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Ibero-American Network as a Collaborative Strategy to Provide Tools for the Development of Phytopharmaceuticals and Nutraceuticals

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Abstract
The increasing trend for using natural ingredients for nutraceuticals and phytopharmaceuticals development triggers the study of non-traditional sources for phytopharmaceuticals development, such as underexploited plants and agroindustrial wastes. The extraction, characterization and stabilization of bioactive compounds are intricate due to their low concentrations and their complex interactions in the vegetable matrix. New simple, ecological and efficient technologies are being developed to overcome the disadvantages of traditional extraction procedures, and many strategies should be developed to preserve their bioactivity. Oxidative reactions and protein glycation are two of the main deteriorative reactions affecting biological molecules and functionality loss *in vitro* and *in vivo*. Thus, efforts have been extended in search of edible plants with antioxidant or antiglycant properties. This chapter is an outcome of the CYTED Iberoamerican network 415RT0495, which task was to promote the valorization of subvaluated sources of bioactive compounds for food and medical uses.

***Keywords*:** Bioactive natural compounds, edible flowers, industrial by-products and wastes, ultrasound and microwave assisted extractions, analytical antioxidant and anti-glycant assays, microencapsulation techniques

2.1 Introduction

Current trends impulse the use of natural ingredients in food, cosmetic and pharmaceutical industries [1]. Nutraceuticals emerged in the last decade as bioactive herbal formulations with health-promoting capacity, and are employed for the development of functional foods [2]. These trends merge as an answer to consumer demands and require the development of ecofriendly extraction, purification and stabilization techniques.

After the observation of health benefits, symptom relief or disease treatment in different ethnic groups, non-traditional sources of phytopharmaceuticals and nutraceuticals, such as underexploited plants and industrial wastes, are being studied [1–3]. The sustainable use of plant resources, supported by the advances of extraction, stabilization techniques will lead to scientific developments of food ingredients and medicines for the benefit of health. Also, the social and environmental conditions of many regions of the world would be favored through the reduced amount of industrial by-products and losses and the recovery of valuable compounds, relevant for sustainable development.

Once the sources of active biocompounds, or matrix ingredients are identified, extraction procedures are necessary, and the functionality of the extracts has to be confirmed. In the last decades, many improvements of the extraction procedures were developed, and also the analytical methodology for the assessment of their functionality was upgraded.

This review is based in the activities of the CYTED network 415RT0495 composed by members of 8 Ibero-American countries. This network focus in the effective valorization of unexplored plant sources of bioactive compounds for food and medical uses. In this Ibero-American network each member of the eight countries has selected plant materials which they considered under-valuated and/or have been poorly studied, and it was not the aim to analyze an exhaustive number of plants. The tools that the network aims to provide consist of guidelines for sources selection, extraction methodologies, analytical methods, stabilizing procedures and data management.

2.2 Some Unexplored Botanicals From Ibero-America as Potential Sources of Bioactive Compounds

The search of bioactive compounds from Latin-American vegetable sources generally begin after the observation and knowledge exchange among different ethnic groups. Native people intake some of these plants for symptom relief, disease treatment as well as for popular belief. Leaves, flowers, fruits, seeds, stems and roots could be sources of bioactive compounds, antioxidant, antitumor, antihypertensive, antidiabetic, antiparasitic, antimicrobial, antihypertensive, cardiodepressive, vasorelaxant, anti-inflammatory, anti-ulcer, antiproliferative, antimalarial, anti-leishmani or antinociceptive properties were reported in the literature [4–6].

2.2.1 South America Regions: Tropical Savanna and Atlantic Forest

The Brazilian Continental Biome (around 8,514,877 km^2) includes a large variety of plants. Nevertheless, few of them have been completely studied. Thus, the bioactive compounds or potential carrier materials of many species remain unexplored and need to be characterized [7].

Among the many Brazilian vegetable species from Asteraceae family, *Baccharis dracunculifolia* (De Candole, D.C.), traditionally named "alecrim do campo", "alecrim vassoura", "carqueja", "chilca", "cilca", "erva-de-são-joão-maria", "suncho", "thola", "vassoureira" ou "vassourinha" (Figure 2.1, right) is one of the main plant sources of bioactive compounds present in Brazilian propolis produced in the South East region [8]. This plant that grows mainly in areas of Cerrado, produces essential oil with strong aroma, characteristic of green Brazilian propolis. More than fourteen compounds belonging to the sesquiterpenes and monoterpenes groups have been found in the volatile profile of the extracts obtained from the leaves of *B. dracunculifolia*. Among them, nerolidol and spathulenol varied seasonally [8], while caffeic acid; 2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran; drupanin; aromadendrin-4-methylether; artepillin C; p-coumaric acid were also identified [9, 10]. Hydroalcoholic extracts of *B. dracunculifolia* showed gastroprotective activity (inhibiting ulcers formation in different animal models with decreased of gastric secretion) [11, 16],

Figure 2.1 Left: Jabuticaba fruits (*M. cauliflora* Berg). Right: Alecrim do campo (*B. dracunculifolia*). Source: Falcksbaum, https://es.m.wikipedia.org/wiki/Archivo:Alecrimdocampo.jpg.

antinociceptive and anti-inflammatory activities [15] and anticariogenic factors and other antimicrobial activities [10], these latter as so as Brazilian green propolis [12].

Another example of a native plant from the Brazilian Atlantic Forest is Jabuticabeira (*Plínia* sp.) and also from Santa Cruz area, Bolivia, where it is known as "guapurú". It belongs to the Myrtaceae family which species *Myrciaria cauliflora* (DC.) O. Berg and *Myrciaria jabuticaba* (Vell.) O. Berg are most frequent in the South Central region [14]. The fruits have around 3 cm diameter and barks with dark red to black coloration (Figure 2.1, left), and are consumed fresh or in jellies and liqueurs [18, 19]. They are rich in phenolic compounds such as anthocyanins, flavonoids, tannins, vitamins, fibers and minerals. The bark and seeds, which are usually discarded after pulp consumption, have higher concentrations of these compounds [13]. Cyanidin and delphinidin glucosides were identified in the bark skin from the wastes of these fruits, which are good sources of vitamin C [13], and have been incorporated into foods [16]. The oil extracted from jabuticaba seeds have unsaturated essential fatty acids with predominance of linoleic and linolenic [18], and highly antioxidant compounds are produced by the plant to protect them. Their high antioxidant capacity has been confirmed by electron transfer methods, and they could potentially be used as additives in the food industry, with possible benefits to consumer health [16]. Particularly interesting are the medicinal properties of the stem bark and the fruit of *M. cauliflora* (DC.) O. Berg, which are very effective against diarrhea, chronic amygdalas inflammation and inductors of skin regeneration [17], attributable to its antioxidants content, especially polyphenols. However, the various biological activities of these materials still lack detailed studies for the development of food products, pharmaceuticals or cosmetics with bioactive properties.

Vitex cymosa (Bertero), from Lamiaceae Family, is a little tree known as "Taruma" or "pechiche" that grows in Amazon areas of Brazil and Bolivia. Although it has been scarcely studied, potential bioactive compounds may be obtained from its essential oils (such as flavonoids, iridoid glycosides, diterpenoides). Some preparations of this plant are used in folk medicine as antidiarrheic and are currently being investigated for use in specific pest control programs [18].

Also from Lamiaceae family, "Vira vira negra" or *Hyptis spicigera*, is employed as febrifuge, expectorant and parasiticide. Its essential oil is used as an insect repellent [19, 20].

2.2.2 Central South America Semiarid Regions

Species from Apocynaceae family are world-wide distributed and have been used since antiquity in South American folk medicine as antifebrifuge against malaria and as antiasthmatic. Chemical studies have shown the presence of several alkaloids in its bark, used in modern medicine, such as reserpine with hypotensive properties or cardiotonic glycosides, and alkaloids of some species (catharantus) have been shown to be effective in the chemotherapy of certain types of cancer [21].

