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An overview of encapsulation technologies for food applications

Viktor Nedovic[a]*, Ana Kalusevic[a], Verica Manojlovic[b], Steva Levic[a], Branko Bugarski[b]

[a] *Department of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*
[b] *Department of Chemical Engineering, Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*

Abstract

Encapsulation is a process to entrap active agents within a carrier material and it is a useful tool to improve delivery of bioactive molecules and living cells into foods. Materials used for design of protective shell of encapsulates must be food-grade, biodegradable and able to form a barrier between the internal phase and its surroundings. Among all materials, the most widely used for encapsulation in food applications are polysaccharides. Proteins and lipids are also appropriate for encapsulation. Spray drying is the most extensively applied encapsulation technique in the food industry because it is flexible, continuous, but more important an economical operation. Most of encapsulates are spray-dried ones, rest of them are prepared by spray-chilling, freeze-drying, melt extrusion and melt injection. Molecular inclusion in cyclodextrins and liposomal vesicles are more expensive technologies, and therefore, less exploited. There are number of reasons why to employ an encapsulation technology and this paper reviews some of them. For example, this technology may provide barriers between sensitive bioactive materials and the environment, and thus, to allow taste and aroma differentiation, mask bad tasting or smelling, stabilize food ingredients or increase their bioavailability. One of the most important reasons for encapsulation of active ingredients is to provide improved stability in final products and during processing. Another benefit of encapsulation is less evaporation and degradation of volatile actives, such as aroma. Furthermore, encapsulation is used to mask unpleasant feelings during eating, such as bitter taste and astringency of polyphenols. Also, another goal of employing encapsulation is to prevent reaction with other components in food products such as oxygen or water. In addition to the above, encapsulation may be used to immobilize cells or enzymes in food processing applications, such as fermentation process and metabolite production processes. There is an increasing demand to find suitable solutions that provide high productivity and, at the same time, satisfy an adequate quality of the final food products. This paper aims to provide a short overview of commonly used processes to encapsulate food actives.

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Keywords: Encapsulation; food application; bioactive compounds.

* Corresponding author. Tel.: +381-11-2615-315; fax: +381-11-2193-659.
E-mail address: vnedovic@agrif.bg.ac.rs

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1. Why to employ encapsulation technologies?

Encapsulation may be defined as a process to entrap one substance (active agent) within another substance (wall material). The encapsulated substance, except active agent, can be called the core, fill, active, internal or payload phase. The substance that is encapsulating is often called the coating, membrane, shell, capsule, carrier material, external phase, or matrix [1,2].

In the food industry, encapsulation process can be applied for a variety of reasons. Encapsulation is a useful tool to improve delivery of bioactive molecules (e.g. antioxidants, minerals, vitamins, phytosterols, lutein, fatty acids, lycopene) and living cells (e.g. probiotics) into foods [1,3]. In most cases, encapsulation refers to a technology in which the bioactive components are completely enveloped, covered and protected by a physical barrier, without any protrusion of the bioactive components [3]. Also, encapsulation has been defined as a technology of packaging solids, liquids, or gaseous materials in small capsules that release their contents at controlled rates over prolonged periods and under specific conditions [4]. Produced particles usually have diameters of a few nm to a few mm [1].

Encapsulation was originally introduced in the area of biotechnology to make production-processes more efficient as the matrix around the cells allows for rapid and efficient separation of the producer cells and the metabolites. Such technologies developed approximately 60 years ago, are of significant interest to the pharmaceutical sector (especially for drug and vaccine delivery), but also have relevance for the food industry. In recent years, the food industry requires the addition of functional compounds in products. These compounds are usually highly susceptible to environmental, processing and/or gastrointestinal conditions and therefore, encapsulation has imposed an approach for effective protection of those. Functional compounds are used to control flavour, colour, texture or preservation properties. Bioactive compounds with various potential health benefits are included, too. There is a multitude of possible benefits of encapsulated ingredients in the food industry. Encapsulation aims to preserve stability of the bioactive compounds during processing and storage and to prevent undesirable interactions with food matrix. Mainly, bioactive food compounds are characterised by rapid inactivation. These compounds would profit from an encapsulation procedure, since it slows down the degradation processes (e.g., oxidation or hydrolysis) or prevents degradation until the product is delivered at the desired sites [5]. Thus, the bioactive component would be kept as fully functional. Also, this technology may provide barriers between sensitive bioactive materials and the environment, and thus, to allow taste and aroma differentiation, mask bad tasting or smelling, stabilize food ingredients or increase their bioavailability.

