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STUDY OF MICROENCAPSULATED BIOACTIVE COMPOUNDS IN FOOD PRODUCTS

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SUMMARY

Nowadays, consumers are increasingly aware of diet related health problems and therefore demand natural and safe ingredients as an alternative to synthetic substances, which are commonly used in the food, pharmaceutical and cosmetic industry. This idea is supported by the consumer's concern about the safety of products containing synthetic chemicals because these synthetic molecules are suspected to cause or promote negative health effects. Recent studies showed that phenolic and carotenoid compounds are important bioactive compounds with human health benefits. However, the development of new functional foods requires technologies for incorporating these ingredients into food in order to use and protect sensitive food components, to ensure protection against nutritional loss, to mask or preserve flavor/aroma and transform liquids into easy to handle solid ingredients. In many cases, microencapsulation can provide the necessary protection for these compounds and among various techniques that can be employed to form microcapsules, spray drying appears to be one of the most well-established and widely used technique.

In this contest, propolis, one of the few natural remedies that have maintained its popularity over a long-period of time, represents a widely available natural substance very rich in bioactive compounds that has biological and pharmacological properties, such as immunomodulatory, antitumor, antiinflammatory, antioxidant, antibacterial, antiviral, antifungal, antiparasite activities.

Moreover, it is well known that by-products of plant origin also represent an abundant source of sugars, minerals, organic acid, dietary fibre and phenolics. With increasing concerns over the use of organic solvents and their disposal, supercritical fluid extraction (SFE), with carbon dioxide (CO_2) as solvent and ethanol (EtOH) as co-solvent, is becoming a promising alternative. In particular, due to low cost and high content of added-

value products, such as ferulic and p-coumaric acids, brewer's spent grain (BSG), the major by-product of brewing industry, produced in large quantities annually and generally used as feeding stuff, can be used as an attractive adjunct in human nutrition. By-products of orange fruits processing industries also represent a promising source of active compounds with valuable technological and nutritional properties.

Hence, the aim of the study was to enhance the antioxidant properties of fish burgers with microencapsulated propolis and extracts from brewer's spent grain and orange by-products.

In particular, spray-drying process was used to microencapsulate propolis (30 g in 100 mL of ethanol 70% v/v) by means of gum Arabic and Capsul in different ratios (1:6 for gum Arabic and Capsul and then 1:20 just for Capsul). Once defined the optimal microencapsulation conditions, an alcohol-free powder able to mask the strong odor of propolis was obtained, thus promoting a potential food application as source of phenolics and antioxidants. Specifically, 5% w/w of spray-dried propolis was incorporated in fish burgers. To improve their sensory properties, new ingredients such as potato flakes (3%, 5%, 7% and 10% w/w) and extra virgin olive oil (9% w/w) were tested and optimized to give a final fish product with good acceptability. Proper tests on burgers also demonstrated an effective increase of both phenolic content and antioxidant activity. Then, to extract bioactive compounds from BSG a proper supercritical fluid extraction (SFE) was found. The effects of three factors including pressure (15–35 MPa), temperature (40–60°C) and ethanol concentration (0–60%, v/v) were investigated. Among the extraction variables, the best conditions (35 MPa of pressure, 40°C of temperature and 60% ethanol) were found considering the criterion of maximum concentration of phenolic compounds (0.35 ± 0.01 mg/g BSG), flavonoids (0.22 ± 0.01 mg/g BSG) and antioxidant activity, evaluated by the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (2.09 ± 0.04%/g BSG). After, the optimal BSG extract was microencapsulated and finally added to the fish-

burger formulation. In particular, microencapsulation was performed by means of a spray-drying, using Capsul as wall material, and modifying inlet temperatures (90-120-150°C) and ratios between extract and carrier (1:2; 1:4; 1:6; 1:8). Lastly, a sensory evaluation on the fish-burgers prepared with the different bioactive powders was carried out. The sample with 5% microencapsulated BSG extract and Capsul solution in ratio equal to 1:2 at 150°C was chosen as the best sample from both the nutritional and sensory point of view. Finally, the potential use of orange by-products, traditionally used as molasses for animal feed, fibre (pectin) and for fuel production, was investigated. Two SFE and spray drying techniques were compared to extract and microencapsulate the bio-active ingredient. Then, different percentages of this powder were added to the fish burger until its overall sensory quality remained acceptable. Fish-burger loaded with 5% microencapsulated orange by-product extract recorded high levels of bioactive compounds and were considered as acceptable as control burgers for their sensory characteristics, thus confirming the ability of proper techniques to mask and protect the bioactive compounds before food addition to enhance the health properties.

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CHAPTER 1

NEW TRENDS IN FOOD INDUSTRY

A background on main changes in food industry and functional food development are reported in this chapter. In addition, an overview of the most important bioactive compounds to enrich food is presented.

1.1 Functional foods and bioactive compounds

In the last decades consumer demands in the field of food production have changed considerably. Today, consumers more and more believe that foods contribute directly to their health (Mollet & Rowland, 2002). In fact, nowadays, foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but to prevent nutrition-related diseases and improve physical and mental well-being of consumers (Nöthlings, et al., 2007). Moreover, the food industry has been facing technical and economic changes both in society and in the manufacturing and food processing, that in turn had a significant impact on the entire food supply chain, up to the distribution of food to end consumers, and forced companies to pay high attention in food products that meet the consumers' demand for a healthy lifestyle. As a consequence, innovation has been widely investigated also within this traditional industry. Innovations introduced in the food industry in recent years mainly refer to new scientific and technical approaches in food processing, and to the introduction of novel foods. In this regard, functional food plays an outstanding role, as demonstrated by their increasing demand derived from the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for an improved quality of life in their later years (Bigliardia & Galati, 2013). Although there is not a regulatory definition for functional foods, these foods include a wide variety of foods and food components believed to improve overall health and well-being, reduce the risk of

specific diseases, or minimize the effects of other health concerns. However, the term "functional food" was first used in 1984 in Japan as a result of a study on the relationships between nutrition, sensory satisfaction, fortification and modulation of physiological systems in order to define those food products fortified with special constituents that possess advantageous physiological effects.

On the other hand, from a product point of view, Spence (2006) has proposed the following classification:

A) fortified products (food fortified with additional nutrients), e.g. fruit juices fortified with vitamin C, vitamin E, folic acid, zinc and calcium ;

B) enriched products (food with added new nutrients or components not normally found in a particular food), e.g. margarine with plant sterol ester, probiotics, prebiotics;

C) altered products (food from which a deleterious component has been removed, reduced or replaced with another substance with beneficial effects), e.g. fibers as fat releasers in meat or ice cream;

D) enhanced commodities (food in which one of the components has been naturally enhanced through special growing conditions, new feed composition, genetic manipulation, or otherwise), e.g. eggs with increased omega-3 content.

It should be emphasized, however, that this is just one of the possible classifications. In fact, according to alternative classification some functional products (1) add good to life or improve children's, like prebiotics and probiotics; (2) reduce an existing health risk problem such as high cholesterol or high blood pressure; (3) makes life easier, such as lactose-free or gluten-free products (Makinen-Aakula, 2006).

Furthermore, new types of products, derived from food, called nutraceuticals have recently been developed. These products, usually employed as food supplements, are marketed as tablets and pills, and can provide important health benefits. Frequently, functional foods are obtained from traditional foods enriched with an ingredient able to provide or promote

a beneficial action for human health. These are the so-called functional ingredients. These ingredients are preferred by consumers to have a natural origin (i.e. non-synthetic origin) being commonly extracted from natural sources. In effect, there is an extremely wide range of bioactive compounds that are associated with beneficial effects on human health, including but not limited to fibers, probiotics and prebiotics, vitamins, minerals, fatty acids, peptides, proteins, and secondary plant metabolites.

The most important types of functional ingredients are presented briefly hereinafter.

Probiotics are defined as live microorganisms that if consumed in adequate numbers, confer a health benefit on the host. Lactic acid bacteria (LAB) and bifidobacteria, normal components of the intestinal microbiota that have a long tradition of safe application within the food industry, are the most studied and widely employed bacteria within the probiotic field. Among probiotics, dairy products (e.g. Actimel, Activia, Yacutl) are the key product sector but fruit juice has also been suggested as a new, appropriate medium for fortification with probiotic cultures since it is already considered a healthy food product, and it is consumed frequently by a large percentage of the consumer population (Tuorila & Cardello, 2002).

Prebiotics are non-digestible food ingredients that beneficially affect the host by stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health. Among them fructo-oligosaccharide (FOS), inulin, isomalto-oligosaccharides (IMO), polydextrose, lactulose and resistant starch are considered as the main prebiotic components. These compounds have shown to have an important role in obesity control, to increase calcium absorption, to influence the formation of blood glucose, and reduce the levels of cholesterol and serum lipids (Sirò, et al., 2008).

Functional drinks are non-alcoholic beverages fortified with vitamins A, C and E or other functional ingredients such as cholesterol-lowering drinks (with combination of omega-3

and soy), “eye health” drinks (with lutein) or “bone health” drinks (with calcium and inulin) (Keller, 2006).

Functional cereals can be used as fermentable substrates for the growth of probiotic microorganisms, as sources of non-digestible carbohydrates since cereals contain water soluble fiber, such as beta-glucan and arabinoxylan, oligosaccharides, such as galacto- and fructo-oligosaccharides and resistant starch, which have been suggested to fulfill the prebiotic concept.

White bread containing the nutritional elements normally available in brown bread including fibers, vitamins B1, B3 and B6, iron, zinc, inulin and functional eggs enriched with omega-3 fatty acids, Se, vitamins D, E, B12 and folic acid are other important examples of functional foods.

Secondary plant metabolites, mainly consisting of phenolic compounds and carotenoids, have aroused particular interest because many of them have been demonstrated to possess an effect on human health (Biesalski, et al., 2009). Specifically their main effects are the reduction in the incidence of some degenerative diseases like cancer and diabetes, reduction in risk factors of cardiovascular diseases, antioxidant, anti-mutagenic, anti-allergenic, anti-inflammatory, and anti-microbial effects (Ignat, et al., 2011).

PHENOLIC COMPOUNDS are widespread groups of substances synthesized by flowering plants and all vegetative organs, as well as flowers and fruits, vegetables, cereals, grains, seeds and drinks, during normal development and in defense to stress conditions as infection, UV irradiation and reactive oxygen species (Beckman, 2000). The quantity and quality of the polyphenols present in plant foods depend on plant genetics and cultivar, soil composition and growing conditions, maturity state and post-harvest conditions (Jaffery, et al., 2003).

Besides being responsible for the color (such as yellow, orange, red, and blue pigments), taste and flavor (such as vanillin and eugenol) of foods, one of the major polyphenol characteristics is radical-scavenging capacity, which is involved in antioxidant properties, and the ability to interact with proteins. In addition with their antioxidant property, phenolic compounds offer a wide range of physiological properties like anti-microbial, anti-inflammatory, anti-allergenic, anti-atherogenic, anti-thrombotic effects. Moreover, the intake of phenolic compounds decreases the propensity to several chronic diseases such as several types of cancer, coronary, artery and cardiovascular diseases (Martins, et al., 2011). Furthermore, polyphenols have many industrial applications, for example, they may be used as natural colourants and preservatives for foods, or in the production of paints, paper, and cosmetic (Ignat, et al., 2011). However, in food application the utilization of microencapsulated polyphenols instead of free compounds can overcome the drawbacks of their instability, alleviate unpleasant tastes or flavors, as well as improve the bioavailability and half-life of the compound in vivo and in vitro (Fang & Bhandari, 2010).

In their general structure, phenolics possess an aromatic ring with a hydroxyl substituent and a functional residue and can be constituted by a simple phenolic molecule or a complex high-molecular weight polymer. Polyphenols are divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings to one another. The main groups of polyphenols are: flavonoids, phenolic acids, tannins (hydrolysable and condensed), stilbenes and lignans (D'Archivio, et al., 2007).

Flavonoids are the largest group of phenolic compounds: more than 4000 flavonoids have been identified. They are low molecular weight compounds, consisting of fifteen carbon atoms, arranged in a C6–C3–C6 configuration. Essentially the structure consists of two aromatic rings joined by a 3-carbon bridge, usually in the form of a heterocyclic ring. Variations in the substitution patterns of heterocyclic ring divided flavonoids into two

classes: a) anthocyanins (glycosylated derivative of anthocyanidin, present in colorful flowers and fruits) and b) anthoxanthins (group of colorless compounds including flavones, flavans, flavonols, isoflavones and their glycosides) (Ozcan, et al., 2014). The main sources of flavonoids are blackberries, black currant, blueberries, grape, strawberries, cherries, plums, cranberry, pomegranate, and raspberry. Flavonoids exhibit important antioxidant activity due to their antiradical (OH) anti-lipoperoxidation (R, ROO, RO) and metal chelating activities (Ignat, et al., 2011). Moreover, if consumed regularly by humans, flavonoids lead to a reduction in the incidence of diseases such as cancer and heart disease (Liu, et al., 2008).

Phenolic acids constitute about one-third of phenols and consist of two subgroups: the hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids are comprised of gallic, p-hydroxybenzoic, protocatechuic, vanillic and syringic acids, which in common have the C6–C1 structure. Hydroxycinnamic acids, on the other hand, are aromatic compounds with a three-carbon side chain (C6–C3), with caffeic, ferulic, p-coumaric and sinapic acids being the most common. The main sources of phenolic acids are blueberry, cranberry, pear, cherry (sweet), apple, orange, grapefruit, cherry juice, apple juice, lemon, peach, potato, lettuce, spinach, coffee beans, tea, coffee and cider (Bravo, 1998).

Tannins constitute the third important group of phenolics, which may be subdivided into hydrolysable (esters of gallic acid: gallo- and ellagi-tannins) and condensed tannins, known as proanthocyanidins (polymers of polyhydroxyflavan-3-ol: flavan-3-ols (-)-epicatechin and (+)-catechin). There is a third subdivision, the phlorotannins that consist entirely of phloroglucinol, isolated from several genera of brown algae, but without significant in the human diet (Bravo, 1998; Porter, 1989). Tannins have diverse effects on biological systems since they are potential metal ion chelators, protein precipitating agents and biological antioxidants. The main sources of tannins are grape (dark/light) seed/skin, apple juice, strawberries, raspberries, pomegranate, walnuts, muscadine grape, peach, blackberry,

olive, plum, chick pea, black-eyed peas, lentils, haricot bean, red/white wine, cocoa, chocolate, tea, cider, coffee and immature fruits (Ozcan, et al., 2014).

Stilbenes are present in low quantities and are structurally characterized by the presence of monomers or oligomers. The best known compound is resveratrol that exists in both cis and trans isomeric forms, mostly in glycosylated forms. The major dietary sources of stilbenes include grapes, wine, soy, peanuts, and peanut products (Ozcan, et al., 2014).

Lignans are produced by oxidative dimerisation of two phenylpropane units; they are mostly present in nature in the free form, while their glycoside derivatives are only a minor form. The interest in lignans and their synthetic derivatives is growing because of potential applications in cancer chemotherapy and various other pharmacological effects (Saleem, et al., 2005).

CAROTENOIDS are a family of red, orange, or yellow colored oil-soluble terpenoid that are located in the chloroplasts and chromoplasts of plants. Carotenoids can be divided into two structural groups based on the elements present in them: carotenes contain only carbon and hydrogen atoms and xanthophylls contain also oxygen in the form of hydroxyl, epoxy, aldehyde and/or keto groups. About 90% of the carotenoids is represented by β-carotene, α-carotene, lycopene, lutein and α-cryptoxanthin (Gerster, 1997).

Based on epidemiological studies, there is a positive correlation between intake of carotenoids and a low risk of chronic diseases; in fact it was shown that β-carotene and lycopene are inversely proportional to the risk of cardiovascular diseases and certain cancers while lutein and zeaxanthin to diseases of the eyes (Johnson, 2002; Ribaya-Mercado & Blumberg, 2004). In addition, Paiva at al. (1999) reported that carotenoids also perform biological actions through the following mechanisms: gap junction communication, cell growth regulation, modulation of gene expression, immune response and antioxidant function (Rao & Rao, 2007). In addition, carotenoids such as α-and β-

carotein and β-cryptoxanthin have the added advantage of being able to be converted to Vitamin A, important for growth and development, for the maintenance of the immune system and good vision (Tanumihardjo, 2011).

Carotenoids have an unsaturated nature, due to conjugated polyene chain, whereby they are subject to changes due mainly to oxidation that have the potential to impair the quality and bioactivity of the product (Boon, et al., 2010). However, other factors such as temperature, light and pH can also produce alterations that can influence the color of foods as well as their nutritional value (Melendez-Martinez, et al., 2004). Therefore, the alternative possibility for the incorporation of carotenoids in foods, without affecting their properties, would be the use of microencapsulation. In effect, Aissa et al. (2012) evaluated antigenotoxic activitiy *in vivo* by comparing β-carotene and microencapsulated β-carotene (mBC). Results have demonstrated that effectively mBC had protective effects in the liver and thus does not lose its protective properties, but higher doses must be used to observe antigenotoxic effects.

Unfortunately, phenolic compounds and carotenoids cannot be biologically synthesized in the human body, and vegetables, fruits and beverages, naturally act as their principal supplier (Beecher, 2003). Hence, more and more people rely on functional foods for nutrient supplement. In order to meet this growing demand, there is a great variability of techniques, listed below, to achieve functional foods (Betoret & Fito, 2011).

- Formulation and blending are traditional, simply and cheap technologies, widely used in food processing. For example, classical enriched milks and yogurts enriched with prebiotics, probiotics, and vitamins have been obtained with these techniques.
- Cultivation has always been traditionally a means to obtain products with high nutrients. In cases where agronomic approaches cannot achieve significant improvement, biotechnology (e.g. molecular biology tools and the development of

genetically modified seeds) offers a modern technique to modify composition of foods.

- Animal breeding offers the possibility to obtain improved food products, by adding new nutrients in animal diets, with subsequent transfer into final products.
- Edible films and coatings have the advantage to carry active ingredients in order to extend product shelf life, reducing the risk of pathogen growth on food surfaces and providing specific nutrients that affect beneficially one or more functions of the body.
- Microencapsulation, described hereinafter, is a specific technology, which has the purpose to prevent the physiological deterioration of bioactive compounds.
- Recent technologies that have the purpose to design personalized functional foods. These technologies are known as nutrigenomics (sometimes called nutritional genomics) and consider the interactions between foods or dietary supplements and an individual's genome, and the consequent effects on their phenotype.

Among the multiple suppliers of these compounds, propolis extract was chosen, representative of the products normally consumed as such and not yet used as a food ingredient. Furthermore, has also been shown that food by-products are rich in bioactive compounds so brewer's spent grain and flavedo of orange were further considered as potential functional ingredients.

1.2 Propolis

Propolis is a product obtained from bud and exudates of plants, transformed in presence of bee enzymes. Its color varies from green, red to dark brown, has a characteristic smell and shows adhesive properties. Raw propolis is processed using water washing and solubilizing in 95% ethanol to remove the wax and organic residues, creating propolis tincture or ethanol extract of propolis. In fact, raw propolis is composed of 30% wax, 50% resin and

vegetable balsam, 10% essential and aromatic oils, 5% pollen, and other substances (Burdock, 1998). However, its chemical composition, dependent upon the source plant and local flora, is very complex: more than 300 components have already been identified (De Castro, 2001). The largest group of compounds isolated from propolis is phenolic compounds and in particular flavonoid pigments, which are ubiquitous in the plant kingdom. Anyway, the substances identified in propolis are familiar constituents of food, food additives and/or GRAS substances. Important among the list of constituents are hydroquinone, caffeic acid (and its esters, above all caffeic acid phenethyl ester - CAPE), and quercetin, each of which have exhibited carcinogenic effects when administered to rodents (Burdock, 1998; Banskota, et al., 2001).

The use of propolis goes back to ancient times, at least to 300 BC as a medicine in many parts of the world. Today propolis is currently used as a popular remedy and is available in the form of capsules, as an extract (hydroalcholic or glycolic), as a mouthwash, in throat lozenges, creams, and in powder form. Anyway, numerous studies have demonstrated several biological and pharmacological properties of propolis, such as immunomodulatory, antitumor, antimicrobial, antiinflammatory, antioxidant, among others (Bankova, et al., 2000). In effect, Tosi et al. (2007) reported that propolis may also be used as a natural food preservative, after showing that the ethanolic extract of propolis may inhibit *E. coli* development while Burdok et al. (1998) showed that propolis exhibited an antitumor effect both *in vivo* and *in vitro*. Thus, the important properties attributed to propolis are valuable to the food industry because of its potential effect in the retardation of lipid oxidation and its positive effect on food product stability and shelf-life (da Silva, et al., 2013). Hence, propolis can be potentially used as a natural food additive and as a functional food ingredient (Mendiola, et al., 2010), also considering the fact that the synthetic antioxidants as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), proplyl gallate (PG) and tertiary butylhidroquinone (TBHQ) have shown carcinogenic activity (Ucak, et al.,

2011; Chen, et al., 1992). Although consumers occasionally consume strongly bitter-tasting foods and beverages such as coffee, tea, wine and dark chocolate, strong taste is generally undesirable and largely contribute to consumer unacceptability (Sun-Waterhouse & Wadhwa, 2013). For this reason, the application of propolis to food is still limited; due to its volatile phenolic acid fraction and its solubility in alcohol; moreover, it presents a strong and unpleasant taste and a characteristic odor that generally alter sensory characteristics of food (Banskota, et al., 2001; da Silva, et al., 2011). Various approaches for minimizing these problems have been developed but the most diffuse is the spray drying through the formation of a physical barrier to the taste buds. Recently, there has been an increasing interest in spray-dried propolis. In fact propolis extract has been encapsulated with different methods as in alginate microparticles by Hay et al. (2002), by atomization methods, using gelatin as encapsulant (Bruschi, et al., 2003), by the emulsification-solvent evaporation technique at microparticles of poly(e-caprolactone) (Durán, et al., 2007), by incorporation in a β-cyclodextrin (β-CD) cavity (Kalogeropoulos, et al., 2009), by complex coacervation using isolated soy protein and pectin as encapsulant agents (Nori, et al., 2011) and by spray-dried using gum Arabic and octenyl succinic anhydride (OSA) starch as carriers (da Silva, et al., 2013). However, no publications on application of microencapsulated propolis extract to food are still available.

1.3 By-products

It is well known that fruit and vegetables are rich in non-digestible components and phytochemicals that individually, or in combination, may act synergistically to contribute to the nutritional and health welfare. Despite the consumption recommendations, the intake of fruit and vegetables remains low and, consequently, both dietary fibre and antioxidant compounds are usually deficient in most diets around the world. Thus, there is a new trend to find innovative functional ingredients. In this contest, by-products, traditionally

undervalued, represent an interesting alternative. Nowadays a growing interest in the recovery of vegetable by-products and their conversion into high-value products was noticed. The use of by-products in the food industry results in benefit to both industry and consumers. The advantages for industry are from an economic point of view since the disposal represents an additional cost to the producer. The benefits of consumers, as well as the increase rejection of synthetic additives, concern the high content of valuable bio-active compounds (proteins, vitamins, pigments, antioxidants, antimicrobials, fragrances, etc.) with a wide range of actions as anti-tumoral, antiviral, antibacterial, cardio-protective and antimutagenic activities (Goni & Hervert-Hernandez, 2011). An important consequential benefit of the use of industrial by-products as raw materials is the generation of more jobs. High amount of by-products are produced by processing fruit such as apples, grapes, citrus, peach, apricot, mango, pineapple, banana, guava, papaya, kiwi and passion fruit, and vegetables as tomato, carrots, onions, olives, potato and red beets (Schieber & Stintzing, 2001). For this thesis, brewer's spent grain and orange flavedo are taken into consideration as potential food ingredients.

1.3.1 Brewer's spent grain

Recently, a particular attention has been paid to by-products of brewing industry after the production of wort: the brewer's spent grain (BSG). A schematic representation of the process to obtain BSG from natural barley is shown in Figure 1.1. Variety, harvest time, malting and mashing conditions, and the quality and type of adjuncts added in the brewing process influence the chemical composition of BSG (Huige, 1994; Santos, et al., 2003). In general, BSG consists of the walls of the husk–pericarp–seed coat and is considered as a lignocellulosic material rich in protein, fibre, minerals, vitamins and amino acids. Until now the main application of BSG has been as an animal feed (mainly for cattle), due both

to its high content of protein and fibre and its possible combination with inexpensive nitrogen sources, such as urea, to provide all the essential amino acids.

