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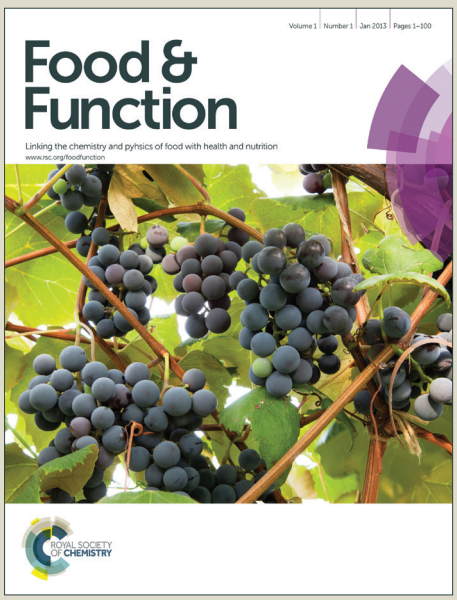

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Microencapsulation of bioactives for food applications

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Abstract

Health issues are an emerging concern to the world population and, therefore, the food industry is searching for novel food products containing health-promoting bioactive compounds, but with little or no synthetic ingredients. However, there are some challenges in the development of functional foods, namely in what respects to the direct use of some bioactives. They can show problems of instability, react with other food matrix ingredients or present strong odours and/or flavours. In this context, microencapsulation emerges as a potential approach to overcome these problems and, additionally, to provide controlled or targeted delivery or release. This work intends to contribute to the field of functional food development by performing a comprehensive review on the microencapsulation methods and materials, the bioactives used (extracts and isolated compounds) and the final application development. Although the existence of several works dealing with microencapsulation of bioactives, they are mainly focused on the process development and the majority lack proof of concept with final applications. These factors, together with a lack of regulation, either in Europe or in the United States, delay the development of new functional foods and, consequently, their market entrance. In conclusion, the potential of microencapsulation to protect bioactive compounds ensuring their bioavailability is shown, but further studies are required, both considering applicability and incentives by regulatory agencies.

Keywords: Bioactive extracts/compounds; microencapsulation; food applications

1. Introduction

1.1. The increasing interest for functional foods

Nowadays, food serves not only to satisfy the primal urge of hunger, but also emerges as a means of promoting consumer's health. In this context, the food industry has focused on avoiding potential harmfulness of synthetic food additives and on developing novel food products containing health-promoting ingredients. Therefore, bioactive natural products are considered as viable and safer substitutes to satisfy the world market demand for new products.[1]

"Functional foods" arise as the frontier between nutrition and health, providing a long-term beneficial physiological/health effect beyond the nutritional properties.[1] The concept of functional food appeared 40 years ago, however, the growing interest for this type of products, either by industry (through patents) or academia (through scientific research articles and reviews), was only observed from the second half of the 1990s, indicating an increasing tendency (Figure 1). The exponential growth of patents and scientific research articles/reviews observed since 2005 was accompanied by the regulation (EC) No 1924/2006 publication by the European Parliament on nutrition and health claims in foods, which was completed and finalized in 2011 by the European Food Safety Authority (EFSA) regarding beneficial health claims in certain food ingredients.[2,3] In the United States (US) the regulation of functional foods is facilitated, as the food industry itself provides the product definition that will be placed on the market supply; food companies are only obliged to follow labelling and safety rules implemented by the Food and Drug Administration (FDA).[4]

Nowadays, consumer's awareness of health issues is growing together with the increasing incidence of chronic age-related diseases, such as neurodegenerative,

diabetes and cancer, usually correlated with the lifestyle and dietary habits of our societies.[5] Moreover, as the life expectancy is rising, with the consequent increase of health costs, pharmaceutical and food industries start to consider functional foods as a new market with huge growth potential. Nowadays, Japan, United States (US) and European Union (EU) are the leading markets for functional foods, representing in total 90% of the world market supply for this type of products.[6] In 2006, US and EU markets were valued at 33 billion US$ and at 15 billion US$, respectively, with tendency to grow. German, France, United Kingdom and Netherlands are considered the most important countries within the European functional foods market.[7]

1.2. The problems related with the use of free bioactives

Despite the known beneficial health effects of natural bioactive matrices and isolated individual compounds, as it will be discussed in this section, they show some fragility that has to be considered regarding their direct use or incorporation into foods.

The main factors limiting the use of bioactives in food applications are shown in Figure 2. Bioactive ingredients are generally prone to degradation, both during storage and food processing, as many of them are physically, chemically and/or enzymatically instable leading to their degradation or transformation with the consequent loss of bioactivity. In many cases the mechanism involved in the degradation of these bioactive molecules is very complex and still unknown.[8,5] Wu *et al*.[9] reported the reduction of the anthocyanins content in blackberries fruits after six months of canned and jam storage and also after drying treatment. Various types of cereals (wheat, barley and oat) were also tested for their content in biologically active compounds, such as tocopherols, phenolic compounds and microelements, and after hydrothermal processing, the concentration of these molecules severely decreased.[10] Rawson *et al*.[11] described major

losses of bioactive compounds after processing exotic fruits such as mangoes, *açaí*, pineapple and *pitanga*, relating them to heating treatments, pasteurizing and drying, canning and even to storage processing steps. All these processes affect, to a lesser or greater extent, the stability, chemical characteristics, concentration, and even antioxidant activity, of a number of compounds such as vitamins and phenolic compounds. Another study that describes the modifications occurring in fruits and vegetables during the processing steps was published by Nicoli *et al*.[12], giving focus to the antioxidant decrease of the food matrix derived from the loss and transformation of the antioxidant compounds, but also due to their interaction with other molecules. The processing steps of a food matrix involves the action of endogenous enzymes, water activity, oxygen pressure and also thermal/mechanical energy, and all of those factors can influence the degradation/transformation of the bioactive molecules leading to the loss of its intended characteristics. Nevertheless, not all the compounds are equally affected; phenolic compounds and vitamins (e.g. vitamin C and E) are more sensitive to blanching and long-term freezing treatments, than minerals or dietary fibres.[13] Despite the processing steps, the perishability of food is also a limitation in their intake in a free form. This is because the shelf life determines whether a particular food maintains its characteristics and bioactive properties. For instance, edible mushrooms have a very short shelf life and the postharvest changes, such as browning, cap transformation, texture and weight loss changes, occur immediately, which decrease their bioactive components.[14]

The ingested amount of the bioactive compound, its structure and chemical form, the interaction with other molecules, but also the organism itself (mucosal mass, intestinal and gastric behaviour, metabolism and protein bonding) will influence the stability and functionality within the human body, and consequently its bioavailability.[15,16] For

instance, phenolic compounds present very low bioavailability due to their poor solubility and stability, especially those with high molecular weight. Furthermore, there are no reports on specific receptors in the small intestinal epithelial cells surface, and thus, the transport mechanism is made by active diffusion and active efflux, lowering the permeability of such compounds.[17] In the case of anthocyanins, they are very sensitive to pH and temperature changes in the medium.[18] Concerning carotenoid compounds, the nature of the food matrix, the particle size and processing method, but also the interaction with other food constituents, will affect their bioavailability; moreover, fibre constituents decrease the absorption of carotenoids. The nutritional state of the organism itself will influence the absorption of these molecules (e.g., protein deficiency affects the bioavailability).[19,20] As an example, the interaction of mineral elements with other molecules can decrease their bioavailability, as is the case of calcium where compounds such as oxalates, tannins and dietary fibres decrease the absorption due to precipitation.[21] Also, the gastrointestinal environment and epithelial transport can also decrease the bioavailability of natural extracts, as described by Vermaak *et al*.[22] who investigated the biological activity of green tea and sage extracts under simulated gastrointestinal conditions; the authors observed an accentuated decrease in the antimicrobial activity. Lipophilic compounds have also low solubility, which restrict their incorporation into many food matrices, especially in water-based carriers. The molecular weight, functionality and polarity seriously influence their solubility, physical state, chemical stability and bioavailability.[8,23] It is very difficult to evaluate the bioavailability of these type of compounds, since once metabolized they reach the systemic circulatory system where they can be stored, utilized or excreted. Depending on the concentration and time of these molecules in a particular tissue, or use in some biological function, the bioavailability can be estimated.[24] For instance, the

bioavailability of lycopene, a highly lipophilic carotenoid compound, is influenced dramatically by the intestinal lymphatic uptake. Faisal *et al*.[25] applied an *in vivo* model to increase its solubility using digestible lipid excipients. A similar study was performed by Balakrishnan *et al*.[26] in order to increase the solubility and bioavailability of the Coenzime Q_{10}, practically insoluble in aqueous medium, by using oil and surfactant compounds for its oral delivery.