In addition to the above, encapsulation can be applied for modification of physical characteristics of the original material in order to (a) allow easier handling, (b) to help separate the components of the mixture that would otherwise react with one another, (c) to provide an adequate concentration and uniform dispersion of an active agent [4].

2. Materials used for encapsulation

A lot of substances may be used to coat or encapsulate solids liquids, or gases of different types and properties. However, regulations for food additives are more rigid than for e.g. pharmaceuticals. Different compounds, widely accepted for drug encapsulation, have not been approved for use in the food industry, because many of these substances have not been certified for food applications as “generally recognized as safe” (GRAS) materials. Actually, the whole food process should be designed in order to meet the safety requirements of governmental agencies such as the European Food Safety Authority (EFSA) or Food and Drug Administration (FDA) in the USA [1].

The most important criteria for selection of an encapsulation material are functionality that encapsulate should provide to the final product, potential restrictions for the coating material, concentration of encapsulates, type of release, stability requirements and cost constrains. Materials used for design of protective shell of encapsulates must be food-grade, biodegradable and able to form a barrier between the

internal phase and its surroundings. The majority of materials used for encapsulation in the food sector are biomolecules. Except to be natural, materials have to provide maximal protection of the active material against environmental conditions, to hold actives within capsules structure during processing or storage under various conditions, not to react with the encapsulated material, to have good rheological characteristics at high concentration if it is needed and to have easy work ability during the encapsulation. Among all materials, the most widely used for encapsulation in food applications are polysaccharides. Starch and their derivates – amylose, amylopectin, dextrins, maltodextrins, polydextrose, syrups and cellulose and their derivatives are commonly used. Plant exudates and extracts – gum Arabic, gum tragacanth, gum karaya, mesquite gum, galactomannans, pectins and soluble soybean polysaccharides are employed, too. Subsequently, marine extracts such as carrageenans and alginate are also present in foods. Microbial and animal polysaccharides like dextran, chitosan, xanthan and gellan are also exploited. Apart from natural and modified polysaccharides, proteins and lipids are also appropriate for encapsulation. Examples of the most common milk and whey proteins are caseins, gelatine and gluten. Among lipid materials suitable for food applications there are fatty acids and fatty alcohols, waxes (beeswax, carnauba wax, candellia wax), glycerides and phospholipids. In addition to the above, other materials are employed such as PVP, paraffin, shellac, inorganic materials [1].

It is impossible to number all criteria to select a proper material for encapsulation. For sure, the type of an active, and its characteristics, and an application where the encapulates are going to be used for are first on the list. Except this, cost constraint stays a key factor for choosing the most appropriate materials. No matter what is the material in question, the conversion of the physico-chemical characteristics of the materials will be the precondition for successful food product development. So, it is prerequisite to study and analyse all properties of potential wall material in order to conclude and predict its behaviour under conditions present in food formulations [1].

3. Encapsulation techniques

There are number of techniques available for encapsulation of food compounds. Since encapsulating compounds are very often in a liquid form, many technologies are based on drying. Different techniques like spray drying, spray-bed-drying, fluid-bed coating, spray-chilling, spray-cooling or melt injection are available to encapsulate active agents [6,7].

Spray drying is one of the oldest and the most widely used encapsulation technique in the food industrial sector. It is a flexible, continuous, but more important an economical operation. It produces particles of good quality, which size is less than 40 μm [7]. This feature is desired from the standpoint of sensorial and textural characteristics of final products. Although spray-dryers are widespread in the food industry, there are several disadvantages of this technique such complexity of the equipment, non-uniform conditions in the drying chamber and it is not always easy to control particle size. About 80–90% of encapsulates are spray-dried ones, rest of them are mostly prepared by spray-chilling, freeze-drying, melt extrusion and melt injection [8,9].

