Microencapsulation of antioxidant compounds through innovative technologies and its specific application in meat processing

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Abstract

Background: Meat has a complex physical structure and chemical composition that is very prone to oxidation. Plants are sources of biologically active compounds (antioxidants) of interest as potential raw materials for meat processing, primary as replacements for synthetic additives. Some examples are essential oils from aromatic plants that are usually unstable under common processing and storage conditions and exhibit strong smell and off flavour. Hence, stable delivery systems like encapsulation are required.

Scope and approach: Encapsulation, and particularly spray-drying, offers protection of active compounds, their controlled and targeted release in food products and ability to mask unacceptable odours in products.

Key findings and conclusions: Albeit current results are promising for microparticles and nanomaterials, more research is needed to evaluate the application of various natural ingredients in meat processing. Direction of future research should address functionality of systems, consumers' health concerns and benefits, better sensory acceptance, reduced operating costs, scalability for industrial needs, and size of environmental footprints.

Keywords: natural antioxidants; meat processing; encapsulation; wall materials; spray-drying; nanotechnology

1. Introduction

Meat, as a food matrix, has a complex physical structure and chemical composition that is very prone to oxidation, depending on the interaction between endogenous anti- and prooxidant substances (Banerjee, Verma, Siddiqui, Naveena & Kulkarni, 2017). Lipid oxidation consists of a chemical process that mainly involves the development of off odours and decreases the acceptability of meat products by deterioration of their colour, texture and nutritional value (Falowo, Fayemi & Muchenje, 2014; Lorenzo, Batlle & Gómez, 2014).

Over the last years, both meat industry and food technologists expressed increased interest regarding meat health aspects. Although it is usually very expensive to develop new food products, great efforts have been made to produce new meat products with low fat and sodium, while being enriched with dietary fibres and/or n-3 PUFAs and keeping an extended shelf life (dos Santos *et al.*, 2017; dos Santos *et al.*, 2016; Lorenzo, Cittadini, Bermúdez, Munekata & Dominguez, 2015). The development of healthier meat products is possible through two strategies: decreasing the undesired substances and increasing the levels of desired components (Hygreeva, Pandey & Radhakrishna, 2014; Toldrá & Reig, 2011). This trend is in accordance with the use of natural compounds, including by-products from the agro-food industry, instead of synthetic antioxidants for the development of stable meat products over the shelf life (Fernandes, Trindade, Lorenzo & de Melo, 2018; Fernandes *et al.*, 2017; Pateiro, Lorenzo, Amado & Franco, 2014). Synthetic antioxidants, including BHA, BHT or TBHQ, have been questioned because of their potential toxic and undesirable side effects (Moure *et al.*, 2001). For instance, Munekata *et al.* (2017) observed positive effects on physicochemical properties, lipid and protein oxidation, volatile

compounds and free fatty acids content after adding natural antioxidants in Spanish salchichón enriched with n-3 fatty acids encapsulated with konjac glucomannan as stabilizer. However, they suggested that more studies are necessary to evaluate the effect on texture and sensory properties of the salchichón.

It has been also reported that extracts from plants, which contribute to consumers' health benefits, have stronger antioxidant activity than that of synthetic antioxidants (Biswas, Chatli & Jairath, 2017). In addition, within the current concern about pesticides, antibiotics, hormones, genetic modification and other food contaminants, natural compounds facilitate to find on the labelling familiar food ingredients and additives that are organic and therefore perceived as healthier (Bhatnagar & Kulshrestha, 2017). Some plants such as beetroot, aloe vera, canola, cocoa, garlic, grapes, citrus, green tea, onion, pumpkin, plum extract or soya sprouts are important sources of antioxidants (Biswas *et al.*, 2017).

In this context, antioxidant compounds can prevent, delay and even inhibit oxidative reactions at relative low concentrations (Lorenzo *et al.*, 2017) and have been reported as a useful tool in the prevention of cancer, diabetes, cardiovascular and neurodegenerative diseases (Aguar, Costa, Rocha, Estevinho & Santos, 2017; Stelmach, Pohl & Szymczycha-madeja, 2015). However, natural antioxidants are limited to their own nature and the processing conditions (exposure to oxygen and light, pH, temperature, storage time, etc.) that are important factors to consider for food technological purposes (Lorenzo *et al.*, 2017; Anandharamakrishnan & Ishwarya, 2015a). Finally, the spoilage of meat is specially related with three aspects: (a) microorganisms, (b) lipid

content, and (c) autolytic enzymes. Thus, meat preservation strategies should take these mechanisms into consideration (Ramachandraiah, Choi & Hong, 2017).

2. Microencapsulation technology

There are several techniques which have been used for microencapsulation of food ingredients to overcome some of their restrictions and increase their bioavailability, solubility, and applicability. These techniques are spray-drying, freeze drying, liposomes, coacervation, molecular inclusion, polymeric micelles, extrusion processes, supercritical fluids, nanostructured lipid matrices or solvent evaporation (Franco *et al.*, 2017; Aguiar, Estevinho & Santos, 2016). Previously, microencapsulation methods were divided into the following basic groups: chemical, physico-chemical and physico-mechanical. No single encapsulation process is adaptable to all core materials or product application (Wilson & Shah, 2007). Each method can present its own advantages and disadvantages, but most of the commonly used ones can present several disadvantages as unfavourable conditions for the core material, complexity in procedure, low encapsulation efficiency or problems in terms of reproducibility (Das *et al.*, 2011). For instance, coacervation presents high encapsulation efficiency and control of particle size but is an expensive method, as emulsification which is also associated with high production of residual solvents and low encapsulation efficiency. On the other hand, fluid bed coating has low operational costs, but it is a time-consuming technique (Carvalho, Estevinho & Santos, 2016).