Another factor that drives researchers to invest their knowledge into the design of novel food delivery systems is the organoleptic behaviour of some bioactive extracts/compounds. They can present unpleasant tastes, odours and/or textures. This is a crucial point for food industry when developing a new product because the consumer not only gives importance to price, but especially to the taste, smell and appearance. Accordingly, consumers will usually choose, even with lower bioactive properties, the non-functional counterpart of a similar product.[16,27] It is known that many people avoid eating fruits and vegetables because most of their compounds such as polyphenols, terpenes and glucosinolates have bitter or astringent tastes, making them unappealing to the consumer.[28]

To overcome the problems related with the direct use of bioactive extracts/compounds, microencapsulation techniques arise as a potential approach to food industry dealing with their incorporation, either to impart additional functional properties or to protect the bioactive itself.

The main goal of the present review is to highlight the use of microencapsulation techniques for food applications, as well as discussing the advantages of microencapsulating bioactive extracts/compounds. Various extracts and compounds that have been encapsulated using different techniques and formulations will be enumerated focusing on the potential for functional foods development. A particular emphasis will

be given to examples where a final application (incorporation in food matrices) is explored.

2. Overview of microencapsulation techniques and materials

2.1. The advantages of using microencapsulated bioactives

Microencapsulation can provide a tool to protect natural extracts and compounds from the action of biotic, abiotic, and biological factors. It emerges as a reliable methodology for the food industry, but also for the fields of nutrition and health, where the stability, efficacy and bioavailability of these extracts and compounds are needed. As described previously, there are several factors affecting the bioactive stability in its free form (Figure 2), however with microencapsulation technology, a protection from factors such as light, moisture, heat and oxygen is provided. Also, the organoleptic characteristics of many food products can be masked, but most importantly functional/biological characteristics can be maintained after ingestion together with a controlled release in a specific target. The success of a delivery system based on microencapsulation can be measured by the bioactives behaviour during food processing and storage, and after ingestion.[8]

From a practical point of view, microencapsulation techniques protect the core material from the outside environment; it increases product shelf life by reducing the transfer between the core and the surrounding medium, and by protecting the molecules from reaction with other food constituents, which can decrease their bioavailability.[29] It also increases solubility, dispersability and flowability of the bioactives.[30]

Depending on the applied technology and encapsulated bioactive, the response of the produced delivery system will be different; each compound has specific characteristics that should be considered in the design of a novel microcapsulation process. For

instance, phenolic compounds are very powerful antioxidant molecules; however they present problems in their bioavailability because they are transformed, after ingestion, in methylated, glucuronated and sulphatated metabolites.[31] Nano- and micro-particles based delivery systems appear as the response to overcome those problems, increasing the phytochemical absorption of phenolic compounds in epithelial cells.[17,32] In particular, Davidov-Pardo & McClements[33] showed that the microencapsulation of resveratrol increased its bioavailability.

Essential oils have also some organoleptic related problems, most of them presenting an unpleasant taste and odour, with very poor water solubility and high volatility. All these limitations can be overcome by using microencapsulation techniques that increase the effectiveness of their biological functions and decrease the sensory impact in food products.[34]

2.2. Microencapsulation techniques

The microencapsulation concept was primarily developed by the pharmaceutical industrial sector; whose goal was to control and/or modify the release of drug substances. Nowadays, it still represents the major field of microencapsulation (68%) while the food sector account for only 13%.[35] The amount of scientific reports and patents regarding microencapsulation for food purposes (Figure 3) is indicative of the growing interest for this technique regarding the incorporation of bioactive extracts and compounds. Nevertheless, the absence of regulation for novel food ingredients, including the ones deriving from using nano- and micro-technologies in their preparation, is still remaining. In US the FDA is currently developing a recognition program for nanomaterials to overcome the existing scarcity of information, and also to assess food safety of these new ingredients.[36] The introduction of microencapsulation

technologies into the food industry allows the incorporation of flavouring agents in certain types of foods, but also the improvement of their functional and health properties.[30,37] Regarding food science and biotechnology, the incorporation of natural ingredients intends to stabilize, protect and preserve the bioactives into a core, surrounded by a wall, or dispersed in a matrix, made of a material chosen to be suitable for the target delivery system.[34] There are already reviews on microencapsulation of bioactive compounds and extracts for food applications,[29,30,34,37-40] nevertheless, they mainly explore the available techniques for microencapsulation, lacking specificity in existing examples of microencapsulated bioactive extracts and compounds together with the applicability of the performed studies. Figure 4 shows the logical chain, from the choice of bioactives, materials and microencapsulation process, to final applications evidencing the crucial points involved in each step.

Microcapsules are particles comprising diameters ranging from 1 to 1000 micrometers. The most common morphology can be divided in two types: (1) Shell type, where the core, the bioactive itself or a carrier containing it (compounds that facilitate the release), is protected by a membrane; (2) Matrix type, where the bioactive is dispersed in a material's matrix. The encapsulation materials, production process, final morphology and ultimate application are the most important factors to be taken into account when designing a novel delivery system based-product. Also, stability and functional properties of the bioactive must be taken into account when selecting the microencapsulation technique. Furthermore, to achieve high encapsulation yields it is necessary to assure process reproducibility, release profile and overcome limiting drawbacks such as microsphere aggregation and adherence.[30]

The encapsulation methods and materials most commonly used in food applications are described in Tables 1 and 2, respectively (as also in supplementary material). The

definition of categories presented in Table 1 was somehow difficult because the microencapsulation processes can be categorized according to the formation mechanism, the consolidation method, and even according to the specific equipment used. A clear distinction among the described possibilities is not always clear in the published works. Therefore, in this work, effort was made to define categories according to the microcapsule formation process and a set of general categories are proposed: coacervation, extrusion-based processes, spray-based processes, emulsion-based processes, liposomes, supercritical fluids based process, ultrasound-based process and others.

2.2.1. Spray-based process

Spray-based processes are by far the most common methods being divided into spray-drying, electrospray, spray-coagulation (according to internal or external gelation) and spray-freeze drying methods. Spray-drying, the oldest microencapsulation process used by the food industry is a very straightforward technique. It can be described as flexible, allowing a continuous production, making it a cost effective process and consequently the most economical among several encapsulation methods. It can be easily industrialized in terms of equipment and materials, which have a low cost, comparatively with other available techniques.[41] The most commonly used shell materials in this technique are carbohydrates which may limit the encapsulation of some bioactives.[39] It produces high quality microcapsules, with a size less than 40 μm, by atomizing a liquid solution or emulsion through a nozzle to a hot gas chamber giving rise to the prompt formation of a powder. The method's speed and effectiveness ensures the production of microbiologically stable products, with lower costs and with specific properties.[37,41] There are several applications dealing with the encapsulation of bioactive

compounds and extracts by spray-drying. Examples in the published literature are crude extracts[42-52], carotenoids[53,54], enzymes[55,56], essential oils[57-62], fatty acids[63-66], phenolic compounds (including anthocyanins)[67-87] and vitamins[88]. It is also noticeable (supplementary material) that the vast majority of used shell materials, as it was previously reported, are carbohydrates and derivatives. However, Medina-Torres *et al*.[72] encapsulated gallic acid in mucilage obtained directly from *Opuntia ficus indica*, while Cortés-Rojas *et al*.[61] encapsulated eugenol in lipid formulations, both obtaining good results and high encapsulation yields. These results show the constant evolution of this method, and the possibility to overcome constraints related with the limited number of available shell materials, as stated by Gouin *et al*.[39]

Coagulation processes are also commonly used to encapsulate bioactive extracts and compounds for food applications, the most common being those based on alginate beads.[89-94] Alginate beads are formed from the polyanionic copolymer derived from the brown marine algae, alginate, which is frequently used as a stabilizer and thickener of many food products. Its coagulation can be promoted by external gelation (e.g. using calcium chloride as the calcium source added to the coagulation solution) or internal gelation (e.g. using calcium carbonate as the calcium source added to the alginate solution). In the first case, gelation occurs mainly at the particle surface and in the second one gelation occurs mainly inside the formed particles. The formed materials, due to their degree of ionic reticulation and functionality, permit the control of water intake and thus the release of the bioactive.[95] The preparation of such alginate beads is easily performed at a lab-scale, and have been used to encapsulate a wide variety of compounds (hydrophilic, lipophilic, oils, among others), and the controlled release is achieved by pH changes.[39,95]

Freeze-drying technology allows the encapsulation of many food constituents, being used on a daily basis to stabilize compounds and increase controlled release.[39] It is mostly used to encapsulate bioactive extracts,[96] phenolic compounds,[97-99] vitamin C[100,101] and even essential oils.[102] To the best of our knowledge the use of electrospray technology for food applications is not very common and only one work was found in the reviewed literature.[103] This works refers to the encapsulation of folic acid (vitamin B_9), and according to the provided description, it is a very appealing technology since the use of organic solvents and high temperatures is not required.