Another proposed use for BSG is in energy production through direct combustion that however requires pre-drainage of the spent grain, causing problems that arise from NOx and dust particle emissions. An alternative possibility for energy production from BSG is by anaerobic fermentation, which is efficient only if it is divided into a hydrolytic and a methanogenic phases. Moreover, due to its low ash content, fibrous nature, low cost and easy availability, BSG has been used in charcoal production, as a brick component, in paper manufacture and as adsorbent. Recently, BSG was considered in biotechnological processes as substrate for cultivation of microorganisms and for enzyme production, too (Mussatto, et al., 2006). However, in spite of all the possible applications described, its use remains still limited. Hence, BSG should be valorized as an ideal ingredient for human foods for its high phenolic compounds, widely recognized to have important antioxidant and antiradical properties (Maróstica Junior, et al., 2010). In fact, several researchers have shown that BSG contains high concentrations of hydroxycinnamic acids such as ferulic, p-coumaric, sinapic, caffeic and syringic acids can exert antioxidant, anti-cancer, anti-atherogenic and anti-inflammatory effects. To embed BSG into foodstuffs is necessary to consider some important aspects. Firstly, there are concerns about appearance since BSG is brown in colour, thus it can be unlikely integrated into products. More importantly, it is imperative that the sensorial properties of the foodstuff which must remain acceptable to consumers. In effect, BSG can impart unpleasant flavors and aromas (Townsley, 1979). For these reasons, the application of BSG to food is still limited. Therefore, to fully exploit the potential of BSG in human food products are necessary to identify a way to mask the undesirable attributes. In particular, the problem related to the particle size and color can be solved by the production of an extract, which in turn can be microencapsulated in order to minimize negative sensory properties.

1.3.2 Orange flavedo

Among the different types of fruit, the orange is one of the most cultivated and consumed and a large amount of by-products is generated when this fruit is destined to the production of fruit juice. It is worth noting that the amount of residue obtained from citrus fruits account for 50% of the original amount of whole fruit, thus the tones of orange by-products produced per day represent a problem for management, pollution, and environmental issues, due to microbial spoilage. Orange juice by-products are mainly constituted by peel (flavedo and albedo), pulp (juice sac residue), rag (membranes and cores) and seeds and are traditionally incorporated into the cattle feed or can be fermented for the production of valuable products like biogas, ethanol, citric acid, various enzymes, volatile flavouring compounds, fatty acids and microbial biomass (Li, et al., 2006). In fact extracted oils can be used in food and pharmaceutical industries or as flavor ingredients to drinks, ice creams or in the preparation of toilet soaps, perfumes, cosmetics and other home care products (Raeissi, et al., 2008). Virot et al. (2008) reported that the d-limonene, major component of the oil extracted from citrus peels, could be used as green solvent instead of hazardous petroleum solvents for fats and oils determination. Moreover, it has been recognized that orange by-products are an interesting source of dietary fiber and natural antioxidants compounds. The chemical composition is affected by factors such as growing conditions, maturity, variety and climate. However, in orange peel, phenolic compounds, including phenolic acids, polymethoxylated flavones, and glycosylated flavanones were identified (Huang & Ho, 2010). It is known that the phenolic compounds own antioxidant activity (Shahidi, 1997), that avoids the oxidative changes in foods, responsible for the development of off flavors compounds that alter their sensory and nutritional quality. Moreover, the extraction of the bioactive compounds from peels might be a good way to turn orange by-product into a valuable ingredient for a wide variety of food products. In addition, microencapsulation might be necessary to protect the stability of flavours (that

are volatile and chemically unstable in the presence of air, light, moisture and high temperatures) or to hide any unpleasant characteristics. Though several works have been done on the application of spray drying technique on orange juice to convert it into a powder (Goula & Adamopoulos, 2010; Phisut, 2012), a few studies have been yet appeared on spray drying of extracts from orange by-products. Just Jun-xia et al (2011) have reported microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic. However, the acquisition of bioactive compounds requires their extraction from brewer's spent grain and orange by-products without altering their natural original properties.

CHAPTER 2

OVERVIEW ON EXTRACTION TECHNIQUES

The following chapter aims to provide a background on different methods of extraction from bioactive compounds. Specifically, conventional and non-conventional methods are described, focusing on supercritical fluid extraction used in this thesis.

2.1 Introduction on extraction methods

Extraction is defined as the separation (isolation, identification and use) of bioactive portions of plant using selective solvents; in fact the purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue). The selection of proper extraction method affects bioactive compounds from qualitative and quantitative point of view. Different techniques, many of them remain almost same through hundreds of years, can also be used to extract bioactive compounds. All these techniques have some common objectives (Smith, 2003):

1. to extract targeted bioactive compounds from complex plant sample,
2. to increase selectivity of analytical methods,
3. to increase sensitivity of bioassay by increasing the concentration of targeted compounds,
4. to convert the bioactive compounds into a more suitable form for detection and separation, and
5. to provide a strong and reproducible method that is independent of variations in the sample matrix.

Most of classical extraction techniques are based on the extracting power of different solvents and the application of heat and/or mixing. In order to obtain bioactive compounds

from plants, the existing classical techniques are: Soxhlet extraction, maceration, infusion, percolation and decoction.

2.2 Conventional extraction methods

Soxhlet extraction or hot continuous extraction was first proposed by German chemist Franz Ritter Von Soxhlet (1879). It was designed mainly for extraction of lipid but now it has widely been used for extracting valuable bioactive compounds from various natural sources. Generally finely ground sample is positioned in a porous bag or thimble, that is then placed in distillation flask which contains the solvent of particular interest. Extraction solvent is heated, vaporizes, condenses in the condenser and drips back. When the liquid content reaches the siphon, the liquid contents are emptied into the bottom flask again. The process runs repeatedly until the extraction is completed. However, the Soxhlet extraction comes with disadvantage such as exposure to hazardous and flammable liquid organic solvents, with potential toxic emissions during extraction. Solvents used in the extraction system need to be of high-purity and this constitutes an additional cost. This procedure is considered not environmental friendly and may contribute to pollution problem. Moreover, dry and finely divided solid temperature, solvent-sample ratio and agitation speed need are limiting factors for this technique (Amid, et al., 2010).

Maceration consists of soaking plant materials with a solvent in a closed container and left under stirring at room temperature for a period of minimum 3 days. Some of the most widely used solvents (also in used in different ratios with water) in this extraction procedure are hexane, ether, chloroform, acetonitrile, benzene, and ethanol and are commonly. The process is directed to soften and break the walls of plant cells in order to allow the release of bioactive substances. After 3 days, the residue is pressed and/or filtered. In this conventional method, heat is transferred through convection and conduction and the choice of solvents will determine the type of compound extracted from

the samples. This technique is advantageous due to low processing cost and ease of operation. However, this method uses toxic solvents that requires proper waste management, requires an evaporation/concentration step for recovery, and usually calls for large amounts of solvent and extended time to be carried out.

Infusion and decoction use the same principle of maceration but include the use of hot or cold water (that is brought to the boil), respectively. Decoction is only suitable for extracting heat-stable compounds, hard plants materials (e.g. roots and barks) and usually resulted in more oil-soluble compounds compared to maceration and infusion. Another method that shares similar fundamental principle is percolation, which involves the use of unique equipment called percolator. Dried powdered samples are packed in the percolator, added with boiling water and macerated for 2 hours. In this contest, the possibility of thermal degradation of bioactive compounds cannot be ignored, due to the high temperatures during the times of extraction.

For classical methods, molecular affinity between solvent and solute, mass transfer, use of co-solvent, environmental safety, human toxicity and financial feasibility should also consider in selection of solvent for bioactive compound extraction (Azmir, et al., 2013).
Hence, to overcome the major problems of conventional extraction (longer extraction time, requirement of costly and high purity solvent, evaporation of the huge amount of solvent, low extraction selectivity and thermal decomposition of thermo labile compounds) new and promising extraction techniques are introduced. Specifically, during the last 50 years have been developed non-conventional methods that are considered as “green techniques” though in conformity with standards set by Environmental Protection Agency (EPA) and allow less hazardous chemical synthesis, designing safer chemicals, safe solvents auxiliaries, design for energy efficiency, use of renewable feedstock, reduce derivatives,

catalysis, design to prevent degradation, atom economy and time analysis for pollution prevention and inherently safer chemistry for the prevention of accident.

Some of the most promising techniques are ultrasound assisted extraction (UAE), enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), pulsed electric field assisted extraction (PEF), pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE).

2.3 Non-conventional extraction methods

UAE involves the use of ultrasound, special type of sound wave beyond human hearing, ranging from 20 kHz to 2000 kHz. Like other waves, it passes through a medium by creating compression and expansion. This process produces a phenomenon called cavitation, which increases the surface contact between solvents and samples and permeability of cell walls. Specifically, when the ultrasound intensity is sufficient, the expansion cycle can create cavities or micro-bubbles in the liquid. Once formed, bubbles will absorb the energy from the sound waves and grow during the expansion cycles and recompress during the compression cycle. Thus, the implosion of cavitation bubbles can hit the surface of the solid matrix and disintegrate the cells causing the release of the desired compounds (Gil-Chàvez, et al., 2013). Summary, the extraction mechanism by ultrasound involves two main types of physical phenomena, (a) the diffusion across the cell wall and (b) rinsing the contents of cell after breaking the walls (Mason, et al., 1996). Moisture content of sample, milling degree, particle size and solvent are very important factors for obtaining efficient and effective extraction. Furthermore, temperature, pressure, frequency and time of sonication are the governing factors for the action of ultrasound. The process is simple, relatively low cost with reduction in extraction time and solvent consumption but use of ultrasound energy more than 20 kHz may have an effect on the active phytochemicals through the formation of free radicals (Azmir, et al., 2013).

It is known that some phytochemical compounds are retained by hydrogen or hydrophobic bonding in plant cell walls and some are dispersed in cell cytoplasm. Some enzymes such as cellulase, β-glucosidase, xylanase, β-gluconase, and pectinase help to degrade cell wall structure and depolymerize plant cell wall polysaccharides, facilitating the release of linked compounds (Chen, et al., 2010). Hence, AEA has been proposed as a novel and an effective method to optimize the extraction of compounds from plant matrix (Wang, et al., 2010).

MAE utilizes microwave that are electromagnetic fields in the frequency range from 300 MHz to 300 GHz. Specifically, when microwaves pass through the medium, its energy may be absorbed and converted into thermal energy (Zhang, et al., 2011). Hence, moisture inside the cells is heated and evaporates, producing a high pressure on the cell wall. The pressure builds up inside the biomaterial which modifies the physical properties of the biological tissues (cell wall and organelles disrupter) improving the porosity of the biological matrix. This allows better penetration of extracting solvent through the matrix and improved yield of the desired compounds. If compared to maceration and Soxhlet extraction, this technique reduced extraction time and solvent volume. However, power, frequency, and time of microwave, moisture content and particle size of sample matrix, type and concentration of solvent, ratio of solid to liquid, extraction temperature, extraction pressure and number of extraction cycles are the main operating parameters to be optimized. Furthermore, this method is not suitable for tannins and anthocyanins as susceptible to thermal degradation and is limited to small-molecule phenolic compounds such as phenolic acids (gallic acid and ellagic acid), quercetin, isoflavin and trans-resveratrol since these molecules are stable under microwave heating conditions up to 100°C for 20 minutes (Trusheva, et al., 2007).

PEF may be a promising alternative to conventional cell disintegration methods. It is based on the application of external electric fields on biological cells. The external field provokes a charging of the membrane and the arrangement of the phospholipid molecules changes. As a result the membrane loses its barrier function and becomes permeable, a phenomenon often referred to as “electroporation” or “electropermeabilization”, enhancing the diffusion of solutes. This permeabilisation of cell membranes can be achieved at moderate electric fields (<10 kV/cm) and low specific energies (<10 kJ/kg). According to literature, the major electrical parameters that influence the electropermeabilization efficiency are field strength of the external field, pulse shape, pulse duration, number of pulses applied, and specific treatment energy (Goettel, et al., 2013).

As reported by Nieto et al. (2010), PLE is known by several names: pressurized fluid extraction (PFE), accelerated fluid extraction (ASE), enhanced solvent extraction (ESE), and high pressure solvent extraction (HSPE) and its operation is based on use of organic liquid solvents (e.g. water or ethanol) at high temperatures (50 to 200°C) and pressures (10-15 MPa) (Dunford, et al., 2010). To ensure the rapid and great extraction of compounds is necessary a proper combination of pressure and temperatures. In comparison to the traditional Soxhlet extraction, with PLE there is a considerable reduction of the consumption time and the solvent use (Richter, et al., 1996). However, this method is not found to be suitable for thermolabile compounds as high temperature can have deleterious effects on their structure and functional activity (Ajila, et al., 2011).

SFE may be defined as efficient and worldwide spread technique to extract bioactive compounds from products that are mixed with supercritical fluid (fluid close to the critical point) to form a mobile phase in order to obtain valuable natural substances. Specifically, raw material is placed in an extractor vessel, which has temperature and pressure controllers to maintain the desired conditions. Then the extractor vessel is pressurized with

the fluid by a pump. Once the fluid and the dissolved compounds are transported to separators, the products are collected through a tap located in the lower part of the separators. Finally, the fluid is regenerated and cycled or released to the environment (Sihvonen, et al., 1999). Among different supercritical fluids, the main used is carbon dioxide (CO_2). In its supercritical state (when both the temperature and pressure equal or exceed the critical point of 31.3°C and 7.39 MPa) has both gas-like and liquid-like quality, an important dual characteristic that helps the fluid diffusion to the matrix (the gas-like characteristic) and provides good solvation power (the liquid-like characteristic). CO_2 is cheap, environmentally friendly and generally recognized as safe by FDA and EFSA. Another advantage is that CO_2 is gaseous at room temperature and pressure, which makes analyte recovery very simple and provides solvent-free analytes. Furthermore, supercritical CO_2 (SC-CO_2) operates at low temperatures using a non-oxidant medium, which allows the extraction of thermolabile or easily oxidized compounds (Herrero, et al., 2010). The main disadvantage of CO_2 is its low polarity, problem that can be overcome using modifiers (co-solvents), capable of hydrogen-bonding, dipole–dipole and others polarity interactions with the analyte of interest. Generally, in SC-CO_2, ethanol is very often used as co-solvent considering its good miscibility with CO_2, non-toxicity and permissible use in the food and pharmaceutical industries. However, to develop a successful SFE, several factors need to be taken in consideration including temperature, pressure, particle size and moisture content of feed material, time of extraction, flow rate of CO_2, and solvent-to-feed-ratio (Gil-Chàvez, et al., 2013). A schematic diagram of SFE is represented in Figure 2.1.

Nowadays, SFE was used for the extraction of carbohydrates, phospholipids, essential oils, fatty acids and/or bioactive compounds from fruits and vegetables. In addition, SFE has demonstrated to be a useful tool to investigate fatty acids profile in fish oils and amino acids profiles in different genetically modified varieties of maize and soybean, and to be employed as a sample treatment technique prior to volatiles analysis in different beverages

and to determinate (Herrero, et al., 2010). It is interesting to note that SFE has been widely used to value food industry by-products; in fact by-products extraction allows the removal of valuable/interesting compounds. Specifically, in the contest of thesis, Fernandez et al. (2008) reported a study of the use of supercritical fluid technology coupled with pretreatment processes for the valorization of brewer's spent grain. Instead Mira et al. (1999) and Berna et al. (2000) studied the influence of SFE operating conditions (pressure, temperature, solvent, mass flow and particle size) on the extract composition and the influence of the height of the particle bed on the kinetics of SFE of essential oil from orange peel. In addition, the extraction of orange oil from *Citrus sinensis* pomace by SFE with CO_2 and CO_2 with co-solvent was investigated by Benelli at al. (2010).

CHAPTER 3

OVERVIEW ON MICROENCAPSULATION

The following chapter aims to provide a background on microencapsulation applied to food science. In particular, the benefits and significance of microencapsulation technology for food fortification, the methods to carry out microencapsulation, the main materials for microencapsulation and the most important microencapsulated food ingredients are reviewed.

3.1 Microencapsulation

As reported by Schrooyen et al (2001), bioactive compounds could slowly degrade and lose their activity, become hazardous (by oxidation reactions), react with other components present in the food system and/or change color or taste of food products. In this contest, microencapsulation technology can be inserted. Microencapsulation is not a new technology but was first commercially applied in 1954 for carbonless copy paper (Desai & Park, 2005). Microencapsulation has been used in the biomedicine and biopharmaceutics industry for the past 30 years for application ranging from cell therapy to drug delivery (Smidsrød & Skjak-Braek, 1990). Recently, microencapsulation has become suitable for food industry applications, in particular for the production of high value aliments and nutraceuticals. One of the most important reasons for microencapsulation of active ingredients is to improve stability in final products and during processing. Many bioactives (e.g. omega-3 oils, carotenes and polyphenols) need to be protected against degradation. For example, omega-3 oils are very susceptible to oxidation, leading to the development of off-flavours and off-odours. Another advantage of microencapsulation is less evaporation and degradation of volatile compounds, such as aroma, which usually contains mixture of volatile and odorous organic molecules. On the other hand, microencapsulation is used to

mask unpleasant feelings during eating, such as bitter taste and astringency of polyphenols and other antioxidant compounds that limit their food application. Moreover, microencapsulation can facilitate or improve handling of ingredients during production processes used in the final applications. The conversion of a liquid ingredient into a powder offers significant convenience, as it is much easier to store, weigh and add a powdered ingredient, compared to its liquid version. The addition of a more homogeneous mixing of the active compounds in the formation of the final product are definitely are improved by microencapsulation. In fact, the addition of small amounts (milligrams or few grams) of the bioactive compound in a product (that weighs hundreds of kilograms or tons) causes a non-uniform distribution within the food matrix. Instead, with microencapsulated compounds distribution becomes more homogeneous because the microencapsulateds have low payloads that provide a larger amount of the microencapsulated ingredient to be added. In summary, Shahidi and Han (1993) listed six reasons for employing microencapsulation in food industry:

- to reduce the core reactivity with environmental factors;
- to decrease the transfer rate of the core material to the outside environment;
- to promote easier handling;
- to control the release of the core material;
- to mask the core taste;
- to dilute the core material when it should be used in only very small amounts.

Microencapsulation involves the incorporation of chemically sensitive molecules within a secondary material to form microcapsules with diameters ranging between a few micrometers and a few millimeters. The material inside the microcapsule is named core, internal phase or fill, while the wall is called shell, coating, wall material or membrane. The shape and type of capsule depends on the properties of core and wall materials and on the encapsulation method used (Dziezak, 1988). Examples of different types of

microcapsules are shown in Figure 3.1. The simplest form of microcapsule is a spherical core particle, which has a uniform layer of wall material surrounding it. The core may also have multiple layers of the same or different wall materials, in which case it is called a multi-wall or multi-layer capsule. The capsule can also have multiple cores, and usually in these kinds of multi-core or aggregate capsules, the cores are embedded in a continuous matrix of wall material. The capsules may also be non-spherical, in which case they are usually irregular-shaped.

3.2 Microencapsulation methods

Many different microencapsulation techniques are available for the food industry, and each has its own advantages and disadvantages. They have different requirements for the core and wall materials, and they can produce different sized microcapsules. A brief description of techniques most commonly used (coacervation, fuidized-bed coating, spray-cooling/chilling, freeze-drying, liposome entrapment, extrusion, spinning disk, emulsion and spray-drying) in food industry is presented here.

3.2.1 Coacervation

Coacervation, known as phase separation, is defined as the separation of colloidal systems into two liquid phases. The basic mechanism consists of the formation of an emulsion and subsequent precipitation of the continuous phase around the droplets of the discontinuous phase. Coacervation process consists of three steps: (i) formation of a three-immiscible chemical phase, (ii) deposition of the coating, (iii) solidification of the coating. Coacervation technique is widely used in the industry; however, the coacervation method possesses some drawbacks. This process is not well suited for producing spheres in the low size range, is very expensive and rather complex (Gibbs, et al., 1999).

3.2.2 Fluidized-bed coating

Fluidized bed coating consists of suspending of solid particles of the core material in a moving stream of heated or cooled air. Once reached the desired temperature atomized particles of wall material were sprayed, depositing onto the core material. Coating material is sprayed into the chamber either as a hot melt, which solidifies onto the core material particles or as an aqueous solution in which water evaporates by hot air. The coating materials can be sprayed from above (top-spray), from below (bottom-spray) or from side (tangential spray). The main disadvantages of fluidized-bed coating concern the type of core material (i.e. solid particles with a size varying between 100 μm and several millimeters), and the type of coating material (i.e. mainly water-soluble biopolymers). In addition, this process is not cheap (Desai & Park, 2005).

3.2.3 Spray-cooling/chilling

In spray-cooling and spray-chilling, the core and wall mixtures are atomized into the cooled or chilled air, causing solidification the wall around the core. Ambient temperature and vegetable oil (melting point of 45 to 122°C) are used for spray cooling while refrigeration temperature and hydrogenated or fractionated vegetable oil (melting point of 32 to 42°C). Microcapsules obtained by spray-cooling and spray-chilling are insoluble in water due to the lipid coating. Consequently, these techniques tend to be utilized for encapsulating water-soluble core materials such as minerals, water-soluble vitamins, enzymes, acidulants, and some flavors. Another disadvantage of spray-cooling and spray-chilling is that special handling and storage conditions can be required.

3.2.4 Freeze-drying

Freeze-drying, also known as lyophilization, is the process of dehydrating frozen foods under a vacuum so the moisture content changes directly from a solid to a gaseous form

without having to undergo the intermediate liquid state through sublimation. This process is used for the dehydration of several heat-sensitive substances that are unstable in aqueous solutions. This drying technique is less attractive than others because the costs of freeze-drying are ups, the storage and transport of particles produced is extremely expensive and the commercial applicability is also severely restricted by the long processing time (Madene, et al., 2006).

3.2.5 Liposome entrapment

A liposome or lipid vesicle is a structure composed of lipid bilayers that enclose some aqueous or liquid compartments. The liposomes form spontaneously when phospholipids are dispersed in an aqueous phase. The mechanism for the formation of liposomes is based on hydrophilic-hydrophobic interactions between phospholipids and water molecules. A major advantage of their use is the target delivery and the ability to control the release rate of the incorporated materials. Bioactive compounds encapsulated into liposomes can be protected from digestion in the stomach, and show significant levels of absorption in the gastrointestinal tract, leading to the enhancement of bioactivity and bioavailability (Fang & Bhandari, 2010). Usually, liposome formulations are kept in relatively dilute aqueous suspensions and this might be a very serious drawback for the large-scale production, storage, and shipping of microencapsulated compounds (Desai & Park, 2005).

3.2.6 Extrusion

In the extrusion process, the core material is mixed into a molten carbohydrate mass, and the mixture is forced through a nozzle (extruder die) into a cooling and hardening solution, usually isopropanol, after which the extrusion product is dried and cut or ground to smaller pieces. High-dextrose equivalent corn syrup and a combination of sucrose and maltodextrin are often used as the encapsulation matrix (Fang & Bhandari, 2010). The

major advantage of this method is that the wall material, providing a good stability against oxidation and therefore prolonging the shelf life, surrounds the material. The limitations of extrusion include its relatively high cost, low flavor loading, low solubility in cold water, and high process temperature. In addition, yield is quite low, since means that high amount of carbohydrate is added to the food product; moreover, particles show high size (up to 1 mm) (Gibbs, et al., 1999).

3.2.7 Spinning disk

Spinning disk or centrifugal suspension separation is a relatively new microencapsulation method. This process involves mixing the core and liquid or dissolved wall materials and then adding of suspension to a rotating disk. While the disk spins, the coated particles or smaller particles of pure coating material are atomized at the edge of the disk. Due to their size difference, the microcapsules are easy to separate from the pure wall material particles, for example, by sieving. Since the process is continuous and quite fast and the equipment is relatively simple, the method is suitable for food applications. However, scaling up the process is challenging due to requirement of multi-head and the small nozzles, which could be subject to frequent problems of clogging (Gouin, 2004).