Extrusion methods consists of dropping droplets of an aqueous solution of polymer (most often this is 0.6-3 wt% sodium alginate) and active into a gelling bath (in case of alginate, gelling bath is 0.05-1.5 M calcium-chloride solution). The dripping tool can be simply a pipette, a syringe, a vibrating nozzle, a spraying nozzle, jet cutter or atomizing disk [1]. In comparison to other extrusion techniques, JetCutter was found to be the best technology for large-scale/industrial applications [10]. Electrostatic extrusion is especially effective for production of very small particles, down to 50 μm. An alternative extrusion technology is co-extrusion. It might be utilized to prepare spherical microbeads with a hydrophobic core and a hydrophilic or hydrophobic shell [11].

Another frequently used technique is emulsification. It is utilised in case of water soluble food active agents and there are two combinations of emulsions: water/oil emulsions or oil/water emulsions and water/oil/water double emulsions. An oil-in-water emulsion can be dried by different drying methods

such as spray- or freeze-drying, and thus to produce a powder. Such dried emulsions might be encapsulates or an instant formulation for numerous food products [11].

Spray-chilling or spray-cooling are technologies to produce lipid-coated active agents. The difference between these two techniques is the melting point of lipids. In case of spray chilling it is in range of 34–42°C and for spray cooling temperature is higher. The agent could be dissolved in lipids, present as dry particles or present as aqueous emulsions. The spray cooling is a technique with possibility to achieve high yields and it can be run in both continuous and batch processing modes. In case of spray-chilling, the particles are kept at a low temperature in a set-up similar to the fluidized bed spray granulation [11,12].

Fluid bed coating is an encapsulation technique where a coating is applied onto powder particles in a batch processor or a continuous set-up. The powder particles are suspended by an air stream at a specific temperature and sprayed with an atomized, coating material. The coating material might be an aqueous solution of cellulose or starch derivatives, proteins and gums [13].

Vacuum and freeze-drying are very similar drying processes, but the first one is faster and cheaper, because it operates at a temperature above the freezing point of the solvent. The major disadvantages of freeze-drying are the high energy input and long processing time. In addition, during processing a barrier with an open porous structure between the active agent and its surroundings is formed; this high-porous wall offers poor protection when prolonged release of an active is required [11].

Also, molecular inclusion in cyclodextrins and liposomal vesicles provide some specific features to bioactives; however these techniques are more expensive, and therefore, less exploited. Cyclodextrins have a lipophilic inner pocket of about 5-8 Å, in which an active molecule with the right size can be reversible entrapped in an aqueous environment. However, the small size of the ring forming hole limits its loading capacity. Liposomes are particles with size ranges from 30 nm to a several microns. The mechanism for the creation of liposomes is basically the hydrophilic–hydrophobic interactions between phospholipids and water molecules.

4. Examples of microenapsulates in food products

One of the most important reasons for encapsulation of active ingredients is to provide improved stability in final products and during processing. For example, probiotics are highly sensitive to variation of pH, mechanical stress, transport conditions, and digestive enzymes in the stomach. Probiotic bacteria are defined as live microorganisms and bioactive food components with serious health benefits in the host, if they are present in adequate amounts [3]. At present, probiotics are the driving force in the design of functional foods, especially in dairy products, maintaining their functional effects for supporting human health. These living cells need to survive the food process, storage, and food intake before they can be useful. Encapsulation increases not only their bioavailability, but more importantly functionality. A choice of an encapsulation system is always crucial, since it has to be efficient and easily incorporated into the food without interfering with the texture and taste of the food. Also, some microbes such as lactobacilli and bifidobacteria seem to benefit from the encapsulation matrix during dehydration and lyophilisation [14]. However, there is no wide choice of encapsulation technologies that can be applied for living cells, as it is case in most molecules which are resistant to heat. One among ‘gentle’ approaches for encapsulation is the extrusion technique and in combination with matrix molecules that preserve or even promotes the functionality; this technology is advisable for probiotics. Except extrusion, mostly used encapsulation techniques are spray-, freeze- or vacuum-drying**.** Typical carrier materials are mixture of carbohydrates and/or (dairy) proteins. Usually, protein isolates, gum Arabic, pectin skim milk powder, non-fat dry milk solids, soy, modified starch, maltodextrin and sugars are employed [1,3].