The different advantages of encapsulating active food components are summarised in **Figure 1**. Among all of the microencapsulation processes, spray-drying has become a successful industrial drying technique that, at the same

time, has evolved as the main technique used for the encapsulation of food ingredients due to its low processing cost, rapid water evaporation or ease of application and scaling (Favaro-Trindade, Santana, Monterrey-Quintero, Trindade & Netto, 2010). Briefly, microencapsulation consists of a technological process to incorporate a compound(s) within another substance(s), commonly known as core/active compound and wall/coating/encapsulating material, respectively, with the formation of very small "packages." The application of these microcapsules could be a viable option to maintain the functional features of bioactive compounds and thus, benefit the consumers, including the possibility of reducing the use of fat, salt and/or additives (Franco *et al.*, 2017). Paulo & Santos (2017) mentioned different examples of microencapsulated ingredients available for food industry, classified according to their application field: (1) flavouring agents (lemon, vanilla and peppermint oils); (2) sweeteners (aspartame, sucralose, xylitol); (3) colorants (annatto, β -carotene); and (4) vitamins (A, D₂, E, C). Moreover, the microencapsulated active ingredients can be living cells like probiotics (*Bifidobacterium*, *Lactobacillus*).

Besides protecting certain target components by the action of one or more wall materials, encapsulation is relevant for the development of final products containing aromatic plant antioxidants (e.g. sage or rosemary extracts) in which their unpleasant smells or flavours are masked (Tavares, Croguennec, Carvalho & Bouhallab, 2014). Furthermore, low water activity powders can be used as additive, ensuring the microbiological stability, facilitating the packaging, transportation, storage and commercialisation.

Selection of appropriate wall material is an extremely important factor for encapsulation of active compounds. That material must protect the core

substance from its deterioration, must have the required mechanical strength, be compatible with the food product, allow controlled release, and/or have thermal properties compatible with those of the product (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Some common wall materials used for encapsulation of food ingredients are gum arabic, maltodextrin, gelatin, whey protein, sodium caseinate, modified starches, and chitosan.

This interdisciplinary technology requires fundamental competences on colloid and interface chemistry, material science, and in-depth understanding of active agents' stabilization (Vinceković *et al.*, 2017). Generally, it is possible to differentiate between two main forms of encapsulated systems, namely core-shell type (capsules) and matrix type (spheres). In the first type, the core material forms a continuous phase enclosed in a shell (liquid or solid), while the matrix type has active compounds uniformly distributed inside a homogeneous solid phase matrix (see **Fig. 2**). The morphologies of the particles will depend on the active and encapsulant materials and the technique used in their preparation.

Currently, numerous ingredients that include different antioxidant compounds are subject to encapsulation. Some of these studies have already been focused on encapsulating additives of benefit to specific meat products, thus demonstrating the suitability of such technology for meat processing and the improvement of products shelf life (Munekata *et al.*, 2017; Heck *et al.*, 2017; Domínguez, Pateiro, Agregán & Lorenzo, 2017).

3. Spray-drying

The principle of the encapsulation by spray-drying is related to its ability for enclosing active components within a protective outer layer while the liquid feed is transformed into a dry, stable form. During spray-drying, the liquid feed is

pumped through a heated nozzle and is atomized into little droplets by using compressed air. The droplets are sprayed into a drying chamber. As it was previously described by Anandharamakrishnan & Ishwarya (2015b), the difference in the drying characteristics between the wall and core compounds rules this encapsulation process. Because of its inherent film-forming properties, the wall component dries out at a much faster rate than the medium (usually water) in which the core material is suspended to obtain a feed solution. This fact allows the wall material to form a coating around the droplet containing the core. Some factors that contribute to choose this technique over others are the ready availability of equipment, economic viability, reproducibility, ease of scale up and process flexibility.

The variables that influence the effectiveness of the spray-drying microencapsulation could be generally classified into two categories: (A) properties of the food emulsion (including the core and the wall material), and (B) conditions of the spray-drying process (Anandharamakrishnan & Ishwarya, 2015b). In each of them, there are numerous influencing parameters to be taken into account: dry content, viscosity, type of solvents and materials, humidity of the gas, inlet and outlet temperature, gas flow, feed rate, type of atomization and geometry of the chamber.

Since the objective is to produce ingredients suitable for their incorporation into the food industry, almost all spray-drying processes are carried out from aqueous formulations, thereby the wall material must be soluble in water at an acceptable level (Gouin, 2004).

The choice of the appropriate wall material is the primary step in the spray-drying encapsulation. Some of the ideal properties include: low viscosity, low

hygroscopy (to avoid agglomeration and easier manipulation), ability to stabilize the core material in emulsion, high solubility, film-forming ability, provide high protection, absence of taste or flavour, and favourable economic costs (Franco *et al.*, 2017).

Table 1 shows examples of antioxidants for microencapsulation by spray-drying, indicating the promising results obtained in terms of efficiency and/or stability. For instance, in order to avoid the degradation of the bioactive compounds and to control their release in the gastrointestinal tract, Indian horse chestnut starch and β -cyclodextrin were used to encapsulate folic acid under an airflow of 140 L/h and an inlet temperature of 130 °C (Ahmad, Qureshi, Maqsood, Gani & Masoodi, 2017).

For industrial applications, different antioxidant compounds (caffeic acid, chlorogenic acid and rosmarinic acid) were spray-dried with sodium carboxymethyl cellulose (SCMC), demonstrating a great encapsulation efficiency, stability and preservation of their antioxidant activity (Aguiar *et al.*, 2017). Other researchers produced *Lannea microcarpa* extract microparticles with enhanced functional and technological characteristics, selecting the same wall material (Sansone *et al.*, 2014). In this last case, even under harsh storage conditions, the bioactive polyphenols and the antioxidant activity did not seem significantly modified.

Furthermore, bacterial exopolysaccharides were also reported as appropriate encapsulation materials. In fact, Lourenço *et al.* (2017) evaluated a fucose-rich saccharide, named FucoPol, which was suitable for encapsulation of gallic acid by the same spray-drying technique at inlet of 198 L/h and at 200 °C.

Bioactive compounds from by-products/ wastes can be successfully used in food industry and also in other applications as cosmetic or pharmaceutical areas. In this sense, extracts rich in phenolic compounds from spent coffee grounds were efficiently microencapsulated (maximum encapsulation efficiency of 63%) using a carrier composition of 80% of inulin and flow rates of 7.5 and 10 mL/ min (Pettinato, Aliakbarian, Casazza & Perego, 2017).