Particularly, it is less known that barks infusions of *Aspidosperma triternatum* (Rojas Acosta), known as "cacha", or "quebracho blanco lagunero", and from *Mandevilla cuspidata* (Rusby) Woodson, known as "comida de socori", both from Apocynaceae family, have been employed against diarrhea in popular Bolivian medicin.

Flowers infusions of "Chichapi" or "tala" *Celtis spinosa* (Gill. et Planchon) from Ulmaceae family, are against diarrhea, and bark extracts are antiseptic [22].

Achyrocline satureioides (Lam.) *DC*, known as "vira vira blanca" or "Marcela" is from Asteraceae family and grows in South Brasil and Uruguay. The flowers have a pleasant aroma and the infusion of their leaves relieves headaches, cramps and stomach problems. It is currently used in infusions such as digestive, carminative, antispasmodic, cholagogue and emmenagogue. They are also attributed properties of reducing cholesterol and antidiarrheic. "Marcela" extracts are components of many "bitter" popular, which is its main commercial use.

2.2.3 Northern South America, Central America and Caribbean

Two species of the family Bignonaceae originated in these tropical regions are considered for pharmaceutical and food uses: *Crescentia cujete* L. (Figures 2.2a–e) and *Crescentia alata* Khunt. The majority of the Colombian Caribbean population only use the bark of the fruit for homemade crafts and utensils (Figure 2.2f). However, they are used by native communities since ancient times for the treatment of alopecia and for animal nutrition, prepared and presented in different ways to treat each human need [23]. In his book of natural medicines Geronimo Pompa, reported the benefits of the fruit pulp (or its syrup), the root and bark of the trunk, to promote menstruation and treat sympthoms of blenorrhea, ulcerative phthisis and apostemas.

The Colombian vademecum of medicinal plants includes *C. cujete* L. (totumo) for infections treatment but it does not mention its traditional use or pharmacological activity against alopecia nor includes *C. alata*, a plant also reported for traditional uses and pharmacological properties. Flavonoids, steroids and triterpenes were analyzed in the fruit epicarp [24]. furanonaftoquinones from *C. cujete* L. have selective activity against the DNA of cancerous tumors [25, 26]. It also contains 16 iridoids among those the derivatives of catalpol and of aucubin, agnuside, ajugol, crescentosides A, B and C and iridoid glycosides with the activity of inhibiting the growth of skin cells keratinocytes, effective to treat psoriasis, having the same effect as the commercial antisoriatic drug anthralin [25]. Iridoids and furanonaftoquinones present in the fruit of *C. cujete* L. were considered the active principles in the alopecia treatment. However, there is still a lack of systematic information on specific bioactive molecules in each part of the plant with medicinal properties [26, 27].

These kinds of plants have a great potential in the production of functional cosmetics, which occupy a prominent place in the consumer interests and large cosmetics

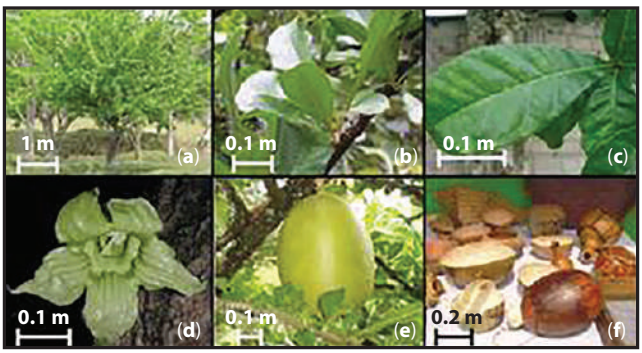

Figure 2.2 *C. cujete* L. tree (a), leaves (b and c), flower (d), fruit (e) and commercial fruit products (f).

manufacturers are introducing more organic and natural ingredients in commercial products [28]. Besides the properties of *C. cujete* L. leaves infusion used for stoping hair loss and promoting hair growth, different communities use these leaves in baths, washes and compresses to control dermatomycosis, dermatitis, bumps, leucorrhoea, scrapes, tumors and other diseases [29, 30]. The immature or green pulp of the fruit has been used to treat cough, headaches and pneumonia and as laxative, antipyretic, bruises, for menstrual disorders, burns, herpes, tetanus, seizures, prostate disorders [31–33]. Other traditional reported uses of the pulp include cervical cancer treatment, and antiproliferative and apoptotic effect on human lymphocytes cultured *in vitro* was observed [33].

Regarding the uses as a food ingredient, an emulsion can be obtained from *C. cujete* L. pulp, which is an authentic vegetable "milk" without lactose or cholesterol, rich in omega 6, omega 9 and protein of good nutritional quality. This milky emulsion represents a healthy alternative with functional properties and with innovative potential for the production of foods and pharmaceuticals from native natural resources [34]. However, according to a work carried out in Nigeria, it also contains antinutritional substances (some alkaloids and phenols) that, if not properly handled, can be toxic [35]. The root and pulp of the fruit are toxic for birds, small mammals and cattle. The pulp has no antibacterial activity, but induces neoplasm of the leukemia lymphoma type in 25% of the mice subjected to the administration of the syrup.

2.2.4 Exploitation of Undervalued Resources From Fabaceae Family to Obtain Hydrocolloids

Gums, or hydrocolloids are currently used worldwide as ingredients in pharmaceutical and cosmetic formulations, additives in many food or pharmaceutical excipients, as emulsifiers, film formers, foaming, thickening, stabilizer, filler agents, binders, diluents, disintegrants in tablets, coatings or carriers for drug delivery, among others [37]. Seeds and exudates from Ibero-America native plants have been recently characterized as sources of gums in order to find local alternatives to commercially available ones [36].

Several of the gum producer trees recently reported in Latin America conform the landscape of economically vulnerable areas [38]. In the following section we will discuss the characteristics and potentiality of hydrocolloids from some of these regional seeds (espina corona and vinal) and plant exudates (from *Prosopis alba* Griseb, Fabaceae Family).

2.2.4.1 Gums From Native Fabaceae Family Seeds

The endosperm of seeds from "Espina corona", *Gleditsia amorphoides* Griseb., Taub., and vinal *Prosopis ruscifolia* Griseb., both belonging to Fabaceae Family, contain gums, ECG and VG, respectively. The structure and functionalities of these gums are similar to those of very common and widely used galactomannans such as guar gum, GG [39].

The genus Gleditsia, belonging to the family of Legumes-Cesalpinoideas, has only few species with a worldwide distribution. *G. amorphoides* Griseb., Taub. and *Gleditsia triacanthos* L. grow spontaneously in forests and jungles in the North of Argentina and adjacent regions of Bolivia, Paraguay, Brazil and Uruguay.

"Espina corona", is one of the closest forest specie of *Ceratonia silicua* L., also form the Fabaceae Family or European carob, whose seeds are used to obtain locust bean gum (LBG)

[40]. The trees reach 12–15 m in height and 30–60 cm in diameter (Figure 2.3a). The fruit is a legume, 6–8 cm long and 2–3 cm in width, containing 6–10 seeds form oblong-ovoid, olive to cinnamon color (Figure 2.3b) [40].

Vinal is a very abundant tree growing in South American semiarid areas [41]. Figure 2.4 shows vinal seeds extracted from pods by milling, their endosperm after alkaline treatment and tegument separation, and the ethanol purified freeze dried VG. It can be noted that the obtained VG has whitish color, and fibrous and scaly appearance. Many interesting functionalities have been reported from galactomannans extracted from *Prosopis* spp. seeds: *Prosopis flexuosa* DC. and *Prosopis pallida* Humb. & Bonpl. as thickening agents [42, 43] and as synergic gel formers [44]; *Prosopis chilensis* (Molina) Stuntz as texturing agent [45] and foam stabilizer [46]; *Prosopis glandulosa* Torr. as emulsifying agent [46]; *Prosopis africana* Guill. & Perr, Taub. as thickening agent [47], as drug release controller [48], as emulsifying agent and film former [46]; *Prosopis juliflora* (Sw.) DC. as thickening and stabilizer agent [49], as encapsulating agent and wall material [50], as synergic gel former [51], and as release controller [52]; *Prosopis velutina* Wooton as thickening and stabilizing agent [53].