Another benefit of encapsulation is less evaporation and degradation of volatile actives, such as aroma, which usually contains mixture of volatile and odorous organic molecules. Besides, flavours are usually expensive and therefore food manufacturers are usually concerned about the preservation of aromatic additives [8]. Thanks to encapsulation a food compound such as aroma is covered with a protective wall

material and protected against evaporation, chemical reactions (such as flavour-flavour interactions, light-induced reactions, oxidation) or migration in a food [15,16]. Flavour encapsulation can be accomplished by a variety of methods: spray-drying, spray-chilling or -cooling, spray bed drying and others. Examples of carrier material used for spray-drying are mono- and disaccharides, maltodextrin, corn syrup solids, modified starches, gum Arabic, larch gum, milk or soy proteins, hydrolysed gelatin and their various combinations. Spray-chilling is a convenient technology to produce lipid particles with aroma [7]. Since the flavour is one of the most important characteristics of food, the ultimate goal of encapsulation is to control aroma release and to improve stability during processing and consumption of the final product. In general, aroma releases from food before and after eating which depends on the aroma features and physical state of the matrix.

Furthermore, encapsulation is used to mask unpleasant feelings during eating, such as bitter taste and astringency of polyphenols and other compounds that show high antioxidant activities. The effectiveness of polyphenols depends on preserving their stability, bioactivity and bioavailability. The utilization of encapsulated polyphenols, instead of free compounds, can effectively alleviate some deficiencies [17]. The unpleasant taste of the most phenolic compounds is one of the reasons which limit their application at higher concentrations. Another problem is that only a small proportion of the molecules remain available following oral administration, due to insufficient gastric residence time, low permeability and solubility within the gut. In addition, their instability under conditions encountered in food processing and storage (temperature, oxygen, light) or in the gastrointestinal tract (pH, enzymes, presence of other nutrients), are limiting for activity and potential health benefits of the components like polyphenols [17]. Therefore, manufacturers have to provide protective mechanisms that can maintain the active ingredients until the time of consumption, enabling delivery to the physiological target in an organism [18]. From the literature, it is clear that the utilization of encapsulated polyphenols instead of free compounds, can lead to improvements in both, the stability and bioavailability of the compounds *in vivo* and *in vitro*. Although most of the encapsulation technologies employed for various compounds is adopted, there are still some technologies which have not been yet applied for polyphenols, including spray cooling/chilling, spinning disk and centrifugal coextrusion. However, this does not necessary mean that these technologies are not suitable for polyphenol encapsulation [2]. Future research of polyphenol encapsulation is likely to focus on aspects of delivery and the potential use of co-encapsulation methodologies, where two or more bioactive ingredients can be immobilised together simultaneously in order to provide synergistic activity of those. It can be foreseen that, with a deep understanding of the health benefits of polyphenols and new strategies for stabilization of fragile nutraceuticals, encapsulated polyphenols will play an important role in increasing the efficacy of functional foods.

Also, another goal of employing encapsulation is to prevent reaction with other components in food products such as oxygen or water, e.g. in case of essential oils. Essential oils are slightly soluble in water and they transfer to the water their odour and taste. Essential oils contain terpenes, phenols, alcohols, aldehydes, esters, ketones and other compounds. Essential oils have a wide spectrum of biological activities, including growth inhibition observed against bacteria, yeasts and fungi. The encapsulation of essential oils into different nanospheres has being used as a controlled release vehicle with site-specific delivery properties to maximize the antimicrobial activity of the oils [19].

Superior handling of an active, for example by conversion of liquid actives (e.g. plant extracts) into a powder also often calls for encapsulation. The most common method for preparing herbal extracts is percolation followed by lyophilisation. Among other techniques, spray- and vacuum- drying have also been employed with good results [20].

Except actives mentioned above, many other ingredients and food-fortifying compounds have been submerged to encapsulation, such as vitamins, micronutrients, fish oils, peptides, etc [7,22].

5. Immobilization of cells and enzymes in food processing

In addition to the above, encapsulation may be used to immobilize cells or enzymes in food processing applications, such as fermentation process and metabolite production processes. Immobilisation of microbiological cells by entrapment within natural or synthetic polymers or by adsorption onto solid (in)organic carrier materials has become an increasing research area. Adsorption, gel entrapment, and covalent binding are the accepted methods of immobilization used in various bioprocesses. Among several different approaches described in literature [21,23], the most useful for food processing is entrapment of cells within matrix of natural polymers like alginate, agarose, carrageenan, chitosan, and pectin. Such natural gelling polysaccharides represent an emerging group due to their advantage of being non-toxic, biocompatible, and cheap [24].