3.2.8 Emulsion

Another frequently used technique is emulsification and is used in case of water-soluble food active agents. Two types of simple emulsions are possible: oil-in-water (o/w) or direct emulsions, in which oil is dispersed in continuous water phase, and water-in-oil (w/o) or inverse emulsions, in which water are dispersed in continuous oil phase. If the system contains three (or more) liquid phases, it is a double (or multiple) emulsion, which can be described as an emulsion within an emulsion. Two major types of double emulsions can be classified – water-in-oil in- water (w/o/w) emulsions, which have water droplets dispersed

into oil droplets dispersed into a continuous water phase, and oil-in-water-in-oil (o/w/o) emulsions, which have oil droplets dispersed into water droplets dispersed into a continuous oil phase. However, emulsions are typically unstable or metastable systems (Nedovic, et al., 2011).

3.2.9 Spray-drying

As compared to all methods, spray drying is the most common method used for microencapsulation thanks to its availability of equipment, low process cost (if compared to freeze-drying, the cost of spray-drying method is 30–50 times cheaper (Desai & Park, 2005), good retention of volatiles, good stability of the finished product and large-scale production in continuous mode. Spray-drying also offers other advantages to other drying methods: the control of particle size, morphology and density of the powder in a single step. Spray-drying can be used for some heat-labile (low-boiling point) materials because of the lower temperatures that the core material reaches (Madene, et al., 2006).

Spray-drying is one of the oldest encapsulation methods used originally in the 1930's to encapsulate flavours using gum acacia (Shahidi & Han, 1993). Spray drying is a unit operation by which a liquid product is atomized by means of a hot gas current (air or more rarely an inert gas as nitrogen) to obtain a powder. Spray-drying process is divided in three basic steps (Dziezak, 1988):

- **preparation of the dispersion**. This stage provides for the formation of a fine and stable emulsion of the core material in the wall solution. The mixture in the form of a solution, a paste or a suspension to be atomized is prepared by dispersing the core material, which is usually of hydrophobic nature, into a solution of the coating agent with which it is immiscible;
- **homogenization of the dispersion**. The dispersion must be heated and homogenized, with or without the addition of an emulsifier depending on the

emulsifying properties of the coating materials. It is important that this emulsion are stable over a certain period of time (Liu, et al., 2001), oil droplets are rather small and viscosity are low (Drusch, et al., 2006). In fact as reported by Rosenberg et al., (1990) spray drying was affected by viscosity and particle size. Specifically high viscosities interfere with the atomization process and lead to the formation of long and large droplets that negatively affect the drying rate;

- **atomization of the mass** into the drying chamber. The atomization is carried out by atomizers, which are generally classified as rotary atomizers, pressure nozzles, pneumatic nozzles and sonic nozzles (Cal & Sollohub, 2010). In this step, the mixture is atomized into a heated air stream supplied to the drying chamber with consequent evaporation of the solvent, usually water that then leads to the formation of microcapsules. Due to the subsequent reduction in particle size and dispersion of the particles in the drying gas, the surface area of the particles increases exponentially, that helps to dry the feed in seconds. With the small size of droplets and the even distribution of the fluid feed, the moisture removal occurs without disturbing the integrity of the material (Orsat & Murugesan, 2012). The final product can be in the form of granules, powders and agglomerates. According to Shahidi and Han (1993) this last step could be further subdivided in other two detailing phases: atomization of the infeed emulsion and dehydration of the atomized particles.

Figure 3.2 presents a schematic diagram involved in a typical spray-drying microencapsulation process. The drying efficiency and the final product properties are influenced by these phases and their operational parameters (Cal & Sollohub, 2010). According to Reineccius (1988) for a microencapsulation is necessary to optimize spray-drying conditions that are mainly feed temperature, air inlet temperature and outlet temperature. In fact, the best spray-drying conditions depend on the right compromise

between following operating parameters. In particular, feed temperature modifies the viscosity of the emulsion, its fluidity and thus, its capacity to be homogenously sprayed: increasing feed temperature viscosity and size of droplets decrease but high increases of temperatures can cause volatilization or degradation of thermolabile ingredients. Inlet temperature is directly proportional to the microcapsule-drying rate and the final water content. Specifically, if this parameter is low, high density and intensive capsule membranes, high water content, poor fluidity, and easiness of agglomeration were occurred (Gharsallaoui, et al., 2007). At the same time, high values of temperature can cause excessive evaporation, with consequent cracks in the membrane, as well as release of flavoring ingredients through volatilization and the thermal degradation. Finally, outlet temperature depends on inlet temperature and cannot be directly controlled; however, for microencapsulation of food products its ideal value should not be too high (50-80°C) (Bhandari, et al., 1992). Despite the numerous advantages of spray drying techniques for food microencapsulation, according to Gharsallaoui et al (2007), there are also some limitations of this technology. The main limitation is the relatively limited number of wall materials available in the market, which also have the property of good solubility at high concentration. The main ones are discussed below.

3.3 Wall materials utilized in microencapsulation

As reported by Desai and Park (2005), an ideal coating should have good rheological properties at high concentration and easy workability during microencapsulation, to be able to disperse or to emulsify the active material and stabilize the emulsion produced and not to react chemically with the core materials. In addition, the wall material must seal and hold the active material within its structure and then release completely the solvent or other materials used during the process of microencapsulation. Finally, inexpensive, food-grade, biodegradable and Generally Recognized as Safe (GRAS) for human health are other

mandatory requirements. Generally, the microencapsulating materials are biomolecules that derive from various origins such as plants, marine, animals, microbial. They are classified into three major categories (Desai & Park, 2005):

- proteins that have an amphiphilic character that offer physicochemical and functional properties required to encapsulate hydrophobic core materials. The most commonly used proteins for encapsulating food ingredients by spray-drying are whey proteins and gelatin (Gharsallaoui, et al., 2007). Whey proteins have been used with success to microencapsulate anhydrous milk fat with a yield greater than 90% through their functional properties (Young, et al., 1993). However, in presence of high temperatures it is very difficult to predict the effect of spray-drying process on the stability of wall proteins (Gharsallaoui, et al., 2007). Likewise, gelatin, a water-soluble protein, consists mainly in glycine, proline and 4-hydroxyproline residues and has all the properties of an effective entrapping agent: high emulsifying activity, high stabilizing activity and a tendency to form a fine dense network upon drying. Finally, as reported by Pierucci et al. (2006) also, pea protein can be considered as a good coating agent. In all cases, it should be noticed that there are sometimes certain issues that may limit the use of proteins as encapsulating agents, for example, allergy and precipitation of protein when microcapsules are added to products having pH near their isoelectric point (Gharsallaoui, et al., 2007).
- lipids such as fatty acids, fatty alcohols, waxes, glycerides and phospholipids. Lipids are generally used as secondary coating materials applied to primary microcapsules or to powdered bioactive cores to improve their moisture barrier properties (Wu, et al., 2005). Lipids can also be incorporated in an emulsion formulation to form a matrix or film around the bioactive core (Crittenden, et al., 2006).

- carbohydrates such as starches, maltodextrins, gums. These materials are good encapsulating agents because they show low viscosities and good solubility, but most of them require more properties for high microencapsulation efficiency. In this contest, chemical modifications of carbohydrates are a novel approach to improve encapsulating properties of common wall materials (Gharsallaoui, et al., 2007). For example, some modified starches have surface-active properties and are widely used in the process of microencapsulation by spray-drying. In effect, Capsul is a modified food starch derived from waxy maize especially suited for the encapsulation of flavors, clouds, vitamins and spices. It appears as an off-white powder, and is an excellent replacement for expensive gums and proteins. It is often used in dry beverage mixes reconstituted by the addition of water, bartender dry mixes, and bakery dry mixes, such as cakes and cookies, but can also be used to encapsulate other water insoluble liquid or solid substances such as vitamins and fatty esters. Among hydrolyzed starches, maltodextrins have the advantages of being low cost, and good flavor protection against oxidation, but lack in emulsifying properties. In fact, when are used as wall constituents, it is necessary to incorporate other wall material such as gelating agent, sodium caseinate, whey proteins, lecithins etc. for improving emulsifying characteristics (Lin, et al., 1995). Gum Arabic is a natural exudates polysaccharide of acacia and is a well-known effective wall material because of its good emulsifying capacity, low viscosity in aqueous solution and good volatile retention. Problems associated with the use of gum arabic in encapsulation are high cost and limited supply (Kuan, et al., 2009).

Anyway, among different wall materials, carbohydrates are the most commonly used if compared to proteins and lipids.

3.4 Microencapsulated food ingredients

Vitamins and minerals are generally added to a range of food products for the following reasons: (a) to replace those that are lost during processing and storage; (b) to meet special nutritional needs, e.g. for infants and elderly; and (c) to prevent disease in specific consumer or at-risk groups. In effect, an inadequate supply of vitamins can lead to many deficiency diseases such as scurvy, pellagra, ariboflavinosis, dermatitis, enteritis while a lack of minerals (iron, iodine, potassium, sodium, phosphorus, magnesium and calcium) can lead to other disorders (anaemia, hypokalemia, hypochloremia, hyponatremia). However, the addition of these ingredients into food matrices is often difficult because are generally sensitive to temperature, moisture, light and pH, and their potency is often compromised by their reaction with other ingredients or premature release. Therefore, it is important to take precautionary measures to preserve them. A number of authors Desobry et al. (1997) Esposito et al. (2002), Desai and Park (2005), Pierucci et al. (2006) and de Oliveira et al. (2009) have described the potential of microencapsulation as a strategy for enhancing stability of vitamins in general. In addition, calcium micro-particles were successfully microencapsulated by Oneda and Ré (2003) while sodium caseinate by Hogan et al. (2001). Furthermore, spray drying process has been used to encapsulate flavors and oleoresins (substances responsible for most of the spice flavours), with examples including cardamom oleoresin, pepper oleoresin, I-menthol, coffee extracts, D-limonene, oil of oregano and many others (Orsat & Murugesan, 2012). The color deterioration of foods is one of the effects of food process, so particular attention was paid to antoxidants' colours. For instance, lycopene, anthocyanin pigments, watermelon juice, bayberries juice were protected with microencapsulation by spray drying (Orsat & Murugesan, 2012).

Probiotics, sensitive to heat and moisture, were microencapsulated employing spray coating, spray-drying, extrusion, emulsification and gel particle technologies. These live microorganisms provide benefits to the immune system, strengthen the mucosal barrier,

suppress intestinal infection, and must remain alive during processing, storage and gastric transit (Anal & Singh, 2007).

Finally, functional fatty acids, particularly docosahexaenoic acid, eicosapentaenoic acid, α-linolenic acid and conjugated linoleic acid whose proper intake ensures normal growth and development. These acids were microencapsulated with emulsion-based technologies and spray-drying to protect them from oxidation and to mask the fishy taste and odour (McClements, et al., 2007). However, extensive research is needed to extend the investigation on microencapsulation of further natural ingredients. For example, phytochemicals, biologically active plant chemicals, can reduce the risk of chronic diseases and, once extracted, generally require microencapsulation to stabilize the active component and mask undesirable tastes, colours and odours. Among phytochemicals, polyphenols (and flavonoids) and carotenoids represent the major classes of interest in this thesis. Therefore, among the various matrices rich in phenolic and carotenoid compounds propolis, brewers's spent grain and orange flavedo, widely available natural substances, could be good candidates. As above mentioned, bioactive compounds have potential in preventing the effect of major diseases. This has led to the development of functional foods and to the increased use of microencapsulation to produce bioactive compounds. For example, microencapsulation has been used to help the delivery of functional food ingredients (probiotics, prebiotics, dietary fibre, fish oil, polyphenols and short-chain fatty acids) into food, to stabilize and control their release during GI transit and to enhance their desired function in the body. Dairy, cereal, fatty and oil, bakery products and beverages are the major food categories containing functional ingredients. Moreover still today, works regarding fish products enriched with microencapsulated ingredients are very limited and even more the use of spray-dried propolis and extracts of by-product, as source of antioxidants.

CHAPTER 4

AIM OF THESIS

The purpose of this chapter is to describe the main aim and the objectives of three case studies of the research in this thesis.

The main aim of the thesis was to enhance the antioxidant properties of a fish-based product by addition of three natural ingredients: propolis, brewer's spent grain extract and orange by-products extract. To achieve this goal, the work was divided into different steps aimed to optimize the extraction conditions, the microencapsulation of extracts and the final application to fish burger. The three case-studies are presented below separately.

PROPOLIS CASE STUDY

The aims of the present case study were (a) to develop the best conditions of spray-drying to mask the pungent odor of propolis to be embed into sea bass fish-burgers, (b) to optimize the formulation of sea bass fish-burger enriched with spray-dried propolis, in order to improve its sensory properties and (c) to evaluate the total phenolic compounds and the antioxidant activity in the optimized fish-burgers with propolis.

BREWER'S SPENT GRAIN CASE STUDY

The aim of this case study was at first to optimize the extraction process of total phenols, flavonoids and free antiradical scavenging activity from BSG with SC-CO_2(EtOH), modifying the temperatures (40-50-60°C), the pressure (15, 25 and 35 MPa) and using only CO_2 and CO_2 + 20, 40 and 60 % ethanol (v/v). Then, after identifying the best SFE conditions, the second objective was to identify the best spray drying conditions to obtain a

powder able to mask the unattractive attributes of extracts. In particular, the microencapsulation by spray drying of polyphenols and flavonoids extracted from BSG was conducted in order to obtain a positive sensory evaluation in a proper fish-burger formulation. Finally, the antioxidant properties in the optimized fish-burgers with microencapsulated BSG extract were also addressed.

ORANGE BY-PRODUCTS CASE STUDY

Also for the by-products of oranges the ultimate aim was to increase the antioxidant properties of fish burgers. To achieve this objective the work was divided into four steps which had the following aims: (1) to investigate the extraction of orange bioactive compounds (polyphenols, flavonoids and carotenoids) by SFE with CO_2 with co-solvent, (2) to identify the best spray drying conditions, (3) determine the highest concentration of powder to be incorporated into fish burgers and (4) to evaluate bioactive compounds in the optimized fish burgers.

CHAPTER 5

MATERIALS AND METHODS

The purpose of this chapter is to describe the chemicals, reagents, equipment and methods used during this study. This is divided by the case study and includes microcapsules, as well as the preparation of extract and fish burgers and their chemical and sensory characterization.

PROPOLIS CASE-STUDY

5.1 Raw materials and chemicals

The commercial solution of propolis (30g dissolved in 100 ml of ethanol 70g/100 ml) was produced and distributed by Dr. Taffi s.r.l. (La California, LI, Italy). Gum Arabic (GA) (Farmalabor s.r.l., Canosa di Puglia, BAT, Italy) and Capsul, a starch chemically modified, (National Starch, USA) were used as carriers during spray-drying. Folin-Ciocalteu reagent, anhydrous sodium carbonate (Na_2CO_3), gallic acid monohydrate, ethanol (EtOH), methanol, 2,2-Diphenyl-1-picrylhydrazyl (DPPH radical) and 2,6-Di-tert-butyl-4-methylphenol (BHT) were supplied from Sigma-Aldrich (Milan, Italy).

5.2 Microencapsulation of propolis

Microencapsulation of commercial propolis (ethanolic extract) was performed by dispersion followed by a drying process, using a mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Switzerland). The Mini Spray Dryer B-290 is a laboratory scale system to perform spray drying processes down to 50 ml batch volume and up to 1 litre solution per hour and functions according to the same principle as the co-current flow atomizer, i.e. the sprayed product and drying air flow are in the same direction. Propolis was microencapsulated as described by da Silva et al. (2013) with slight modifications. In a

first step, gum Arabic and Capsul were dissolved in distillated water at the concentration of 30 g/100 mL, respectively (carrier solution). Propolis (propolis extract to-carrier solution ratios equal to 1:6 and then 1:20 just for Capsul; w/w) was dispersed into carrier solution with a homogenizer (Ultra-Turrax, T 25, D IKA, Germany) for 2 min at 15 000 rpm. The resulting formulations were spray-dried at the following conditions: inlet temperature of 120°C, outlet temperature of 88±2°C, aspiration rate of 100% and pump flow rate 25%. At the end of each drying session, the powders were collected, placed in closed vials and kept at room temperature in a dry and dark place until analysis. The samples were named SDP-1/6GA (powder of propolis extract and gum Arabic solution, 1/6), SDP-1/6C (powder of propolis extract and Capsul solution, 1/6) and SDP-1/20C (powder of propolis extract and Capsul solution, 1/20). In addition, the relative amount of extract of propolis used during microencapsulation was used as reference and named as EP. The microencapsulation process was conducted in triplicate.

5.3 Fish-burger preparation

Among the numerous fish species, European sea bass (*Dicentrarchus labrax*) is of major economic importance in the Mediterranean Sea. It is well known that fish flesh has some unique characteristics because it is a good source of protein, minerals and polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), very important for the prevention of coronary heart disease (Mozaffarian, et al., 2005). Conversely, Carlsen et al. (2010) reported that fish products are foods low in antioxidants. Hence, in order to enhance antioxidant content and due to modern trends to consume foods that contain no chemical preservatives, new food products with natural additives have become popular. In recent years, changes in socioeconomic factors including rapid urbanization and increase in working women population, have resulted in increased consumer's preference for ready-to-cook and ready-to-eat. Among them, one of the

important sea products is fish burger. Fish burger has been suggested as popular and tasty products in fast food industry and is preferred to traditional preparations.

So, fresh sea bass fillets (*Dicentrarchus labrax*) without removing the skin were obtained from a local seafood company (Lepore Mare S.r.l., Fasano, Brindisi, Italy). On arrival at the laboratory, they were trimmed to remove bones and skin. The minced fish was mixed with 5% (w/w) of SDP-1/6GA, SDP-1/6C and SDP-1/20C, shaped in a circular mould to produce fish-burgers (named SDP-1/6GA/FB, SDP-1/6C/FB and SDP-1/20C/FB, respectively) and cooked in a electric convention oven (H2810, Hugin, Italy) at 180°C for 15 min. In addition, fish-burgers embedding the same amount of extract of propolis contained in 5% of powder (named EP-1/6/FB and EP-1/20/FB, respectively) were also prepared to better highlight the difference with samples containing microencapsulated propolis. The control fish-burger (CTRL) was composed only by minced sea bass.

The formulation based on SDP-1/20C was optimized using different concentrations of potato flakes (3%, 5%, 7%, 10%; w/w) (Digei s.r.l., Foggia, Italy) and extra virgin olive oil (9%; w/w) (Olearia Desantis s.p.a, Bitonto, Bari, Italy). Three independent replicates were used for each fish-burger formulation.

5.4 Sensory evaluation

In accordance with the standard UNI EN ISO 13299:2010, seven testers of the Food Packaging laboratory were selected on the basis of international standard ISO 8586:2012. To the aim, three-digit numbers were used for each sample offered to the panelist in individual cabins under controlled conditions of light, temperature and humidity. Unsalted crackers and water at room temperature were provided to clean the palate between samples. The panelists were asked to assess odor, color, texture and overall quality for uncooked fish products. Moreover, juiciness, tenderness and taste were evaluated for the cooked ones. Then, the judges were also asked to identify the best fish-burger between the

different theses. According to the method of Paulus et al. (1979), a 9-point scale was used to quantify each attribute, where a score of 9 corresponded to "very good quality," scores of 7–8 to "good quality" and a score of 6 to "sufficient quality." The value equal to 5 represented the acceptability threshold, while a score of 1–4 corresponded to "unacceptable quality."

5.5 Extraction of phenolic compounds from fish-burgers

The extraction of polyphenols was based on method described by Sun et al. (2007) with slight modifications. Raw and cooked fish-burgers were dried, minced and extracted with methanol (50 mL). The solution was thoroughly shaken at room temperature for 20 min and centrifuged at 6000 rpm for 15 min (5804R, Eppendorf, Milan, Italy); this procedure was performed three times. After extraction, the supernatant was evaporated to dryness in a rotary evaporator (R200, BŰCHI Labortechnik AG, Switzerland), under vacuum at 40°C. The dry residue was dissolved in 10 mL of ethanol and filtered through a 0.45 µm syringe filter (Teknokroma PTFE 0.45 µm, Sant Cugat del Vallés, Barcelona, Spain). Extractions were performed in triplicate.

5.6 Chemical determination of samples

5.6.1 Total polyphenolic compounds

The Folin-Ciocalteu method was used to determine total phenolic compounds of the propolis based samples. This method uses gallic acid as standard and is based on the oxidability of phenols at basic pH, while the Folin-Ciocalteu reagent works as an oxidant agent. Phenols are responsible for most of the antioxidant capacity in most plant-derived products, so that their determination can be very informative (Woisky & Salatino, 1998; Singleton, et al., 1999). The total phenolic compounds were determined as described by da Silva et al. (2013) with slight modifications. Briefly, a 0.5 mL of sample and 2.5 mL of

Folin-Ciocalteu reagent diluted in water in a 1:10 ratio were left to rest for 5 min. An amount of 2 mL of Na_2CO_3 (4g/100mL) was then added, and the mixture was allowed to rest again for 2 h in darkness. The absorbance was then read using a spectrophotometer (UV1800, Shimadzu Italia s.r.l) at 740 nm. Total phenols content was expressed as mg of gallic acid equivalents (GAE) per g of powder or per g of fish-burger, according to a calibration curve. For each sample, the analyses were carried out in triplicate.

5.6.2 Antioxidant activity

Among the antioxidant evaluation methods, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, based on the reaction of the stable free-radical (DPPH radical) with components of the sample to evaluate a change in color of solution, is the most frequently used. The DPPH radical has a deep violet color; due to its unpaired electron and radical scavenging capability, it can be followed spectrophotometrically by absorbance loss at 517 nm when the pale yellow non-radical form is produced (Chen, et al., 1993). The antioxidant activity was determined using a method described by Hidalgo et al. (2010) with slight modifications. Briefly, 0.1 mL of BSG extract was mixed with 2.9 mL of 100 μM DPPH in ethanol and after 1 h of incubation in the dark, absorbance was measured at 517 nm using absolute ethanol as blank in a spectrophotometer (UV1800, Shimadzu Italia s.r.l). All samples were run in triplicate. The radical scavenging activity was expressed as the inhibition percentage using the following equation, where A_s was the absorbance of sample and A_c consisted in the absorbance of the control solution (solution in which ethanol was used in place of the extract): % Inhibition of DPPH = $(1-A_s/A_c)*100$.

5.7 Statistical analysis

All experimental data were subjected to one-way analysis of variance (ANOVA). To the aim, a Fisher's test with the option of homogeneous groups ($P<0.05$) was carried out to

determinate significance differences between samples. STATISTICA 7.1 for Windows (StatSoft Italia s.r.l.) was used.

BREWER'S SPENT GRAIN CASE STUDY

5.8 Raw materials and chemicals

Brewer's spent grains (BSG) were supplied by a local brewery industry located in Puglia (Italy). They were dried overnight in a dryer (T2 – Namad, Rome, Italy) at 35°C reaching a moisture of 5.62 ± 0.18% (according to AACC method 44-19). The particles size was determined and was found <500 µm. As carrier of spray drying, Capsul (Ingredion Incorporated, Westchester, USA), was used. Food grade ethanol (EtOH) was provided by Perrin's Chemicals (Triggiano, Italy). Sapio (Monza, Italy) supplied CO_2 with purity degree of 4.5 for SFE. All solvents were purchased from Sigma Aldrich (Milan, Italy).