Regarding the future development of spray-drying microencapsulation, there are still challenges to overcome as to minimize the use of organic solvents, incorporating the concept of green food processing, and to produce microcapsules that are able to resist harsh processing conditions at the same time that all the parameters (responsible for the microparticles final properties) are optimized (Vinceković *et al.*, 2017). Additionally, another interesting challenge reported by Anandharamakrishnan & Ishwarya (2015b) is to increase the flavour load of encapsulates by use of suitable wall materials, as well as to evaluate the consumer's acceptability. In connection with this last point, Çam, İçyer & Erdoğan (2014) determined the acceptability of ice cream samples enriched with microencapsulated phenolics considering the opinion of 60 regular ice cream consumers. The positive results in sensory evaluation supports to such products for commercial introduction to the general public with the potential as functional foods.

4. Freeze-drying

Freeze-drying is based on the dehydration by sublimation of a frozen product and the process is technically divided into three main stages: freezing, primary drying (or sublimation), and secondary drying (or desorption). It is a widely used technique due to simplicity, flexibility and scalability. Moreover,

freeze-dried products preserve their initial nutritious characteristics since substances are not exposed to high temperatures (Wilkowska, Ambroziak, Czyżowska & Adamiec, 2016). Nevertheless, compared to spray-drying, the cost of freeze-drying is 30-50 times more expensive (Gharsallaoui *et al.*, 2007). This fact can be explained because of the operational conditions, that generally requires from 8 to 24 hours. This results in a great incidence of energy costs, necessary to maintain set vacuum and refrigeration levels. Huang *et al.* (2017) reported that although freeze-drying remains the preferred technique to preserve probiotic bacteria, it is expensive and time-consuming process. Some examples of antioxidants encapsulated by this technique are presented in **Table 2**.

Regarding the preservation of the antioxidant capacity of phenolic compounds, Ballesteros, Ramirez, Orrego, Teixeira & Mussatto (2017) found that freeze-drying was better than the spray-drying, where total phenolic and flavonoids compounds were retained in 62% and 73%, respectively. Ursache *et al.* (2018) successfully used coacervation and freeze-drying for the valorisation of sea buckhorn carotenoids by microencapsulation with whey proteins and gum acacia as wall materials (formulation of high-added value food products). In this study, the muffins with microencapsulated powder was preferred by all panellists, as the added microencapsulated powder gave a nice yellow colour. Another interesting result for preserving the bioactives after encapsulation with this technique was reported for goldenberry juice (Dag, Kilercioglu & Oztop, 2017) that was mixed with maltodextrin, gum arabic, alginate and pectin.

5. Nanoscale manufacture

Nanotechnology offers a wide range of opportunities for the development and application of structures, materials, or systems with new properties for

various industries such as food, agriculture or pharmaceutical (Singh, Shukla, Kumar, Wahla & Bajpai, 2017). According to Gouin (2004), the term 'nanoencapsulation' involves the formation of a complex between a polymeric active ingredient (typically a protein) and a polymeric vector, without the actual coating of the active ingredient by a shell layer.

Nanometric-size encapsulation systems generally have the matrix type structure (nanoemulsions, micelles, microemulsions, and molecular complexes), because of the current limitations in the capability to manipulate structures at the nanoscale levels, whereas micro and micrometric size systems are more appropriate for core/shell or even more complex structures (Vinceković *et al.*, 2017). Nanoparticles are suitable for encapsulation and have an increased release efficiency when compared to traditional encapsulation systems. In this sense, the application of nanotechnology involves effective delivery systems with availability of encapsulated compound at a target time with specific rate. Furthermore, nanotechnology is efficient to maintain the active compounds at suitable levels for long periods, to exhibit compatibility with other compounds in the matrix and to penetrate deeply into target sites. Moreover, the importance of nanotechnology in food processing is evident for sensory properties in terms of food texture, appearance and taste (Singh *et al.*, 2017).

Various synthetic and natural nanoencapsulating delivery systems have been elaborated for the improved bioavailability and preservation of active food components. For example, edible coatings such as gelatin containing cellulose nanocrystal (Fakhouri *et al.*, 2014) or chitosan with nano-SiO₂ (Shi *et al.*, 2013; Yu *et al.*, 2012) were reported as suitable to preserve the quality of fresh foods during extended storage. In the same way, Sari *et al.* (2015) demonstrated that

encapsulated curcumin, the most active and least stable bioactive component of turmeric (*Curcuma longa*), was stable, although with a total antioxidant activity slightly lower to the original one (3.33 vs 3.53 for curcumin nanoemulsion and curcumin, respectively), to pasteurization, different ionic strengths and pH ranging from 3.0 to 7.0. Additionally, nanoemulsions had greater stability to droplet aggregation and gravitational separation, as well as increased oral bioavailability (Kong, Chen, Kweon, & Park, 2011; Yuan, Gao, Zhao & Mao, 2008).

Since liposome surrounds an aqueous solution inside a hydrophobic membrane, nanoemulsions can be used as delivery vessels for both hydrophobic and hydrophilic molecules (contained within the bilayer or in the aqueous interior, respectively) (Yoshizaki *et al.*, 2014). **Table 3** includes several examples of the effective use of nanotechnology for encapsulation of a variety of compounds (e.g. *Rosmarinus officinalis* essential oils, pomegranate peel extract, clove essential oils, and *Zataria multiflora* Boiss essential oil).

6. Other novel microencapsulation techniques

6.1. Supercritical fluid technology

In order to reduce or avoid the use of organic solvents in extraction, new techniques with supercritical fluids have been tested (Tsai & Rizvi, 2016). Briefly, in the supercritical fluid extraction process, an emulsion (where the material of interest is dissolved in the organic phase) is contacted with a supercritical fluid, in order to rapidly extract the organic phase from the emulsion. Supercritical fluid must be chosen for showing great affinity with the organic solvent, but negligible affinity with the active compound. Because of the rapid supersaturation of the active compound promoted by the extraction of the solvent, this compound is

precipitated in sub-micrometric scale, encapsulated by the surfactant material used to prepare the emulsion. Therefore, the product obtained by this technique is an aqueous suspension of the encapsulated material (Lévai *et al.*, 2017).

