fats and oils are important components in many foods and have personal care and pharmaceutical applications. They can serve as carriers for functional components such as vitamins, aromas, colors, nutraceuticals, and pharmaceuticals (41).

Inorganic encapsulants

Inorganic encapsulants are also implemented, mainly in ionic gelling technology such as sodium alginate and calcium chloride. Sodium alginate is one of the most widely used natural anionic biopolymers and exhibits a great ability to chelate ions with divalent cations (such as Ca^{2+}), forming a 3D network with a unique reservoir-model encapsulation structure (42).

MICROENCAPSULATION TECHNIQUES

Microencapsulation is a process of encapsulating active compounds to better protect them and increase their stability by limiting their interaction with air and food constituents (43, 44, 45). There are several techniques applied to this process; however, due to their particularities, they can be classified into three main classes (46, 47, 48):

(I) Physical-Mechanical Methods: spray-drying, lyophilization, supercritical encapsulation, spray chilling, extrusion, co-crystallization and fluidized bed coating.
(II) Physical-Chemical Methods: complex coacervation, ionic gelation, and organic phase separation, emulsion systems and liposome formation.
(III) Chemical Methods: molecular inclusion by complexation, interfacial and emulsion polymerization.

A summary of the main techniques used for the microencapsulation of bioactive compounds derived from fruits is presented in Table 1.

Table 1 Main microencapsulation techniques used for the encapsulation of bioactive compounds present in Brazilian fruits.

ENCAPSULATING TECHNOLOGIES	OPERATING PRINCIPLE	ADVANTAGES	DISADVANTAGES	INFLUENCING FACTORS	REFERENCES
PHYSICAL METHOD					
Spray drying	- Preparation of solution, dispersion, emulsion (bioactive material + encapsulant);- Submission of the solution in an atomizing nozzle and pulverized in the form of droplets in a chamber with hot air.	- High encapsulation efficiency;- Low cost;- Possibility of scale-up.- Economic;- Possibility of using a wide variety of encapsulants;- Process with short time;- Ability to produce good quality powder products.	- Thermal degradation of certain encapsulated compounds;- Difficulty controlling particle size;- Non-uniform conditions in the drying chamber can result in agglomeration and material losses in processing;- Inability to dry solids.	- Encapsulating material;- Temperature of the inlet air;- Supply flow.	(49, 50, 51, 52, 53)
Freeze drying	- Preparation of solution, dispersion, emulsion (bioactive material and encapsulant);- Freezing of the solution, emulsion, dispersion;- Sublimation for solvent removal at low temperature and vacuum pressure.	- Simplicity;- Absence of air in the process, generating products of superior and prolonged quality;- Production of highly porous and light powders;- Preservation of physical-chemical, sensorial properties and biological activity of the compounds;- Improved encapsulation efficiency.	- Long process time (more than 20 h);- High capital and operating costs in comparison with other techniques;- The products tend to shrink and crack;	- Encapsulating material;- Primary drying temperature;- Concentration of solute in the initial solution;- Ratio of core matrix to solution.	(53, 54, 49, 55, 56)
Spray chilling	- Preparation of solution, emulsion or dispersion (bioactive material + encapsulant), in molten lipid encapsulating materials;- Submission of the solution by atomizer and sprayed in a refrigerated chamber, where the droplets solidify when in contact with cold air, originating solid lipid microparticles.	- Less consumption of time and energy;- No use of solvents;- Formation of free-flow microparticles;- High encapsulation efficiency and asset retention;- Ease of incorporation in different food matrices and non-toxic;- Possibility of encapsulating hygroscopic and water-sensitive ingredients;- Possibility of scale-up.	- Encapsulating materials of lipid origin that meet the required characteristics, such as food grade, melting point, oxidative stability and maintenance of the structure during storage;– Stability of the encapsulant at the melting temperature of the lipid matrix, with the possibility of physical changes during the process;- The clogging of the atomizer when using highly viscous mixtures.	- Melting temperature of lipid compounds, atomization air and cooling chamber;- Atomization air pressure;- Feed flow of the molten mix	(57, 58, 59, 60)
Electrospinning	- Preparation of the polymeric solution;- Application of an electric charge in the polymeric solution present in a syringe that will be spun to obtain fibers.- Evaporation of the solvent and collection of the hardened polymer on a fixed or rotating metallic screen.	- Requires room temperature, preserving thermosensitive compounds, physical-chemical properties and biological functions;- Easy, low cost and flexible method.	- Slow process;- Low profit.	- Concentration of polymer in the solution;- Applied electrical potential and feed rate;- Distance between the tip of the syringe and the collector.	(61, 62, 63)
PHYSICO-CHEMICAL METHOD					
Complex coacervation	- Preparation of homogeneous colloidal solution with two different types of polymers;- Separation into two phases resulting from the interaction of oppositely charged polyions.	- Simplicity, low cost, scalability and reproducibility;- Good retention and encapsulation efficiency;- Low concentration of encapsulant;- Reduction of losses due to evaporation or thermal degradation;- Compatibility in controlling the release of active materials such as encapsulants.	- High cost of storage and transportation;- Difficult to control its size;- Particles are sensitive to pH and ionic strength;- Limited stability in aqueous matrices;- Crowd;- Inadequate method for encapsulating heat-sensitive compounds.	- Processing conditions (pH, ionic strength and temperature);- Active and encapsulating material.	(64, 65, 66, 67)
Ionic gelation	- Preparation of a polymeric or hydrocolloid solution;- Dropping of the solution into an ionic solution under constant agitation.	- Low cost, simple and feasible;- Does't use organic solvents and high temperatures.	- Low stability in acidic pH;- Difficulty trapping high molecular weight compounds.	- Polymeric material used;- Concentration of the polymeric material.	(68, 69, 45)
CHEMICAL METHOD					
Interfacial-polymerization	- Dispersion of an immiscible phase containing a reactive monomer in a continuous phase containing a second monomer;- The monomers react at the interface to form polymeric membranes.	- Simplicity, reliability and low cost;- Possibility of controlling the dimensions of the microcapsule and wall thickness;- Direct control of conditions and adjustable delivery rates;- Versatile mechanical and chemical properties of the capsule wall;- Possibility of scale-up.	- Large amounts of solvents are required;- Formation of fragile encapsulating matrix;- Presence of reactive monomer in contact with the active agent can be harmful.	- Process parameters such as pressure, reaction temperature, time and presence of reaction initiator.	(70, 71, 72, 67, 73, 74)