Immobilisation of cells provides ease of biomass separation and recovery, lower risk of microbial contamination, better use of equipment and, as a consequence of these and other benefits, higher productivity and efficiency. Immobilised cell technology offers other numerous potential advantages compared to free cell systems, such as higher cell densities and cell loads, shorter reaction times, smaller bioreactor sizes thereby lower capital costs, reuse of the same biocatalysts for prolonged periods of time, development of continuous processes which may be performed beyond the nominal washout rate, better substrate utilisation, reduced risk for microbial contamination, simplified process design, constant product quality and protection of cells, and faster fermentation rates [25]. However, the two most important disadvantages should be kept on mind, such as complexity of production process and cost constraints.

Nowadays, immobilized cell technology is well established at commercial scales in secondary beer fermentation, alcohol-free, low-alcohol beer and sparkling wine production. In order to meet the increasing demand for alcohol-free beer, several methods have been developed including alcohol removal from the product or limited fermentation of wort. In the second case, production is much better when immobilized cells are used [25]. In other processes like primary beer fermentation, wine and cider fermentation, immobilisation technology is still under scrutiny on the lab or pilot levels. These processes are significantly more complex with various side reactions important for flavour formation and final beverage quality. At the moment, the major challenge for successful application of immobilized yeast cell technology on an industrial scale is yeast phisiology control and fine-tuning of the flavour formation during fermentation processes [26].

Beer production with immobilised yeast has been the subject of extensive researches over the last 30 years. Traditional beer fermentation systems uses freely suspended yeast cells to ferment wort in an unstirred batch reactor. The traditional primary fermentation for lager beer takes about 1 week with secondary fermentation of few weeks. The treatment with a higher fermentation temperature and a selected specific yeast strain allows the production of lager beer in 12–15 days. Immobilised cell technology is able to produce lager beer in a much shorter period, usually 1–3 days, and the ultimate goal is the production of beer with satisfied final quality in that period of time [27,28]. Nowadays, as a result of detailed and comprehensive research, immobilised yeast technology is a well established technology for beer maturation and alcohol-free and low-alcohol beer production. However, the situation is more complex in primary fermentation and this process is still under searching on the lab and pilot levels [25,28]. An important dilemma of this research area is whether beer can be produced by immobilised yeast in continuous culture with the same properties as the traditional method. Achieving of satisfactory sensory characteristics in such a short time is a major difficulty. In some cases, cell proliferation and activity can be limited and it can result in deficient free amino nitrogen consumption and therefore an unbalanced flavour profile of the final beer, because of reduced cell growth. Therefore, different approaches for the adaptation of immobilized systems were investigated in order to correct the final beer quality. The researches aim to explore new materials as potential carriers for microbial cells and to identify and characterise changes in cell physiology and metabolism. Another direction will be to use

yeast strains that have been genetically modified to develop a capability to produce larger or lower amounts of one or more flavour compounds.

Applications of immobilized yeast cells in wine production have been explored in a view to reduce labour requirements, to simplify time-consuming procedures, and thereby to reduce costs. To be convenient in wine production, the method has to be economical, easily performed in industrial conditions and not to cause oxidation and contamination of wine [29]. In wine- or in cider-making, the main objective is to achieve an adequate quality of the product. Microbial cell immobilization can improve efficiency of malolactic fermentation, ability for cell recycling, cell stability and viability and improvement of product quality [33]. The use of immobilized cells in wine and cider production offers some other advantages as well, such as: simplified systems for removing microbial cells from batch processes, greater tolerance to inhibitory substances, smaller scale fermentation facilities, and possibilities of using a variety of microbial strains including genetically modified organisms. Immobilization in different materials aims to increase tolerance of malolactic bacteria and to speed up the process. In cider-production, researches have been focused on simultaneous alcoholic and malolactic fermentation by co-immobilization of two different species or by the same microorganism, often using genetic modification. However, some potential disadvantages must be also mentioned, like cell overgrowth which increases turbidity of the fermented beverage, mechanical stability of the matrix used to immobilize microbial cells, and loss of activity on prolonged operation. The selection of the suitable carrier and bioreactor system is a challenge and many factors should be taken into account, such as product quality, safety and stability, investments, operating costs and legality [30].