The advantages of supercritical fluids are primarily due to their physical and chemical properties that are intermediate to liquids and gases and easily adjustable with changes in temperature and pressure (Silva & Meireles, 2014). In this context, among others, supercritical fluid processing was positioned at the frontiers of food processing, chemistry, and microbiology. Additionally, it was identified as becoming a hot research topic for the design of green and sustainable processing for the food industry, avoiding the problem of solvent residues and restrictions on the use of conventional organic solvents.

For instance, Lévai *et al.* (2017) used supercritical fluid extraction in the processing of encapsulated quercetin to increase its bioavailability for pharmaceutical or cosmeceutical products. Supercritical fluid extraction has been used for emulsions to produce aqueous suspensions with encapsulated valuable materials like antioxidants. It must be noted that supercritical fluid has to be chosen by selecting a higher affinity for the organic solvent and with negligible affinity for the bioactive compound(s). Supercritical carbon dioxide (SC-CO₂) is the substance most widely used for micro/nanoparticles due to its unique characteristics (low toxicity, low cost, easy removal and non-flammability) (Silva & Meireles, 2014).

Recently, the microencapsulation process of carotenoids was reported for CO₂ supercritical fluids sea buckthorn extracts and anthocyanins from cherries or red grapes (Mihalcea *et al.*, 2017; Oancea *et al.*, 2017). Supercritical carbon dioxide (SC-CO₂) created microspheres of different types of starch wrapping

oregano essential oil (EO). This process was attractive as an effective alternative for protection of the EO, keeping its antioxidant activity under mild operating conditions (Almeida *et al.*, 2013).

The application of the supercritical encapsulation is restricted in food products because of the moderate solubility of substances such as fats, vegetable oils and vitamins with SC-CO₂ as a solvent. Therefore, it is suggested to use SC-CO₂ as the antisolvent (Visentin, Rodríguez-Rojo, Navarrete, Maestri, & Cocero, 2012; Sosa, Rodríguez-Rojo, Mattea, Cismondi, & Cocero, 2011). **Fig. 3** provides a simplified scheme of this process.

6.2. Inclusion complexation

As we mentioned above, no single encapsulation process is adaptable to all core materials or product applications. In this sense, another available alternative technique is inclusion complexation. The strong prevalence of this technique for microencapsulation is possibly due to the versatility and availability of β -cyclodextrins (β -CD) (Aguiar *et al.*, 2016). β -cyclodextrins (β -CDs) are cyclic carbohydrates made of seven glucopyranose units joined by α -1,4-glycosidic bonds in a ring. The β -CD has its hydroxyl group on the outside of the doughnut-shaped molecule and a relatively nonpolar hole in the middle. This hole can accommodate another molecule, called as “guest”, with appropriate size and polarity, form “host-guest” inclusion complexes in aqueous solution (Guan, Xu, Hu, & Peng, 2015). The formation of the inclusion compounds greatly modifies the physical and chemical properties of the host molecule, mostly in terms of water solubility, attracting much interest in many fields. Inclusion complex with cyclodextrins is the most attractive technique to enhance aqueous solubility of poorly soluble drugs. Patil, Kadam, Marapur & Kamalapur (2010) summarized

detailed information on the use of cyclodextrins as complexing agents, the different technologies adopted to prepare the inclusion complexes and the potential applications, technical and economical limitations associated with these approaches.

Zhao, Wang, Yang & Tao (2010) opted for this alternative to develop and characterize β -CD complexes with chlorogenic acid for industrial applications. Likewise, Shao, Zhang, Fang & Sun (2014) investigated the effects of chlorogenic acid/ β -CD complexes on preservation of anthocyanins and colour quality of grape juices. As a result of adding inclusions to the juice, researchers achieved reduction in anthocyanins degradation. β -CD and its modified derivative hydroxypropyl- β -CD were reported as appropriate wall materials for encapsulation and release of chlorogenic acid since they form stable complexes with higher antioxidant capacity (Chao, Wang, Zhao, Zhang & Zhang, 2012). Previously, Zhang, Li, Zhang & Chao, (2009) performed the microencapsulation of caffeic acid with this technique and by hydroxypropyl- β -CD as encapsulating agent.

6.3. Ionic gelation

There are two gelling techniques: external and internal gelation. In the first one, the soluble calcium salt is added in the emulsion, the particle size cannot be well controlled and the particles tend to coagulate in large masses before acquiring consistency. On the other hand, internal gelation is based on the release of calcium ion from an insoluble complex in a sodium alginate solution. This is carried out by acidification of a soluble oil-acid system, with participation in the aqueous phase of the alginate (Villena, Hernández, Lara & Martínez, 2009). According to this technique, the oil phase is added to the aqueous phase,

generally formed by alginate and calcium carbonate. Meanwhile, Ahirrao, Gide, Shrivastav & Sharma (2014) described the ionotropic gelation (polyelectrolyte complexation) as a technique that involves interaction of a cation (or an anion) with an ionic polymer to generate a highly cross linked structure.

Ionic gelation process is a method quite frequently used for synthesis of chitosan microparticles and nanoparticles (Kunjachan, Jose & Lammers, 2014). The method allows the use of mild conditions at lower temperatures with vigorous stirring or organic solvents. This is an advantage because it enables the encapsulation of substances sensitive to degradation if using other harsher techniques (de Moura, Berling, Germer, Alvim & Hubinger, 2018). However, the main limitation is for hydrophilic or low molecular materials because they can easily diffuse and can be rapidly released through the ionic-gel network regardless of the surrounding pH (Kim, Lee & Lee, 2016). Hence, to keep the activity of the hydrophilic compounds, different strategies are necessary (i.e. emulsion systems, coating material). The use of oil in the gelation technique reduces the loss of hydrophilic compounds because a barrier is created (Kim *et al.*, 2016).