5.9 Supercritical fluid extraction (SC-CO_2)

Extraction experiments were carried out using the supercritical fluid extractor Speed SFE-2 (Applied Separation, Allentown, USA), capable of pressures up to 69.0 MPa and temperatures up to 240°C, static and dynamic extraction with flow from 0 to 10 L/min (gaseous CO_2 at atmospheric pressure) and extraction vessels from 1 to 20 L. The unit includes two pumps: a solvent pump that delivers the fluid throughout the system, driven by air compressed obtained from a compressor, and a modifier pump for the addition of organic co-solvent. This instrument provides for application of gases above their critical points to extract selective soluble components from a raw material (Cavero, et al., 2006). However, CO_2 is not suitable for the extraction of polar compounds because it behaves as non-polar fluid for certain conditions of temperature and pressure. Therefore, this main drawback can be overcome with the use some food grade modifiers like ethanol (Lang & Wai, 2001). The system was set to the desired temperature and pressure with suitable

switches, while a metering valve is used to vary the CO_2 flow rate. Extractions were performed at different conditions: temperatures 40, 50, 60°C, pressures, 15, 25, 35 MPa and CO_2+ 20, 40 and 60% ethanol (v/v). The CO_2 flow rate used for all the experiments was fixed at 2 L/min; while the collection condition was at room temperature and atmospheric pressure. Based on the performed curve overall extraction (OEC). The whole process was divided into 6 cycles for a total duration of 240 min. 1 cycle corresponded to 30 min of static phase in order to try to maximize the contact of the supercritical solvent with sample material and 10 min of dynamic phase, in order to avoid an excessive consumption of CO_2 and thus reduce operating costs. Briefly, 35 g of BSG were loaded into the 50 mL extraction vessel that was placed in the extractor and was allowed to equilibrate to the desired temperature. Upon reaching the desired temperature, pressurization was initiated and the fluid (CO_2 or CO_2 + ethanol) flowed through the extraction vessel from the bottom to the top. The extract-laden gas from the extractor was passed through a heated metering valve, where the supercritical CO_2 was depressurized, and the extract was collected in a separator vessel while CO_2 was vented by a flow meter. The extract (E_BSG) was placed overnight in vacuum oven at 30°C in order to remove ethanol. The residue was dissolved in 20 mL of absolute ethanol and filtered through a 0.45µm syringe filter (Teknokroma PTFE 0.45 µm, Sant Cugat delVallés, Barcelona, Spain).

5.10 Microencapsulation of extract from brewer's spent grains

E_BSG microencapsulation was performed by a drying process using a mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Flawil, Switzerland). At first, Capsul was dissolved in distillated water at the concentration of 30g/100 mL, named carrier solution. The E_BSG was added to the carrier solution at proportions of 1:2, 1:4, 1:6 and 1:8 (w/w) and homogenized by an Ultra Turrax IKA T25 basic homogenizer (IKA Works Inc., Staufen,

Germany) for 2 min at 15,000 rpm. The resulting formulations were spray dried at different inlet drying air temperatures (90, 120, 150°C). The aspiration rate and pump flow used for all the experiments were fixed at 100% and 25%, respectively. At the end of each drying session, the powders were collected, placed in closed vials and kept at room temperature in a dry and dark place until analysis.

The powders were named SD2/90, 120 or 150 (BSG extract to C solution ratio equal to 1:2 at different temperatures), SD4/90, 120 or 150 (BSG extract to C solution ratio equal to 1:4 at different temperatures), SD6/90, 120 or 150 (BSG extract to C solution ratio equal to 1:6 at different temperatures) and SD8/90, 120 or 150 (BSG extract to C solution ratio equal to 1:8 at different temperatures). The microencapsulation process was conducted in triplicate.

5.11 Fish-burger preparation

A local seafood company supplied us fresh sea bass fillets (*Dicentrarchus labrax*) (Tortuga srl, Manfredonia, Italy). In accordance with the optimized recipe in a previous work of propolis, fish-burgers were prepared using minced fish mixed with 5% of spray-drying powders, 10% potato flakes (Digei s.r.l., Foggia, Italy) and 9% extra virgin olive oil (Olearia Desantis s.p.a, Bitonto, Bari, Italy). Thus, fish-burgers were cooked in an electric convention oven (H2810, Hugin, Milan, Italy) at 180°C for 15 min. Three independent replicates were used for each fish burger formulation. In addition, a fish burger control (CTRL) was composed by minced sea bass enriched with 5% of wall material, 10% of potato flakes and 9% of extra virgin olive oil. In addition, for a further comparison a fish-burger with 5% spent grain as that, 9% of potato flakes and 10% of extra virgin olive oil (FB/Spent grain) and a fish burger enriched with the corresponding not microencapsulated extract, 10% of potato flakes and 9% of extra virgin olive oil (FB/E_BSG) were prepared.

5.12 Sensory evaluation

Sensory analysis was conducted as for the propolis study (paragraph 5.4).

5.13 Extraction of phenolic compounds from fish-burgers

The extraction of polyphenols was carry out using the method previously described (paragraph 5.5).

5.14 Chemical determination of samples

5.14.1 Total Polyphenolic compounds

Total polyhenolic compounds were measured as reported in the previous paragraph 5.6.1.

5.14.2 Total flavonoid compounds

Flavonoids were quantified by aluminium trichloride method as described by Silva et al. (2012), with slight modifications. Briefly, 0.5 of mL of sample was mixed with 2 mL of distilled H_2O and 0.15 mL of $NaNO_2$ (5% w/v) solution. Subsequently (after 6 min), 0.15 mL of $AlCl_3$ solution (10% w/v) was added. After 6 min of reaction, 1 mL of 1 M NaOH and 1.2 mL of absolute EtOH were added to the mixture and filtered through a 0.22 µm syringe filter (Teknokroma Nylon 0.22 µm, Sant Cugat del Vallés, Barcelona, Spain). The solution was measured at 415 nm using a spectrophotometer (UV1800, Shimadzu Italia s.r.l). Quercetin standard solutions were used for constructing the calibration curve (6.25-200 mg/L; R2 = 0.9993). Total flavonoids content were expressed as mg of quercetin equivalents (QEs) per g of E_BSG or per gram of powder or per gram of fish burger. For each sample, the analyses were carried out in triplicate.

5.14.3 Antioxidant activity

See paragraph 5.6.2.

5.15 Statistical analysis

See paragraph 5.7.

ORANGE BY-PRODUCTS CASE STUDY

5.16 Raw materials and chemicals

Biological Valencia oranges were distributed by BIA consortium (Cosenza, Italy). The flavedo (the orange peripheral surface of the peel or epicarp) was carefully removed with a manual peeler and cut into small pieces, dried at 30°C overnight (T2 – Namad, Rome, Italy) and ground before extraction. The particles size was determined and it was found to be about 500 μm. Food-grade ethanol (EtOH) was provided by Perrin's Chemicals (Triggiano, Italy). Sapio (Monza, Italy) supplied CO_2 with purity degree of 4.5 for SFE. Capsul (Ingredion Incorporated, Westchester, USA) was used as carrier of spray drying. All solvents were purchased from Sigma Aldrich (Milan, Italy).

5.17 Supercritical fluid extraction (SC-CO_2)

The extraction process was carried out using the supercritical fluid extractor Speed SFE-2 (Applied Separation, Allentown, USA). For the recovery of bioactive compounds from ground-dried flavedo of orange two supercritical fluid extractions have been used and then compared. In particular, Benelli et al. (2010) have achieved good results (in terms of yield, concentration of phenols and carotenoids) using ethanol in concentrations of 2%, 5% and 8%, temperature of 50°C and pressure of 25 MPa. Since the yield differences were statistically significant only using 8% (w/w), this percentage of ethanol was finally fixed for this study. On the contrary, in previous work of brewer's spent grain, a proper supercritical fluid extraction, to give bioactive compounds from brewer's spent grain, was found. The effects of different values of pressure, temperature and percentage of ethanol were studied. Results indicated that temperature of 40°C, pressure of 35 MPa and 60%

ethanol (v/v) were the best operating conditions to achieve a high phenolic and flavonoid content. Therefore, it was decided to apply these parameters also for the extraction of the bioactive compounds from ground-dried flavedo of oranges. The methods were named SFE_1 and SFE_2, while the extracts were named E_1 and E_2, respectively.

The system was set to the desired temperature (40 or 50°C) and pressure (25 or 35MPa) with suitable switches, while a metering valve was used to vary the CO_2 flow rate. The CO_2 flow rate used was set at 2 L/min, whereas the collection condition was at room temperature and atmospheric pressure. The process lasted 240 minutes. Briefly, 28 g of ground-dried flavedo was loaded into the 50 mL extraction vessel with diameter of 15 mm that was placed in the extractor and was allowed to equilibrate to the desired temperature. Upon reaching the desired temperature, pressurization was initiated and the fluid (CO_2 +8% or 60% ethanol, v/v) flowed through the extraction vessel from the bottom to the top. The extract-laden gas from the extractor was passed through a heated metering valve, where the supercritical CO_2 was depressurized, and the extract was collected in a separator vessel while CO_2 was vented by a flow meter. The extract was placed overnight in vacuum oven at 30°C in order to remove ethanol. The residue was dissolved in 20 mL of absolute ethanol and filtered through a 0.45µm syringe filter (Teknokroma PTFE 0.45 µm, Sant Cugat del Vallés, Barcelona, Spain). To obtain an appropriate quantity of extract, several extractions were carried out.

5.18 Microencapsulation of extract from orange by-products

The microencapsulation process was performed using two different procedures by means of a mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Flawil, Switzerland). At first, Capsul was dissolved in distillated water at the concentration of 30 g/100 mL (carrier solution). The extract was added to the carrier solution at proportions of 1:2 (w/w) and

homogenized by an Ultra Turrax IKA T25 basic homogenizer (IKA Works Inc., Staufen, Germany) for 2 min at 15.000 rpm.

At first, this mixture was spray-dried using parameters reported by Romo-Hualde et al. (2012) that have microencapsulated bioactive compounds from red pepper by products. It was decided to use these same parameters (inlet temperature of 185°C, aspiration rate 90% and pump flow rate 10%) as the pepper is rich in phenolic compounds and carotenoids such as orange. Then, the same mixture was further spray dried using optimized parameters in a previous study, setting inlet temperature, aspiration rate and pump flow at 90°C, 100% and 25%, respectively. At the end of each drying session, the powders were collected, placed in closed vials and kept at room temperature in a dry and dark place until analysis. With SD_1 and SD_2 were indicated two microencapsulation methods, while the resulting powders were named SDE_1 and SDE_2, respectively. The microencapsulation processes were conducted in triplicate.

5.19 Fish-burger preparation

A local seafood company supplied us fresh sea bass fillets (*Dicentrarchus labrax*) (Tortuga srl, Manfredonia, Italy). In accordance with the optimized recipe in a previous work of propolis, fish-burgers were prepared using minced fish mixed with spray-dried extract (SDE_2), 10% potato flakes (Digei s.r.l., Foggia, Italy) and 9% extra virgin olive oil (Olearia Desantis s.p.a, Bitonto, Bari, Italy). SDE_2 was added at various concentrations: 2%, 5%, 10% and 15%, named FB_2, FB_5; FB_10 and FB_15, respectively. Thus, fish-burgers were cooked in an electric convention oven (2810, Hugin, Milan, Italy) at 180°C for 15 min. Three independent replicates were used for each fish-burger formulation. In addition, a fish-burger control (CTRL) was composed by minced sea bass enriched with 10% of potato flakes and 9% of extra virgin olive oil. In addition, for a further comparison a fish-burger with 5% of ground dried flavedo, 10% of potato flakes and 9% of extra virgin

olive oil (FB_GDF), and a fish burger enriched with the corresponding not microencapsulated extract, 10% of potato flakes and 9% of extra virgin olive oil (FB_E) were prepared.

5.20 Sensory evaluation

Sensory analysis was conducted as for the propolis study (paragraph 5.4).

5.21 Extraction of bioactive compounds in fish-burgers

The extraction of polyphenols was carry out using the method previously described (paragraph 5.5).

5.22 Chemical analyses

5.22.1. Total polyphenolic compounds

See paragraph 5.6.1.

5.22.2 Total flavonoid compounds

See paragraph 5.14.3.

5.22.3 Total carotenoid compounds

The absorbance at 450 nm of sample was measured using a UV-vis spectrophotometer (Shimadzu UV spectrophotometer UV-1800, Milan, Italy). Total carotenoid content (TCC) were calculated according to the method of Li et al. (2013) and expressed as micrograms per sample.

5.23 Statistical analysis

Sensory analysis was conducted as for the propolis (paragraph 5.4).

CHAPTER 6

RESULTS AND DISCUSSION

This chapter describes and discusses the results obtained for the three different cases-studies.

PROPOLIS CASE-STUDY

6.1 Optimization of spray drying conditions

The resulted spray-dried propolis was fine and pale yellow colored powder. It is well-known that propolis presents a strong and characteristic smell due to its volatile phenolic acid fraction (Thomson, 1990; Banskota, et al., 2001; da Silva, et al., 2011) and this is the main discriminating attribute that affects the overall quality and limits propolis application in foods. So, in order to establish the optimal conditions for microencapsulating propolis, an odor assessment on the fish-burger enriched with propolis as such (EP-1/6/FB) and with the two different powders of microencapsulated propolis (SDP-1/6GA/FB and SDP-1/6C/FB) was carried out. The effect of adding different powders of propolis to fish-burger is shown in Fig. 6.1 (a, b). All the samples presented a characteristic odor of propolis with a score under the acceptability threshold (score = 5) even though several authors assessed that the spray drying is generally used to mask unpleasant feelings, bitterness and astringency of polyphenols and other compounds (Re, 1998; Favaro-Trindade, et al., 2010; da Silva, et al., 2013). In this case, it is likely that the concentration of encapsulating material is unable to hide the pungent smell of propolis. On the other hand, a good microencapsulation efficiency is generally achieved when the maximum amount of core material is microencapsulated inside the powder particles (Seid, et al., 2008) so, in the current study, total phenolic compounds of the EP, SDP-1/6GA and SDP-1/6C samples

were determined. Significant differences in phenolic content were observed between the tested carriers; in fact, for SDP-1/6AG, 12.73 ± 0.15 mg of GAE per gram of powder against 13.86 ± 0.13 for SDP-1/6C were recorded. In addition, no difference appeared between the C and the EP (13.95 ± 0.06 mg of GAE in the relative amount of extract of propolis contained in 1 g of powder). This showed a slightly higher capacity of the Capsul to retain propolis, compared to gum Arabic. Although the gum Arabic presented many desirable characteristics to be a good encapsulating agent (high solubility, low viscosity and good emulsifying properties), Capsul appeared to be the best to microencapsulate the propolis extract during atomization. Most likely, the presence of the lipophilic component (octenylsuccinate) in the formulation of the Capsul gave the carrier a major capacity for retaining compounds during atomization in the spray-dryer. Moreover, low viscosity, good film-forming properties and thermo-protective effect during the exposure to high temperatures (Marchal, et al., 1999; Reineccius, 1991; Shahidi & Han, 1993) may also contribute to the general good performances of Capsul if compared to gum Arabic. In addition, the oscillation in supply, as well as the increasing prices of gum Arabic has directed still more the preference towards the Capsul (Charve & Reineccius, 2009) modifying, however, the propolis extract-to-carrier ratio to 1:20. As can be seen in Fig. 6.2 (a, b), increasing the amount of wall material positively affected the sensory attribute. Specifically, the score for the odor of the SDP-1/20C/FB sample was slightly over the acceptability threshold (5.67 and 6.21), unlike the SDP-1/6C/FB sample was 4.33. In addition, Fig. 6.3 (a, b) highlights that the SDP-1/20C/FB had an odor score statistically different from samples prepared with the appropriate amount of propolis, such as the EP-1/20/FB. Therefore, on the basis of the above considerations, the best propolis powder was that microencapsulated with Capsul in the ratio 1/20.

6.2. Optimization of fish-burger formulation

The sensory evaluation of SDP-1/20C/FB is reported in the Table 6.1. As can be seen, while the cooked samples received good judgment from panel test (all the sensory attributes were higher than 5 with a global quality score of 6.00), the uncooked ones showed an unpleasant overall quality (score = 4.50) because of lack of texture. Specifically, the samples appeared little compact, sticky and difficult to form. Most likely, the low consistency was due to the fact that the spray-dried propolis was partially dissolved in fish-burger (the sea bass is made up of about 70% water; (Erkan & Özden, 2007), though the solubility of propolis in water sometimes increases its potential application to be used as a natural additive (da Silva, et al., 2013). Therefore, to improve the fish-burger sensory quality, dried potato flakes (PF) were added to the formulation. In particular, potato flakes were added to the patties at various amounts (3%, 5%, 7% and 10%; w/w) up to reach a significant quality improvement. As can be seen in Table 6.1, among all uncooked samples, the highest pleasant score was obtained for the sample containing 10% of potato flakes (6.00). In particular, this sample showed a good color (6.50) and texture (6.58) and a smell equal to that of the SDP-1/20C/FB sample (5.67). On the contrary, the fish-burgers enriched with 3%, 5% and 7% of PF did not appear to have an acceptable quality (but no significant different from the SDP-1/20C/FB samples). In fact, these concentrations negatively affected color and texture, due to not homogenous and still little firm dough. Concerning the odor attribute, instead, no significant differences between samples were found. Regarding the cooked patties, 3% PF, 5% PF and 7% PF samples recorded again the lowest overall quality score (4.14, 4.21 and 4.79, respectively). Specifically, texture and, above all, taste scores were below the limit of acceptability, although the evaluation of the odor and color were found to be positive. Moreover, their low juiciness and tenderness scores were statistically similar to that of the 10% PF sample. These results were due to the fact that the flakes can retain water better than substitute

flours (potato flour, cornstarch), and, if not properly dosed, can reduce the juiciness and tenderness of the product. Hence, among all the samples (raw and cooked), the burger enriched with 10% PF was chosen as the best one; in fact, as can be noted from the Table 6.1, this sample appeared to have a pleasant odor (6.71), color (6.29), texture (5.29) and taste (5.93), that positively influenced the final quality (5.57). However, it was necessary to further improve formulation of the selected sample by adding extra virgin olive oil, being able to enhance the product texture. An amount of 9% (w/w) represented the highest concentration of oil that was absorbed by the potato flakes.

The sensory properties on the final optimized fish-burgers are listed in Table 6.2. Results on the uncooked samples showed that no differences were observed in the overall quality among the last two formulations (10% PF with and without oil). In particular, it is worth noting that the texture was significantly increased with respect to the original sample (SDP-1/20C/FB). Concerning the cooked samples, the sensory data highlighted that the incorporation of the oil positively induced a general enhancement in all sensory attributes. Particularly, higher values of texture (6.29), juiciness (6.21), tenderness (6.07) and taste (6.79) were found when compared to the sample without oil (5.29, 4.79, 4.79 and 5.93 respectively).

6.3. Characterization of sample antioxidant properties

The total phenolic content and the sequestrating activity on DPPH of the optimized fish-burger (10% PF and 9% oil) were determined and compared to those of the control (CTRL). The results are presented in Table 6.3. As expected, the active sample demonstrated higher sequestrating activity on DPPH than the CTRL, 28.43% against 7.41% for uncooked samples and 29.69% against 6.64% for cooked samples. This antioxidant activity turned out to be in agreement with the total phenolic content of the

fish-burgers. In fact, the optimized sample showed a higher concentration of polyphenolics (1.28 and 1.13 mg/g sample) than the control (0.42 and 0.36 mg/g sample). The total phenols content is considered the main responsible factor for the antioxidant capability; several studies have been carried out to correlate polyphenolic composition of propolis with its antioxidant properties (Russo, et al., 2002; Kumazawa, et al., 2004; da Silva, et al., 2006; Gregoris & Stevanato, 2010). It is worth noting that the samples cooking did not affect these two parameters. In addition, as a mean of comparison, the sequestrating activity on DPPH for different concentrations (3.125 – 50 µg/mL) of a synthetic antioxidant, BHT, was determined (R^2 = 0.9992). In particular, the percent of inhibition of raw optimized fish-burger corresponded to a same inhibitory effect of BHT at concentration of 30.65±2.01 µg/ml and 32.14±3.53 µg/ml for cooked sample. Since this compound added to food is synthetic, undoubtedly it is justified the growing interest of introducing natural additives to food as propolis, whose components generally are constituents of food, and are recognized as GRAS (Generally Recognized As Safe) (Chen, et al., 1992; Burdock, 1998; Ucak, et al., 2011).

BREWER'S SPENT GRAIN CASE-STUDY

6.4 Supercritical carbon dioxide extraction from brewer's spent grain

To select the best SFE operating conditions, the effects of three extraction parameters were studied in term of total phenolic con-tent (TPC), total flavonoid content (TFC) and antioxidant capacity, generated by the free radical DPPH, of extracts from BSG. The experiments were divided into three groups maintaining constant the extraction temperatures (40, 50 or 60°C) and varying the extraction pressures and the CO_2+ ethanol (v/v) (15, 25 or 35 MPa, 0, 20, 40 or 60%, respectively). At first, to know the time required for the extraction process in order to obtain an economical advantageous process in terms of time and extraction yields, the overall extraction curve (OEC) has been performed. The

OEC generally is divided in three phases: the constant extraction rate period (CER), where the solute is easily transferred from solid to fluid phase, followed by the falling extraction rate period (FER) and, finally, the diffusion controlled rate period (DC) (Brunner, 1994; Leal, et al., 2008). In our case, OEC was obtained by taking into consideration the amount extracted (g) in relation to number cycles (1 cycle corresponded to 30 min of static phase plus 10 min of dynamic state) and using the following process parameters: 40°C, 35 MPa, only CO_2 and CO_2+ 20, 40 and 60% ethanol (v/v). As can be seen from Figure 6.4, the yield increased with the addition of co-solvent and with increasing time, until reaching a balance between the sixth and seventh cycle. Based on this result, it was assumed that an extraction time of 240 min could also represent a good compromise for all other experimental trials. Table 6.4 shows the experimental results of the chemical determination of BSG extracts, derived from SC-CO_2 performed at 40°C and modifying values of pressure and ethanol concentration. As can be seen, in the case of SC-CO_2 without co-solvent, the pressure had not significant effect ($P<0.05$) on both phenolic and flavonoid content and antioxidant capacity. This could be explained by considering that the BSG extracts contain many polar substances with high molecular weight, which appear to have a low solubility in neat CO_2 (Maróstica Junior, et al., 2010). The results indicated that it was necessary to increase the polarity of the supercritical fluid by adding a polar modifier such as ethanol, safer and less toxic as compared to acetone, methanol and other organic solvent. In this case, the proportion of co-solvent was the variable that significantly interfered on the extraction. In fact, ethanol adding provoked a strong effect, probably due to the rupture in solute/matrix interactions and consequent substitution with co-solvent molecules in solid active sites (Hollender, et al., 1997; Benelli, et al., 2010). In effect, increasing the percentage of modifier, SC-CO_2 the TPC, TFC and antioxidant activity increased. Specifically, at 40°C the best TPC, TFC and antioxidant potential values were obtained at 35 MPa with CO_2 + 60% ethanol (v/v) (0.35 ± 0.01 mg of gallic acid

equivalents per g of BSG, 0.22 ± 0.01 mg of quercetin equivalents per g of BSG and 2.09 ± 0.04 inhibition percentage of DPPH, respectively). It is worth noting that the antioxidant activity of phenols is linked to their ability to act as hydrogen-donating radical scavengers (Chew, et al., 2011) most likely, the treatment with CO_2 + 60% ethanol (v/v) at 40°C and 35 MPa allowed the phenolic compounds to act more easily as hydrogen-donating radical scavengers. The findings achieved at constant temperature of 50°C are listed in Table 6.5. Even in this experimental condition, it was necessary to pump ethanol as co-solvent to increase the solvent strength since with only CO_2, low TPC, TFC and DPPH values were obtained. As in the first group, high values of TPC and TFC were obtained at 35 MPa and with CO_2+ 60% ethanol (v/v) (0.29 ± 0.00 and 0.16 ± 0.01, respectively); however, good results have been achieved with a lower pressure of 25 MPa (0.28 ± 0.01 and 0.16 ± 0.01, respectively). In addition, in term of antioxidant capacity, the highest DPPH were achieved with CO_2+ 60% ethanol (v/v) and with 15 and 25 MPa (1.91 ± 0.10 and 1.91 ± 0.02, respectively). Thus, increasing the temperature and decreasing the pressure it was possible to obtain satisfactory results. The chemical composition of SC-CO_2 BSG under 60°C, different pressure and ethanol conditions is reported in Table 6.6. As can be seen, the highest TPC (0.35 ± 0.01) was obtained at 15 MPa and with CO_2+ 40% ethanol (v/v) despite of the highest TFC (0.20 ± 0.01) was achieved at 25 MPa and with CO_2+ 40% ethanol (v/v), while the highest DPPH inhibition (1.99 ± 0.06) at 35 MPa and with CO_2+ 40% ethanol (v/v). As observed, there was no single process condition that could give high values for all the three evaluated chemical parameters. Most likely, it can be suggested that the temperature of 60°C was able to detect the presence in the BSG extract of several compounds with different polarity and volatility (Wang, et al., 2008). Moreover, as reported by Huang et al. (2005), a high content of phenolic compounds is not necessarily associated with a high antioxidant activity, which however depends on the structure and interaction of extracted phenolic compounds. Finally, the highest values of phenols,

flavonoids and antioxidant activity resulting from the different extraction trials conducted were summarized and compared. The lowest values were recorded at 50°C for all the three parameters considered, while the highest TPC was achieved both with the combination of the minimum temperature/maximum pressure (40/35) and with maximum temperature/minimum pressure (60/15) using in both cases CO_2+ 60% and 40% ethanol (v/v), respectively. This is in agreement with supercritical CO_2 extraction fundamentals: tuning pressure and/or temperature of the supercritical fluid, it is possible to modify its density that is directly proportional to its solubility (Raventós, et al., 2002). In addition, the supercritical carbon dioxide at 40°C, with CO_2+ 60% ethanol (v/v) and at 35 MPa (SC-CO_2 60/35) also appeared to be a good condition to extract flavonoids and to have the highest antioxidant activity compared to the other experimental conditions tested.