2.2.2. Coacervation

Coacervation is the second most commonly used encapsulation technique for food applications, not only because it provides high encapsulation efficiency, but also due to the triggered controlled release that can be based on temperature, mechanical or biological mechanisms, providing the needed versatility to support the development of a wide range of food products.[39] It can be divided into complex and simple coacervation; the first is based on the complexation of two opposite charged polymers that form a strong polymeric shell or matrix.[104] For the complex coacervation, chitosan is the preferable wall material, and alginate is the most commonly used polymer in all the mentioned studies.[92,93,105-107] Chitosan has low toxicity, antimicrobial activity, biocompatibility, but it is mainly muco-adherence that allows transmucosal absorption and better release of the bioactive.[107] In simple coacervation the initially soluble polymer is precipitated by changing pH or temperature.[34] Milk proteins[108,109] and pectins with PGPR (polyglycerol polyricinoleate)[110] are some examples of wall materials used in simples coacervation.

2.2.3. Emulsion based process

Emulsion based processes are also commonly used for food encapsulation applications. It allows the encapsulation of both water and oil soluble food ingredients.[34,37] Emulsion based techniques have been successfully used to encapsulate bioactive compounds including fatty acids,[111,112] vitamins,[113] phenolic compounds,[109,114-117] anthocyanins,[110-118] oils[119,120] and bioactive extracts.[106,121] This technique is sometimes coupled with a second one, in most cases a spray-drying based process, which gives rise to a dry powder that, can be promptly introduced into a food matrix.[37] In fact, several of the common used encapsulation processes start with a first step comprising the preparation of an emulsion. This is the reason why a straightforward division of the encapsulation techniques is not easy to achieve and some superimposition exists. In this work, and given the importance of spray-based processes, the cases dealing with emulsion coupled with spray techniques were included in the spray-based processes category.

2.2.4. Extrusion based process

Extrusion methodologies, unlike the above described methods are not so usual. They can be divided in electrostatic extrusion and co-extrusion. The extrusion method comprises the passage of the polymer melt with the solubilized bioactive through a nozzle, or the polymer melt and bioactive through concentric nozzles, leading to the formation of particles with high density and encapsulation efficiency.[30,37] This technique is primarily used for the encapsulation of volatiles and unstable flavours.[39] Belščak-Cvitanović *et al.*[105] and Barbosa-Pereira *et al.*[122] demonstrated the efficiency of this method for the encapsulation of phenolic compounds. Co-extrusion is used to prepare spherical microbeads with hydrophobic core,[37] nevertheless it can also be used

for the encapsulation of hydrophilic compounds in alginate beads as it was done by Piazza & Rioversi.[123]

2.2.5. Lipossomes

Liposomes technology has been mostly used in pharmaceutical and cosmetic fields, for targeted delivery of therapeutic agents and inclusion of stabilizers in creams and lotions, respectively. For food applicability they represent a high valuable resource due to their high encapsulation efficiency, stability and easy production.[39] Foremost, liposomes have been used to stabilize and increase bioavailability of bioactive molecules.[124-127] Moreover it is widely used to encapsulate compounds that are poorly soluble in certain solvents. Coimbra *et al*.[128] demonstrated the efficacy of liposomes for the encapsulation of resveratrol, caffeic acid, carvacrol, among others (compounds poorly soluble in water). While Rasti *et al*.[129] increased the oxidative stability of polyunsaturated fatty acids by means of its encapsulation in liposomes.

2.2.6. Supercritical fluids based process

Supercritical based processes have major advantages for the encapsulation of sensitive substances such as essential oils or enzymes, always being coupled with other encapsulation techniques. Almeida *et al*.,[62] used supercritical fluid impregnation technique to encapsulate oregano essential oil into a starch matrix, achieving a homogenous product in a faster way due to the low viscosity and higher diffusion of supercritical CO_2. On the other hand, Santos *et al*.,[94] by using rapid extraction of supercritical solution, and Sosa *et al*.[130] and Visentin *et al*.[87] by using supercritical antisolvent process, applied this technique to encapsulate bioactive extracts with high encapsulation efficiencies. The main advantages of supercritical fluids are related to

384 their physical properties such as viscosity, density, solvating power, diffusion and mass
385 transfer. The solubilisation of the core and shell materials are therefore faster as
386 microcapsule formation is facilitated, i.e. they are formed by using lower temperatures
387 and without the presence of water.[39]
388
389 **2.2.7. Ultrasound based process**
390 Ultrasound based processes, such as sonification and ultrasound, are also reliable
391 techniques for food applications, mostly being used with the double function of
392 extracting the bioactive and forming the microcapsules.[131,132] Otherwise,
393 Kalogeropoulus *et al.*[133] used sonification to aggregate the inclusion complex of
394 propolis extract and β-cyclodextrins to form the microcapsules.
395
396 **2.2.8. Others**
397 Despite all the above described, there are other methods not so common for food
398 applications. An example is the fluidized bed, a microencapsulation technique for
399 powder compounds. It needs the preparation of a suspension with the coating material
400 (polysaccharides, proteins, emulsifiers and fats) and subsequent spray, offering a more
401 effective controlled release of the core material than with other existing
402 technologies.[30,37,39] Li *et al.*[134] used this technology achieving good integrity and
403 stability of the core compound after the drying process. Molecular inclusion is another
404 process that is not so commonly used, generally referred to as a supramolecular method
405 in the sense that the bond between the encapsulated compound and the shell material
406 occurs in a cavity-bearing substrate by hydrogen bonds, Wander-Wall forces or entropy-
407 driven hydrophobic effects. Cyclodextrins and hydrophobic vitamins are the most
408 common used shell materials in molecular inclusion methods.[39] Spinning-disk and

centrifugal co-extrusion appeared as new atomisation methods, possibly used in modified spray encapsulation methods; the difference relies on the formation of the capsule, involving the creation of a film with much smaller dimensions than those obtained in common atomisers.[39] Aktar *et al*.[135], showed that the reduction of the particle size using spinning-disk reactor to encapsulate flavonoids by means of a double emulsion technique, reaching a better stabilization of the prepared emulsions by this technique. Other microencapsulation methods that are not commonly used in the food sector are co-crystallization,[136,137] core-shell printing,[138] nanoprecipitation,[111,139] lyophilisation,[140,141] microwave,[142] phase separation method,[143] response surface methodology[144] and solvent evaporation method.[145,146]

2.3. Encapsulation materials

When designing an experiment protocol for the development of encapsulated products (Figure 4), the shell material choice is one of the most important steps, firstly because it has to be non-toxic to the organism, its preparation has to respect environment issues and use clean solvents (water soluble materials are therefore preferable) and, finally, because it plays a crucial role in the bioactive release behaviour. Conditions such as pH, temperature, salts and ions concentration also have to be taken into account and defined in accordance with the ultimate objective of the developed microcapsules. In this work the materials were divided into four categories (Table 2), according to Kuang *et al*.[30] which discriminate them as water and non-water soluble materials, and as polymer and non-polymer materials. Within each category it was also possible to sub-divide into carbohydrate and its derivatives, protein and its derivatives, synthetic polymers and other type of materials.

The coating material and its physical structure strongly influence the product development; nevertheless there are some constraints since law does not allow the application of some materials in food. They must be considered "generally recognized as safe" (GRAS), biodegradable and efficient as the protective barrier between the nucleus and the surrounding medium. Both EU through the EFSA and the US through FDA have many strict rules about material usage for food applications.[37,147] The most commonly used materials are carbohydrate polymers (starch and cellulose and their derivatives), plant exudates and extracts (gum, galactomannans, pectins and soybean polysaccharide), marine extracts (carragenin and alginate), microbial and animal derived polysaccharides (xanthan, gellan, dextran and chitosan), and also proteins, lipids and others (paraffin and some inorganic materials).[148] This is in accordance with our survey, where it can be observed that water soluble materials, both polymer (e.g. alginate and chitosan) and non-polymer (e.g. cyclodextrins) types, are the most commonly used, followed by non-water soluble polymers (e.g. starch and caseins) and, finally, non-water soluble non-polymers (e.g. sucrose and lecithin).