Anthocyanin has been successfully encapsulated with the ionic gelation technique (Belščak-Cvitanović *et al.*, 2016; Santos, Albarelli, Beppu & Meireles, 2013; Yamdech, Aramwit & Kanokpanont, 2012). The unfavourable aspects of this technique are the larger size and low stability of particles (mainly for hydrophilic active compounds). The main advantages include low polydispersity and high encapsulation efficiency. Yamdech *et al.* (2012) studied the anthocyanin stability of a blackberry extract with temperature change when it was encapsulated by ionic gelation at different alginate concentrations (1.0-2.5%).

The stability of encapsulated anthocyanins increased up to 40% as compared to the control sample. However, the stability of anthocyanin decreased up to 92% and to 24% after being exposed for 10 h to 40 and 100 °C, respectively, demonstrating the degradation of the microencapsulated anthocyanins with temperature increase.

On the other hand, microparticles resistant to higher temperatures may be produced through microencapsulation by external ionic gelation using alginate as microencapsulating agent (Onwulata, 2013) and, further, allowed the controlled release of active compounds in the human intestine (Soliman, El-Moghazy, El-Din & Massoud, 2013). Both, de Moura *et al.* (2018) and Zhao, Sun, Torley, Wang & Niu (2008) reported that larger microcapsules usually provide better protection than smaller ones, in terms of higher stability of anthocyanin. However, they could exhibit poor dispersion in final food products.

According to Yoo, Song, Chang & Lee (2006), who used sodium alginate as coating material for the microencapsulation of α -tocopherol by ionic gelation, *in vitro* release was significantly affected by pH. These results for a simulated gastric fluid showed that the sodium alginate microparticles release was reduced almost three fold with exposure to the fluid.

7. Applicability of microencapsulated antioxidants in meat

In the last few years, meat consumers have been concerned with possible health problems caused by high saturated fats or added nitrite salts (Ramachandraiah *et al.*, 2017). Animal products are the primary source of saturated fat and the consumption of meat in the high-income countries exceeds nutritional needs, which contributes to high rates of chronic diseases such as cardiovascular disease and diabetes mellitus. Moreover, meat itself is also associated with increased risk of some cancers (Walker, Rhubart-Berg, McKenzie, Kelling, & Lawrence, 2005). For this reason, and despite all of

the positive aspects of the consumption, the innovative research in meat industries is focused on preventing negative effects associated with the consumption of processed and red meats by finding new and healthier additives (Toldrá & Reig, 2011). For instance, Hadian, Rajaei, Mohsenifar & Tabatabaei (2017), nano-encapsulated *Rosmarinus officinalis* essential oils to improve their antioxidant and antimicrobial activity in beef cutlet, and they concluded that the produced nanogel reduced foodborne pathogens like *Salmonella* and can be a suitable tool to extend meat shelf life. A similar reducing effect on pathogens and good antioxidant activity was reported with microencapsulated essential oils (EO) from Mexican oregano (*Lippia graveolens* Kunth) being thymol and γ -terpinene the main EO (Hernández-Hernández *et al.*, 2017). Furthermore, nanoparticles from lyophilized pomegranate peel (LPP) prevented lipid oxidation and improved microbial quality and cooking characteristics of meatballs of minced beef meat (Morsy, Mekawi, & Elsabagh, 2017). Based not only on oxidative studies, but also on sensory evaluation, the shelf-life of meatballs was extended up to 15 days in LPP nanoparticles treated samples and the results did not show any negative impact on sensory properties of both raw and cooked meatballs. Therefore, the LPP nanoparticles assayed could be successfully added to meatball products as a natural antioxidant and antimicrobial additives.

Instability of active compounds, immiscibility and other challenges may be overcome by the use of nanoemulsions. In this context, the oxidative stability of sausages containing 50 mg/kg of oil-in-water emulsions loaded with rosemary extract improved significantly during the first 21 days of storage at 7 °C (Erdmann, Lautenschlaeger, Zeeb, Gibis & Weiss, 2017). However, reduction of particle size to nanoscale might be ineffective, as reported by Erdmann *et al.* (2017) for rosemary extract in sausages.

Examples of microencapsulated antioxidants specifically incorporated in meat products are given in **Table 3**. As shown in such table, there are not so

many studies considering the applications of spray-dried microcapsules in meat products. Nonetheless, it is important to provide new research data on the subject due to recent positive results. For instance, Ghaderi-Ghahfarokhi, Barzegar, Sahari and Azizi (2016) noticed that the encapsulation of thyme essential oil in chitosan nanoparticles is a promising technology for the control of undesirable lipid oxidation, and sensory changes in beef burgers. In this regard, Baldin *et al.* (2016) evaluated the addition of microencapsulated jabuticaba extracts (MJE) to fresh sausage as a natural antioxidant. Authors observed significant reduction in lipid oxidation of fresh pork sausages over 15 days of storage and after using MJE at both, 2% and 4%. However, the inclusion of 4% MJE negatively affected colour, texture and overall acceptability of the sausages, but such effect did not happen at 2% MJE addition. Further, microencapsulated propolis co-product extract (MPC) by Capsul[®] resulted in an effective inhibition of oxidation in burger meat. On the other hand, sensory analyses using an ideal scale provide information about whether the products are accepted or rejected by each consumer. For the burger meat containing MPC, the attributes color, appearance and texture had ideal grades, while the attributes aroma and flavour resulted below than the ideal scale (dos Reis *et al.*, 2017).

A two-step process oil-in-water emulsification and ionic gelation was used to prepare cinnamon essential oil (CEO) in chitosan nanoparticles (Ghaderi-Ghahfarokhi, Barzegar, Sahari, Gavlighi & Gardini, 2017). CEO had good preservative properties, but its strong flavour and adverse effect on red colour limited its utilization in beef patties. This study revealed that encapsulated CEO could be a useful strategy to overcome negative sensory attributes of the final patties and supported further research and use of nanoencapsulation techniques.