6.5 Microencapsulation of brewer's spent grain extracts

E_BSG presents an unpleasant flavor and aroma and this limits its application in foods. According to Ktenioudaki et al. (2013), BSG altered the odour profile in the baked snacks, as a number of compounds present in high levels in the BSG flour were also observed in high levels in the BSG-containing snacks. In particular, BSG flour was associated with high quantities of compounds such as 2-butyl-1-octanol, 3-methyl-butanal, 2-heptane, butanal, benzene and 2,3-butanedione, responsible for its characteristic odor. Therefore, in order to minimizing this problem, a microencapsulation process was performed, using different ratios between mass of core and mass of wall material (Mc:Mw - 1:2, 1:4, 1:6 and 1:8). The resulting formulations were spray dried at different inlet temperatures (90, 120 and 150°C), obtaining a dry and slightly brown-coloured powder. As expected and as can be seen in Table 6.7, increasing the amount of wall material decreased the values of TPC and TFC per gram of powder; in fact, significant differences were observed. On the contrary, it is important to underline that, for the same ratio, the temperature did not affect

the values of the polyphenols and flavonoids. Most likely, this is due to the thermo-protective effect of Capsul; this capacity, together with the low viscosity and the good film-forming properties, enhances effectiveness and the general performances as encapsulating agent. It is worth noting that using spray drying with a suitable wall material at the proper concentration, it is possible to maintain the stability of ingredients that are sensitive to environmental stresses. Therefore, in order to establish the best combination of operating parameters analyzed, it was necessary to perform a sensory analysis on the fish burger prepared with 10% of potato flakes and 9% of extra virgin olive oil and enriched with 5% of the different spray dried E_BSG.

The sensory evaluation of uncooked (A) and cooked (B) fish-burger is reported in Figure 6.5. Although the loss of some volatiles including flavours during spray drying encapsulation is inevitable, the attribute that mainly influenced the panelist's acceptability of raw samples was predominantly the odour. In particular, is interesting to note that, in all cases, the acceptability of the odor increases with the increase of the inlet temperature. It is shown that a high enough inlet air temperature leads to a rapid and easy formation of a crust on the microcapsule surface, so that the flavours cannot evaporate outwards (Jafari, et al., 2008). Most probably, with inlet temperature at 150°C the volatile compounds, responsible for the unpleasant odor of threshing, were retained within the microcapsule and therefore not detected by panelists. Concerning the color and texture attributes, instead, no differences between samples were found and were statistically similar to that of the CTRL. The cooked samples showed high scores and were appreciated by the judges. In fact, the general evaluation of the sensory attributes was found to be positive. However, it is noteworthy that increasing the amount of Capsul used, decreased juiciness and tenderness of fish-burgers, but improved taste. Finally, the fish-burger with 5% microencapsulated BSG extract and Capsul solution in ratio equal to 1:2 at 150°C was chosen as the best compromise according to the evaluation and sensory. (Figure 6.6 A and B). In particular, it

appeared to have good sensory properties that positively influenced the final quality of raw and cooked sample (6.30±0.41 and 6.40±0.22, respectively). Furthermore the relative powder used for the preparation of fish burger (SD2/150) presented a high phenolic and flavonoid content (1.70±0.06 and 1.25 ±0.06 per g of powder, respectively; Table 6.7).

To better highlight the difference and to demonstrate the efficacy of the method of microencapsulation, this sample was compared to fish burger enriched with the corresponding not microencapsulated extract (FB/E_BSG) and to fish burger with 5% of spent grain as is (FB/Spent grain) (Figure 6.7). As can be seen, FB/Spent grain showed an unpleasant overall quality (3.50 ±0.00 for uncooked samples and 3.50±0.00 for cooked samples). Specifically, the sample showed mostly a bad odor, a brown color and gritty texture. In cooked the situation worsened due to a persistent bitter taste. The FB/E_BSG sample, instead, showed a good colour (6.80±0.27), texture (7.00±0.00), juiciness (6.50±0.27) and tenderness (6.50±0.00) but its global quality score remained below the limit of acceptability (4.30±0.30 for uncooked and 4.20±0.27 for cooked) because of the strong odor and bitter taste. The potential successful spray drying microencapsulation is thus demonstrated in addition to the fact that it is an economical and flexible process, uses equipment that is readily available, and produces powder particles of good quality.

6.6 Characterization of fish-burgers: antioxidant properties

It was shown that BSG contains hydroxycinnamic acids including ferulic acid, p-coumaric acid and caffeic acid, which act as antioxidant, anti-inflammatory, antiatherogenic and anti-cancer. Given that these phenolic acids are some of the major phenolics in BSG, it is expected that phenolic extracts from spent grain may also exhibit similar properties, which are then preserved during their microencapsulation and incorporation into fish burgers (McCarthy, et al., 2013).

Table 6.8 presented the antioxidant properties of the 1:2 150°C fish burger and compared with those of the control. The active sample showed a higher concentration of polyphenols (0.125 and 0.136 mg/g sample) and flavonoids (0.132 and 0.143 mg/g sample) than the control (0.035 and 0.036 mg/g sample for TPC, 0.065 and 0.063 mg/g sample for TFC). These data turned out to be in agreement with the sequestrating activity on DPPH. Specifically, the fortified sample demonstrated higher antioxidant potential than the CTRL: 44.93% against 21.57% for uncooked samples and 44.49% against 21.19% for cooked samples. Lastly, it is worth noting that cooking had no deleterious effect on total antioxidant activity and total phenolics and flavonoids content indeed in the cooked sample was noted a significant increase of these values (0.125 ± 0.00 against 0.136 ± 0.00 for TPC; 0.132 ± 0.01 against 0.143 ± 0.00 for TFC and 44.93 ± 1.07 against 48.49 ± 1.45 for % DPPH inhibition). Most likely, this could be due to hydrolysis of conjugate molecules after cooking sample (Turkmen, et al., 2005).

ORANGE BY-BRODUCTS CASE-STUDY

6.7 Supercritical carbon dioxide extraction from orange by-products

In Table 6.9 are listed chemical properties of extracts obtained by two different methods. As can be seen, significant differences in phenolic, flavonoid and carotenoid content were observed between two extracts. In particular, for E_2 have been achieved higher values of these components compared to E_1. As expected, the different operating parameters have significant influence over the extraction. In fact, one of the supercritical extraction fundamentals states that it is possible to tune pressure and/or temperature and/or co-solvent amount in order to modify density of CO_2 that is directly proportional to its solubility, thus affecting the extraction of bioactive compounds (Raventós, et al., 2002). Moreover, as reported by Wang et al. (2008), increasing temperature decreases CO_2 density, with a consequent reduction in solubility while high pressure can lead to an increase in CO_2

density. The high CO_2 density increases its solvent power and therefore more substances were extracted. This is in agreement with our results since with lower temperature and higher pressure (40°C and 35 MPa against 50°C and 25MPa) an improved extract was obtained. As well, due to different solubility of different kinds of carotenoids it was necessary to achieve a selective extraction by adjusting the temperature and pressure of SC-CO_2 (Shi, et al., 2010). However, also the different concentration of ethanol interferes statistically on the extraction. In fact, it is known that with the addition of co-solvent, the extraction efficiency increases (up to a certain value and thereafter decreases). Co-solvent causes the increase of analyte solubility, which in turn causes variations in the characteristics of the matrix and concomitant enhancement of interaction with the supercritical fluid so as to improve the desorption of the analytes from the matrix of sample (Montero, et al., 2005). Specifically, as reported by Hollender e al. (1997), the addition of ethanol provoked a strong effect, due to the rupture in solute/matrix interactions and consequent substitution with co-solvent molecules in solid active sites.

Therefore, on the basis of the results obtained, E_2 was chosen and used in the subsequent step of microencapsulation.

6.8 Microencapsulation of orange by-products extracts

E_2 was spray-dried using two methods described above. Fine and orange-colored powders were produced (SDE_1 and SDE_2) and their chemical compositions are reported in Table 6.10. As can be seen, SDE_1 presented lower values of TPC (and therefore also of TFC) than SDE_2. This result can be attributed to thermal degradation when the sample was exposed to high temperature (185°C). Hence, high inlet air temperature of SD_1 could have a significant effect on the total polyphenol and flavonoid content. These findings are in agreement with those of Georgetti (2008) who found that the increase in the spray drying temperatures led to a product with lower concentration of polyphenol in soybean

extract. In addition, TCC in SDE_1 was lower than SDE_2 (1.24±0.07 and 7.40±0.03 mg β-carotene per g powder, respectively). Also for carotenoids, it was observed that dehydration of air considerably influenced their concentration (Phisut, 2012). Hence, inlet temperature at 185°C exhibited carotenoids to oxygen causing their oxidation because of presence of large number of conjugated double bonds. Finally, the powder produced at 90°C temperature and powder produced by the second method was used to enrich fish burgers.

6.9 Sensory evaluation of fish-burgers

Sensory data of fish burgers (uncooked and cooked) showed that different percentage of added powder (SDE_2) significantly influenced the overall quality. In particular, as regard the uncooked sample (Fig.6.8a), the high amount of powder (10 and 15%) used for FB_10 and FB_15, made the dough enough compact thus negatively affected the texture and the overall quality that became under the limits of acceptability (4.60±0.42 and 3.60±0.55, respectively). Moreover the panelists had not considered much pleasant the persistent odor of orange in these samples (5.80±0.27 and 5.40±0.42). On the contrary, the overall quality of the FB_5 sample was statistically similar to that of the CTRL sample (7.50±0.35 and 7.10±0.55, respectively). This was due to a pleasant and proper odor of orange (7.50±0.35), an appreciated color (7.5±0.35) and a good consistency (7.50±0.35). FB_2 sample had also received a good judgment but its overall quality (6.60±0.22) was nevertheless statistically different from that of the sample FB_5. Concerning the cooked burgers (Fig. 6.8b), samples with the high percentages of powder showed low sensory attributes scores. In particular, the main discriminating attribute was the taste because in fish burger persisted the bitter taste of flavedo (4.30±0.27 for FB_10 and 3.20±0.27 for FB_15). The situation improved with FB_2 but especially with FB_5 in which the sensorial properties were not affected by the powder addition. It is worth noting that FB_5

seemed the best sample, recording an overall quality not statistically different from the control one (7.40±0.22 and 7.10±0.22, respectively). To prove the efficacy of the spray-drying, the sample FB_5 was compared to fish-burger enriched with the corresponding not microencapsulated extract (FB_E) and to fish burger with 5% of ground dried flavedo (FB_GDF) (data not shown). Briefly, FB_E sample showed a low overall quality score due to a persistent bitter taste while FB_GDF sample was rejected by the judges because of grainy texture, poor juiciness and tenderness.

6.10 Chemical characteristics of fish burger

In Figure 6.9 is reported the comparison between the fish burger enriched with 5% of powder and the control sample. As can be seen, the main contribution is observed on the carotenoids that increased of about twelve times (0.274 and 0.213 mg β-carotene/g sample) compared to the control (0.022 and 0.017 mg β-carotene/g sample). In addition, the active burger showed a higher concentration of polyphenols (0.371 and 0.318 mg GAEs/g sample) and flavonoids (0.243 and 0.263 mg QEs/g sample) than the control (0.200 and 0.189 mg/g sample for TPC, 0.121 and 0.151 mg/g sample for TFC). It is worth noting that cooking had no deleterious effect on antioxidant compounds content, indeed in the cooked sample was noted a slight increase of TFC values, most likely due to the hydrolysis of conjugate molecules after cooking (Turkmen, et al., 2005).

CHAPTER 7

CONCLUSIONS

As regard propolis case-study, results indicated that by using Capsul with a mass ratio of core to wall material equal to 1:20 as carrier during the spray-drying technique, it is possible to retain a greater amount of propolis and mask the characteristic smell. In particular, the study showed that the addition of 5% of spray-dried propolis to a proper fish burger formulation (10% of PF and 9% extra virgin olive oil) showed a positive sensory evaluation (overall quality scores of 6.00 and 6.50 for raw and cooked fish burgers, respectively) and resulted to about three times greater phenolic content and about four times higher sequestrating activity on DPPH compared with the control.

In the case of BSG, an efficient supercritical carbon dioxide with co-solvent process (SC-CO_2(EtOH)) was optimized. Results indicated that an extraction time of 240 min, temperature of 40°C, pressure of 35 MPa and with CO_2+ 60% ethanol (v/v) were the best operating conditions to achieve a high phenolic (0.35 ± 0.01 mg/g BSG) and flavonoid (0.22 ± 0.01 mg/g BSG) content and good antioxidant properties (2.09 ± 0.04%/g BSG). Then, to enhance the antioxidant properties of fish-burgers the microencapsulation of BSG extract was carried out. In fact, in order to mask the pungent odour and the bitter taste of BSG extract a spray drying process was performed. The results of this step showed that using an extract to Capsul ratio of 1:2 and a temperature of 150°C, it was possible to obtain a positive sensory evaluation of the fish burger formulated. Moreover, this sample showed to have a higher phenolic and flavonoid content and greater antioxidant potential compared than the control sample.

As regard the orange by-products, different extraction methods were proposed. It was shown that different operating conditions affected the quality of the extract. Subsequently, the best extract was microencapsulated with two methods. Also in this case the powder

obtained by the same method adopted for BSG extract presented a high concentration of bioactive compounds. Among different percentages used, 5% microencapsulated extract seemed the best to enrich the fish burger because these new products were acceptable from a sensory point of view and showed high concentration of β-carotene polyphenols and flavonoids.

Nowadays, the use of propolis, brewer's spent grain and orange by-products is rather limited even if several studies have shown that they are a valuable source of compounds with potential interest for food, pharmaceutical and/or cosmetic industries. For this reason, the development of new techniques to valorize these products is of particular interest. In particular, SFE has proven to be an efficient technique to extract bioactive compounds. Furthermore, results suggest that spray-drying microencapsulation is quite successful in masking the bad smell and bitter taste of these products. In addition, it protects bioactive compounds from high temperature exposure during cooking. Finally, the finding of this study highlighted the potential re-use of propolis, brewery and orange by-products and the possibility of embedding these products as natural ingredients in various foods, including fish, to enhance the antioxidant content by using an economical and flexible process with the possibility to realize a promising system for a large-scale.

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FIGURES AND TABLES

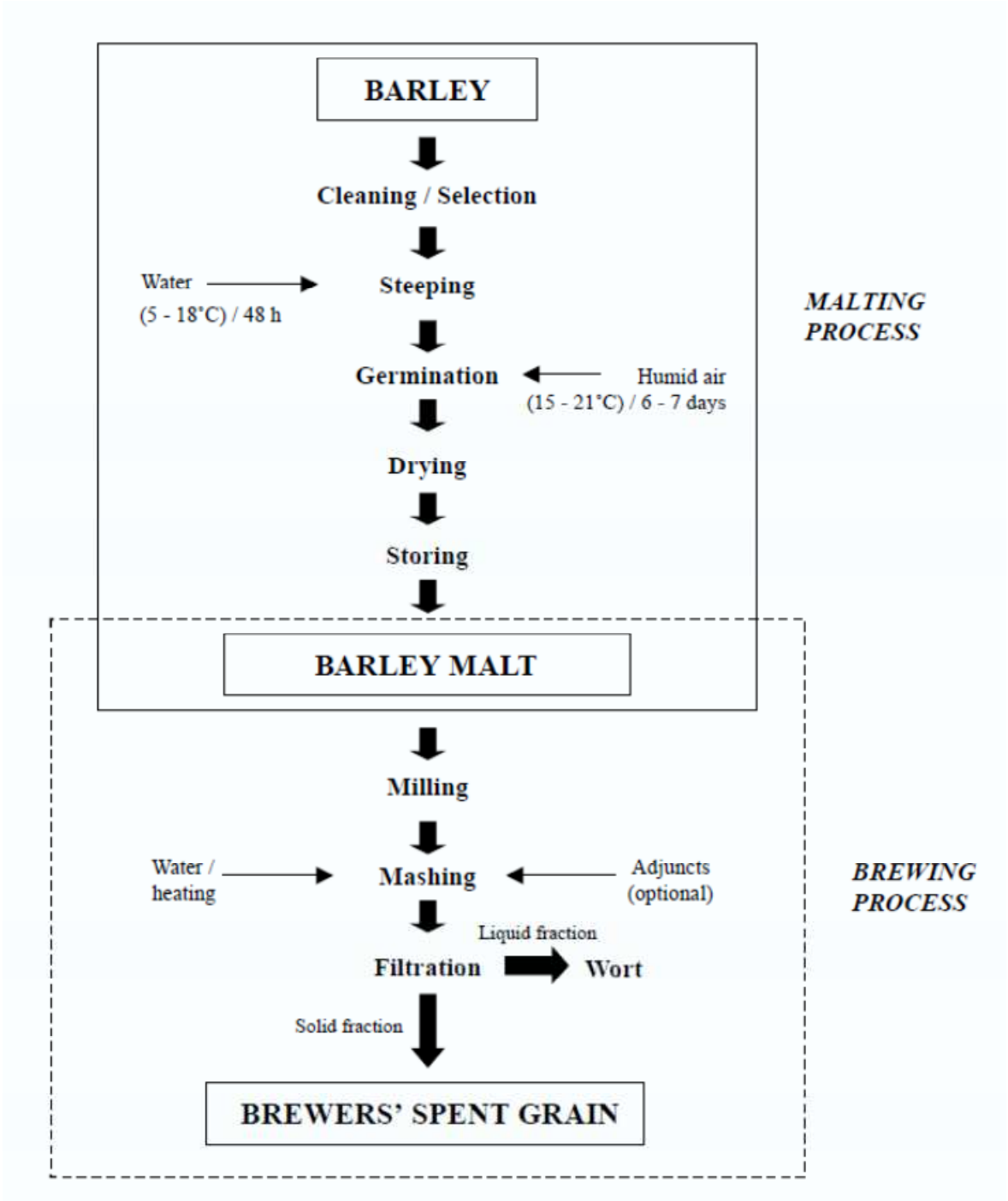

Fig 1.1. Schematic representation of the process to obtain BSG from natural barley.

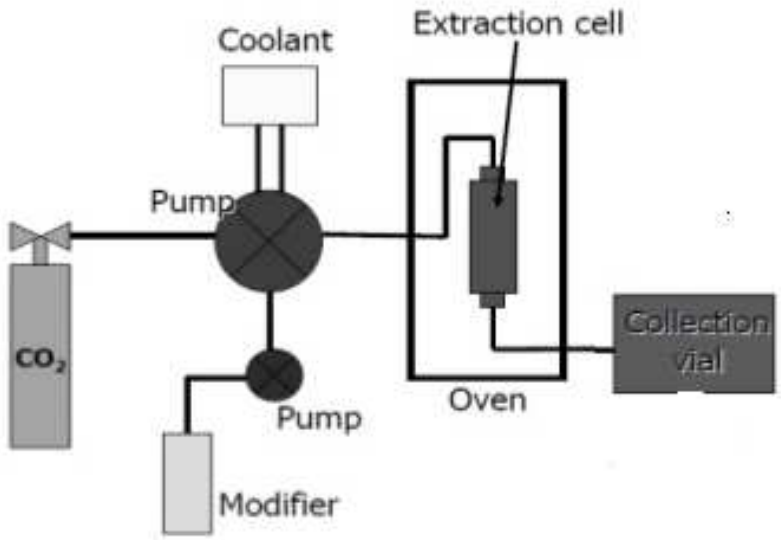

Fig 2.1. Schematic diagram of supercritical CO_2 extraction.

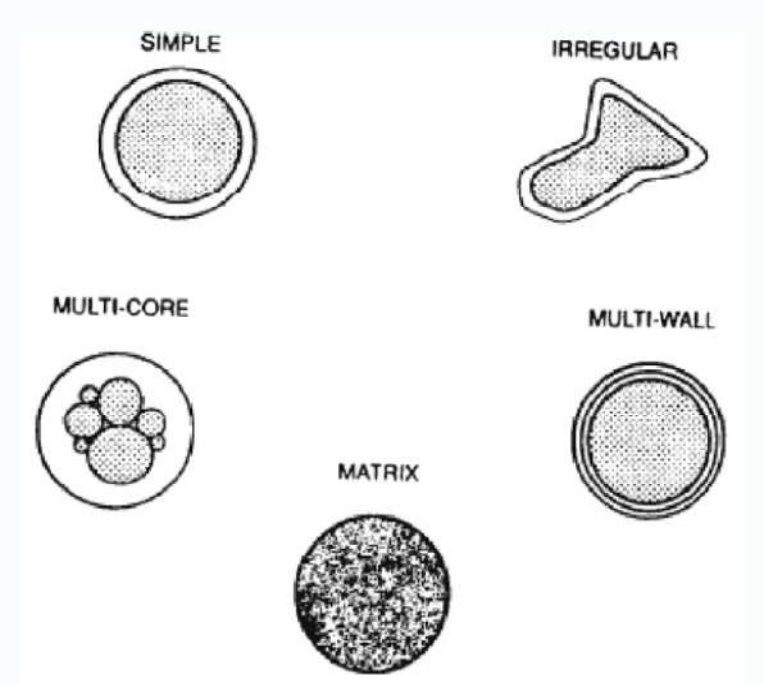

Fig. 3.1 Morphology of different types of microcapsules.