ECG and VG are galactomannans, with a molecular weight of about 1,400 kDa and mannose/galactose (M/G) ratio of 2.5 and 1.6, respectively. Both galactomannans consist of b-mannose units (1→4) linked with α-D-galactose residues at C-6 [54]. The reference gum, guar gum (GG) has a M/G of 1.8 [55]. Since linear polysaccharides occupy a greater volume than branched polymers, at the same concentration and similar molar mass, they exhibit higher viscosity [55]. Therefore, ECG and VG form highly viscous dispersions in hot and cold water, but they are non-gelling biopolymers.

VG and ECG display different rheological behavior according to the deformation rates and concentrations. At low deformation rates they develop thixotropic behavior, while at

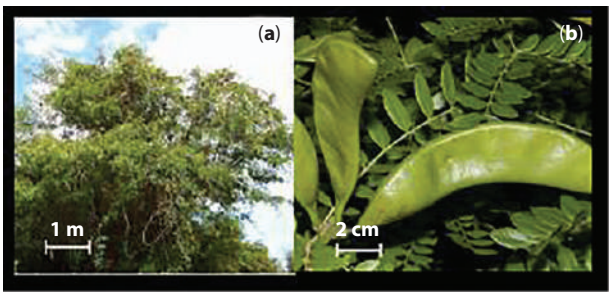

Figure 2.3 *G. amorphoides* Griseb., Taub. (a) tree and (b) pods.

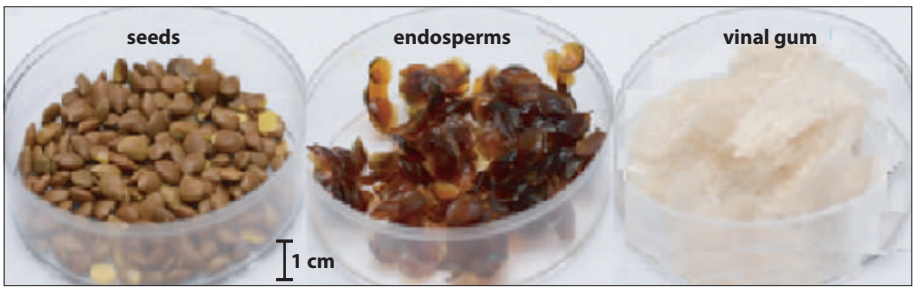

Figure 2.4 *P. ruscifolia* Griseb. seeds, endosperm after alkaline treatment, and the obtained vinal gum are shown.

high deformation rates the behavior becomes Newtonian. At all deformation rates and concentrations tested, ECG solutions were more viscous than those of guar gum [56].

When dispersed in water, ECG and VG shows a pseudo-plastic behavior [57] in the concentration ranges 0.25–1.50% w/w and above 0.04% (w/v). At intermediate concentrations, in high frequency range there is a cross point where the solid behavior (represented by the elastic modulus G') becomes higher than the viscous behavior (represented by the loss modulus, G"). Meanwhile, in the low frequency range, G" prevails over G' [57].

Zeta potential is an important property in coacervation processes and other applications, including molecular interactions with other components. VG, as GG and other galactomannan gums, is nearly neutral with weak acid behavior in solution and high stability to pH changes [58].

Galactomannans (such as ECG, GG and VG) are able to produce stable emulsions and reduce oil in water interfacial tensions. The emulsifying activity of galactomannans is not completely explained. The possible reasons are their non-polar regions, their protein content, or the protein moieties bounded to the gum, besides to their ability to thicken the aqueous phase [59, 60].

2.2.4.2 Gums From Fabaceae Family Exudates

The international market of exudate gums comprises only few varieties: arabic gum (*Acacia Senegal* Willd.), karaya (*Sterculia urens* Roxb.), tragacanth (*Astragalus gummifer (Labill.) Podl.*) and gatti (*Anogeissus latifolia* Roxb.) Wall., which producer trees are confined to specific Asian regions [61]. Certain trade difficulties related to sustained supply, variable quality and high importation costs has focused the researchers' attention on the search and exploitation of non-conventional exudate gums to find local alternatives to commercially available gums [36]. The environment particularities and botanical identity of gum producer trees, impart distinctive functional properties resulting in gums with different applications.

Exudate gums are generated in response to stress conditions, such as mechanical injuries, dehydration, microorganisms and insects attack [62]. Their main components are different carbohydrates, and also proteins, minerals and also polyphenols, among other antioxidant compounds [63].

Their appropriate physical properties and biocompatibility favored their use from ancient times [63]. Particularly, the exudate gum obtained from *P. alba* Griseb. trees or "algarrobo blanco", PAEG (Figure 2.5) is comparable to arabic gum [37]. It grows in South America arid and semi-arid regions [38], and its seeds and pods are often employed as ingredient for a variety of food products [64]. The exudate gum is hand collected in several locations in Chaco, Argentine, and purified by dissolution in water, heat treatment, filtration to remove impurities and finally dried [37]. Obtained gum powder exhibits a bright brown color and transparent shiny appearance (Figure 2.5).

Protein fraction in PAEG is markedly higher than in arabic gum, with better interfacial and emulsifying properties. PAEG is able to lower the interfacial tension and to stabilize emulsions based on high charge distribution (negative ζ-potential) and good interfacial rheological properties [37]. When PAEG was included in beads obtained by ionic gelation, fish oil oxidation was inhibited [65–67]. Additionally, PAEG affected water–solid interactions of the beads, favoring the formation of glassy matrices and improving the protection against lipid oxidation [68].

Figure 2.5 *P. alba* exudate gum, spontaneous exudation, raw gum samples and purified powder.

In 2011 the Argentine Administration of Drugs and Foods (ANMAT) accepted ECG to be used as thickener and stabilizer agent [69] and, more recently, in 2018, the flour of vinal seed has also been included. In pharmaceutical formulations, ECG and VG could be used as binder, suspending, thickening and stabilizing agents due to their capability of fast hydration.

Gums may also be used in alginate pH-dependent hydrogels for controlled release of drugs [66], with minimal release in the stomach, increasing in the intestinal tract [67].

2.2.5 Healthy Fatty Acid Sources From Ibero America

Besides representing a major energy supply in the human diet, vegetable oils are an excellent source of essential unsaturated fatty acids, which participate extensively in the metabolism, generating many bioactive molecules which are fundamental mediators of multiple signalling pathways, and as components of biomembranes [70]. The intake of saturated fatty acids has been associated with cardiovascular diseases, obesity, and related diseases. Whereas, monounsaturated and polyunsaturated fatty acids have health benefits as cardio protectors due to their anti-inflammatory, antiarrhythmic, and antithrombotic effects [71]. Industry and consumers have shown interest in vegetable oils from different sources, such as olive, canola flaxseed, chia, avocado and moringa since the discovery of their health benefits (Table 2.1).

Oils with higher unsaturation degree are very susceptible to oxidation reactions during which their fatty acids decompose to small volatile molecules that generate unpleasant aromas and toxic compounds (aldehyde, ketones, epoxides, hydroxyl compounds). This leads to decrease the shelf life, functionality, nutritional value, and add safety concerns, as well as the non-acceptance by consumers. The strategy to reduce oils deterioration is microencapsulation, as will be explained in Section 2.3.3.

