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Review

Natural phytochemicals and probiotics as bioactive ingredients for functional foods: Extraction, biochemistry and protected-delivery technologies

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ABSTRACT

Background: The well-known correlation between diet and physiology demonstrates the great possibilities of food to maintain or improve our health, increasing the interest in finding new products with positive physiological effects. Nowadays, one of the top research areas in Food Science and Technology is the extraction and characterization of new natural ingredients with biological activity that can be further incorporated into a **functional food,** contributing to consumer's well-being. Furthermore, there is a high demand for effective encapsulation methodologies to preserve all the characteristics of bioactive compounds until the physiological action site is reached.

Scope and approach: In this review, the relevance of developing standard approaches for the extraction of the highly diverse bioactive compounds was described, as it defines the suitability of the following steps of separation, identification and characterization. Special attention was also dedicated to the encapsulation techniques used on hydrophilic and/or lipophilic compounds (*e.g.*, emulsification, coacervation, supercritical fluid, inclusion complexation, emulsification-solvent evaporation and nanoprecipitation).

Key findings and conclusions: Some useful conclusions regarding the selection of the best extraction methodology (Soxhlet extraction, ultrasound-assisted extraction, supercritical fluid extraction, accelerated solvent extraction, or shake extraction) were achieved, considering important aspects such as cost, required technical skills, extract integrity, green chemistry principles, solvent type, sample size, pH, temperature and pressure. In addition, this comprehensive review allowed defining the best protective approach to solve the limitations related to the extremely low absorption and bioavailability of bioactive phytochemicals, overcoming problems related to their low solubility, poor stability, low permeability and metabolic processes in the GI tract.

Abbreviations: ADA, American Dietetic Association; AP1, activator protein 1; APC, antigen presenting cell; ARE, antioxidant response elements; ASE, accelerated solvent extraction; CAT, catalase; DAG, diacylglycerol; DLS, dynamin light scattering; ELK-1, [ETwenty-six (ETS)-like transcription factor 1]; EM, electron microscopy; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinases; FDA, Food and Drug Administration; FOSHU, food for specific health use; GDP/GTP, guanosine di-/triphosphate; GI, gastrointestinal; GPX, glutathione peroxidase; GSH, reduced glutathione; HRE, hormone response elements; IP3, inositol trisphosphate; LDL, Low Density Lipoprotein; MAPK, mitogen activated protein kinase; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor kappa-B; Nrf2, nuclear erythroid-derived 2-related factor; PIP2, phosphatidylinositol bisphosphate; PLC, phospholipase C; PPAR, peroxisome proliferator activated receptor; RAF, rapidly activated fibrosarcoma oncogene analog; RAR, retinoic acid receptor; RAS, rat sarcoma subfamily of small GTPases; ROS, reactive oxygen species; RXR, retinoid X receptor; SFE, supercritical fluid extraction; SNF, solid not fat; SOD, superoxide dismutase; Th, T-helper cells; UAE, ultrasound assisted extraction; WHO, World Health Organization.

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1. Introduction

Plants and their constituents have a key position in the progression of modern studies and knowledge on biological activity or active substances. Plant species are important sources of food, medicinal and supplementary health products, and their bioactive compounds are themselves products of metabolism, acting in similar ways to those operating in humans and animals (Gurib-Fakim, 2006).

The emergency of dietary compounds with health benefits offers an excellent opportunity to improve public health (Chen, Remondetto, & Subirade, 2006). Despite the dynamics of nutraceutical substances in physiological functions is not yet fully understood, their addition in food matrices is acknowledged as holding high potential to decrease disease risk. Accordingly, the scientific community should provide the necessary bases to develop innovative functional foods with the potential to produce physiological benefits or reduce the long-term risk of diseases onset (Elliott & Ong, 2002).

The effectiveness of nutraceutical products in preventing diseases depends on preserving the stability, bioactivity and bioavailability of the active ingredients (Fang & Bhandari, 2010). This represents a formidable challenge because only a small proportion of molecules remains available after oral administration, usually due to insufficient gastric residence time, low permeability and/or solubility in the gut, as well as instability under conditions encountered in food processing or in the gastrointestinal (GI) tract (Leonard, 2000).

Besides phytochemicals, probiotic delivery systems have also been widely used with the main target of reinforcing the natural intestinal flora. These systems are usually categorized in conventional (pharmaceutical formulations) and non-conventional (mainly food-based) products. Their effectiveness in improving the health status depends mainly on their ability to deliver viable functional bacteria, overcoming the harsh effects of GI. Probiotics have been increasingly used, but they present two sets of problems: i) their size, and ii) the need to be kept alive (Champagne & Fustier, 2007). The delivery of these agents will therefore require food formulations and production techniques to provide protective mechanisms that maintain the active molecular form until the time of consumption and its release in the physiological target within the organism (Chen et al., 2006; Leonard, 2000). Once encapsulated in a biodegradable polymer, cells are easier to handle than in a suspension and their presence in microparticles can be quantified, allowing the control of their dosage. In addition, their incorporation enhances the survival of cells during processing and storage (Anal & Singh, 2007).