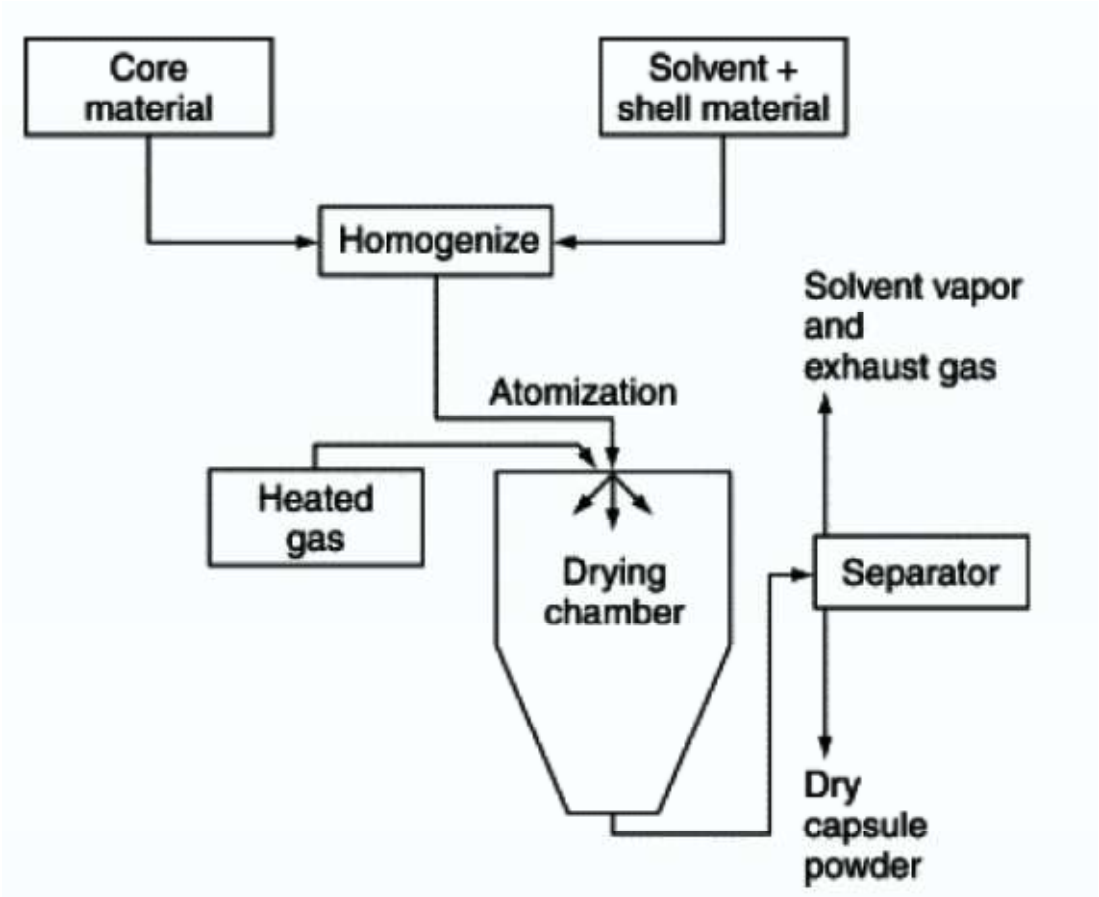

Fig. 3.2 Schematic diagram and steps involved in a spray-drying encapsulation process.

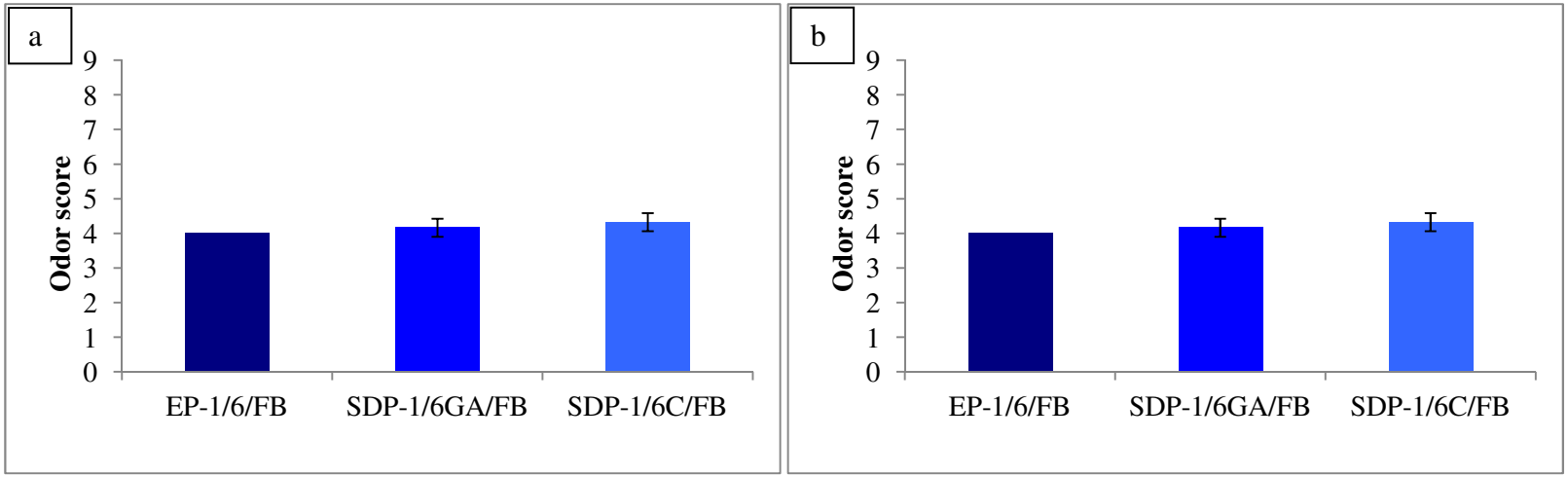

Fig. 6.1. Odor evaluation of raw (a) and cooked (b) fish burgers enriched with the corresponding not microencapsulated propolis (EP-1/6/FB), fish burgers enriched with microencapsulated propolis (SDP-1/6GA/FB; propolis extract to arabic gum solution ratio equal to 1:6 w/w) and fish burgers enriched with microencapsulated propolis (SDP-1/6C/FB; propolis extract to Capsul solution ratio equal to 1:6 w/w).

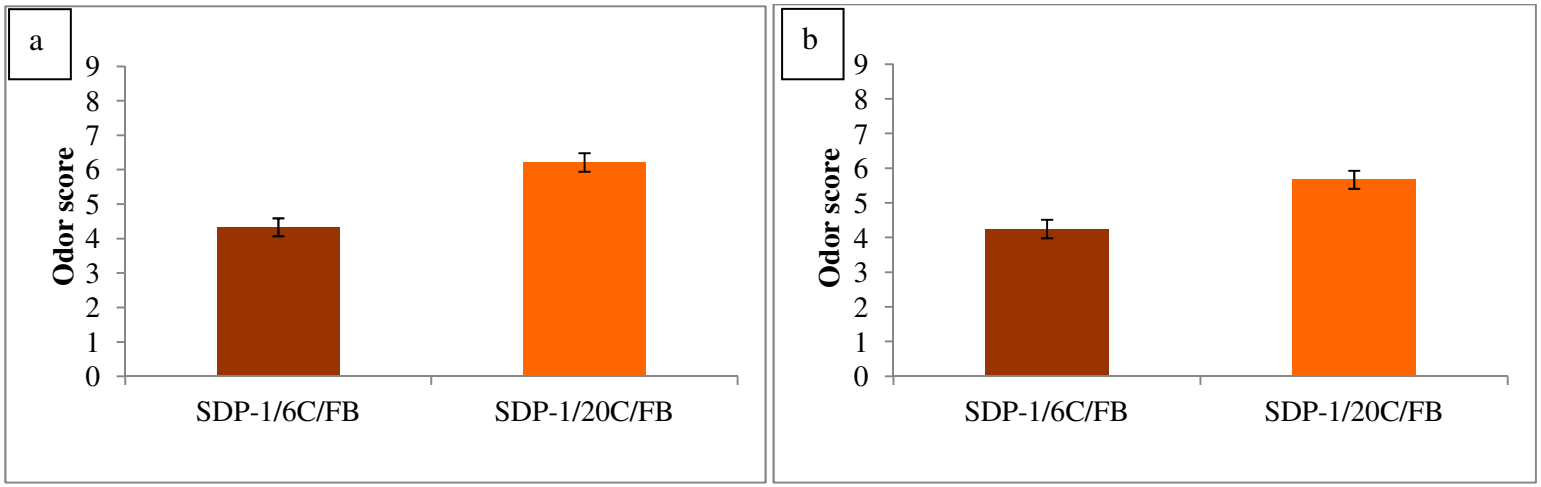

Fig. 6.2. Odor evaluation of raw (a) and cooked (b), fish burgers enriched with powder of propolis extract and Capsul solution, 1/6 (SDP-1/6C/FB) and fish burgers enriched with powder of propolis extract and Capsul solution, 1/20 (SDP-1/20C/FB).

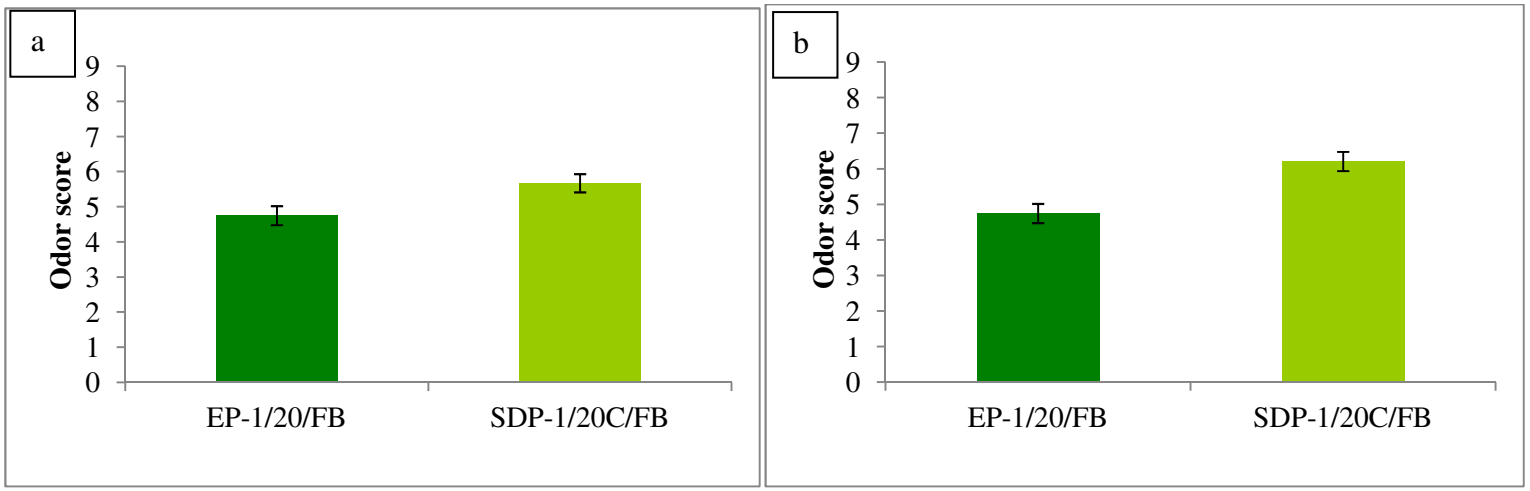

Fig. 6.3. Odor evaluation of raw (a) and cooked (b) fish burgers enriched with the corresponding not microencapsulated propolis (EP-1/20/FB) and fish burgers enriched with microencapsulated propolis (SDP-1/20C/FB; propolis extract to Capsul solution ratio equal to1:20 w/w).

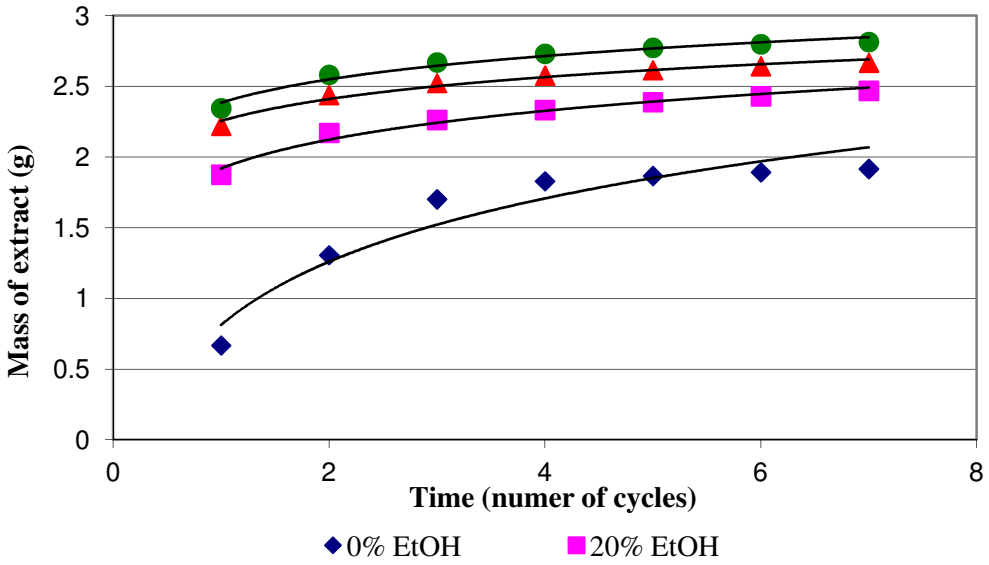

Fig. 6.4. Overall extraction curves of supercritical carbon dioxide employed at 40°Cand at 35 MPa with and without ethanol (0, 20, 40 and 60%, v/v). The curves were obtained by taking into account the amount extracted (g) during time (number of cycles).

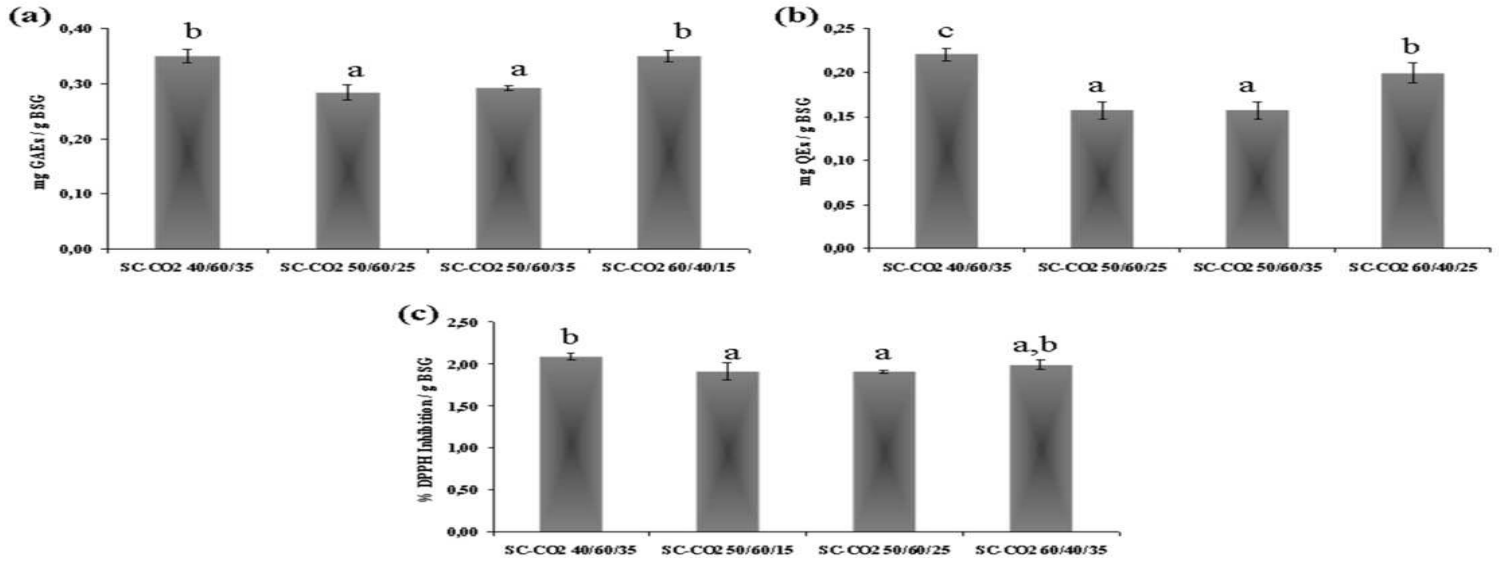

Fig. 6.5. Maximum values of total phenolic content (a), total flavonoid content (b) and antioxidant capacity (c) obtained by the different analytical trials. Values marked by different lower case letters are significantly different. Error bars represent the standard deviation.

Note:

SC-CO2 40/60/35 = supercritical carbon dioxide at 40°C, with CO2+ 60% ethanol (v/v) and at 35 MPa.

SC-CO2 50/60/15 = supercritical carbon dioxide at 50°C, with CO2+ 60% ethanol (v/v) and at 15 MPa.

SC-CO2 50/60/25 = supercritical carbon dioxide at 50°C, with CO2+ 60% ethanol (v/v) and at 25 MPa.

SC-CO2 50/60/35 = supercritical carbon dioxide at 50◦C, with CO2+ 60% ethanol (v/v) and at 35 MPa.

SC-CO2 60/40/15 = supercritical carbon dioxide at 60°C, with CO2+ 40% ethanol (v/v) and at 15 MPa.

SC-CO2 60/40/25 = supercritical carbon dioxide at 60◦C, with CO2+ 40%ethanol (v/v) and at 25 MPa.

SC-CO2 60/40/35 = supercritical carbon dioxide at 60◦C, with CO2+ 40% ethanol (v/v) and at 35 MPa.

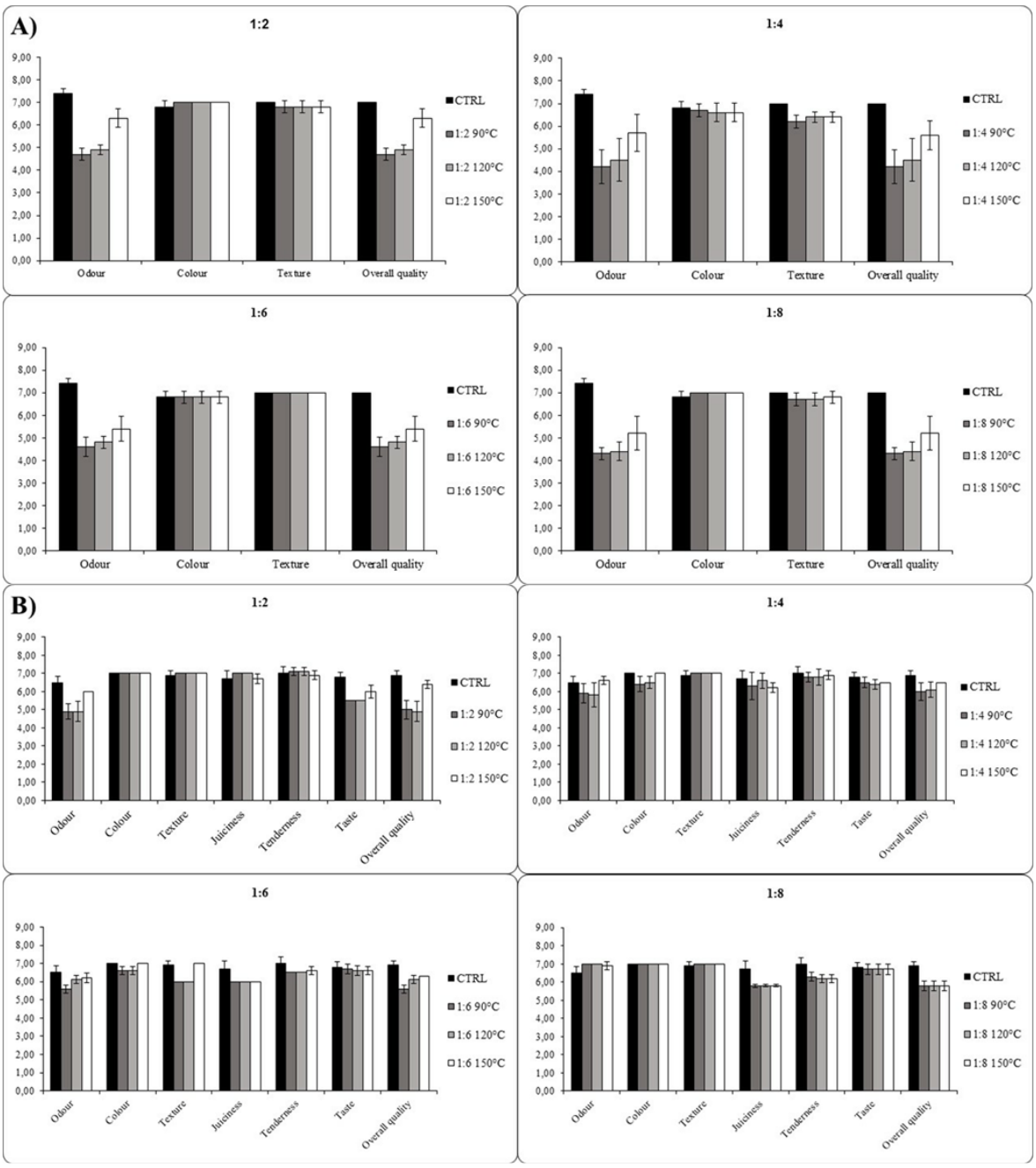

Fig. 6.6. Sensory properties of uncooked (A) and cooked (B) fish-burger samples enriched with different powders.

Note:

1:2 90, 120 or 150°C = fish-burger with 5% microencapsulated BSG extract and Capsul solution in ratio equal to 1:2 at 90 or 120 or 150°C;

1:4 90, 120 or 150°C = fish-burger with 5% microencapsulated BSG extract and Capsul solution in ratio equal to 1:4 at 90 or 120 or 150°C;

1:6 90, 120 or 150°C = fish-burger with 5 % microencapsulated BSG extract and Capsul solution in ratio equal to 1:6 at 90 or 120 or 150°C;

1:8 90, 120 or 150°C = fish-burger with 5% microencapsulated BSG extract and Capsul solution in ratio equal to 1:8 at 90 or 120 or 150°C.

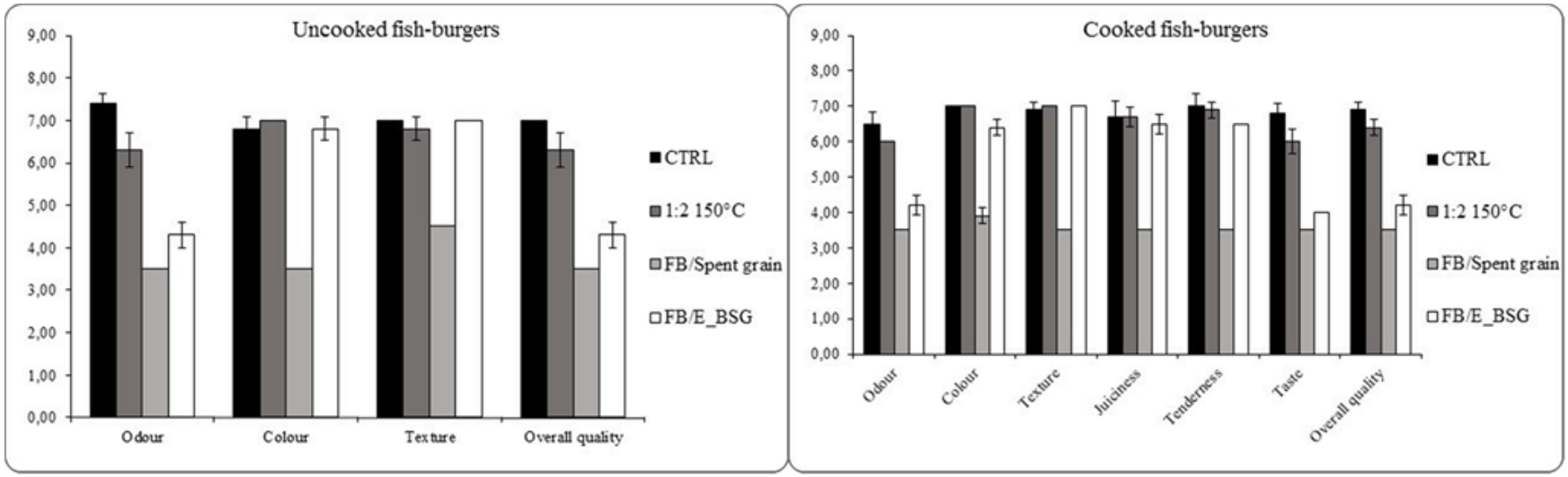

Figure 6.7. Comparison between CTRL, 1:2 150°C, fish burger spent grain and fish burger E_BSG.

Note. 1:2 150°C = fish-burger with 5% microencapsulated extract (Mc:Mw = 1:2, T inlet = 150°C), potato flakes and extra virgin olive oil;

FB/Spent grain = fish-burger with 5% spent grain as that, potato flakes and extra virgin olive oil; FB/E_BSG = fish burger enriched with the corresponding not microencapsulated extract, potato flakes and extra virgin olive oil.