The tree crucial factors for the implementation on an industrial level are carrier materials, immobilization technology and bioreactor design. Natural polysaccharides such as alginate, chitosan, pectate and carrageenan, then synthetic polymers like polyvinyl alcohol and proteins like gelatine and collagen can be gelled into hydrophilic matrices under mild conditions, allowing cell entrapment with minimal loss of viability. As a result, very high biomass loadings can be achieved. Gels are mostly used in the form of spherical beads with diameters ranging from about 0.3 to 5 mm. However; mechanical stability is an important disadvantage of gels. It has often noticed that the gel structure is being destroyed due the growth of the cells and not only because of that, also because of intensive carbon dioxide production.

Probably the oldest and simplest method for enzyme and cell immobilization is adsorption. Immobilization by adsorption includes reversible surface interactions between enzymes or cells and support material. The typical adsorbent materials are ion-exchange materials such as ion-exchange celluloses. They are more suitable than traditional ion-exchange resins, since the high degree of ionic substitution of the resins often results in protein denaturation. Some advantages of adsorption techniques include (a) little or no damage to enzymes/cells, (b) simple and quick immobilization, (c) no chemical changes of support or enzyme/cells and (d) reversible process to allow regeneration with fresh enzymes/cells. Among disadvantages are nonspecific binding and overloading on the support and the most significant one is leakage of enzymes/cells from the support [21,23].

Except in beverage production processes, immobilized cell/enzyme technology has been used in dairy and meat fermentations or enzymatic processes. Immobilization may be very useful in improving the stability of probiotics and protective cultures in fermented foods. Immobilised cell technology can provide protection of cells during fermentation and drying, protection against bacteriophage attack, inhibition of undesirable flora, enhance survival of cells to heating and freezing, improve stability of cells during storage, accelerate of flavour development. In fermented meat and milk, the main microorganisms used are lactic acid bacteria. Basically, cells are micro entrapped into gel particles and added to the growth medium. Extrusion and emulsion techniques are commonly used for immobilization of lactic cultures in gels. A specific feature is that most of the biomass is located on the surface of gel beads, principally because of mass-transfer limitations of substrates and fermentation products. Cells are therefore released from the beads into the surrounding medium. This feature is undesirable when the

immobilised cell technology process is used for biomass production, however from the standpoint of industrial manufacturers of probiotics, it becomes desirable when system is used for continuous inoculation of milk such as in a dairy plant.

However, there are also some disadvantages referring to this biomass production. The two most important are higher investment costs and lower yields than it is expected. Another important disadvantage is the attacks of the released cells by bacteriophages. This often occurs, when milk for cheese making has not been enough sterilized by pasteurization; then bacteriophages from raw milk can survive and contaminate the bioreactor [31,32].

6. Conclusion

Encapsulation provides an effective method to cover an active compound with a protective wall material and thus, offers numerous advantages. These bioactive components include lipids, vitamins, peptides, fatty acids, antioxidants, minerals and living cells such as probiotics. Some of the main benefits are protection of various actives against evaporation, chemical reactions or migration in food, controlled delivery and preservation of stability of the bioactive compounds during processing and storage, prevention of undesirable interactions with other components in food products and masking unpleasant feelings during eating. Encapsulation is an important approach to meet all demands by delivering bioactive food components at the right time and right place. An attractive possibility is to use a methodology where two or more bioactive components can be combined to have a synergistic effect. It may be foreseen that encapsulated bioactives will play a significant role in increasing the efficacy of functional foods over the next period. With advanced strategies for stabilization of food ingredients and development of new approaches, we will be able to improve nutritional properties and health benefits of food compounds.

The main advantages of immobilised cell technology in the beverage industries are high-productivity of continuous fermentation and efficiency. Although research on immobilised cells is now approximately 30 years old, many difficulties related to the application at industrial scales have not been solved yet. In fact, engineering problems linked to choice of the carrier and reactor design are complicated by the effects of immobilization on the flavour profile of the final product. There is a growing need to find suitable solutions that provide high productivity, and at the same time, satisfy an adequate quality of the final food products. Future researches should be focused on overcoming the gap between conditions at research level and demands for large-scale applications, improving existing manufacturing technologies, choosing new processing conditions and new carrier materials. Also, future studies should be oriented to preservation and storage techniques that could be easily adopted at the industrial level.

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