Table 2 Studies developed involving the encapsulation of bioactive compounds from some Brazilian fruits

FRUIT	BIOACTIVE	ENCAPSULATION METHODS	MAIN RESULTS	REFERENCES
Pineapple (*Ananas comosus*)	Enzyme bromelain	Chitosan-coated alginate microcapsules by ionic gelation	86% efficiency and 8.59% dissolution at pH 1.2	76
	Polyphenol and antioxidante compounds	Maltodextrin and gum arabic powder by spray-drying	Retention of bioactive up to 15 mg/g dry powder	77
Bacuri (*Platonia insignis*) and Tucumã (*Astrocaryum vulgare*)	Fucoxanthin carotenoid	Lipid nanoparticles – SLN with Bacuri butter and Tacumã oil	Efficiency up to 98% and adequate for skin application	22
Ticazo (*Plukenetia volubilis*)	Tocopherol and sterols	Oil microencapsulated by the combination of Arabic gum and maltodextrin. A nanoemulsion with alginate and chitosan by ionotropic gelation	Efficiency up to 67% and 35%, respectively. Increased antioxidant activity	25
Taperebá (*Spondias mombin*)	Flavonols, phenylpropanoids, coumarins, ellagic acid and quercetin	Chitosan microparticles obtained by spray-drying	Encapsulation efficiency up to 55%	16
Sapota (*Manilkara zapota*)	Gallic acid and fumaric acid	Powder with maltodextrin and gum arabic by spray-drying	Oxidation of the compound was avoided	78
Pitanga (*Eugenia uniflora*)	Anthocyanins	Microparticles by spray-drying using fructans and maltodextrin as wall material	High retention power and protection of antioxidant compounds	18
Pinhão (*Araucaria angustifolia*)	Phenolic compounds	Pectin, hydrolyzed collagen, polydextrose, and partially hydrolyzed guar gum were used as wall materials, by spray-drying	80.97% retention	79
Pequi (*Caryocar coriaceum*)	Oleic acid and palmitic acid	Microparticles of Pequi oil in alginate and chitosan by ionic gelation	Encapsulation efficiency of 96.17%.	80
Passion fruit (*Passiflora edulis*)	Piceatannol	The dispersions of lipid nanoparticles – NLC were prepared by glyceryl distearate and passion fruit seed oil	The efficiency of 94.91%.	14
	Phenolic compounds	Microencapsulation by spray-drying used the combination of gelatin with maltodextrin	High retention and probiotic effect	81
Juçara (*Euterpe edulis*)	Anthocyanins and phenolic compounds with probiotic	Microparticles with maltodextrin and modified starch by spray-drying	Retention is higher than 92%.	19
Jabuticaba Sabará (*Plinia cauliflora*)	Polyphenols	Chitosan microparticles obtained by the spray-drying	Storage efficiency of 79%.	13
Açaí (*Euterpe oleracea*)	Anthocyanins and phenolic compounds	Chitosan and alginate microparticles	Efficiency higher than 99%.	12

ENCAPSULATION STUDIES OF BIOACTIVE COMPOUNDS OF BRAZILIAN FRUITS

Fruits native to Brazil are excellent sources of nutrients and bioactive compounds because they can exert antioxidant, anti-lipidic, anti-inflammatory, and antiproliferative effects. Açaí (*Arecaceae*), Jaboticaba (*Mirtaceae*), and Murici (*Malpighiaceae*) are examples of fruits that can be used in food, pharmaceutical, and cosmetic products (75).