2.2.6 Bioactives From Agroindustrial Wastes

2.2.6.1 Commercial Edible Flowers

The climate conditions in many regions of Latin America enable the development of commercial flower crops, which is a fruitful business that combines the best of agriculture with refined commercial services. The main world flower producers are Colombia and Ecuador,

Table 2.1 Composition and health benefits of some vegetable oils from Ibero-America.

Oil source	Fatty acids composition (%w/w)			Benefits	Ref.
	Oleic acid	Linoleic acid	Linolenic acid		
Olive	66.4	16.4	<1.0	Anti-ulcer, anti-aging and plasma cholesterol-lowering properties. Increment of bone mineral density.	[72]
Canola	59.5	18.8	11.9	Reduction of type 2 diabetes, osteoporosis risk, and cardiovascular disease.	[70]
Flaxseed	18.1	15.3	58	Reduction of tumour growth at the later stage of carcinogenesis and LDL cholesterol level.	[71]
Chia	5.4	19.7	65.4	Improvement in the fetal and infant growth and prevention of cardiovascular diseases.	[73]
Avocado	74	9.7	<1.0	Improvement in the blood lipid profiles (lowering LDL-cholesterol and triglycerides).	[74]
Moringa	78.2	1.29	<1.0	Reduction of rheumatism, hypertension and arthritis.	[75]

but in the last decades, in countries such as Peru, Mexico, Chile or Bolivia floriculture has become an economic alternative to traditional regional productions and in Argentina the sector merged as a new agricultural actor. Floriculture is an intensive rural activity, located mainly in suburban areas, developed by almost entirely small and medium producers, with a strong social impact. The importance of this productive sector, lies in the contribution to the territorial development, the transfer of knowledge and the need for labor intensive. Through agricultural techniques with different degrees of technological development a great diversity of products is available for the community.

Flowers contain natural nutrients, vitamins and bioactive compounds like phenolic acids, anthocyanins, betalains, and carotenoids [76]. The three latter are associated with the different colors of their petals, in a wide chroma spectrum [77, 78]. Rose petals have been used since ancient times in food preparation of salads, cakes, teas, desserts, drinks and innumerable meals [79]. These condiments have been maintained in the traditional kitchen in countries like China, Mexico and Brazil, and they have acquired notorious relevance due to their medicinal qualities and healthy properties.

Roses (*Rose* spp L., Family Rosaceae) occupy one of the first places in the world market. There is a huge variety of rose cultivars (more than 30,000) from different hybridizations, and new ones appear every year. The progenitor species mostly involved in the cultivars are: *Rosa moschata* Herrm., *Rosa gallica* L., *Rosa damascene* Mill., *Rosa wichuraiana* Crép., *Rosa californica* Cham. & Schltdl. and *Rosa rugose* Thunb. They are cultivated in commercial

farms for the cut flower industry or to be sold as garden flower. In the time that it takes to obtain a plant of commercial size, several blooms are produced, which are discarded, leaving the material in the field. Therefore, in the search for an adequate use of discarded flowers, food, pharmaceutical and cosmetic industries are potential destinations [80].

Rose petals contain phenolics (flavonoids, tannins), carotenoids (β-carotene, lycopene), ascorbic acid, tocopherol and essential oils [81, 82]. Flavonoids, gallic acid, protocatechinic and chorogenic acid from rose petals extracts have shown anti-proliferative effect against cancer cells [83–85].

The predominant anthocyanins in red rose petals are pelargonidin and cyaniding glucosides [86]. These water-soluble pigments are associated to many health-promoting activities (against cancer, diabetics, and oxidative damage). They also arouse the interest of their potential use as natural food colorants [87].

Antimicrobial activity of *Rosa Rugosa* Thunb. methanolic extracts against eight bacteria has been reported [51]. Besides, ethanolic extracts of rose petals showed greater inhibition zone of *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* than the maximum concentration of the antibiotic streptomycin [88].

On the other hand, the hexane fraction of *R. rugose* Thunb. was able to prevent oxidative damage by free radicals scavenging and inhibited lipid peroxidation [89].

In orange pink color rose varieties, important carotenes content was detected [90]. Some of them, such as β-carotene, and β-cryptoxanthin are vitamin A (retinol) precursors when ingested by mammals. The contribution of this vitamin to alleviate blindness, illness and premature death among children under five years of age and pregnant women [91] is well known. Carotenoid-rich diets have been also shown to be associated with a lower development rate of different cancers and chronic diseases [92].

2.2.6.2 Coffee Grounds as Source of Prebiotics

Due to the high consumption of coffee drinks, after extraction with hot water, coffee grounds are valuable primary products. During coffee processing, different by-products are generated such as: a) silver skin produced during the roasting of the coffee beans, after the previous removal of the outer skin and pulp of the dried fruit; b) coffee grounds generated after grinding and extraction with hot water of roasted coffee beans. Coffee grounds formed by small particles, while the silver coffee skin has a much larger particles and a large amount of soluble dietary fiber [93].

The composition of the coffee grounds comprises polysaccharides such as hemicelluloses, lignins, cellulose, lipids, proteins, caffeine and polyphenols [69]. Moreover, they contain melanoidins, generated by chemical browning during roasting. Due to the high organic matter in coffee grounds, (annual production in Spain in 2014 was estimated at 270,000 tons) there is a high biological oxygen demand with the genesis of up to 51,000 tons of CO_2/year. Therefore, this increases the content of greenhouse gases, aggravating the problem of global warming of the atmosphere by preventing adequate reflection of the sun rays. Coffee grounds may also be very polluting when added directly to the lithosphere, since they contain toxic compounds such as caffeine, and polyphenols, especially tannins. Thus, coffee processing by-products may induce toxicity in the ecosystem of the cultivation soils and air, causing a great negative environmental impact [94]. Recently, different investigations have been carried out looking for possible uses of coffee by-products [95]: as composting

fertilizers; for adsorption-removal of heavy metals; for the production of enzymes and non-fossil fuels such as bioethanol, biodiesel and hydrogen; as a source of polysaccharides with immunostimulant activity; as biosurfactants for the removal of pesticides from cultivation soils; as substrates in fermentation technology; as potential functional ingredients with antioxidant, prebiotic, antimicrobial or anti-hypertensive capacities [96].

Coffee by-products can be potentially used as prebiotics, since they provide components that match prebiotics definition: "nondigestible food components beneficial to the consumer (soluble fiber) that cross the gastrointestinal tract without being digested until reaching the colon, where they are fermented by selective components of the microbiota (lactic acid bacteria, mainly bifidobacteria and lactobacilli), selectively increasing their growth and/or activity with the strengthening of consumer health". In addition to these requirements, prebiotics must be stable during food processing [97]. The beneficial effects on health include the generation of acetic, propionic and butyric acids in the colon, which lower the pH that inhibits the development of pathogenic and putrefactive bacteria in the colon microbiota. Low molecular weight fatty acids stimulate the development and differentiation of the colonocytes, improving the intestinal barrier function and decreasing the bacterial translocation associated to inflammatory bowel disease problems [97]. While butyrate facilitates the energy supply in the colonocytes, acetate and propionate do so in the liver, where propionyl-CoA also facilitates the inhibition of the enzyme that directs the endogenous synthesis of cholesterol, hydroxy-methyl-glutaryl-CoA-reductase, causing blood cholesterol levels decrease and lipid profile improvement [97].

2.2.6.3 Healthy Compounds From Olive Oil Wastes

For thousands of years, olive trees (*Olea europaea* L.) have been cultivated mainly for the production of olive oil, with high economic value. Leaves, mills—alpechin—and pomace oil—orujo-, which are wastes from this industry, have a high content of valuable phenolic compounds, being leaves around 10% of the total collected matter [98].