In this sense, Heck *et al.* (2017) also found positive results of applying ionic gelation technique in meat products, concluding that the microencapsulation of n-3 PUFA-rich oils is effective for production of healthier burgers. Additionally, Rajaei, Hadian, Mohsenifar, Rahmani-Cherati & Tabatabaei (2017) observed that the stability of the particles prepared by physical crosslinking (based on ionic gelation) was negatively affected by the changes in the pH of the environment due to electrostatic interactions. Thus, authors chose covalent crosslinking chitosan-myristic acid nanogels to encapsulate clove essential oils for addition to beef cutlets and exert its beneficial antioxidant effects. Recently, hamburger patties were incorporated with encapsulated β -carotene (0.5%) by crosslinked chitosan with sodium tripolyphosphate, being more effective in reducing the lipid oxidation its use as an edible coating than its use as an additive (Ozvural and Huang, 2018).

8. Conclusions/ Perspectives

Most biological compounds from plant sources, such as essential oils and other antioxidants, often exhibit remarkable positive features, which can be exploited in the production of healthy and quality food products. However, these compounds can exhibit low water solubility, strong off-flavours/odours, and are generally unstable and easily degraded under common processing and storage conditions. Encapsulation is a technology that offers the delivery of bioactive compounds in food systems, their protection, as well as their controlled and targeted release. In particular, spray-drying is one of the most economic and common micrometric encapsulation technologies. However, manufacturers and society in general, demand more research in this area and innovative processes for enabling the use of different types of natural ingredients and better

522 preservation of their healthy properties. In this sense, promising results have
523 been achieved in food preservation by microparticles and nanomaterials, where
524 they might provide benefits with reference to weight control, anti-ageing or
525 disease-prevention (diabetes or cardiovascular diseases).

526 Although the advances in this kind of technology are opening new paths in
527 research, there are many challenges and available opportunities for
528 improvement. Furthermore, various issues about the consequences of
529 encapsulation, at both microscopic and nanoscopic levels, must be addressed in
530 order to alleviate consumer concerns, and to respect the green food processing
531 concepts with transparency of safety issues. The benefits that are driving
532 implementation of innovative strategies and non-conventional methods in
533 production include reduced operating costs and environmental impact, improved
534 scalability and the functionality of the encapsulation systems. Furthermore, it is
535 important to evaluate the impact of encapsulates incorporation from formulation
536 to delivery, considering the sensorial features of the final product and the
537 consumer preferences/ acceptability (Aguiar *et al.*, 2016).

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Figure 1.- Advantages of the encapsulation of food ingredients

Figure 2.- Different forms of encapsulated systems: a) simple core-shell or capsule, b) multi-core capsule, (c) multi-walled structure, and (d) matrix type or sphere

Figure 3.- Schematic representation of the supercritical antisolvent encapsulation process

936 **Table 1.** Summary of recent studies about microencapsulation of antioxidants by spray-drying

Antioxidant compound/s	Wall material/s	Operating conditions in the encapsulation	Target/ Applications	Results	Reference
Folic acid	Indian horse chestnut starch and β -cyclodextrin	Air flow ~140 L/h; inlet/ outlet temperatures of 130/ 80°C	Avoid degradation of the compound	Encapsulation efficiency of starch and β -cyclodextrin was 57.29% and 76.10%, respectively	Ahmad <i>et al.</i> (2017)
Caffeic acid, chlorogenic acid and rosmarinic acid	Sodium carboxymethyl cellulose	Feed flow rate of 4 mL/min (15%); inlet/ outlet temperatures of 115/ 60°C; 100% aspiration rate; and nozzle cleaner set to 3	Overcome their limitations in industrial applications	The Na-CMC spray-dried microparticles showed great encapsulation efficiency (>90%), product yield values around 40%, total release ability and compounds stability without loss of antioxidant activity	Aguiar <i>et al.</i> (2017)
Gallic acid	Bacterial exopolysaccharide named FucoPol	Rate of 3.3 mL/min and inlet temperature of 200°C	Explore the potential of a bacterial fucose-rich polysaccharide as encapsulation material	Good ability of FucoPol for encapsulation of bioactive compounds by spray-drying	Lourenço <i>et al.</i> (2017)
Extract rich in phenolic compounds from spent coffee grounds	Inulin and maltodextrin	Air flow rate of 30 m ³ /h; inlet temperature 160°C; flow rate 5-10 ml/min	Preserve the activity of bioactive compounds that can have potential industrial applications	Microencapsulated dried powders rich in polyphenols were produced	Pettinato <i>et al.</i> (2017)
Peanut skin	10, 20, or 30% of maltodextrin	Spray-dryer equipment with a 2.0 mm nozzle, air flow at 40 l/min, and 44 ml/min of feed flow. Three feeds were prepared and atomized at 130, 150, and 170°C	Produce and evaluate a spray-dried extract from peanut skin	Higher temperatures (150 and 170°C) and the 30% of maltodextrin were the best conditions	do Valle Calomeni <i>et al.</i> (2017)

Heme iron ingredients and ascorbyl palmitate	Calcium caseinate and mixture of modified starch: maltodextrin	Not detailed	Overcome the nutritional iron deficiency through delaying the oxidation of a palm oil matrix fortified with heme iron	The combination of ascorbyl palmitate at 400 mg/kg and the co-spray-dried heme iron in a 1:1 ratio was the best treatment	Alemán <i>et al.</i> (2016)
Extract from <i>Lannea microcarpa</i> (Lm)	Sodium-carboxymethylcellulose	Inlet/outlet temperatures 100/ 65°C; flow feed rate 5 mL/min; drying air flow 600 L/h; and aspirator 100%	Produce Lm extract microparticles	The produced microparticles showed satisfying encapsulation efficiency, good functional stability and enhanced technological properties	Sansone <i>et al.</i> (2014)
Phenolics of pomegranate peels	Different types of maltodextrins	Inlet temperature 160°C; air flow rate 600 L/h; and rate of feeding 8 mL/min	Enrichment of the functional properties of regular ice cream	Addition of phenolics at 0.5 and 1.0% (w/w) showed significant improvement of the antioxidant activity, as well as most of the panellists accepted the phenolic enriched ice creams in sensory evaluation	Çam <i>et al.</i> (2014)
β-carotene	Maltodextrin and alginate and chitosan	Inlet/outlet temperatures 170/ 95°C; pump speed 25%; feed rate 7.5 mL/min; and aspirator rate of 100%	Determine the effect of microencapsulation method on physical properties and <i>in vitro</i> release and bioavailability of β-carotene	Spray drying β-carotene is validated as an acceptable method to preserve β-carotene while, whereas microencapsulation with alginate and chitosan is not recommended	Donhowe, Flores, Kerr, Wicker & Kong (2014)
Vitamins: α-tocopherol and ascorbic acid	Native and modified soy protein isolate (SPI)	Inlet/ outlet air temperatures 124/ 74°C ± 4 °C, drying air flow rate of 35 m ³ , spray flow	Encapsulation of vitamins with native	It was demonstrated that SPI modification resulted in decreased emulsion droplet size and viscosity and can improve the	Nesterenko, Alric, Silvestre &