Concerning the EU, no access is provided to a list of authorized materials for food product development by EFSA. There is a lack of information, as the existing list is under construction. They include only food additives and nutrient sources, listing only those who are not considered food additives (e.g. starch), but without any reference to whether they are authorized or not.[149] Regarding the US, the FDA has a list of approved food ingredients that allows the companies and academia to design microencapsulation protocols more suitable to serve food industry purposes. Despite the listed above compounds, identified as the most commonly used, not all have been approved by the FDA (or they were not considered for review or the assessment is pending). From Table 2, and following the guidelines of FDA, it can be observed that the approved materials

458 are: stearic acid, sucrose, amylopectin, maize starch, calcium caseinate, casein, FHCO
459 (fully hydrogenized canola oil), PGPR, β-ciclodextrin, ethanol, lactose, PEG
460 (polyethylene glycol), alginate, chitosan, whey protein, cellulose, xanthan, ethyl
461 cellulose, soy protein, inulin, pectin and lysozyme. The materials with pending requests
462 for assessment are: lecithin, caffeine, arabic gum, milk proteins and poloxamer. For the
463 remaining materials no information is available. It is also necessary to understand that
464 some investigations are conducted to find new encapsulation materials, meaning that
465 although they are not currently present in the FDA list, they could be added in the
466 future. Many of them are of natural origin such as starch from *Araucaria angustifolia*
467 (Bertol.) Kuntze seeds,[100,101] mucilage extract form *Opuntia ficus* Indica[72] and
468 gelatinized sweet potato starch[150] and, therefore, further studies are need to establish the
469 safety of these materials.
470

471 **3. Incorporation of microencapsulated bioactives in food matrices**

472 **3.1. Bioactive extracts**

473 The main reason to consider a bioactive extract is related with synergistic effects
474 occurring among their components that often result in increased bioactive
475 characteristics. The information regarding microencapsulated bioactive extracts
476 obtained from different plant materials and other natural matrices, after extraction with
477 various solvents is summarized in Table 3. Crude extracts represent a significant part of
478 the microencapsulation studies, followed by polyphenols (as also anthocyanins),
479 essential oils, vitamins, proteins and fat extracts.
480 The majority of the microencapsulation studies for food purposes have focused on the
481 technique development itself which includes the definition of the best suitable materials
482 and the achievement of microcapsules with the adequate morphology, encapsulation

efficiency, stability and release behaviour. The studies calling up the development of final applications, i.e., the test of the microencapsulated materials with real food matrices is much scarcer. Chiou & Langrish[47] used the crude extract (water) of *Hibiscus sabdariffa* L. for encapsulation with the fibres extracted from the same fruit as the wall material, aiming at developing a novel nutraceutical product using a by-product usually not consumed. A similar study conducted by Berg *et al.*[70] in which pectin (natural polysaccharide) was used as the encapsulation wall material to protect anthocyanins extracted from *Vaccinium* genus fruits, showed that the addition of gelling substances gave a higher encapsulation efficiency. The optimization of encapsulation methodologies is constantly evolving, as is the case of supercritical-based processes, which were used to encapsulate green tea extract from *Camellia sinensis* L. leaves with polycaprolactone (PCL), by high pressure antisolvent coprecipitation demonstrating a high retention of catechins in the co-precipitates, and also to encapsulate ethanolic extracts from *Rosmarinus officinalis* L. leaves with proloxamer polymers, with similar results.[87,130] With a different goal, but intending to improve encapsulation and delivery of bioactive extracts, Averina & Alléman[111] developed pH sensitive micro- and nanoparticles containing natural sources of polyunsaturated fatty acids namely, oils extracted from *Thymallus baikalensis* Dybowski muscle and *Pinus sibrica* Du Tour seeds, and commercial fish oil, by using the emulsification-diffusion and nanoprecipitation techniques with promising results. Barras *et al.*[124] developed lipid nanoparticules loaded with polyphenol extracts to enhance their solubility and stability. Many of the studies with phenolic compounds are performed with the main objective to optimize the encapsulation process,[80, 118, 125, 131] using different types of extracts (e.g. alcoholic, aqueous, hydroalcoholic etc.). In fact, there is no specific standard protocols for the extraction of each class of phenolic compounds, depending on the nature of the

sample and the objective of the work (structure elucidation and quantification).[151] In terms of proteins,[138,152] vitamins,[88] phytosterols[153] and essential oils,[57,59,60] the majority of the studies was also conducted with the aim of developing new encapsulating methodologies and materials, and to optimize the process.

After optimization of the encapsulation process, it is necessary to establish whether the extracts maintain, reduce or increase their bioactive characteristics. Therefore, several bioactivity assays can be conducted to evaluate the antioxidant and antimicrobial activities, and quantify total phenolic compounds. To assess the antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity is the most commonly used assay, not only to characterize a given sample, but also to evaluate the bioactivity maintenance. The studies performed by López- Córdoba *et al*.[136] and Chan *et al*.[154] with crude extracts of *Ilex paraguarensis* A. St. Hil. aerial parts and *Piper sarmentosum* Roxb., respectively, showed that encapsulation did not affect, positively or negatively, the antioxidant activity of the extracts. On the other hand, in the studies conducted by Igual *et al*.[49] and Parthasarathi *et al*.[43] with *Solanunm quitoense* L. pulp and *Garcinia cowa* Roxb. fruit, respectively, the encapsulation proved to be very effective, since an increase in the antioxidant activity of the extracts was observed, which can be explained by a protection of the bioactives from degradation. Anthocyanin extracts obtained from *Garcinia indica* Choisy fruit pulp,[68] *Euterpe oleracea* Mart. fruit pulp[71] and *Daucus carota* L. roots[67] were encapsulated with maltodextrins, which proved to be efficient at protecting these extracts whose stability and antioxidant activity increased after microencapsulation. With another goal Deladino *et al*.,[90] used DPPH assay to assess the diffusion and kinetic behaviour of the produced microencapsulated system. Oxygen radical absorbance capacity (ORAC), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid assay (ABTS) and trolox equivalent antioxidant capacity (TEAC) assays have also

been used to evaluate antioxidant activity of microencapsulated extracts.[50,62,76,82,105,115,117] As previously mentioned, the quantification of phenolic compounds is also a very common methodology to assess the effectiveness of the encapsulation process.[42,44,46,48,64,78,79,85,92,98,141,155] Some studies also describe the use of carotenoids to infer the efficacy of the microencapsulation process.[94,141]

Antibacterial and antifungal properties are among the most studied and important bioactivities. Not only due to the increasing resistance of the microorganisms to commercially available synthetic antibiotics, but also because natural matrices present high potential for the discovery of new drugs. There are several studies focusing on the microencapsulation of natural extracts presenting antibacterial and antifungal activities. Sansone *et al*.[52] and Fernandes *et al*.[45] reported the antifungal activity of *Paeonia rockii* (S.G.Haw & Lauener) roots and *Lippia sidoides* Cham. leaves, respectively, showing the advantage of their microencapsulation since and enhancement of the antifungal activity was obtained comparatively with the extracts in the free form. The antibacterial activity of the essential oil extracted from *Citrus hydrix* D.C. fruit skins was assessed by Adamiec *et al*.,[58] also reporting the enhancement of the activity in the microencapsulated extract. Souza *et al*.,[86] studied the antimicrobial effect of *Vitis labrusca* L. ethanol/water (67.6%) encapsulated extract, showing a very good growth inhibiting capacity of *Staphylococcus aureus* and *Listeria monocytogenes*.

Studies considering the improvement of bone quality in rats[121] and *in vitro* cytotoxicity[107] were performed with microencapsulated *C. sinensis* tea. The antioxidant α-glucosidase inhibitory activity of microencapsulated aqueous extracts from *Punica granatum* L. peel and the anti-inflammatory effect of commercial polyphenols and oil extracts were also studied.[77,128]

As can be observed in Figure 4, *in vitro* release studies are one of the most important steps to consider when developing and validating a microencapsulated product. A successful microencapsulated system has to protect the bioactive compound assuring bioavailability maintenance but also needs to guarantee the intended release behaviour (temporal and target oriented). *In vitro* release studies can be made by simulating the gastrointestinal environment using pH buffers mimicking the conditions of digestion,[106,156] or using *in vitro* gastrointestinal models comprising enzymes and pH buffers.[110, 133,143,150] Tavano *et al*.[156] showed, by *in vitro* released studies, that curcumin and quercetin when microencapsulated in niosomes had improved solubility after gastrointestinal digestion. Frank *et al*.[110] and Park *et al*.[150] reported that after *in vitro* gastrointestinal digestion, microencapsulate anthocyanin extracts of *V. myrtillus* and a commercial oil extract, respectively, presented good resistance to pH changings during digestion, being released only at intestinal conditions. This corroborates the interest and efficacy of microencapsulation to design adequate delivery systems for water and non-water soluble compounds to be incorporated in innovative food products.