Besides the benefits of encapsulating living cells, incorporation of bioactive compounds such as vitamins, prebiotics, bioactive peptides, non-nutrient carotenoids, phenolic compounds, phytoestrogens, glucosinolates, phytosterols, fatty acids or structured lipids into food systems, provides a way to develop novel functional foods that may have physiological benefits or reduce the disease' risk (Chen et al., 2006).

1.1. Bioactive compounds isolated from plants

Since the beginning of mankind, people use plants for their nutritional purposes. However, after discovering their medicinal properties, flora became a useful source of compounds with important roles in preventing/treating diseases and health improvement in several geographical locations. Actually, the ancestral use of herbal plants might be considered as the basis for using naturally bioactive molecules. In addition, the World Health Organization (WHO) predicts that 80% of the world population depends on traditional medicine as primary healthcare, mainly through the use of plant extracts and their bioactive compounds (Azmir et al., 2013).

All living things, from the bacterial cell to the millions of cells in plants, produce chemicals for their survival and livelihood. The compounds produced by biological systems are usually divided in two distinct groups: i) primary metabolites - chemical substances (*e.g.*, carbohydrates, amino acids, proteins and lipids) essential for cell maintenance, growth and development; ii) secondary metabolites - substances with low-molecular-weight (*e.g.*, phenolic acids, alkaloids or terpenes) relevant to increase the overall surviving and protection ability, by interacting with their surroundings (Azmir et al., 2013; Scalbert & Williamson, 2000). The production of secondary metabolites in different species depends on their growth process and the particular requirements of the species. Several studies have shown that the production of secondary metabolites depends also on the climate, soil and crop conditions (for instance, plants growing in harsh environments produce a greater number of antioxidants) (Azmir et al., 2013). Furthermore, and independently of the raw material, the qualitative and quantitative studies of bioactive compounds from plant materials depend mostly on the selection of proper extraction methods. These methods are usually affected by common factors, such as the matrix properties of the botanical source, solvent, temperature, pressure and time (Hernández, Lobo, & González, 2009). The extraction conditions are so determinant, that the bioactive plant compounds might be classified according to the type of extraction: i) hydrophilic or polar compounds (*e.g.*, phenolic acids, flavonoids, organic acids, sugars); ii) lipophilic or nonpolar compounds (*e.g.*, carotenoids, alkaloids, terpenoids, fatty acids, tocopherols, steroids). Another common classification criterion is categorizing the bioactive plant compounds according to their distribution in nature: i) shortly distributed (simple phenols, pyrocatechol, aldehydes); ii) widely distributed (flavonoids, phenolic acids); and iii) the least abundant polymers (tannin and lignin) (Bravo, 1998; Sánchez-Moreno, 2002).

In general, bioactive compounds of plants might be defined as secondary metabolites that cause pharmacological or toxicological effects in human and animals, which can be identified and characterized from extracts of roots, stem, bark, leaves, flowers, fruits and seeds (Bernhoft, 2010).

In the next section, special attention will be given to the particular cases of phenolic compounds and sterols, which represent two of the main classes of phytochemicals with a proven track of success as ingredients in functional foods formulations.

1.1.1. Phenolic compounds

Phenolic compounds are plant secondary metabolites commonly found in plants and derived products such as berries, apples, citrus fruit, cocoa, grapes, onions, olives, tomatoes, broccoli, lettuce, soybeans, grains and cereals, green and black teas, coffee beans and red and white wines (Birt, Hendrich, & Wang, 2001). It has been estimated that about 8000 compounds naturally occurring in plants are phenols (Arceusz, Wesolowski, & Konieczynski, 2013). Their characteristic structural feature is an aromatic ring with varying hydroxyl-substitutions. Despite occurring in free form, these compounds appear mainly in their bound forms, for example as glycosides or esters (Morton, Caccetta, Puddey, & Croft, 2000). Phenolic compounds might be produced from two distinct pathways: i) shikimic acid (phenylpropanoids); ii) acetic acid (phenols) (Sánchez-Moreno, 2002). According to their carbon chain, phenolic compounds can be divided in 16 major classes (Table 1). These compounds have diverse biological activity, being mainly acknowledged for their preventing action against the damage caused by oxidative stress. Other relevant functions in