Gallic acid	Extract of nopal mucilage	rate of 0.47 m ³ /h, liquid feed flow rate of 0.33 x 10 ⁻³ m ³ /h and aspiration of 100%	and modified soy protein	microencapsulation efficiency of suitable active cores	Durrieu (2014)
		Inlet/outlet temperatures 130/ 170°C; speed atomization (14000 and 20000 rpm)	Produce microcapsules of gallic acid	A stable powder with small particle size and, consequently higher viscosity, was produced. These microcapsules represent a promising food additive for incorporation into functional foods	Medina-Torres <i>et al.</i> (2013)

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Table 2. Examples of studies about microencapsulation of antioxidants by the freeze-drying technique

Antioxidant compound/s	Wall material/s	Operating conditions in the encapsulation	Target/ Applications	Results	Reference
Carotenoids from sea buckthorn extract	Whey proteins isolate and gum acacia	Coacervation and freeze drying. The coacervates were freeze dried at -42 °C under a pressure at 0.10 mBar for 48 h	Valorization of sea buckhorn carotenoids extract by microencapsulation and formulation of value-added products	The microencapsulated powder was evaluated in food products as colorant and functional ingredient causing positive effects and being appreciated by panelists	Ursache <i>et al.</i> (2018)
Phenolic compounds extracted from spent coffee grounds	Maltodextrin, gum arabic and a mixture of these components	Freeze-drying: chamber at -60°C under pressure of 0.05 bar, 48 h Spray-drying: flow rate of 108 ml/h, inlet temperature of 100°C, air flow-rate of 600 L /h and aspiration 75%	Preserve their antioxidant activity	The best results were achieved by freeze-drying using maltodextrin, retaining 62% and 73% of phenolic compounds and flavonoids, respectively	Ballesteros <i>et al.</i> (2017)
Goldenberry juice (an exotic fruit with high antioxidant activity and phenolic content)	Maltodextrin mixed with gum arabic, alginate and pectin.	Different core to coating ratios were used and homogenized before freeze-drying for 48 h (with a condenser at -50°C and could drop the internal pressure to 1 Pa)	Preserve the bioactive contents of goldenberry juice	Goldenberry juice powder obtained could be used as a functional food ingredient due to its high antioxidant activity and the encapsulation efficiency	Dag <i>et al.</i> (2017)
Anthocyanin-rich extract of a blackberry by-product	Maltodextrins with 10 and 20 dextrose equivalent (DE)	Total solids concentration of 30%, freeze-drying at a constant temperature of -20°C	Add value to fruit by-products	Blackberry by-products contain valuable biocompounds (anthocyanins) whose microencapsulation presented a potential approach as food colorants and healthy ingredients	Yamashita <i>et al.</i> (2017)

Caffeic acid (red wine cabernet sauvignon)	Maltodextrin (DE 10) and gum arabic	A laboratory-scale freeze dryer was used with a freezing plate and condenser at -40 °C and a vacuum of 100 µm Hg during 40 h	Evaluate the stability of phenolics encapsulated in a lower concentration of wall material (9%)	Water activity affected phenolics stability during storage. Antioxidant activity of the wine powder remained constant over 145 days at accelerated storage conditions.	Rocha-Parra, Lanari, Zamora & Chirife (2016)
Flavonoid-rich <i>Hypericum perforatum</i> (HP) extract	β-cyclodextrin (β-CD)	HP extract was suspended in aqueous solution containing β-CD (mass ratio 1:4). The suspension was subjected to sonication for 4 h, and was subsequently filtered. The previously frozen water-soluble filtrate was freeze-dried. The powder thus obtained was stored under nitrogen in a gas-tight glass container at -40 °C	Characterize purified methanolic extract from HP encapsulated in β-CD	The thermal stability of flavonoids was improved	Kalogeropoulos, Yannakopoulou, Gioxari, Chiou & Makris (2010)

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944 **Table 3.** Current studies of microencapsulated antioxidants specifically incorporated in meat products

Antioxidant compound/s	Wall material/s	Meat product	Operating conditions in the encapsulation	Target/ Applications	Results	Reference
<i>Rosmarinus officinalis</i>	Chitosan-benzoic acid	Beef cutlet	Nanoencapsulation. The REOs were dissolved in ethanol (1:1, v/v) and mixtures of the CS-BA nanogels	Improve antioxidant and antimicrobial activity as well as stability of the REOs	The nanogel-encapsulated REOs coating was more effective compared with the free REOs in reducing Salmonella population	Hadian <i>et al.</i> (2017)