3.2. Bioactive compounds

The importance of studying individual bioactive compounds relies on their powerful bioactivities, with different applications, including in pharmaceutical and food industry fields. In this context their isolation from the original matrix is an interesting topic of study and brings added valued to the developed products. A set of microencapsulated individual bioactive compounds used for food application purposes, is described in Table 4. The number of articles concerning the encapsulation of individual compounds is markedly lower than that of bioactive extracts. However, phenolic compounds are once more the individual molecules most commonly used in microencapsulation

582 experiments. Most of those studies are focused on the development and optimization of
583 microencapsulation techniques,[74,82,132,140,144,145,157] including new encapsulation
584 materials. An example is the work performed by Medina-Torres *et al*.[72], in which
585 commercial gallic acid was encapsulated using mucilage extracted from *O. ficus Indica*.
586 Robert *et al*.[73] also encapsulated gallic acid using acetylated starch and inulin, obtaining
587 higher encapsulation efficiency with the first material. On the other hand, for quercetin
588 and vanillin phenolic compounds, inulin gave the best results[81]. Despite the beneficial
589 health effects of phenolic compounds, their stability and bioavailability is severely
590 compromised during food processing, storage and digestion, as mentioned in the
591 previous sections. So, microencapsulation of individual phenolic compounds could
592 provide a way to maintain or increase their antioxidant activity,[114,139] stability[75,97] and
593 bioavailability.[96,127] The antimicrobial activity was also tested in microcapsules
594 containing chlorogenic acid isolated from *Nicotiana tabacum* L. leaves, indicating that
595 its activity was not affected by microencapsulation, being an alternative in the
596 development of food products with antimicrobial properties.[158]
597 Polyunsaturated fatty acids were also the target of microencapsulation studies. Their
598 known beneficial health effects make them very appealing to enrich food matrices.
599 However, their lipophilic nature and tendency for rancidity are obstacles for the
600 development of efficient delivery systems. Naik *et al*.,[102] developed an encapsulation
601 technique for the delivery of α-linoleic acid isolated from the seeds of *Lepidium sativum*
602 Linn. using freeze drying to achieve a stable and bioavailable compound. On the other
603 hand, Shaw *et al*.[66] and Rasti *et al*.[129] developed different lipophilic delivery systems for
604 commercial ω-3-fatty acids. Shaw *et al*.,[66] used spray-drying technique with lecithin and
605 chitosan as wall material, to prevent lipid oxidation and to study the reconstruction of
606 the enriched microcapsules in aqueous medium, showing that this multilayer system

607 was very promising. Rasti *et al*.,[129] used liposomes based delivery systems to
608 microencapsulate the ω-3-fatty acids, using soybean phospholipids as the wall material.
609 The authors demonstrated that the formation of liposomes in aqueous medium,
610 combined with the antioxidant protection of the phospholipids, increased the stability
611 and prevented fatty acids peroxidation. Other compounds, also very unstable and
612 therefore benefiting from microencapsulation, are essential oils or their constituents. In
613 addition to the lipophilic character they are also very volatile, needing the protection
614 assured by microencapsulation. Lipid carriers involve the formulation of a lipidic
615 solution containing the solid lipids, surfactants and drying carriers (e.g.
616 polysaccharides) and have provided high encapsulation efficiencies for eugenol and
617 eugenyl acetate isolated from *Syzygium aromaticum* L. buds.[61] Microencapsulation by
618 co-crystallization of cardamom oleoresin also protected their major components, 1,8-
619 cineole and α-terpinyl acetate; nevertheless, some degradation occurred during
620 packaging and storage.[137]
621 Carotenoids are a family of compounds largely used for food coloration in substitution
622 of synthetic dyes, presenting additionally antioxidant and antiangiogenic effects.
623 Nevertheless, their tendency for oxidation and isomerization is high. Qv *et al*.[104] and Xu
624 *et al*.,[159] studied the stability of lutein and curcumin, respectively, after
625 microencapsulation by complex coacervation with Ca-alginate/k-carragening, and Ca-
626 alginate/lysozyme, respectively. Both achieved good encapsulation efficiencies and
627 demonstrated the efficacy of the used method. Spada *et al*.,[100,101] microencapsulated
628 commercial β-carotene in starch obtained from *Araucaria angustifolia* (Bertol.) Kuntze
629 seeds, and concluded that a modified gelation form of this starch conducted to higher
630 carotenoid encapsulation efficiency ensuring protection against adverse conditions.
631 Aissa *et al*.,[54] tested microcapsules enriched with β-carotene for its genotoxic and

antiangiogenic effects, using arabic gum as wall material. The authors observed a preservation of the gentoxic effects, but a decrease in antiangiogenic activity, maybe due to the loss of bioavailability during microencapsulation.

Organic acids,[83,84,99,142] enzymes[55,56] and proteins[91,93] are examples of other individual compounds that have been subjected to microencapsulation.

Vitamin B_2 (riboflavin) and vitamin B_9 (folic acid) have also been microencapsulated for food purposes. Due to their known beneficial health effects, coupled with a high tendency to degradation and loss of bioavailability, *in vitro* release tests were used to evaluate new delivery systems. Chen & Subirade,[113] tested the release of riboflavin using simulated gastric, intestinal and pancreatic fluids, concluding that riboflavin microcapsules made of alginate/whey protein are semi-destroyed by the intestinal fluid and completely released with pancreatic fluid. To estimate product shelf life, Wichchukit *et al*.[89] studied the release of riboflavin incorporated into a food product, a model beverage. Prasertmanakit *et al*.[146] studied the *in vitro* release of folic acid from ethyl cellulose microcapsules, material that had good encapsulation efficiency. The addition of water soluble polymer, sucrose, originated the swelling of the polymer matrix, which allowed a better controlled release of folic acid.

An improvement in delivery systems development is the encapsulation of a mixture of bioactive compounds within the same microcapsule, thereby obtaining several beneficial effects. Augustin *et al*.,[112] developed an oil-in-water emulsion to stabilize commercial fish oil, resveratrol and tributyrin using caseinate, glucose and starch, to study their behaviour in the grastrointestinal tract, obtaining increased bioavailability for all the compounds. Pan *et al*.,[109] studied the oxidative stability of curcumin (carotenoid) and retinol (essential oil) in oil-in-water emulsions, with very satisfactory results.

3.3. Incorporation in food matrices

Some examples of applicability studies with microencapsulated bioactive extracts or individual compounds are described in Table 5. After an exhaustive search in literature, it was confirmed that the vast majority of the studies do not include the validation of the developed microencapsulated bioactives through their incorporation into food matrices. Only twelve studies were found where this final step, so important for the food industry, was included. In general, milk and dairy products such as cheese and yoghurt, and ice creams were the preferable food matrices under study. The sector of cereals, bread and pasta, has also significant weight on applicability studies. Tea, soup and meat are also food matrices than have been tested for incorporation of bioactive microcapsules. Phenolic extracts of *Punica granatum* L. peels were studied by Çam *et al*.[77] and added to ice cream to enhance antioxidant and α-glucosidase inhibitory activities. Martins *et al*.[92] and Robert *et al*.,[85] also incorporated phenolic extracts in yogurt using *Rubus ulmifolius* Schott. flowers and *Punica granatum* L. fruits, respectively. Martins *et al*.,[92] obtained higher antioxidant activity in yogurts with microencapsulated extracts, comparatively with the use of extracts in the free form and with the control (yogurts without extracts); on the other hand, Robert *et al*.[85] also reported a higher content of phenolic compounds and anthocyanins in yogurt with microencapsulated extracts. The incorporation technique developed by Barbosa-Pereira *et al*.[122] to add phenolic extracts in active packaging to extend shelf-life of meat products gave promising results retarding lipid oxidation and microbial growth. In terms of individual phenolic compounds, a water soluble isoflavone was microencapsulated in a polyglycerol monostearate emulsion and further incorporated in milk to study its stability during

storage and after *in vitro* digestion. It was demonstrated that the microencapsulated isoflavone did not affect milk taste and that its absorption in the intestine increased.[116] Citric acid and its derivative, (-)-hydroxycitric acid, were also used in incorporation studies; in particular, the derivative extracted from the fruits of *Garcinia cowa* Roxb. was incorporated into bread[83,99] and pasta;[84] in both cases, bread and pasta enriched with microencapsulated bioactives showed good sensory and quality attributes, which proves the viability of using such strategies in food products development. Citric acid was also incorporated in chewing gum at a micronized scale, using a technique based on casein and inulin to form bioactive microcapsules, to develop chewing gums with health promoting properties.[142] Soups, one of the most highly consumed food products worldwide, also served as the matrix for the incorporation study developed by Rubilar *et al*.[65] Microcapsules containing fatty acids (linseed oil) were added to an instant soup in powder form in order to develop a new functional product; moreover, since the linseed oil was microencapsulated in a polymeric matrix consisting of arabic gum and maltodextrin, a higher controlled release of the lipophilic core was successfully achieved. Sardar *et al*.,[137] also encapsulated a lipophilic compound, cardamom oleoresin. Since the stability of this compound to spray-drying was very poor, a sucrose wall matrix was used with a co-crystallization method giving rise to small flavouring sugar cubes for tea beverages. The produced cubes were stable to storage when packed in a three-layer metalized laminate. Cheese, although appreciated by many consumers, is rich in fat and, therefore, there have been efforts in the addition of vegetable oils to this matrix. However, oils degrade very quickly, benefiting from the addition of antioxidants such as vitamins A and E and coenzymes. In this context, the work of Stratulat *et al*.[160] intended to inhibit lipid peroxidation (rancidity), by formulating emulsions, stabilized with calcium caseinate, containing vitamins A and E, and