Oleuropein (OE) is an ester of hydroxytyrosol and elenolic acid, and it is the most abundant polyphenol in olive leaves. It has several health-promoting benefits, against oxidative damage, as cardioprotective, anti-inflammatory, hypoglycemic and hypo-cholesterolemic agent [98]. Especially OE, but also other phenolic compounds that have also been identified in olive leaves [99], could be incorporated as food ingredients in functional and/or healthy food design. However, when the polyphenols are extracted from leaves, they are susceptible of degradation from environmental (light, oxygen, humidity and temperature, etc.), food (pH, enzymes) and gastrointestinal conditions (pH and digestives enzymes) [100], and the encapsulation of olive leaves extracts (OLE) represents an alternative to protect and control release the phenolic compounds in a site and/or at a specific rate (Section 2.3.3).

OE degrades during *in vitro* digestion [101], animal models [102] and human models [103]. Although OE metabolism has not been elucidated yet, it seems that only a small amount of it reaches the blood circulation [103]. OE that is not hydrolyzed in the stomach, could be metabolized, and its metabolites absorbed and distributed through the bloodstream. On the other hand, the OE absorption mechanism in the small intestine could be through the intercellular junctions of the small intestine by passive diffusion, involving a glucose transporter, or as OE aglycone by the action of the enzyme lactase florocin hydrolase (LPH) [104]. However, the simple diffusion of OE through the

cellular lipid bilayer or the intestine intercellular junctions is unlikely, due to the high molecular weight and polarity [104]. It has been reported that after intestinal digestion the unaltered OE could reach the colon, where a fermentation process generated by bacterial strains, could lead into HT [103]. Unaltered OE molecules that reach the colon are the most suitable precursors of HT [104], but its degradation products may provide a beneficial effect [105] in gastrointestinal illnesses, for example, in colon cancer and ulcerative colitis [101]. An increase of OE bioaccessibility and bioavailability during intestinal or colonic digestion is a challenge for OE encapsulation, as will be discussed in Section 2.3.3.

2.3 Technologies for Obtaining Stable Natural Bioactive Extracts

The valuation of natural sources is primordially dependent on technologies developed in the extraction stage, in which viable yields and levels of desired selectivity to biodiversity-sourced processes are established.

2.3.1 Extraction Techniques

Bioactive compounds are present in low concentrations and are often associated with complex chemical structures, complicating the extraction process. Traditional extraction methodologies include maceration with different solvents, heat reflux and Soxhlet extraction [106]. However, these processes are associated with the consumption of large volumes of solvents, energy, long extraction times, and degradation of bioactive compounds. Recently, new technologies have been developed to solve these disadvantages. Among them, extraction assisted by ultrasound (UAE), enzymes (EAE) or microwaves (MAE) are recognized as simple, ecological and efficient processes [107, 108].

Ultrasound waves propagate through a fluid with a frequency between 20 kHz and 10 MHz. This movement generates cavitation bubbles that cause cell disruption, allowing a better penetration of the solvent, thus, increasing the mass transfer of bioactive compounds to it. Extraction efficiency improves by decreasing extraction time and energy consumption in comparison with traditional processes [109].

UAE equipment is simpler than other extraction techniques and more economical. In the extraction of bioactive phenolic compounds from olives UAE showed a higher yield than the obtained by maceration, in lesser time (30 min vs. 4.7 h, respectively) [110]. Hydroxytyrosol, oleuropein and rutin, with excellent antioxidant activity.

The implementation of these techniques requires the optimization of the experimental variables (UAE frequency, solvent type, temperature, time), which affect the extraction efficiency [111].

EAE is based on the enzymatic hydrolysis of cell wall components, which improves the extraction of compounds that are associated to the fiber. Phenolic compounds, functional oils and proteins present in by products of vegetable origin have been extracted by EAE. At industrial level, the main advantage is the reduction in the use of organic solvents, with a direct effect on the environment, and a reduction in the loss of bioactivity [112]. The most used enzymes as extraction agents are pectinases, cellulases and hemicellulases, mainly from bacteria and fungi, but they can also be from animal or vegetable origin [113].

By means of EAE a significant increase in polyphenols, flavonoids, and tannins contents from white grape marc (*Vitis vinifera* L.) was observed [114], with greater antioxidant and anti-tyrosinase activities than the extracts without enzyme treatment. The same enzymatic complex increased up to 3 times the content of protocatechuic and vanillic acids in extracts from rice bran [115].

Commercial combination of cellulolytic and xylanolytic enzymes (Viscozyme L and CeluStar XL) improved the extraction of bioactives from chokeberry pomace [76], increasing the free radicals scavenging capacity. Xylanase-assisted extraction alone did not change the yield of soluble phenols from guava leaves (PGL), whereas cellulase and β-glucosidase did improve PGL extraction. The use of glucoamylase, protease and cellulose in rice bran increased the release of free and conjugated phenolic compounds [116].

The extraction yields and the antioxidant and antiviral capacity of green seaweeds extracts significantly increased employing a mixture of glycosyl-hydrolases and exo-glucanases [117].

UAE combined with EAE improved the extraction of polysaccharides from a freshwater bivalve mollusk, considered to display hypocholesterolemic, hepato-protective, anti-hypertensive properties [118].

MAE extraction process is based on the effects of the microwave energy absorption by the food matrix. This results in a sudden temperature increase inside the material and high pressures within the vegetable cells, causing their expansion and rupture, releasing bioactive compounds in the extraction medium [119]. The efficiency depends on the conversion of microwave energy into heat by the water molecules. The frequencies allowed for industrial, scientific, and medical uses (ISM frequencies), being the most used the range from 0.915 to 2.45 GHz.

MAE advantages over conventional methods include: extraction time reduction, environmental friendly, low cost and automation or online coupling to other analytical procedures. In the dynamic microwave assisted extraction (DMAE), the extraction performance improves, decreasing the possible degradation of labile compounds. This continuous, fast and automatic method was successfully employed for flavonoids extraction from different sources [120].

Enzyme-based ultrasonic/microwave-assisted extraction, (EUMAE) improved the extraction of several natural products, such as orcinol glucoside (with antidepressant activity) from the rhizomes of herbals, more efficiently than conventional techniques [121].

A very important aspect in UAE and MAE is the selection of the extraction solvent. Organic solvents increase the extraction yield, but they promote environmental risks [122]. In this regard, cyclodextrins (CDs) serve as green extracting agents since they allow the aqueous extraction of both polar and non-polar compounds from natural sources in a safely and in a non-contaminating technique. CDs are cyclic oligosaccharides, containing six, seven or eight glucose units that may host many types of compounds [123]. Molecular encapsulation of bioactive compounds in inclusion complexes, improves their aqueous solubility, stability and/or bioavailability [124] and facilitates their extraction from the natural matrices efficiently and eco-friendly [125].

2.3.2 *In Vitro* Tests for Assessing Antioxidant and Antiglycant Activities

Once the bioactive sources have been extracted, the functionality of active extracts has to be measured quantitatively in order to rank candidate species. Much effort is devoted to

the search of plants and fruits with antioxidant or antiglycant properties. Thus, a great part of the search efficiency lays in the availability of fast and efficient methods to evaluate the functionality of the extracts and also of their stability during storage.

Given the worldwide trend towards the reduction of the use of synthetic additives in foods, and of the incorporation of health promoting components, there is a special interest in adding value to plant resources (undervalued subproducts or unexploided vegetable sources), from which extracts with antiglycant or antioxidant activity can be obtained for technological applications [126]. Besides, there is much interest in using natural antiglycating compounds for alleviating diabetic complications. Many studies have revealed a vital role for protein glycation in the pathogenesis of age-related diseases, such as diabetes, atherosclerosis, end-stage renal disease, and neurodegenerative diseases. Protein glycation is related to the reactions between reducing sugars, oxidized products from lipids or sugars, and protein amino groups, resulting in irreversible loss of protein functionality. Thus, antioxidants may also display antiglycant activities [126].