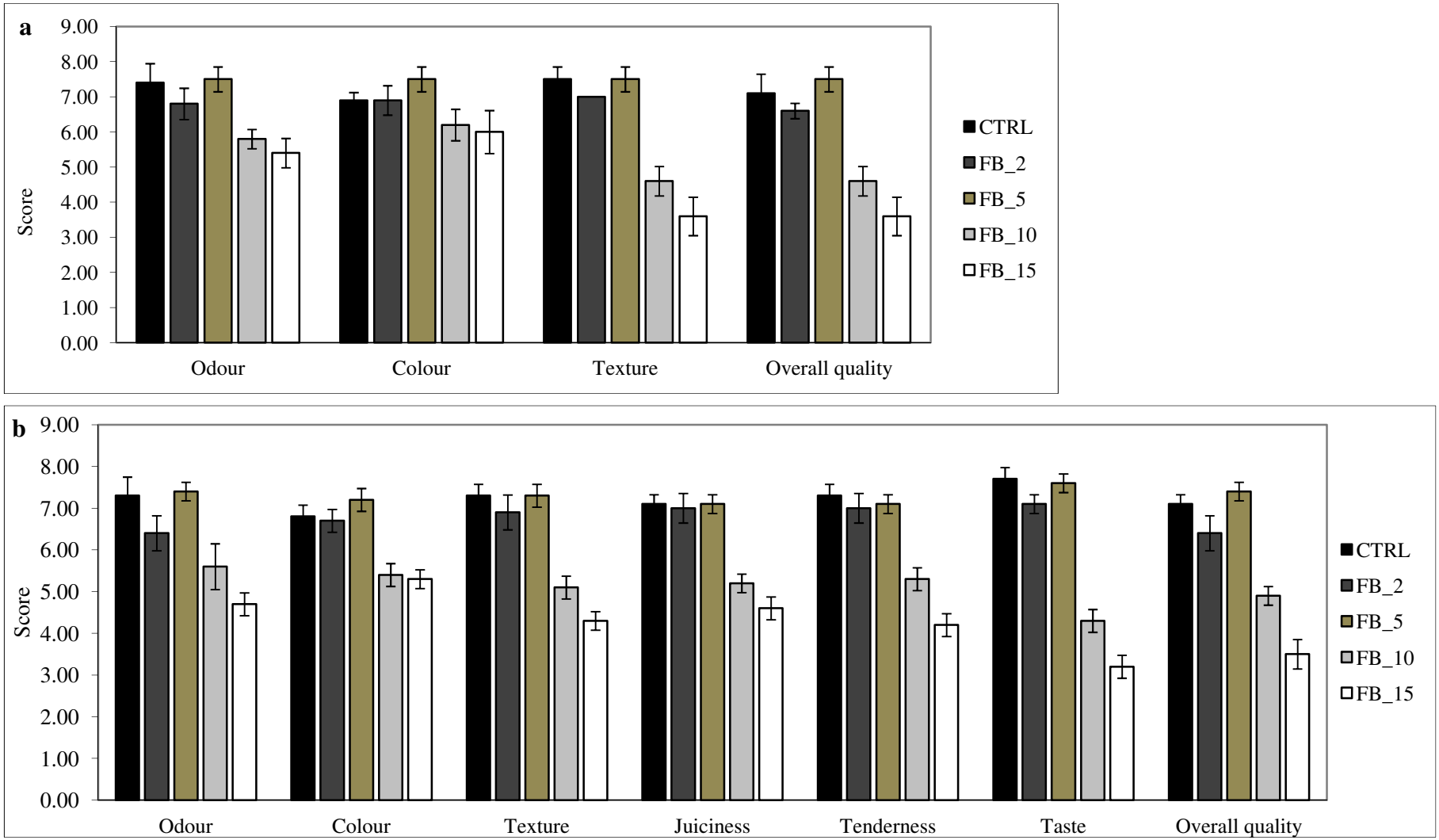

Fig. 6.8. Sensory properties of uncooked (a) and cooked (b) fish-burger samples enriched with different percentage of powders.

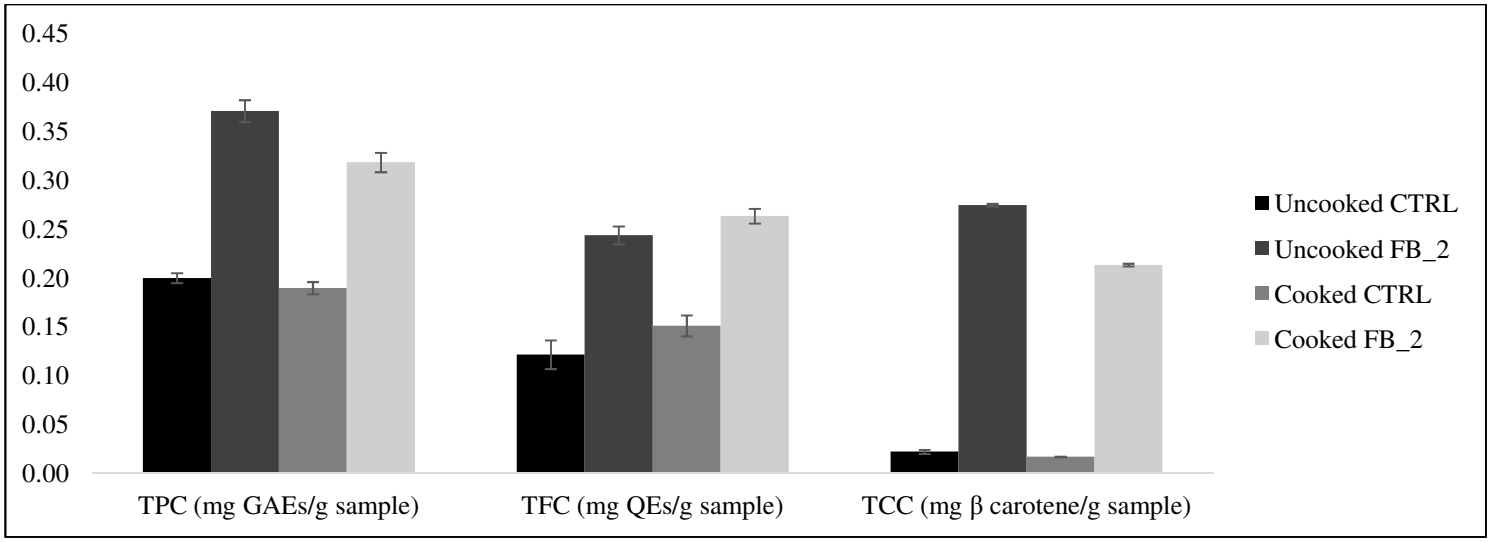

Fig. 6.9. Chemical composition of CTRL and optimized fish burger.

Note: TPC = total phenolic content; TFC = total flavonoid content; GAEs = gallic acid equivalents; QEs = quercetin equivalents; TCC = total carotenoid content.

TABLES

Table 6.1. Sensory properties of uncooked and cooked fish-burger samples.

	Uncooked samples				Cooked samples						
Sample	**Odor**	**Color**	**Texture**	**Overall quality**	**Odor**	**Color**	**Texture**	**Juiciness**	**Tenderness**	**Taste**	**Overall quality**
CTRL_1	6.75±0.27^{a}	7.00±0.00^{a}	7.00±0.00^{a}	6.75±0.27^{a}	7.57±0.19^{a}	7.00±0.00^{a}	7.29±0.27^{a}	7.29±0.27^{a}	7.29±0.27^{a}	7.36±0.24^{a}	7.29±0.27^{a}
SDP-1/20C/FB	5.67±0.26^{b}	6.00±0.00^{c}	4.50±0.00^{d}	4.50±0.00^{c}	6.21±0.27^{c}	7.00±0.00^{a}	6.71±0.27^{b}	7.00±0.41^{a}	7.00±0.41^{a}	5.36±0.48^{c}	6.00±0.00^{b}
3% PF	5.58±0.38^{b}	4.75±0.27^{d}	5.75±0.27^{c}	4.58±0.38^{c}	5.93±0.35^{c}	5.71±0.27^{c}	4.71±0.27^{d}	4.79±0.27^{b}	4.71±0.39^{b}	4.21±0.27^{e}	4.14±0.24^{e}
5% PF	5.33±0.42^{b}	4.75±0.27^{d}	5.75±0.27^{c}	4.33±0.41^{c}	5.93±0.19^{c}	5.71±0.27^{c}	4.50±0.50^{d}	4.86±0.24^{b}	4.79±0.27^{b}	4.29±0.27^{e}	4.21±0.27^{e}
7% PF	5.42±0.20^{b}	4.67±0.26^{d}	5.42±0.38^{c}	4.25±0.27^{c}	6.07±0.19^{c}	5.79±0.27^{c}	4.57±0.19^{d}	4.93±019^{b}	4.86±0.24^{b}	5.00±0.00^{d}	4.79±0.27^{d}
10% PF	5.67±0.26^{b}	6.50±0.00^{b}	6.58±0.58^{b}	6.00±0.00^{b}	6.71±0.27^{b}	6.29±0.27^{b}	5.29±0.27^{c}	4.79±0.27^{b}	4.79±0.27^{b}	5.93±0.19^{b}	5.57±0.19^{c}

Mean in the same column followed by different superscript letters differ significantly ($P < 0.05$).

PF=potato flakes

Table 6.2. Sensory properties of the CTRL, SDP-1/20C/FB, 10% PF and 10% PF 500 – 9% oil samples.

	Uncooked samples				Cooked samples						
Sample	**Odor**	**Color**	**Texture**	**Overall quality**	**Odor**	**Color**	**Texture**	**Juiciness**	**Tenderness**	**Taste**	**Overall quality**
CTRL_1	6.75±0.27^{a}	7.00±0.00^{a}	7.00±0.00^{a}	6.75±0.27^{a}	7.57±0.19^{a}	7.00±0.00^{a}	7.29±0.27^{a}	7.29±0.27^{a}	7.29±0.27^{a}	7.36±0.24^{a}	7.29±0.27^{a}
SDP-1/20C/FB	5.67±0.26^{c}	6.00±0.00^{c}	4.50±0.00^{c}	4.50±0.00^{c}	6.21±0.27^{c}	7.00±0.00^{a}	6.71±0.27^{b}	7.00±0.41^{a}	7.00±0.41^{a}	5.36±0.48^{d}	6.00±0.00^{c}
10% PF	5.67±0.26^{c}	6.50±0.00^{b}	6.58±0.58^{a}	6.00±0.00^{b}	6.71±0.27^{b}	6.29±0.27^{c}	5.29±0.27^{d}	4.79±0.27^{c}	4.79±0.27^{c}	5.93±0.19^{c}	5.57±0.19^{d}
10% PF - 9% oil	6.10±0.22^{b}	5.90±0.22^{c}	6.00±0.35^{b}	6.00±0.00^{b}	6.50±0.00^{b}	6.50±0.00^{b}	6.29±0.27^{c}	6.21±0.27^{b}	6.07±0.19^{b}	6.79±0.27^{b}	6.50±0.00^{b}

Mean in the same column followed by different superscript letters differ significantly ($P < 0.05$)

Table 6.3. Values of total polyphenols and antioxidant activity of CTRL and 503 10% PF – 9% oil.

Sample	Uncooked samples		Cooked samples	
	Polyphenols (mg GAE/g fish-burger)§	**Antioxidant activity DPPH (%)**	**Polyphenols (mg GAE/g fish-burger)§**	**Antioxidant activity DPPH (%)**
CTRL_2	0.42±0.03[b]	7.41±0.67[b]	0.36±0.02[b]	6.64±0.40[b]
10% PF - 9% oil	1.28±0.03[a]	28.43±1.70[a]	1.13±0.03[a]	29.69±2.98[a]

§ milligram of gallic acid equivalents per gram of sample.

The different letters show significant difference between means of triplicate determinations ($P < 0.05$).

DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalents.

Table 6.4. Chemical composition of SC-CO_2 BSG at40°C, under different process conditions.

Extraction conditions at 40°C (EtOH/MPa)	**TPC (mg GAEs/g BSG)**	**TFC (mg QEs/g BSG)**	**Antioxidant Potential (% DPPH inhibition)**
SC-CO_2 0/15	0,05±0.00a	0,04±0.00a	0,25±0.01a
SC-CO_2 0/25	0,06±0.00a	0,05±0.00a	0,43±0.01b
SC-CO_2 0/35	0,05±0.00a	0,05±0.00a	0,35±0.03b
SC-CO_2 20/15	0,20±0.01c	0,12±0.01c	1,34±0.01c
SC-CO_2 20/25	0,17±0.00b	0,10±0.01b	1,35±0.01c
SC-CO_2 20/35	0,21±0.02c	0,10±0.01b	1,41±0.02c
SC-CO_2 40/15	0,25±0.01d	0,14±0.00e	1,76±0.02d,e
SC-CO_2 40/25	0,27±0.01d,e	0,12±0.01c	1,74±0.03d
SC-CO_2 40/35	0,27±0.02e	0,13±0.01c,d	1,70±0.07d
SC-CO_2 60/15	0,29±0.02e,f	0,13±0.01d,e	1,78±0.02d,e
SC-CO_2 60/25	0,30±0.01f	0,13±0.00c,d	1,83±0.02e
SC-CO_2 60/35	0,35±0.01g	0,22±0.01f	2,09±0.04f

Values are means of three replications ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).

SC-CO_2 = supercritical carbon dioxide; TPC = total phenolic content; TFC = total flavonoid content; BSG = brewer's spent grain; GAEs = gallic acid equivalents; QEs = quercetin equivalents.

Table 6.5. Chemical composition of SC-CO_2 BSG at 50°C, under different process conditions.

Extraction conditions at 50°C (EtOH/MPa)	TPC (mg GAEs/g BSG)	TFC (mg QEs/g BSG)	Antioxidant Potential (% DPPH inhibition)
SC-CO_2 0/15	0,04±0.00a	0,06±0.00a	0,26±0.02a
SC-CO_2 0/25	0,07±0.00b	0,07±0.01b	0,78±0.06b
SC-CO_2 0/35	0,06±0.00b	0,07±0.00a,b	0,69±0.04b
SC-CO_2 20/15	0,17±0.00c	0,13±0.01d	1,42±0.03c
SC-CO_2 20/25	0,18±0.00c	0,12±0.01c	1,37±0.06c
SC-CO_2 20/35	0,18±0.00c	0,12±0.01c,d	1,35±0.06c
SC-CO_2 40/15	0,24±0.01d	0,13±0.00c,d	1,59±0.01d
SC-CO_2 40/25	0,27±0.00f	0,12±0.01c,d	1,78±0.06e
SC-CO_2 40/35	0,25±0.00e	0,13±0.01c,d	1,74±0.07e
SC-CO_2 60/15	0,26±0.00e	0,12±0.01c,d	1,91±0.10f
SC-CO_2 60/25	0,28±0.01g	0,16±0.01e	1,91±0.02f
SC-CO_2 60/35	0,29±0.00g	0,16±0.01e	1,80±0.08e

Values are means of three replications ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).

For abbreviations see Table 6.4.

Table 6.6 Chemical composition of SC-CO_2 BSG at 60°C under different process conditions.

Extraction conditions at 60°C (EtOH/MPa)	TPC (mg GAEs/g BSG)	TFC (mg QEs/g BSG)	Antioxidant Potential (% DPPH inhibition)
SC-CO_2 0/15	0,04±0.00a	0,03±0.00a	0,11±0.01a
SC-CO_2 0/25	0,07±0.00b	0,06±0.01b	0,62±0.03b
SC-CO_2 0/35	0,08±0.00b	0,07±0.01c	0,82±0.04c
SC-CO_2 20/15	0,17±0.01c	0,12±0.00d	1,11±0.04d
SC-CO_2 20/25	0,19±0.01d	0,14±0.01e	1,41±0.01e
SC-CO_2 20/35	0,17±0.01c	0,15±0.00f	1,64±0.07f
SC-CO_2 40/15	0,35±0.01i	0,18±0.01h	1,82±0.04g,h
SC-CO_2 40/25	0,28±0.01f	0,20±0.01i	1,88±0.06h
SC-CO_2 40/35	0,24±0.01e	0,17±0.01g	1,99±0.06i
SC-CO_2 60/15	0,34±0.00h,i	0,19±0.01h,i	1,76±0.07g
SC-CO_2 60/25	0,33±0.01h	0,18±0.01g,h	1,85±0.05h
SC-CO_2 60/35	0,31±0.01g	0,18±0.01g,h	1,83±0.05g,h

Values are means of three replications ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).

For abbreviations see Table 6.4.

Table 6.7. Chemical composition of microencapsulated BSG under different ratios between extract and Capsul and different inlet temperatures.

Sample	TPC (mg GAEs/g powder)	TFC (mg QEs/g powder)
SD2/90	1.77±0.04a	1.32±0.08a
SD2/120	1.73±0.03a	1.26±0.08a
SD2/150	1.70±0.06a	1.25±0.06a
SD4/90	1.25±0.04b	0.85±0.08b
SD4/120	1.26±0.08b	0.83±0.08b
SD4/150	1.21±0.02b	0.83±0.03b
SD6/90	1.02±0.08c	0.59±0.03c
SD6/120	1.03±0.01c	0.57±0.06c,d
SD6/150	1.00±0.05c	0.56±0.05c,d,e
SD8/90	0.66±0.04d	0.51±0.00d,e,f
SD8/120	0.68±0.02d	0.47±0.03f
SD8/150	0.69±0.02d	0.49±0.03e,f

Values are means of three-replication ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).

Note: TPC = total phenolic content; TFC = total flavonoid content; BSG = brewer's spent grain; GAEs = gallic acid equivalents; QEs = quercetin equivalents.

SD2/90, 120 or 150 = BSG extract to C solution ratio equal to 1:2 at 90 or 120 or 150°C

SD4/90, 120 or 150 = BSG extract to C solution ratio equal to 1:4 at 90 or 120 or 150°C

SD6/90, 120 or 150 = BSG extract to C solution ratio equal to 1:6 at 90 or 120 or 150°C

SD8/90, 120 or 150 = BSG extract to C solution ratio equal to 1:8 at 90 or 120 or 150°C.

Table 6.8. Chemical composition of CTRL and 1:2 150 fish-burgers.

Fish-burger	TPC (mg GAEs/g fish-burger)	TFC (mg QEs/g fish-burger)	Potential Antioxidant properties (% DPPH inhibition)
Uncooked CTRL	0.035±0.00a	0.065±0.00a	21.57±1.36a
Cooked CTRL	0.036±0.00a	0.063±0.00a	21.19±0.70a
Uncooked 1:2 150	0.125±0.00b	0.132±0.01b	44.93±1.07b
Cooked 1:2 150	0.136±0.00c	0.143±0.00c	48.49±1.45c

Values are means of three-replication ± standard deviation.

Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).

Note: TPC = total phenolic content; TFC = total flavonoid content; GAEs = gallic acid equivalents; QEs = quercetin equivalents; DPPH = 2,2-diphenyl-1-picrylhydrazyl; 1:2 150 = fish-burger enriched by 5% of spray-dried brewer's spent grain extract in ratio 1:2 with Capsul and inlet temperature at 150°C.

Tab 6.9. Chemical comparison between extracts produced by two different extraction methods.

Extracts	TPC (mg GAEs/g extract)	TFC (mg QEs/g extract)	TCC (mg β carotene/g extract)
E_1	3.61±0.11a	1.67±0.02a	5.15±0.02a
E_2	4.07±0.07b	3.22±0.30b	8.40±0.01b

Values are means of three-replication ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).

E_1 = extract from method 1; E_2 = extract from method 2; TPC = total phenolic content; TFC = total flavonoid content; TCC = total carotenoid content; GAEs = gallic acid equivalents; QEs = quercetin equivalents.

Tab 6.10. Chemical comparison between powders produced by two different microencapsulation methods.

Powders	TPC (mg GAEs/g extract)	TFC (mg QEs/g extract)	TCC (mg β carotene/g extract)
SDE_1	1.97±0.10a	0.63±0.07a	1.24±0.07a
SDE_2	4.71±0.10b	3.69±0.39b	7.40±0.03b

Values are means of three-replication ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).

SDE_1 = powder from method 1; SDE_2 = powder from method 2; TPC = total phenolic content; TFC = total flavonoid content; TCC = total carotenoid content; GAEs = gallic acid equivalents; QEs = quercetin equivalents.

POSTERS

STUDY OF MICROENCAPSULATED BIOACTIVE COMPOUNDS IN FOOD PRODUCTS

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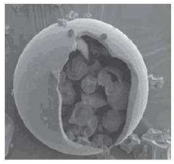
This PhD thesis research project is focused on the optimization of parameters of microencapsulation techniques, to enrich several food matrices with different functional and preserving compounds.



In the last decades, consumer demands in the field of food production have changed considerably. Consumers more and more believe that foods contribute directly to their health (Mollet *et al.*, 2002); in fact, nowadays, foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental well-being (Nöthlings *et al.*, 2007).

Furthermore, shelf-life of food products and beverages has become increasingly important in recent years due to technological developments and the increase in consumer interest in eating fresh, safe and high quality products.

In this regard, functional foods play an outstanding role.

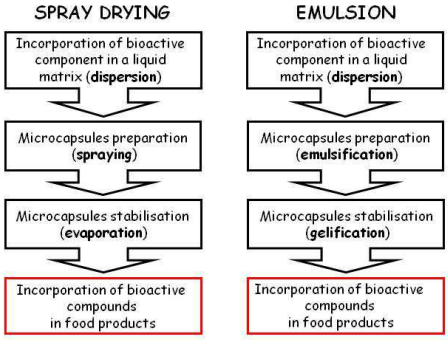
The ingredients need to be incorporated into food systems, in which they can slowly degrade and lose their activity, become hazardous by oxidation reactions and/or can also react with components present in the food system, altering the color or taste of a product (Desai *et al.*, 2005).

Microencapsulation can be used to overcome these challenges: it involves the incorporation of bioactive molecules (e.g. antioxidants, antimicrobial, minerals, vitamins, essential fatty acids, flavors) and living cells (e.g. enzymes, bacteria) in small capsules, giving them the chance to be stable, protected and preserved against nutritional and health loss (Nedovic *et al.*, 2011; Murugesan et al., 2012).

Although wide research has been done on the microencapsulation of functional food components, the use of microencapsulated bioactive compounds in food, as ingredient, is still minimal.

On the basis of previous studies and in order to incorporate the microencapsulated active compounds in different foods (such as fish products, dairy foods, meat and cereal-based products), process parameters of two techniques of microencapsulation will be optimized. To improve nutritional properties and health benefits and to extend shelf-life of foods, three main categories of bioactive compounds are chosen (nutraceutical compounds, probiotic microorganisms and other important antimicrobial/antioxidant compounds).

ORGANIZATION OF RESEARCH ACTIVITIES



Activity \ Months	1-4	5-8	9-12	13-16	17-20	21-24
Optimization of microencapsulation parameters						
functional compounds						
probiotic microorganisms						
antimicrobial/antioxidant						
Incorporation of bioactive compounds in food products						
functional compounds						
probiotic microorganisms						
antimicrobial/antioxidant						
Statistical analysis						
Thesis and Paper Preparation						

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STUDY OF MICROENCAPSULATED BIOACTIVE COMPOUNDS IN FOOD PRODUCTS

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Tutor: Prof. Matteo Alessandro Del Nobile; Co-tutor: Dott. Salvatore Germinara

This work shows the main results of the activities planned and developed during the second year of PhD. The main aim of the project is the study of different bioactive compounds for their incorporation in food matrixes by microencapsulation technique. Specifically, the activity of the second year was focused on the enhancement of antioxidant properties of fish-burgers using microencapsulated propolis.

INTRODUCTION

Propolis exhibits strong antioxidant properties due to its high content of polyphenols (da Silva et al., 2011). Thus, it can be potentially used as natural food additive and functional food ingredient, but its application to food is still limited, due to its strong and unpleasant taste and odor that generally compromise food acceptability (da Silva et al., 2011). Various approaches for minimizing this problem have been developed but the most diffuse is the microencapsulation with spray drying. However, no publications on the application of spray-dried propolis to food are available.

According to the PhD project, this study reports the main results of the activities concerning (i) the best conditions of spray drying to mask the pungent odor of propolis to be embed into sea bass fish-burgers (ii) the optimal formulation of sea bass fish-burger with spray dried propolis to improve the sensory properties and (iii) the evaluation of the total phenolic compounds and the antioxidant activity in the optimized product with propolis.