706 Coenzyme Q10. The results showed that the vegetables oils did not affect the cheese
707 stability, increasing the presence of antioxidants.
708
709 **4. Conclusion**
710 Nowadays, food serves not only to satisfy the primal urge of hunger but is intended to
711 overcoming dietary flaws and/or impart health benefits. Bioatives are sources of
712 functional molecules with recognized health effects in populations that otherwise would
713 not be able to benefit from them. Nevertheless, they can comprise organoleptic
714 constraints and instability to food process, storage and ingestion, which led to a research
715 in the filed of bioactives protection and controlled release. Among the proposed
716 technologies, microencapsulation emerged as a viable route to valorise natural
717 bioactives in functional foods, thus extending their benefits to a wider population.
718 According to the present review, there are available several examples with
719 microencapsulation of bioactives using a wide range of processes and encapsulating
720 materials. Among the various possibilities, the spray-based processes, e.g. spray-drying,
721 are the most commonly used techniques. The advantages refer its easy implementation,
722 namely at industrial level, and the fact of being inexpensive. Nevertheless, green
723 techniques, such as supercritical and ultrasound based processes, are nowadays
724 attracting much attention.
725 Water soluble materials, both polymer and non-polymer ones, are the most commonly
726 used encapsulation materials. They include carbohydrate polymers (starch and cellulose
727 and its derivatives), plant exudates and extracts (gum, galactomannans and pectins),
728 marine extracts (carragenin and alginate), and microbial and animal derived
729 polysaccharides (xanthan, gellan, dextran and chitosan). In most of the cases, the

industrial applicability in the field of food production is prevented by current regulations.

Crude and phenolic extracts, together with individual phenolic compounds, are the most studied bioactives for food purposes. Nevertheless, studies dealing with final food applications are scarce, demanding investment from academia, industry and regulatory agencies. Finally, the consumers have also a crucial contribution on the acceptance of new products in the market.

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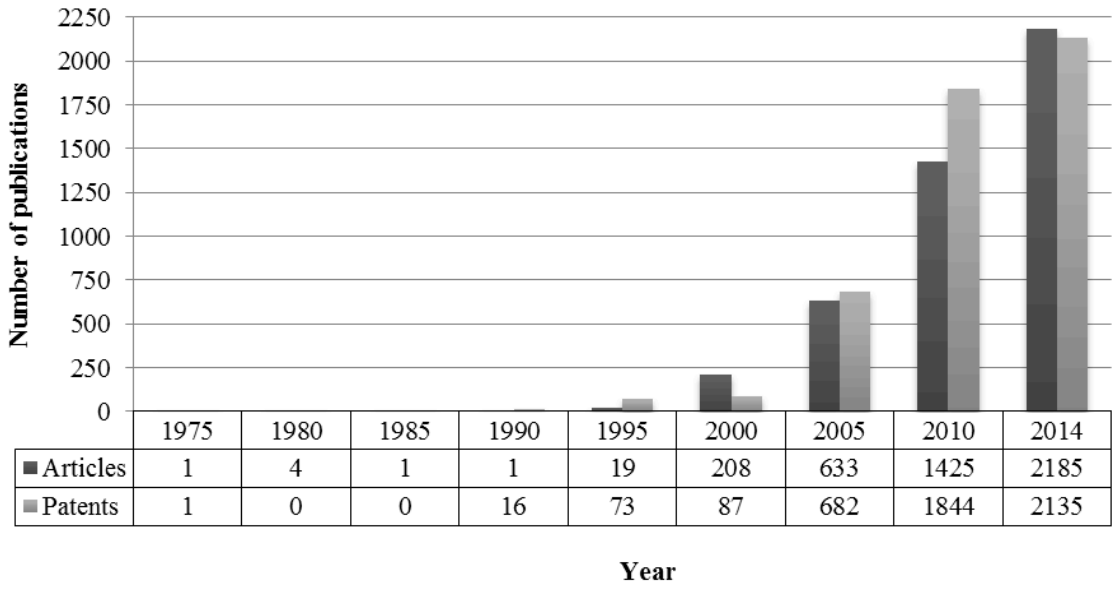


	1975	1980	1985	1990	1995	2000	2005	2010	2014
Articles	1	4	1	1	19	208	633	1425	2185
Patents	1	0	0	16	73	87	682	1844	2135

Figure 1.Number of research articles and reviews, and patents published in the period from 1970 to 2014 regarding functional foods (obtained on web of science, October 2014; keyword: functional food).

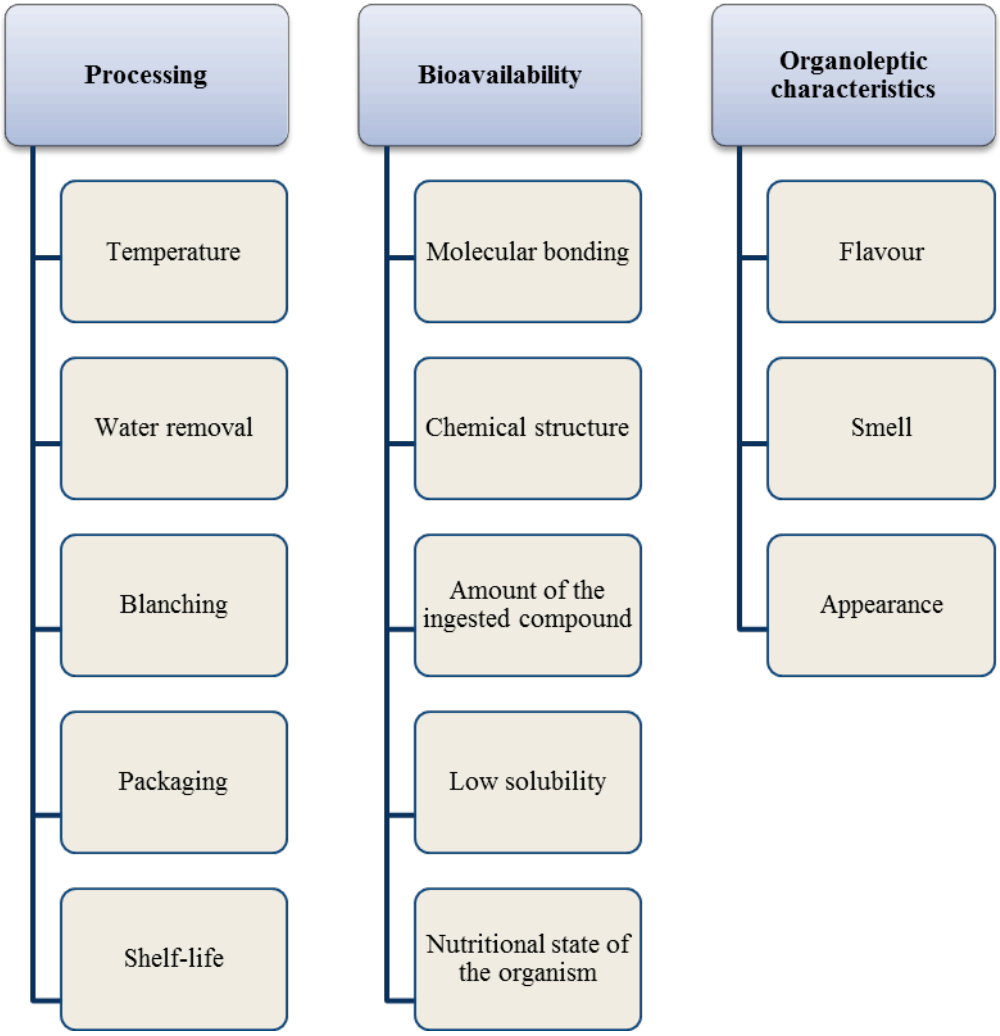
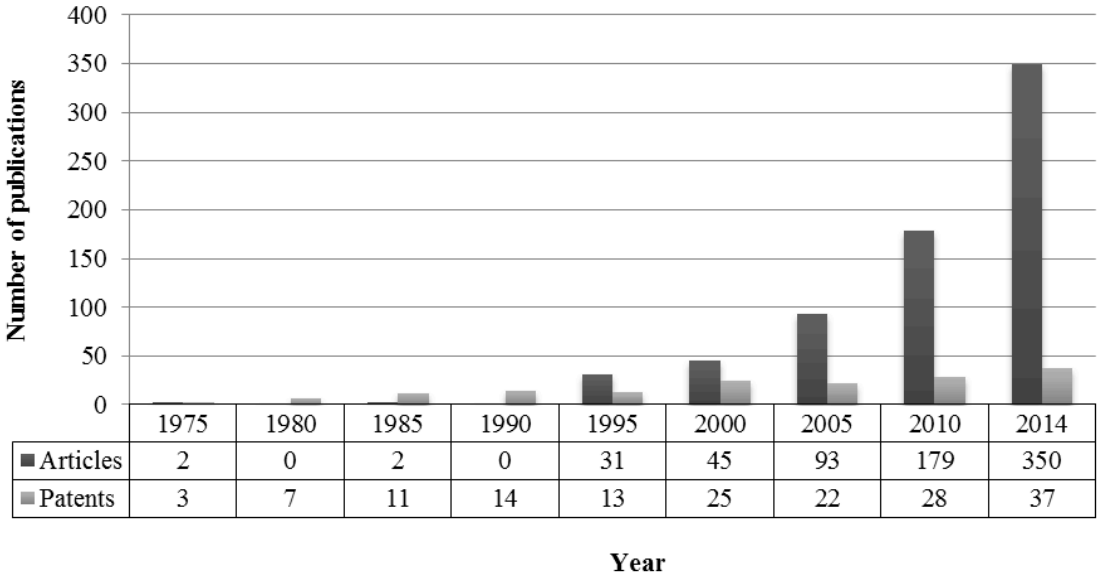


Figure 2. Limiting factors to the use of free bioactives in food applications.