2.3.2.1 Antioxidant Activity

Besides the high complexity of vegetable matrices, it is important to consider the multifaceted and plurality of antioxidant mechanisms (hydrogen transfer or simple electron transfer or both) and the variety of assays conditions (pH, temperature, solvent, ionic strength). Moreover, the effectiveness of an antioxidant compound depends on many parameters: medium polarity, temperature, type of substrate and physical condition. As a result, there is no single antioxidant quantification universal test. Consequently, many authors recommend to determine antioxidant ability by several tests, varying the conditions of extraction and quantification as much as possible to estimate the antioxidant capacity in a better way [127].

Some assays, such as DPPH and ABTS, extensively described, measure the ability of antioxidants to destroy the DPPH• (diphenyl picryl hydrazyl) radical or the pre-formed radical monocation ABTS+ (2,2-azinobis 3-ethyl-benzothiazoline-6-sulfonic), respectively. The radicals scavenge is determined spectrophotometrically at 515 or 734 nm, respectively, after reaction with the test extract, and related to the amount of antiradical compounds [127–129].

Phenolic compounds develop anti-radical antioxidant capacity through their hydroxyl groups, and it may correlate or not with total phenolic content [130].

Figure 2.6 shows the total phenols (TP) content and the antioxidant activity (AA) as Trolox (an analog of tocopherol used as standard) equivalents of infusions made with rose petals of different varieties or with two commercial teas, selected for their high antioxidant activity. Kardinal (K) and Gran Gala (GG) rose varieties presented the highest TP contents ($P < 0.05$), even higher than green tea. King's Ransom (KR) and Cristóbal Colón (CC) showed lower TP content similar to that of black tea ($p > 0.05$). The high content of total phenols in red petal varieties is associated with a higher concentration of the phenolic compounds anthocyanins. On the other hand, the yellow and orange petals are generally linked to the content of non-phenolic antioxidant compounds as carotenoids. For example, marigold flower is a very good source of carotenoids, mainly lutein [131].

The infusions made with the GG and K rose petals presented greater AA ($p < 0.05$) than Green and black teas. Bella Epoca (BE) and CC also showed higher values than Black Tea.

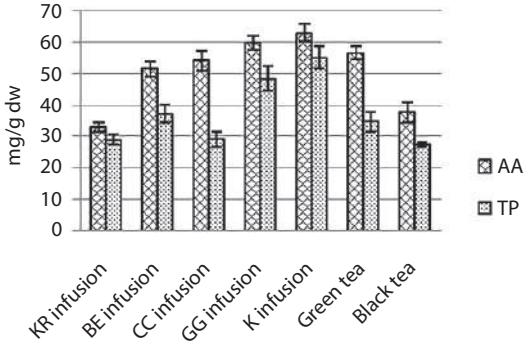

Figure 2.6 Antioxidant activity (AA) and total phenols content (TP) of infusions of rose petals of different varieties and two commercial teas: black and green (results expressed in mg TROLOX acid/g solids for AA and mg chlorogenic acid/g solids for TP).

KR was the only variety in which a lower AA ($p < 0.05$) was observed than commercial teas [132].

Figure 2.7a shows the correlation between the TP content and the AA of the evaluated petal infusions. When comparing all the samples, a low correlation was found between the evaluated parameters ($R^2 = 0.5876$), which improved when excluding the yellow and orange

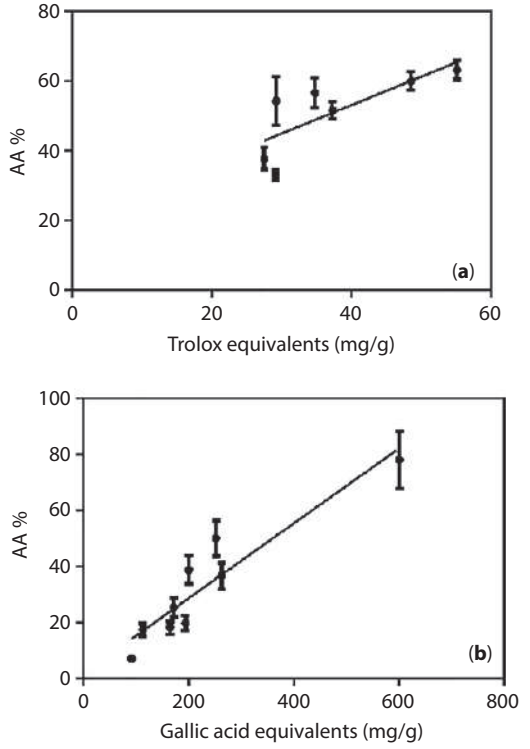

Figure 2.7 Correlation between the antiradical activity (%AA) and total polyphenol content (expressed as trolox/gallic acid equivalents) in rose petals and tea infusions (a), and in nine aqueous extracts of wild herbal species from Argentina (b).

varieties (R^2 = 0.7543) [133], indicating that in yellow and orange roses the antioxidant activity is associated with non-phenolic compounds.

Infusions made with edible flowers have a nutritional advantage over teas because they do not contain caffeine, the latter representing a factor that promotes a transient increase in blood pressure [134]. In turn, infusions made with rose petals are of high AA compared to other medicinal plants.

Nine herbal species native from Argentina were extracted in boiling water for 10 min and extracts were filtered and freeze dried until used. Figure 2.7b shows a plot of antiradical activity vs. total polyphenol content measured by Folin–Cicolteu method. Through a calibration curve using different concentrations of gallic acid, the results were expressed as gallic acid equivalents per gram of extract [135]. A positive correlation was observed (R^2 = 0.828) strongly driven by *Lantana camara* L. (Verbenaceae Family) extract which showed the highest values for both AA% and gallic acid equivalents, indicating that 82.8% of the antirradical activity observed for the evaluated samples results from natural occurring phenolic compounds contribution.

While antioxidant capacity and TP relationships have a positive correlation, it may diverge from ideal linearity since Folin–Ciocalteu method is specific for phenolic compounds and antioxidant activity can also be due to a variety of other non-phenolic compounds [136].

The reducing power of bioactive compounds may be quantified by determining the ferrric reducing (FRAP) or on the capacity to reduce cupric compounds (CUPRAC). FRAP measures the absorbance change at 593 nm produced by Fe (III) in the 2,4,6-tripyridyl-s-thiazine ferric complex TPTZ to the colored Fe (II) derivative [137]. Similarly, CUPRAC uses 2,9-dimetil-4,7-diphenil-1,10-fenantroline, batocuproine and the absorbance change at 490 nm [138]. In the oxygen radical absorbance capacity (ORAC) assay, a probe is measured (β-PE or fluorescein) and its decay by the presence of radicals that are generated by thermic decomposition of azonitriles. Finally, an interesting antioxidant assay is the co-oxidation of β-carotene by lipoxygenase where the oxidation of linoleic acid is monitored spectrophotometrically at 234 nm and the real antioxidant activity of compounds is quantified [128].

Thin layer chromatography (TLC)-coupled method combine de advantage of separative techniques with chemical activity detection which make them a useful tool in assaying natural extracts. The approach consists on an initial screening by dot blot to select positive extracts. TLCs with different mobile phases are then run on selected samples in order to separate active compounds. Chromatography is revealed by a DPPH assay on the plate surface. TLC coupled autographic assays have the advantages of being simple and several samples could be assayed on the same run with minimal sample handling and high repeatability.

Figure 2.8 shows a DPPH autography on TLC [138]. Samples were aqueous extracts of two species from the Brassicaceae family, *Rapistrum rugosum* (RR) and *Sinapis arvensis* (SA). Sulfur containing compounds such as glucosinolates are present in this family. Different types of naturally occurring glucosinolates with different biological activities have been described and many of them have antioxidant capacity [139]. Active compounds appear as yellow spots against purple background. RR showed several active compounds that could be separated along the plate. On the other hand, active compounds on SA remain mainly at the origin while others migrated with low resolution to R_f 0.2.