MATERIALS AND METHODS

Microencapsulation of propolis
- spray drying using:
- propolis extract to-Arabic gum solution ratio equal to 1:6
- propolis extract to-Capsul® solution ratio equal to 1:6
- propolis extract to-Capsul® solution ratio equal to 1:20

Fish-burger preparation
- sea bass fillets (*D. Labrax*)
- microencapsulated propolis (5%, w/w)
- different concentrations of potato flakes (3%, 5%, 7%, 10% w/w)
- extra virgin olive oil (9% w/w)

Total phenolic compounds and antioxidant activity determination
- extraction with pure methanol
- centrifugation at 6000 rpm
- evaporation under vacuum at 40°C
- recovery in ethanol (60%)
- filtration
- Folin-Ciocalteu method
- 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Sensory evaluation
- panel test using a nine-point scale:
- score of 9 = very good quality
- score of 7-8 = good quality
- score of 6 = sufficient quality
- score of 5 = acceptability threshold
- score of 1-4 = unacceptable quality

RESULTS AND DISCUSSION

Optimization of spray drying conditions

Among the two materials used to encapsulate propolis, Capsul® appeared to be the best one. The slightly higher capacity of the Capsul® to retain propolis compared to gum Arabic, was most likely due to the presence of the lipophilic component (octenylsuccinate) in the formulation of the Capsul® that gave the carrier a major capacity for retaining compounds during atomization in the spray-dryer (Seid et al., 2008). As a fact, significant differences in phenolic content were observed between tested powders with gum Arabic and with Capsul® (12.73±0.15 and 13.86±0.13 mg of Gallic acid equivalents per g of powder, respectively), while no difference appeared between the Capsul® and the reference (13.86±0.13 and 13.95±0.06, respectively). Since from a sensory point of view, the products with microencapsulated propolis remained unacceptable, it was necessary to modify the propolis extract-to-carrier ratio to 1:20. The sensory evaluation with propolis microencapsulated according to the new conditions highlighted better results. As can be seen in Fig. 1 (a, b), increasing the amount of wall material positively affected the sensory attribute.

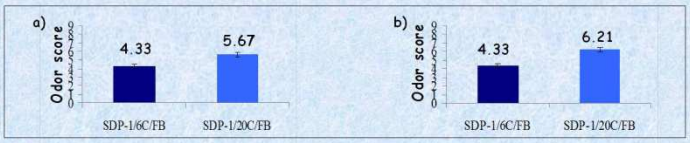

Fig. 1. Odor evaluation of raw (a) and cooked (b), fish-burgers enriched with powder of propolis extract and Capsul® solution, 1/6 (SDP-1/6C/FB) and fish-burgers enriched with powder of propolis extract and Capsul® solution, 1/20 (SDP-1/20C/FB).

Optimization of fish-burger formulation

The fish-burger was mixed with microencapsulated propolis (5%) (SDP-1/20C/FB). As can be seen in Table 1, to improve the sensory quality, dried potato flakes and extra virgin olive oil were added to the patties up to reach a significant quality improvement.

	Uncooked samples	Cooked samples
Sample	Overall quality	Overall quality
Control	6,75±0,27^{a}	7,29±0,27^{a}
SDP-1/20C/FB	4,50±0,00^{e}	6,00±0,00^{e}
3% potato flakes	4,58±0,38^{e}	4,14±0,24^{f}
5% potato flakes	4,33±0,41^{e}	4,21±0,27^{f}
7% potato flakes	4,25±0,27^{e}	4,79±0,27^{e}
10% potato flakes	6,00±0,00^{b}	5,57±0,19^{d}
10%potato flakes+9%oil	6,00±0,00^{b}	6,50±0,00^{b}

Table 1. Overall quality of uncooked and cooked fish-burger samples.

Characterization of sample antioxidant properties

The active sample (microencapsulated propolis, 10% potato flakes and 9% oil) demonstrated a higher sequestrating activity on DPPH than the control (28.43% against 7.41% for uncooked samples and 29.69% against 6.64% for cooked samples). This antioxidant activity turned out to be in agreement with the total phenolic content of the fish-burger. In fact, the optimized sample showed a higher concentration of polyphenolics (1.28 and 1.13 mg/g sample) than the control (0.42 and 0.36 mg/g sample). It is worth noting that the samples cooking did not affect these two parameters.

CONCLUSIONS

Results indicate that using Capsul®, with a mass ratio of core to wall material equal to 1:20, as carrier during the spray-drying technique, it is possible to retain a greater amount of propolis and to mask its characteristic smell. Thus, the finding of this study increases the possibility of embedding the propolis as a natural ingredient in various foods, including fish to enhance their antioxidant content. In particular, the study showed that the addition of 5% of spray-dried propolis to a proper fish-burger formulation (10% of potato flakes and 9% extra virgin olive oil) showed a positive sensory evaluation and resulted in high phenolic content and high sequestrating activity on DPPH, compared to the control.

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XIX WORKSHOP
ON THE DEVELOPMENTS IN THE ITALIAN
PHD RESEARCH ON FOOD SCIENCE
TECHNOLOGY AND BIOTECHNOLOGY

Bari, September 24th-26th, 2014

Brewer's spent grain to enhance food nutritional quality

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INTRODUCTION

Nowadays, there is a constant research to find new alternatives regarding the food fortification. In particular, there is a growing interest in the recovery of vegetable by-products and their conversion into functional compounds. Recently, a particular attention has been paid to the brewer's spent grain (BSG), a by-product of brewing industry, considered an excellent source of bioactive compounds although its application to food is still limited, since it can impart unpleasant flavors and aromas. Thus, to obtain valuable natural substances, supercritical carbon dioxide extraction (SC-CO_2) represents an efficient and worldwide spread extraction process. The main disadvantage of CO_2 is its low polarity, problem that can be overcome using co-solvents (ethanol), capable of hydrogen-bonding, dipole–dipole and others polarity interactions with the analyte of interest (Herrero et al., 2010). Subsequently, to mask the undesirable attributes and simultaneously to preserve the stability of bioactive compounds, the spray drying turns out an valid technique for minimizing these problems. Among the numerous wall materials that are available for food application, a chemically modified starch (Capsul®) was used thanks to its low viscosity, good film-forming properties and thermo-protective effect during the exposure to high temperatures.

The main purpose of this work was to enhance the antioxidant properties of fish burgers with microencapsulated brewer's spent grain extract. To this aim different aspects has been studied:

The effects of supercritical fluid extraction conditions in terms of maximum concentration of phenols (TPC), flavonoids (TFC) antioxidant activity (% DDPH inhibition).	**The identification of the best spray drying conditions to obtain a powder able to mask the unattractive attributes**, modifying the temperatures (90-120-150°C) and extract to carrier ratios (1:2, 1:4, 1:6, 1:8).	**The embedding of microencapsulated BSG extract** in order to obtain a burger with antioxidant properties without compromising consumer acceptance.

MATERIALS AND METHODS

BSGs were supplied by a local brewery industry located in Puglia (Italy). Capsul® was supplied by Ingredion Incorporated (Westchester, USA). The various chemical reagents were supplied by Sigma-Aldrich (Milan, Italy). For SFE, Sapio (Monza, Italy) supplied CO_2 with purity degree of 4.5.

Extraction experiments were carried out using the supercritical fluid extractor Speed SFE-2 (Applied Separation, Allentown, USA). To select the best SFE operating conditions, the effects of three extraction parameters were studied in terms of total phenolic content, total flavonoid content and antioxidant activity of extracts. The experiments were divided into three groups maintaining constant the extraction temperatures (40, 50 or 60°C) and varying the extraction pressures (15, 25 or 35 MPa) and the percentage of ethanol (0, 20, 40 or 60%).

E_BSG microencapsulation was performed by a drying process using a mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Flawil, Switzerland). Capsul® was dissolved in distilled water at the concentration of 30 g/100 mL, named carrier solution. The E_BSG was added to the carrier solution at proportions of 1:2, 1:4, 1:6 and 1:8 (w/w) and homogenized by an Ultra Turrax IKA T25 basic homogenizer (IKA Works Inc., Staufen, Germany) for 2 min at 15,000 rpm. The resulting formulations were spray dried at different inlet drying air temperatures (90, 120, 150°C).

In accordance with the optimized recipe in a previous work (Spinelli et al., 2014) fish-burgers were prepared using minced fish mixed with 5% of spray-drying powders, 10% potato flakes (Digei s.r.l., Foggia, Italy) and 9% extra virgin olive oil (Olearia Desantis s.p.a, Bitonto, Bari, Italy). Thus, fish-burgers were cooked in an electric convention oven at 180°C for 15 min.

In order to choose the best spray-drying conditions, a sensory evaluation was performed in accordance with the standard UNI 10957:2003. Seven trained panelists has been selected and asked to assess odor, color, texture and overall quality for uncooked fish patties and juiciness, tenderness and taste were evaluated for cooked ones. Panelists used a scale from 1 to 9, where 5 was the minimum threshold for acceptability.

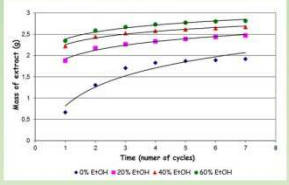
Chemical determination of samples

Extraction of Bioactive Compounds
Total Polyphenolic compounds **(Folin-Ciocalteu method)**
Total Flavonoid compounds **(Aluminium trichloride method)**
Antioxidant Activity **(DPPH assay)**

Statistical analysis → One-way analysis of variance **(ANOVA)**

RESULTS AND DISCUSSION

Fig. 1. Overall extraction curves of supercritical carbon dioxide employed at 40°C and at 35 MPa with and without ethanol (0, 20, 40 and 60%, v/v).



To know the time required for the extraction process, the overall extraction curve was obtained by taking into consideration the amount extracted (g) in relation to number cycles (1 cycle corresponded to 30 min of static phase plus 10 min of dynamic state). The yield increased with the addition of co-solvent and with increasing time, until reaching a balance between the sixth and seventh cycle. Based on this result, it was assumed that an extraction time of 240 min could also represent a good compromise for all other experimental trials.

RESULTS AND DISCUSSION

Table 1, 2, 3. Chemical composition of BSG extracts.

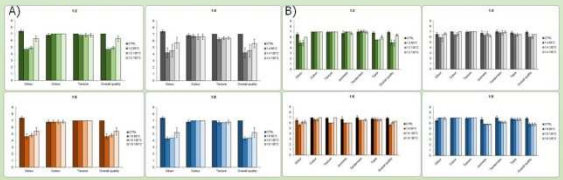
Extraction conditions at 40°C (EtOH/MPa)	TPC (mg GAEs/g BSG)	TFC (mg QEs/g BSG)	Antioxidant Activity (% DPPH inhibition)
$SC\text{-}CO_2$ 0/15	0.05±0.00a	0.04±0.00a	0.35±0.02a
$SC\text{-}CO_2$ 0/25	0.06±0.00a	0.05±0.00a	0.33±0.02a
$SC\text{-}CO_2$ 0/35	0.05±0.00a	0.05±0.00a	0.35±0.03a
$SC\text{-}CO_2$ 20/15	0.20±0.01c	0.12±0.01c	1.34±0.01b
$SC\text{-}CO_2$ 20/25	0.17±0.00b	0.20±0.01b	1.35±0.01b
$SC\text{-}CO_2$ 20/35	0.21±0.01c	0.20±0.01b	1.41±0.01b
$SC\text{-}CO_2$ 40/15	0.35±0.01d	0.34±0.00e	1.76±0.02c,d
$SC\text{-}CO_2$ 40/25	0.27±0.01d,e	0.12±0.01c	1.74±0.03d
$SC\text{-}CO_2$ 40/35	0.25±0.02e	0.23±0.01c,d	1.70±0.07d
$SC\text{-}CO_2$ 60/15	0.39±0.02e,f	0.23±0.01d,e	1.78±0.02e
$SC\text{-}CO_2$ 60/25	0.30±0.01f	0.23±0.00c,d	1.83±0.01f
$SC\text{-}CO_2$ 60/35	0.35±0.01g	0.22±0.01f	2.09±0.04g

Extraction conditions at 50°C (EtOH/MPa)	TPC (mg GAEs/g BSG)	TFC (mg QEs/g BSG)	Antioxidant Activity (% DPPH inhibition)
$SC\text{-}CO_2$ 0/15	0.04±0.00a	0.03±0.00a	0.31±0.01a
$SC\text{-}CO_2$ 0/25	0.07±0.00b	0.06±0.00b	0.62±0.03b
$SC\text{-}CO_2$ 0/35	0.08±0.00b	0.07±0.00c	0.82±0.04c
$SC\text{-}CO_2$ 20/15	0.17±0.01c	0.12±0.00d	1.11±0.04d
$SC\text{-}CO_2$ 20/25	0.19±0.00d	0.14±0.01e	1.41±0.01e
$SC\text{-}CO_2$ 20/35	0.17±0.01c	0.15±0.00f	1.64±0.07f
$SC\text{-}CO_2$ 40/15	0.35±0.01i	0.18±0.00h	1.82±0.04g,h
$SC\text{-}CO_2$ 40/25	0.28±0.01f	0.20±0.01i	1.88±0.06h
$SC\text{-}CO_2$ 40/35	0.24±0.01e	0.17±0.01g	1.99±0.06i
$SC\text{-}CO_2$ 60/15	0.34±0.00h,i	0.19±0.00h,i	1.76±0.07g
$SC\text{-}CO_2$ 60/25	0.33±0.01h	0.18±0.00g,h	1.85±0.05h
$SC\text{-}CO_2$ 60/35	0.31±0.01g	0.18±0.00g,h	1.83±0.05g,h

Values are means of three-replication ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).
GAEs = gallic acid equivalents; QEs = quercetin equivalents.

The results indicated that it was necessary to increase the polarity of the supercritical fluid by adding a polar modifier. In effect, increasing the percentage of modifier TPC, TFC and antioxidant activity increased. **The supercritical carbon dioxide at 40°C, with 60% of ethanol (v/v) and at 35 MPa appeared to be a good condition** to extract polyphenols and flavonoids and to have the highest antioxidant activity compared to the other experimental conditions tested.

Fig. 2. Sensory properties of uncooked (A) and cooked (B) fish-burger samples enriched with different powders.



The attribute that mainly influenced the panelist's acceptability of raw samples was predominantly the odor, that improved with the increase of temperatures. It is shown that a high enough inlet air temperature leads to a rapid and easy formation of a crust on the microcapsule surface, so that the flavours can not evaporate outwards (Jafari et al., 2008). The cooked samples showed high scores and were appreciated by the judges. **The fish-burger with 5% microencapsulated BSG extract and Capsul® solution in ratio equal to 1:2 at 150°C was chosen as the best compromise according to the evaluation and sensory.**

Table 4. Chemical composition of CTRL and 1:2 150°C fish burgers.

Fish-burgers	TPC (mg GAEs/g fish-burger)	TFC (mg QEs/g fish-burger)	Antioxidant Activity (% DPPH inhibition)
Uncooked CTRL	0.03±0.00a	0.06±0.00a	21.57±1.36a
Cooked CTRL	0.03±0.00a	0.06±0.00a	21.19±0.70a
Uncooked 1:2 150°C	0.12±0.00b	0.13±0.01b	44.93±1.07b
Cooked 1:2 150°C	0.13±0.00c	0.14±0.00c	48.49±1.45c

Values are means of three-replication ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).
GAEs = gallic acid equivalents; QEs = quercetin equivalents.

The active sample showed a higher concentration of polyphenols and flavonoids than the control. These data turned out to be in agreement with the sequestrating activity on DPPH. Specifically, the fortified sample demonstrated higher antioxidant potential than the CTRL: 44.93% against 21.57% for uncooked samples and 44.49% against 21.19% for cooked samples.

CONCLUSIONS

The finding of this study highlighted the potential re-use of BSG and the possibility of embedding as a natural ingredient in various foods, including fish, to enhance their antioxidant content.
Results indicated that a process time of 240 min, temperature of 40°C, pressure of 35 MPa and 60% of ethanol (v/v) were the best extraction conditions for bioactive compound, while a ratio between extract and Capsul® of 1:2 and a temperature of 150°C were the best spray-drying parameters to prepare a fish-burger with a high overall quality and with a good antioxidant properties.

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Spinelli S., Conte A., Lecce L., Incoronato A.L., Del Nobile M.A. Microencapsulated propolis to enhance the antioxidant properties of fresh fish burgers, Journal of Food Process Engineering (2014), http://dx.doi.org/10.1111/jfpe.12183.

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MICROENCAPSULATED PROPOLIS TO ENHANCE THE ANTIOXIDANT PROPERTIES OF FRESH FISH BURGERS

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ABSTRACT

The aim of the study was to enhance the antioxidant properties of fish burgers with microencapsulated propolis. Spray-drying process was used to microencapsulate propolis (30 g in 100 mL of ethanol 70% v/v) by means of gum Arabic and Capsul in different ratios (1:6 for gum Arabic and Capsul and then 1:20 just for Capsul). Once defined the optimal microencapsulation conditions, an alcohol-free powder able to mask the strong odor of propolis was obtained, thus promoting a potential food application as source of phenolics and antioxidants. Specifically, 5% w/w of spray-dried propolis was incorporated in fish burgers. To improve their sensory properties, new ingredients such as potato flakes (3%, 5%, 7% and 10% w/w) and extra virgin olive oil (9% w/w) were tested and optimized to give a final fish product with good acceptability. Proper tests on burgers also demonstrated an effective increase of both phenolic content and antioxidant activity.

PRACTICAL APPLICATIONS

Propolis exhibits strong antioxidant properties because of its high content of polyphenols. Thus, it can be potentially used as natural food additive and functional food ingredient, but its application to food products is still limited because of its strong and unpleasant taste and odor that generally compromise food acceptability. The results of this study increase the possibility to embed the propolis as a microencapsulated natural ingredient in various foods to enhance antioxidant properties and phenolic contents.

INTRODUCTION

In the last decades, consumer demands in the field of food production have changed considerably. In particular, foods are no more intended to only satisfy hunger and to provide the necessary nutrients but used to prevent nutrition-related diseases and improve physical and mental well-being (Mollet and Rowland 2002; Nöthlings *et al.* 2007; Takachi *et al.* 2008). Among the numerous bioactive compounds possessing properties for health targets, the antioxidants have aroused great interest because these compounds can protect the human body by reducing the frequency of oxidative chemical reactions. Polyphenols are one of the most important groups of natural antioxidants (Yasin and Abou-Taleb 2007) and propolis represents a widely available natural substance very rich in phenolic compounds (Pietta *et al.* 2002). Specifically, propolis is a bee product composed of more than 300 chemical compounds including the caffeic acid phenethyl ester that exhibits strong antioxidant properties (Sud'ina *et al.* 1993). In addition, several authors (Marcucci 1995; Burdock 1998; Banskota *et al.* 2001) have shown that the phenolic composition of propolis is responsible for other biological and therapeutic properties: antiseptic, antimycotic, antibacterial, anti-inflammatory and antitumor activities. Thus, propolis can be potentially used as a natural food additive and as a functional food ingredient (Mendiola *et al.* 2010), also considering the fact that the synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole, propyl gallate and tertiary butylhydroquinone have shown carcinogenic activity (Chen *et al.* 1992; Uçak *et al.* 2011).

Although consumers occasionally consume strongly bitter-tasting foods and beverages such as coffee, tea, wine and dark chocolate, strong taste is generally undesirable and

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Supercritical carbon dioxide extraction of brewer's spent grain

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ABSTRACT

Brewer's spent grain (BSG) is the major by-product of brewing industry, produced in large quantities annually. Generally, it has been mainly used as feedingstuff but it was demonstrated that it contains a number of potentially high-value components. The aim of this work was to find proper supercritical fluid extraction (SFE) conditions to give bioactive compounds from BSG. The effects of three factors including pressure (15–35 MPa), temperature (40–60 °C) and CO_2 + ethanol (0–60% ethanol concentration, v/v) were investigated. Among the extraction variables, the best conditions were found considering the criterion of maximum concentration of phenolic compounds (0.35 ± 0.01 mg/g BSG), flavonoids (0.22 ± 0.01 mg/g BSG) and antioxidant potential, evaluated by the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (2.09 ± 0.04%/g BSG): 35 MPa of pressure, 40 °C of temperature and CO_2 + 60% ethanol (v/v).

1. Introduction

Nowadays, there is a growing interest in the recovery of vegetable by-products and their conversion into high-value products to obtain functional compounds because of a constant research to find new alternatives regarding the food fortification. The use of by-products in the food industry results in added value to industry and consumers; the industry benefits from an economic point of view since the disposal represents an additional cost to the producer, while the advantages of consumers, as well as the increase rejection of synthetic additives, concern the high content of valuable bio-active compounds (proteins, vitamins, pigments, antioxidants, antimicrobials, fragrances, etc.) with a wide range of actions as anti-tumoral, antiviral, antibacterial, cardio-protective and anti-mutagenic activities [1–3].

High amount of by-products are produced by processing fruit such as apples, grapes, citrus, peach, apricot, mango, pineapple, banana, guava, papaya, kiwi and passion fruit, and vegetables as tomato, carrots, onions, olives, potato and red beets [4]. Recently, a particular attention has been paid to by-products of brewing industry after the production of wort: the brewer's spent grain (BSG). This product is usually used as animal feed, composted or disposed as landfill [5,6]; nevertheless, it could be valorized for its phenolic compounds, widely recognized to have important antioxidant and antiradical properties [7]. Several researchers have reported that BSG is an excellent source of bioactive ingredients (phenolic acids, flavonoids, vitamins and minerals), as well as protein, fat, cellulose, hemi-cellulose and lignin [8–10]. Thus, the low cost and large availability of BSG have stimulated the development of several techniques for extracting the antioxidant components from BSG [11], that generally use the basis of either acid hydrolysis or saponification (with 1–4 M NaOH) and liquid–liquid or liquid–solid extraction with polar solvents [9]. Nowadays, more efficient environmentally sound alternatives for extraction from solid matrices, such as supercritical fluid extraction (SFE) and microwave-assisted extraction (MAE), have been developed. Recently, a novel application of MAE to BSG for extracting polyphenols has been adopted [12]. Nevertheless, studies on the extraction from BSG employing SFE are still limited. Supercritical fluid extraction is an efficient and worldwide spread technique to obtain valuable natural substances. Among compounds that can be used as supercritical solvents, it was chosen carbon dioxide (CO_2). In its supercritical state (when both the temperature and pressure equal or exceed the critical point of 31.3 °C and 7.39 MPa) has both gas-like and liquid-like qualities, an important dual characteristic that helps the fluid diffusion to the matrix (the gas-like characteristic) and provides good solvation power (the liquid-like characteristic). In addition, CO_2 is cheap, environmentally friendly and generally recognized as safe by FDA and EFSA [13,14]. The main disadvantage of CO_2 is its low polarity, problem that can be overcome using modifiers (co-solvents), capable of hydrogen-bonding, dipole–dipole and others polarity interactions with the analyte of interest [7,14]. It is worth noting that the type of co-solvent affects the efficiency of extraction of antioxidant compounds [10]. Generally, in SC-CO_2, ethanol is very

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Abstract: Nowadays, the use of the brewer's spent grain (BSG) is rather limited. Generally, it is employed as animal feed; however, recent studies have shown that BSG is an excellent source of bioactive compounds with potential interest for food, pharmaceutical and/or cosmetic industries. In this work bioactive compounds were extract from BSG, microencapsulated, and finally added to the fish-burger formulation. In particular, micro-encapsulation was performed by means of a spray-drying, using Capsul® as wall material, and modifying inlet temperatures (90-120-150°C) and ratios between extract and carrier (1:2; 1:4; 1:6; 1:8). Finally, a sensory evaluation on the fish-burgers prepared with the different bioactive powders was carried out in order to establish the best combination of operating parameters. The sample with 5% microencapsulated BSG extract and Capsul® solution in ratio equal to 1:2 at 150°C was chosen as the best compromise according to chemical characterization of active powder and sensory evaluation of sample. Finally, the antioxidant properties of fish burger with microencapsulated BSG extract were compared to the control. Results confirmed the potential use of BSG as food ingredient to increase the nutritional quality of fish burgers.

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Ed eccomi qua alla fine di questa tesi.

Ed eccomi qua pronta a ringraziare le persone che mi sono state vicine in questi anni..
che mi hanno aiutato..
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(Marcel Proust)