Based on this observation, studies have been carried out to encapsulate bioactive compounds present in Brazilian fruits to preserve the integrity of these substances so that they can exert their specific function in the human body. Among the most studied Brazilian fruits, pineapple (*Ananas comosus*) contains the proteolytic enzyme bromelain. Bromelain can treat infections, sinusitis, osteoarthritis, and cancer. Still, oral use is not feasible because it is easily degraded by proteases and by the acid pH of the stomach so that its conformation and function are lost. Thus, the use of an encapsulation technique for this substance could prevent its degradation (76). Based on this fact, a literature survey of the encapsulation techniques of bioactive compounds from some Brazilian fruits was performed, highlighting the main results obtained with encapsulation (Table 2).

However, there are few studies with encapsulation of bioactive Brazilian fruits, considering their potential for application in therapies, food, and chemicals. For example, there is the Inajá (*Maximiliana maripa*), a fruit characteristic of the Brazilian Amazon, which is composed of phenolic and anti-inflammatory antioxidants, such as catechins and procyanidins, obtained from the fruit oil (82). Another example is the native species of the Brazilian Cerrado, such as mangaba (*Hancornia speciosa*) and pequi (*Caryocar brasiliense*), which have a high potential for activity against metabolic syndrome because of the presence of carotenoids, polyphenols, and phytosterols (83).

However, in addition to these Brazilian regions, there are others like the Restinga, the Atlantic Forest, and the Caatinga (84). Fruits such as tajuva (*Maclura tinctoria*), saraguají (*Rhamnidium elaeocarpum*), and pitomba-da-baía (*Eugenia luschnathiana*) are found in these regions. These fruits contain the bioactive antimicrobials eleocarpanthraquinone and bornesitol, for example (85, 86, 87-88). In addition to these reports, although not extensively studied, other fruits are excellent sources of carotenoids, anthocyanin, phenolic compounds, ellagitannin, flavonoids, diarylketone derivatives, and iridoids. These compounds have been found in cambuci (*Campomanesia coubaril*), pitanga, cagaita (*Eugenia dysenterica*), jabuticaba, araçá (*Psidium cattleianum*), jatobá (*Hymenae coubaril*), mangaba and pequi, which are fruits characteristic of the Amazon or Brazilian Cerrado. Among the phenolic compounds detected are gallic acid, quinic acid, quercetin, ellagic acid, chlorogenic acid, and rutin (89).

Other fruits, such as araçá-boi (*Eugenia stipitata*), biri-biri (*Averrhoa bilimbi*), abiu grande (*Pouteria caimito*), yellow mangosteen (*Garcinia xanthochymus*) and araticum (*Annona crassiflora*), are employed in the study of the treatment of renal and metabolic problems (90). Cocoa (*Theobroma cacao*) has been characterized as a source of flavonoids, amino acids, and phenolic acid derivatives (91-92). Umbu (*Spondias tuberosa*) is rich in flavonoids, tannins, arabinogalactans, xyloglucans, arabinoxylans, and rhamnoarabinogalactans (93). In ouixabeira (*Sideroxylon obtusifolium*), the anticonvulsant *N*-methyl-(2S,4R)-trans-4-hydroxy-L-proline was detected (94). However, cytoprotective and antioxidant effects (95) were observed for murici (*Byrsonima crassifolia*), and genipap (*Genipa americana*) was characterized as a source of citric acid, quinic acid, and other organic acids (96). Vitamin C and antioxidant activities (97) were found for other fruits, like gabiroba (*Campomanesia pubescens*) and marolo (*Annona crassiflora*). Thus, the studies of encapsulation of bioactive substances must be extended to the various native fruits from other regions of Brazil so that the emergence of new therapies, foods, prebiotics, and drugs can be stimulated.

CONCLUSIONS

Several bioactive compounds are found in Brazilian fruits and require encapsulation techniques that depend on the application of interest. Many studies have already developed nano- or microencapsulation of bioactive compounds from Brazilian fruits, mainly those from the cerrado. Studies like these guarantees greater visibility both for Brazilian fruits and for the regions where they originate.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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