	1975	1980	1985	1990	1995	2000	2005	2010	2014
Articles	2	0	2	0	31	45	93	179	350
Patents	3	7	11	14	13	25	22	28	37

Figure 3.Number of research articles and review, and patents published in the period from 1970 to 2014 regarding microencapsulation for food purposes (obtained on web of science, October 2014; keywords: microencapsulation and food).

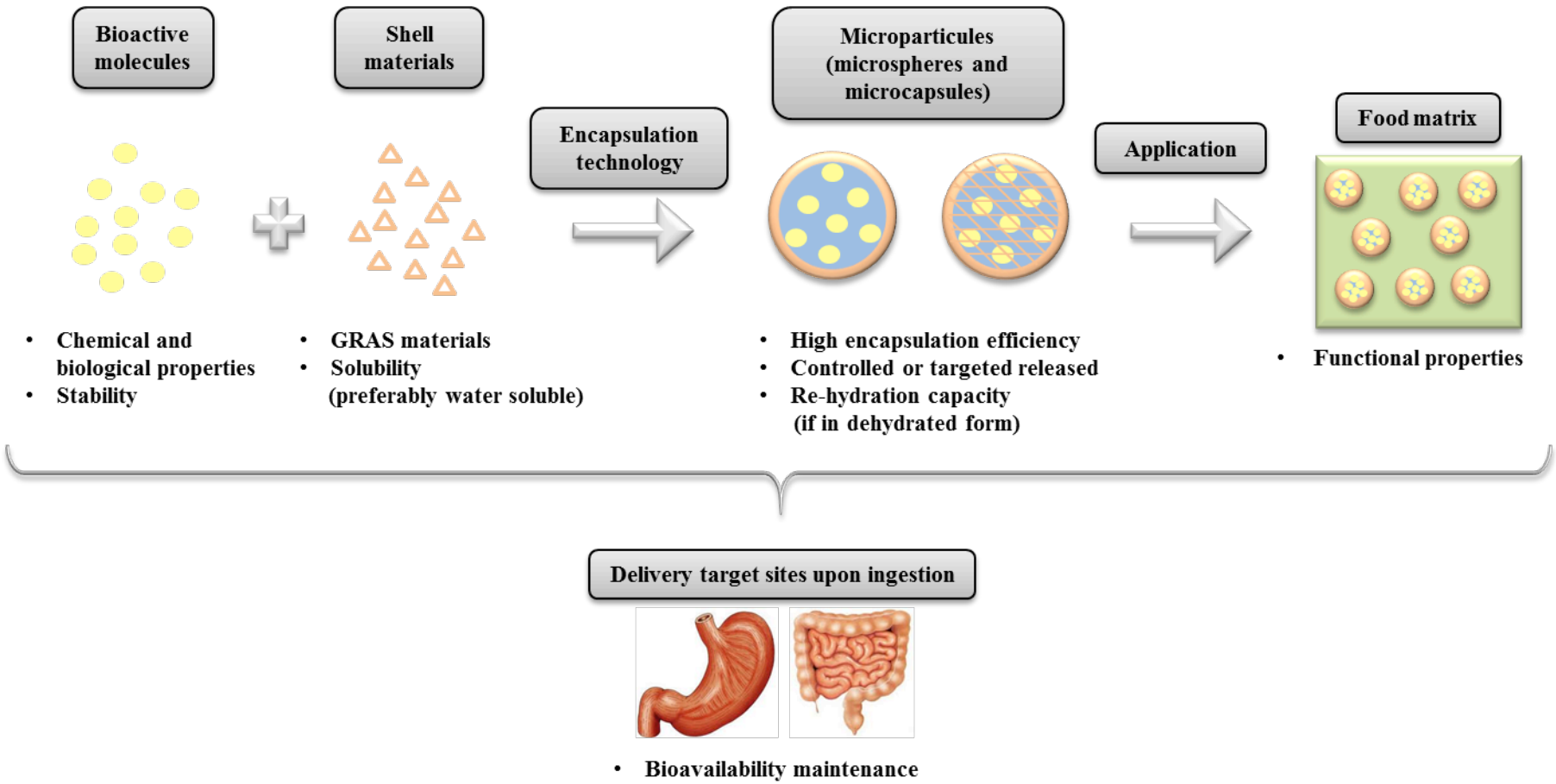


Figure 4 Schematic procedures for the development of microencapsulation protocols (GRAS, generally recognized as safe)

Table 1. The encapsulation methodologies most used for food applications, and corresponding examples.

Method category	Examples	References
Coacervation	Complex Coacervation	90, 93, 102, 104-107, 125, 126, 159
	Simples Coacervation	108-111, 120, 128, 139
Extrusion-based processes	Electrostatic extrusion	105, 122
	Co-extrusion	123, 154
Spray-based processes	Spray drying	42-88, 108, 150, 152, 157
	Electrospray	103
	Spray-coagulation*	89-94, 107
	Spray-freeze drying	96-102
Emulsion based processes		106, 109-121, 160
Lipossomes	Lipossomes and niossomes	124-129, 156
Supercritical fluids based processes	Supercritical antisolvent process	87, 130
	Rapid Extraction of Supercritical Solution	94
	Supercritical fluid impregnation	62
Ultrasound based processes	Sonification	131, 133
	Ultrasound	132
Others	Co-crystallization	136, 137
	Core–Shell Printing	138
	Nanoprecipitation	111, 139
	Fluidized bed	134

Inclusion	153, 158
Lyophilization	140, 141
Microwave	142
Molecular inclusion	155
Phase separation method	143
Response surface methodology	144
Solvent evaporation	145, 146
Spinning disc reactor	135

* Coagulation mostly achieved with internal or external gelation.

Table 2. Main materials used for encapsulating bioactive extracts and compounds for food applications (based on Kuang *et al*.[30]).

Material category	Encapsulation Material	References
Water soluble polymers	Carbohydrate and carbohydrate derivatives (e.g.: alginate, gums, chitosan, amylose, k-carragenin and pectin), protein and protein derivatives (e.g.: whey, milk and soybean proteins), synthetic polymers (e.g: polyethylenoglycol) and others (e.g: ethyl cellulose and mucilage extract of *Opuntia ficus indica*)	43-60, 63-66, 70-79, 81-85, 87-94, 99, 102, 100-108, 110, 111, 11-115, 117-119, 123, 126, 131, 134, 140-144, 146, 153, 154, 157, 159
Water soluble non-polymers	Carbohydrate and carbohydrate derivatives (e.g.: cyclodextrin, maltodextrin, inulin and lactose), synthetic polymers (e.g.:PEG2000-DSPE, polyvinyl alcohol and High and low HLP lypophilic polimeric emulsifiers) and others (Tween, buffer and alcoholic solutions and ascorbic acid)	42, 44-46, 48, 49, 51, 53, 56, 57, 59, 63-65, 67-71, 76-80, 82,83, 94, 96-99, 101, 104, 111, 112, 114, 120, 121, 128, 131-135, 139, 140, 143, 155, 156, 158
Non-water soluble polymers	Carbohydrate and carbohydrate derivatives (e.g.: starch), protein and protein derivatives (e.g.: casein), synthetic polymers (e.g.: low-density polyethylene, poly(ε-caprolactone) and Poly-D,L-lactide (PLA),) and others (liquid vaseline)	51, 56, 59, 62, 71, 73, 100, 101, 103, 109, 110, 112, 115, 122, 129, 139, 142, 145, 150, 152, 153, 160
Non-water soluble non-polymers	Carbohydrate and carbohydrate derivatives (e.g.:sucrose) and others (lecithin, supercritical CO2, stearic acid and wax)	61, 62, 66, 114, 116, 124-129, 136-138, 144

Table 3. Microencapsulated bioactive extracts.