Two-dimensional TLC can be exploited for qualitative activity analysis in complicated mixtures of several plant extracts at the same time more efficiently and with better resolution

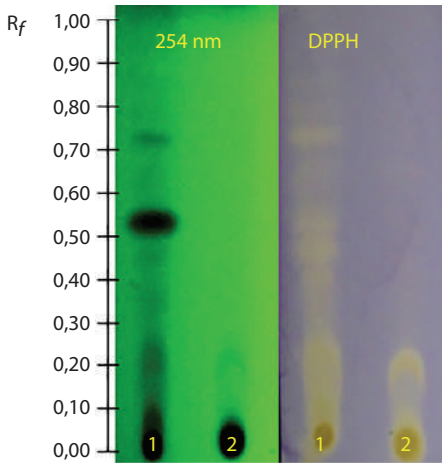

Figure 2.8 Right: TLC coupled DPPH autography. Left: TLC plate revealed using UV light (254 nm). Line 1: *Rapistrum rugosum* extract. Line 2: *Sinapis arvensis* extract. Mobile phase: toluene-acetone-methylene chloride (40:25:35). Rf: retention factor.

than one-dimensional TLC [140]. An innovative technique is based on running high resolution mass spectroscopy (HRMS) in extracting with solvent a autographic separated spot from the TLC (that could be standard low-resolution), allowing the identification of bioactive components in complex systems [141].

2.3.2.2 Antiglycant Agents Detection

The *in vitro* analysis of antiglycant extracts are based on measuring their inhibitory effect on proteins glycation and related markers in control reactions. The most used models are bovine serum albumin (BSA) and a reducing sugar in a phosphate buffered saline solution (PBS) so the potentially inhibiting extracts are challenged. The systems with and without the extract under analysis are incubated at 37°C or 55°C, or higher temperatures, according to the aim of the study.

After the isomerization of the Schiff bases, in the advanced phases of the Maillard reaction, very reactive dicarbonyl compounds are formed from proteins residues, called AGEs (advanced glycosilation end products). The main reaction markers for AGEs formation can be crosslinked compounds, fluorescent (such as pentosidine) or nonfluorescent (such as imidazolium dilysine), and not-crosslinked nonfluorescent AGEs, (such as Nε-carboxyethyllysine, CEL and Nε-carboxymethyl-lysine, CML) [142]. Reactive dicarbonyl compounds can also be generated in the lipid oxidation reactions, and some publications stated the correlation between anti-glycant activity and antioxidant activity of natural species [143]. The anti-glycation properties of extracts can be studied by several techniques analyzing different steps of the reaction:

- Fluorescence (excitation/emission l pair 370/440 nm)
- Protein conformation changes detected by polyacrylamide gel electrophoresis using sodium dodecyl sulfate (SDS-PAGE), Coomassie Brilliant Blue R-250 can be used as staining agent to detect proteins [144] and periodic acid Schiff's staining reveals glycosilated proteins.

- Furosine determination by HPLC [145].
- UV-absorbance and browning at 294/340 nm [146].
- Spectrophotometric analysis of nitro-blue tetrazolium (NBT) reductive assay [147].
- Nε-carboxyethyllysine (CEL) and Nε-carboxymethyl-lysine (CML) (available ELISA Kits).

In the last years several natural aqueous and/or ethanol extracts with antiglycant capacity have been obtained from different natural sources as *Allium sativum*, *Zingiber officinale*, *Thymus vulgaris*, *Petroselinum crispum*, *Murraya koenigii* Spreng, *Mentha piperita*, *Curcuma longa*, *Allium cepa*, *Piper nigrum* [148]. Different polyphenols, mostly phenylpropanoids and flavonoids, which could be present in high concentrations in tea, cinnamon, rosemary, mate and other herbal plants, are efficient trapping agents of a- dicarbonyl compounds, very active glycating agents which are intermediates of the Maillard reaction [146–148].

2.3.3 Biocompounds Conservation and Controlled Delivery Systems

Most bioactive compounds from natural sources with health benefits are difficult to incorporate in food, cosmetics or pharmaceutical matrices. Some of the reasons include incompatibility with ingredients and low stability under processes and storage conditions (light, oxygen, temperatures, enzymes, acid or alkali mediums, and humidity), which may lead to the loss of their activity and beneficial effects [149].

Several methods have been developed for encapsulation to preserve bioactive compounds at industrial scale. These methods can be classified into three main groups (Figure 2.9): chemical, physicochemical and physical–mechanical. For the selection of the most adequate encapsulation method, it is necessary to consider the production and cost of the process, the desirable morphology and the coating materials [150]. We are presenting an

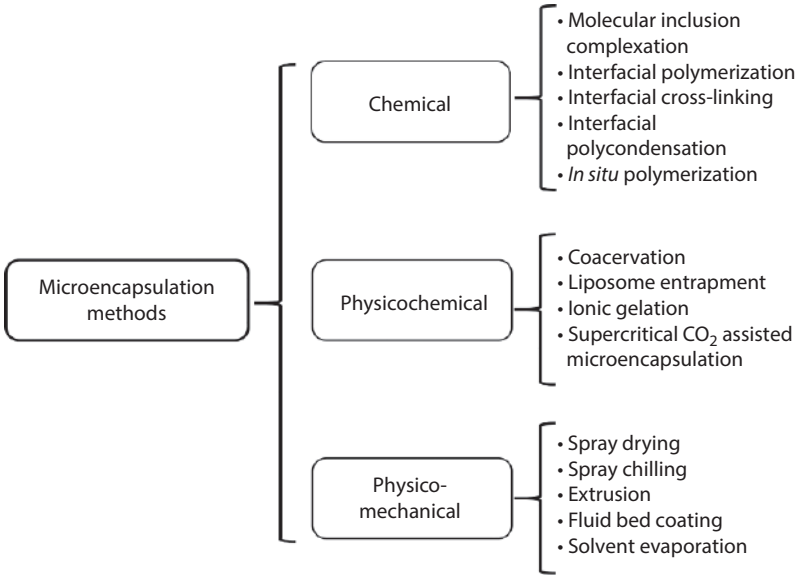

Figure 2.9 Microencapsulation techniques [149–155].

overall view of the recent studies on encapsulation using two of the more simple and widely employed microencapsulation methods, spray drying and coacervation, and protein systems as bioactives delivery and controlled release media.

2.3.3.1 Spray Drying

Microencapsulation is defined as the entrapment of tiny particles of an active agent inside coating materials [151]. Spray drying is the most commonly used microencapsulation technique, due to its low cost and high efficiency, fast and continuous operation and significant increment of the bioactive shelf life [149–151].

Spray drying has different stages: first, the coating materials are solubilized, generally in aqueous solution and then the bioactive compound is added to this coating dispersion. In case of oils, this dispersion is homogenized to generate a stable emulsion. Then, this emulsion is atomized into the spray dryer chamber where hot dry air circulates and quickly dries the droplets and the powder (microcapsules) is collected in a mechanical cyclone. Drying rate is influenced by the emulsion characteristics, such as viscosity and particle size. Emulsions with high viscosity form elongated and bigger droplets, which interfere with the atomization process, affecting adversely the drying rate [149]. The emulsion viscosity can be modified by varying the feed temperature. Some process conditions that have to be controlled are the inlet and outlet air temperature, atomization pressure, feed rate, the concentration of fed flow [150, 151]. The selection of the atomizer type (nozzle or disc) is also important.

The selection of appropriate coating materials or encapsulating agents has a direct impact on encapsulation efficiency and is critical in order to design microparticles with site-specific release properties and stability during storage. The coating material needs to have high water solubility, film-forming and emulsifying capacity, diffusibility, and low cost. Some of the most common coating or wall materials are gums, starches, gelatines and maltodextrins [152], which are water soluble and the elease mechanism of encapsulated compounds is by dissolution. Some results of vegetable oils spray drying microencapsulation are shown in Table 2.2.