essential oils (REOs)	(CS-BA) nanogel		(10,000 mg/l) and the REOs (5000 mg/l), were prepared by sonication for 5 min		on beef cutlets and in extending meat shelf life	
Chia oil and linseed oil with high n-3 PUFAs levels	2.0% sodium alginate solution and 0.1 M CaCl ₂ solution	Burgers	External ionic gelation technique: 25% chia oil and linseed oil were mixed with 2.0% sodium alginate solution and then atomized in 0.1 M CaCl ₂ solution using a dual fluid atomizer nozzle under air pressure of 0.125 kg/cm	Produce n-3 PUFA-rich oil microparticles by external ionic gelation for use as a fat substitute in burgers	Mmicroencapsulation by external ionic gelation seems to be a promising alternative to incorporate vegetable oils with high n-3 PUFA levels in burgers, without affecting their quality	Heck <i>et al.</i> (2017)
Pomegranate peel extracts	-	Meatballs of minced beef meat	The lyophilized pomegranate peel (LPP) was reduced to nanoparticle size. Firstly, LPP was ground with a Moulinex grinder to microparticles range of 100–150 µm, and then crushed by a high-energy planetary ball-mill at a rotation speed of 320 rpm for 2 h	Evaluate the impact of LPP nanoparticles as an antioxidant and antimicrobial in meatballs during storage	LPP nanoparticles were effective in retarding lipid oxidation and improving the microbial quality and cooking characteristics of meatballs	Morsy <i>et al.</i> (2017)
Clove essential oils (CEOs)	Chitosan (CS)-Myristic acid (MA) nanogel	Beef cutlet	Nanoencapsulation. The CEOs were dissolved in ethanol and mixtures of the CS-MA nanogels (10000 mg/l) and the CEOs (5000 mg/l), were prepared by sonication for 5 min	Enhance the shelf-life of beef cutlets	The encapsulation could considerably improve the performance of CEOs that possessed antioxidant and antimicrobial activity and was effective in preserving the natural color of the beef	Rajaei <i>et al.</i> (2017)
Propolis co-product extract	The Capsul®	Burger meat	Spray drying: flow of 1 L h ⁻¹ , inlet and outlet temperatures of 150 and 100 °C, respectively. The encapsulating material (50 g) was dispersed in water until dissolution and then water at 90 °C were added under stirring for 2 min. Finally, the propolis co-product extract (20% soluble solids) was added constantly stirring for 5 min	Evaluate the effects of the microencapsulated propolis co-product extract (MPC) on the oxidative stability and sensory acceptability of burger meat during storage	The encapsulation by the spray dryer proved to be efficient to produce a food ingredient containing six phenolic compounds with antioxidant activity. The burgers elaborated showed an acceptance rate of 63.8%	dos Reis <i>et al.</i> (2017)
<i>Zataria multiflora</i> Boiss. essential oil (ZMEO)	-	Beef burger	Nanoliposomes were prepared by using thin film evaporation method (Bangham method). Soyphosphatidylecoline (10 mg/mL) and cholesterol (2 mg/mL) were dissolved with methanol and chloroform. Then, ZMEO was solved in methanol and mixed with phosphatidylecoline and cholesterol. Then, the organic solvents were removed by using rotary evaporator. After this step, thin layer of lipid was solved in soluble phosphate buffered saline. In the next phase, sonication of final solution was done for 10 min at 4 °C	Evaluate microbial and chemical properties of beef burger incorporated with different concentration of ZMEO in the free and nanoliposomal forms (NZMEO)	Both, ZMEO and NZMEO can be used as preservative agent to extend shelf life of meat products such as burgers in refrigerator condition. Nanoencapsulation of essential oil can increase of antioxidant and antimicrobial activity	Torab, Basti & Khanjari (2017)

Cinnamon essential oil (CEO)	Chitosan	Beef patties	Two-step process: oil-in-water emulsification and ionic gelation	Evaluate the applicability and acceptability of beef patties containing encapsulated CEO	Free CEO has unfavorable effects on color and odor but the incorporation of encapsulated CEO improved, not only the microbial population of patties, but also the consumer acceptability	Ghaderi-Ghahfarokhi <i>et al.</i> (2017)
Thyme essential oil (TEO)	Chitosan	Beef burger	TEO loaded chitosan nanoparticles were prepared by a two-step process including oil/ water emulsion and ionic gelation	Improve antimicrobial and antioxidant activity of TEO in beef burgers during storage	The investigated microbial counts were significantly lower in samples treated with TEO than in the control, as well as the TBARs amount	Ghaderi-Ghahfarokhi <i>et al.</i> (2016)
Jabuticaba (<i>Myrciaria cauliflora</i>) extract (JE)	Maltodextrin	Fresh sausage	Spray-dryer at an air flow of 40 L/min. The independent variables were 150 °C inlet drying air temperature and 30 mL/ min feed flow rate	Evaluate the addition of microencapsulated JE to fresh sausage as natural dye with antioxidant activity	Addition of MJE at 2% and 4% contributed to both a reduction in lipid oxidation and microbial populations. However, the inclusion of 4% MJE negatively influenced the sensory properties	Baldin <i>et al.</i> (2016)
Sodium tripolyphosphate (STP), sodium hexametaphosphate (HMP) and sodium pyrophosphate (SPP)	A commercially available hydrogenated vegetable oil	Ground chicken and ground beef	Encapsulation was accomplished by a commercial coating company. The encapsulate (coating) was designed to achieve a melting release point of 68°C	Investigate the effects of encapsulated phosphates on lipid oxidation in both ground chicken and beef during uncooked and cooked storage	STP and SPP with or without encapsulation were the most effective phosphate types for inhibiting lipid oxidation in raw and cooked ground chicken and ground beef. Encapsulation significantly enhanced the oxidative stability	Kılıç, Şimşek, Claus & Atılğan (2014)
Rosemary extract (RE)	-	Cooked emulsion-type sausages rich in n-3 fatty acids	Coarse emulsions were manufactured mechanically by mixing 10% (w/w) oil phase with 90% (w/w) emulsifier solution (2% (w/w) Tween 80 in 10 mM sodium phosphate buffer, pH 5.0) with a standard unit homogenizer at high speed for 5 min	Investigate the effects of processing and the formulation of oil-in-water emulsions containing RE on the oxidative degradation of n-3 fatty acids of fish oil-in-water emulsions in cooked pork sausages	The addition of 50 mg/kg encapsulated RE to cooked emulsion-type sausages rich in n-3 fatty acids significantly retards lipid oxidation and enhances color stability during refrigerated storage. However, the droplet size of Miglyol oil-in-water emulsions loaded with RE did not influence the formation of secondary oxidation products in the pork sausages	Erdmann <i>et al.</i> (2017)

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