Bioactive extract	Source	Extraction solvent	References
Anthocyanin extracts	*Bactris guineensis* L. fruits	Methanol/acetic acid (19:1, *v/v*)	69
	Daucus carota L. roots	Ethanol	67
	Euterpe oleracea Mart. fruit pulp	Juice	71
	Garcinia indica Choisy fruit pulp	Acidified water	68
	Myrciaria cauliflora (Mart.) fruit peels	Acidified ethanol	82, 94
	Vaccinium genus fruits	n.a	70, 110, 118
Crude extracts	*Bidens pilosa* L. aerial parts	Ethanol	48
	Camellia sinensis L. leaves	Acetone; ethanol	121, 130
	Eugenia uniflora L. fruits	Juice	141
	Fadogia ancylantha Schweinf. aerial parts	Ethanol/water (70:30, *v/v*)	44
	Garcinia cowa Roxb fruits	Water	43
	Hibiscus sabdariffa L fruits	Water	47, 50
	Ilex paraguariensis A. St. Hil. aerial parts	Water	136
	Ipomoea batatas L. Lam variety, Sinjami tuber	n.a	42
	Lippia sidoides Cham. leaves	Ethanol/water (50:50, *v/v*)	45
	Melissa officinalis L. aerial parts	Ethanol/water (70:30, *v/v*)	44
	Morinda citrifolia L. fruits	Ethyl acetate	46
	Paeonia rockii (S.G.Haw & Lauener) roots	Polar	52
	Five herbs: *Paeonia suffruticosa* Andrews, *Phellodendron chinense* Schneid, *Lonicera japónica* Thunb, *Mentha Spicata* L. and *Atractylodes lancea* Thunb.	Water	106
	Piper sarmentosum Roxb.	Water	154
	Propolis	Ethanol	133
	Quercus resinosa Liebm. leaves	Water	51
	Solanum quitoense L. pulp	n.a	49

	Tussilago farfara L.	n.a	44
Crude and fatty acids extracts	Fish oil	Hydrolysis	111
	Pinus sibirica Du Tour seeds	n.a	111
	Thymallus baikalensis Dybowski muscle	Ethanol	111
Essential oil extracts	*Citrus hydrix* D.C. fruit skins	Water	58
	Cymbopogon nardus G. aerial parts	n.a	57
	Majorana hortensis L. aerial parts	n.a	57
	Origanum vulgare L. aerial parts	n.a	57
	Origanum vulgare L. flowers and leaves	Water	59, 60, 62
Fatty acid extracts	Commercial	n.a	63, 65,119
	Hibiscus cannabinus L. seeds	Hexane	64
Phytosterols ester extracts	Commercial	n.a	153
Polyphenol extracts	*Achillea millefolium* L. aerial parts	Water	105
	Cabernet Sauvignon fruits	Juice (wine)	98
	Camellia sinensis L. leaves	Ethanol	107
	Commercial	n.a	122, 124, 156
	Crategus laevigata (Poir.) Dc. aerial parts	Water	105
	Glechoma hederacea L. aerial parts	Water	105
	Hypericum perforatum L. leaves and flowers	Methanol	155
	Ilex paraguariensis A. St. Hil. aerial parts	Water	90
	Myrica genus fruits	Ethanol	143
	Olea europea L. leaves	Water	105
	Orthosiphon stamineus Benth leaves	Methanol/water (50:50, *v/v*)	79
	Prunus cerasus L. pomace	Ethanol/water (50:50, *v/v*)	131
	Punica granatum L. fruits	Ethanol and juice	85
	Punica granatum L. peels	Water	77

	Quercus resinosa Liebm. leaves	Water	78
	Ribes nigrum L. pomace	Ethanol/water/citric acid (80:20 *v/v*; 5%)	76
	Rosmarinus officinalis L. leaves	Ethanol	87
	Rubus chamaemorus L. fruits	Water/acetone (70:30, *v/v*)	97
	Rubus idaeus L. leaves	Water	105
	Rubus ulmifolius Schott flowers	Methanol/water (80:20, *v/v*)	92
	Urtica dioica L. leaves	Water	105
	Vaccinium myrtillus L. fruits	n.a	117
	Vitis labrusca L. seeds and fruits	Water/ethanol (67.6:32.4, *v/v*)	86
	Vitis vinifera L. seeds	Buffer acetate	125
	Aristotelia chilensis [Molina] Stuntz leaves	Ethanol/water (40:60, *v/v*)	115
Polyphenol and betalain extracts	*Opuntia ficus-indica* fruits	Juice and ethanol	80
Polyphenol and oil extracts	Commercial	n.a	128
Protein extracts	Commercial	n.a	138
	Pisum sativum L. grain	n.a	152
Vitamin extracts	*Capsicum annum* L. variety Piquillo seeds, skins and stems	CO_2	88
Vitamin and enzyme extracts	Commercial	n.a	160
Oil extracts	Commercial	n.a	120, 150

n.a. –not available information.

Table 4. Microencapsulated individual bioactive compounds.

Class	Individual bioactive compounds	Source	References
Carotenoids	Curcumin	Commercial	114, 127, 159
	Lutein	Commercial	104
	β-carotene	Commercial	48, 100,101
	β-carotene	*Capsicum annuum* L. fruits	53
Carotenoids and vitamins	Curcumin and retinol	Commercial	109
Enzymes	Cellulases and xylanases	Commercial	55
	Coenzyme Q10	Commercial	56
Essential oil	Cardamom oleoresin	Commercial	137
	Engenol and eugenyl acetate	*Syzygium aromaticum* L. buds	61
Fatty acid	α-Linolenic acid	*Lepidium sativum* Linn. seeds	102
	ω-3 Fatty acids	Commercial	66, 129
Phenolic compounds	Caffeine	Commercial	157
	Catechins	*Camellia sinensis* L. leaves	96
	Chlorogenic acid	*Nicotiana tabacum* L. leaves	158
	Ellagic acid	Commercial	126
	Gallic acid	Commercial	72, 73, 140
	Isoflavone	Commercial	116
	Mangiferin	*Mangifera indica* L. bark	74
	Naringenin and quercetin	Commercial	75
	Quercetin	Commercial	139
	Quercetin and vanillin	Commercial	81

	Quercitrin	*Albizia chinensis* L. flowers (90:10, *v/v*)	145
	Resveratrol	*Arachis hypogaea* L. sprout	144
	Resveratrol	*Polygonum cuspidatum* Siebold & Zucc roots	132
	Rutin and anthocyanins	*Hibiscus sabdariffa* L. dried calyx	135
Proteins	Albumin and hirudin	Commercial	93
	Papain	Commercial	91
Organic acids	Citric acid	Commercial	123
	(−)-Hydroxycitric acid	*Garcinia cowa* Roxb fruit	142
Organosulfur compound	Allicin	*Allium sativum* L. buld cloves	83, 84, 99
Vitamins	Folic acid	Commercial	134
	Riboflavin (Vitamin B_2)	Commercial	123
Mixtures of bioactives	Fish oil, resveratrol, tributyrin	Commercial	123
	Glucose, vitamin B12, olive oil	Commercial	103, 146
	Fish oil, phytosterols (5α-cholestane, β-sitosterol, campesterol and stigmasterol) and limonene	Commercial	89, 113

n.a. –not available information

Table 5. Examples of studies with microencapsulated bioactive extracts or individual compounds incorporated in food matrices.

Food matrix	Bioactive	Source	Encapsulation method	Encapsulation Material	References
Bread	(−)-Hydroxycitric acid	*Garcinia cowa* Roxb fruit rinds	Spray-drying	Whey protein and maltodextrin	83
	(−)-Hydroxycitric acid	*Garcinia cowa* Roxb fruit rinds	Freeze-drying	Whey protein and maltodextrin	99
Cheese	Vitamins E and A; Coenzime10	Commercial	Emulsion	Calcium caseinate	160
Chewing gum	Citric acid	Commercial	Microwave	Casein and inulin	142
Ice cream	Phenolic extracts	*Punica granatum* L. peels	Spray drying	Maltodextrin	77
Meat	Phenolic extracts	Residues from brewing industry	Extrusion	Ethylene vinyl acetate and LDPE	122
Milk	Isoflavone	Commercial	Emulsion	Polyglycerol monostearate	116
Pasta	(−)-Hydroxycitric acid	*Garcinia cowa* Roxb. fruits	Spray-drying	Whey protein	84
Soup	Fatty acid (Linseed oil)	Commercial	Spray-drying	Gum arabic and maltodextrin	65
Tea	Cardamom oleoresin	Commercial	Co-crystallization	Sucrose	137
Yougurt	Phenolic extract	*Rubus ulmifolius* Schott flowers	Atomization/coagulation	Alginate	92
	Phenolic extract	*Punica granatum* L. fruits	Spray-drying	Maltodextrin or soybean protein	85