To obtain a gradual release, it is necessary to include other hydrophobic compounds, such as proteins or other polymers in the coating material formulation [148–154]. Chitosan and alginate may be used to generate pH-sensitive encapsulation systems. In the intestinal tract (pH >6) alginate (a polyanionic water-soluble polysaccharide) disintegrates, and the bioactive compounds are released [155]. Although it is extensively used in food and pharmaceutical industry, alginate is barely been used as wall material for spray-drying encapsulation [156]. Moreover, other polysaccharides that are described for their health benefits as prebiotic or bifidogenic, such as inulin, have been used as colonic release polymers. Their glycosidic linkages are stable during the human digestive enzymatic action but they are fermented by colonic bacteria, releasing the bioactive compounds properly [157].

Oleurepin bioaccesibility (from olive leaves) is influenced by the type of the encapsulation system, polymer type and process conditions [158]. In this context, works in this line are scarce but necessary because the digestive mechanisms related to phenolic compounds remain unknown. Moreover, the most consumed compounds are not necessarily the most active in the organism, since their concentrations in the bloodstream depend on its modifications during metabolism in the gastrointestinal tract.

Table 2.2 Vegetable oils microencapsulation by spray drying.

	Coating material	Spray dryer	Process conditions	Results	Ref.
Flaxseed oil	MD, GA, WPC, Hi-Cap 100, Capsul TA	Laboratory scale spray dryer	Flow rate 12 ± 2 g/min. I/O T: 180 °C/ 110 °C.	High encapsulation efficiency: Good stability.	[159]
Chia oil	WPC, MG, GA	Nichols/Niro spray-drier, Turbo spray-drier PLA	Feed rate 40 ml/min I/O T: 135 °C/80 °C. Pressure 4 bar	WPC:MG and WPC:GA blends promoted good encapsulation efficiency	[160]
Olive oil	MD, agave inulin (IN), AG	Niro Minor pilot scale spray dryer	Flow rate 57.6 g/min I/O T 180 ±5 °C/ 90 ± 5 °C. Pressure 5 bar	MD-AG and IN-AG blends generate high microencapsulation yield.	[161]
Pomegranate oil	Skimmed milk powder	Pilot scale spray dryer, Buchi, B-191	Feed rate 1.75 ± 0.05 g/ min Inlet temperature 150–190 °C Pressure 5 bar	Core to wall material ratio defined the encapsulation yield.	[162]
Avocado oil	WPI and MD	Small scale spray dryer, Model SL10, Saurin Group of Companies	I/O T 180 °C/ 80 °C	Microencapsulated avocado oil showed a good oxidative stability.	[163]

AG, acacia gum; GA, gum Arabic; Hi-Cap 100 and Capsul TA, modified starchs; I/O, Inlet/outlet MD, maltodextrin; MG, mesquite gum; WPC, whey protein concentrate; WPI, whey protein isolate; T, temperature.

2.3.3.2 Coacervation

Coacervation is a physicochemical microencapsulation method, in which one polymeric solution is separated into two or more liquid phases, one of them rich in the polymeric material. The bioactive agent (core) is surrounded by the coating material, forming particles with a polymeric cover. The particles suspended in the solution are separated from the initial polymeric solution, and subsequently, solidify. The barrier created by the polymers

around the core allows the encapsulation of the interest compounds, which can be oils. The coacervation can occur in either aqueous or organic liquid. The factors which have influence on the coacervation process are pH, ionic strength, biopolymers concentration, biopolymers ratio, biopolymers molecular weight, temperature, and homogenization degree [164].

Coacervation may be simple or complex, according to the involved phase separation. In simple coacervation, only one polymer is in aqueous solution, occurring the polymer separation when electrolytes or water-miscible solvents are added, a temperature change is produced, or an inorganic salt is added. In complex coacervation, two or more polymers are in aqueous solution, occurring the separation phenomenon when electrostatically oppositely charged biopolymers are brought together, under certain specific conditions [165].

The coacervation is regarded as one of the simplest, low cost and reproducible microencapsulation method for hydrophobic substances (vitamins and oils), due to its high encapsulation efficiency and high oxidative stability [166]. The biopolymers used as wall materials have hydrophilic colloidal properties, adequate charge density, and high water solubility. The coating materials most used for this method are sodium alginate, protein isolates and gum Arabic [165]. In Table 2.3 some studies of vegetable oils microencapsulation by complex coacervation are shown.

Table 2.3 Vegetable oils microencapsulation by complex coacervation.

Active agent	Wall material	Process conditions	Results	Ref.
Chia oil	Protein and gums from chia seeds	Dropwise addition of citric acid into the emulsion (pH change)	High encapsulation efficiency; oil shelf life increased significantly.	[167]
Stearidonic acid soybean oil	Gelatine, gum Arabic maltodextrin	pH was adjusted with citric acid	Improved oil stability against oxidation reactions.	[168]
Flaxseed oil	Flaxseed protein (FPI) and flaxseed gum (FG)	pH adjusted with HCl	Crosslinked FPI-FG complex coacervates showed high oil microencapsulation efficiency and high oxidation stability.	[169]
Sunflower Oil	Fish gelatine and Arabic gum	pH adjusted with lactic acid; glutaraldehyde as crosslinker	Controlled microcapsules size with low energy at production scale consumption.	[170]
Olive Oil	Gelatine and sodium alginate	Dropwise addition of glacial acetic acid to pH 3.75, crosslinked with glutaraldehyde.	Encapsulation efficiency increased with olive oil, glutaraldehyde and polymer concentration.	[164]

Bioactive extracts encapsulation can be also be performed by freeze drying, formation of inclusion complexes with cyclodextrin and electrostatic extrusion [166].

2.3.3.3 Management of Protein-Hydrocolloid Interactions for Designing Bioactive Delivery Systems

The gums from seeds or extrudates can be employed in delivering systems and encapsulation matrices looking forward the modification of release kinetics and preservation of bioactive compounds.

For example, by controlling the formation of mixed gels of proteins and polysaccharides, different microstructures can be obtained in order to serve as delivery materials.

When aqueous solutions of two biopolymers of equal charge and/or neutral biopolymers are mixed above a given concentration, phase separation or thermodynamic incompatibility may occur [171]. When proteins are involved, this phenomenon is observed at pH higher than their isoelectric point [171]. Macroscopically separated phases are obtained, in the absence of gelation, each of one enriched in one of the two biopolymers. The obtained microstructure and the rheology behavior are defined by the relative rates of gelation and phase separation processes, representing a good alternative to control microstructure. For example, gels with different pore sizes or interstices can be obtained.

Aqueous systems of mixtures with low ECG concentrations and milk proteins exhibit Newtonian flow behavior, and at higher ECG concentrations the mixtures show pseudoplastic behavior. If chymosin is added, the clots formed by enzymatic gelation of these mixtures showed a less continuous and interconnected protein phase while the non-protein phase, which constitutes the pore, occupies greater volume [172]. This is due to phase microseparation that would compete with the gelation process. During acid gelation of milk protein, induced by glucono-β-lactone (GDL), there is a decrease in the protein coagulation time and an increase in the pH at which coagulation occurs. The increase of ECG concentration may favour the electrostatic destabilization of milk protein, leading to the formation of gels with a lower degree of compactness [172].

Thermodynamic incompatibility was studied in mixtures of soy protein isolates and ECG. When the ECG concentration increased in cold-set gels formed upon acidification after GDL addition, a less interconnected gel network with larger pores was formed. On the other hand, by adequate microstructure management though phase separation, ECG-protein cold-set gels could be useful to obtain products with reduced amounts of sugar and/or salt [173]. By increasing serum release from 2 to 12%, around 25% sugar reduction can be attained.

The presence of vinal gum in maltodextrin encapsulating matrices increased the percentage of propolis antioxidants retention and the physical stability against humidification improving particles integrity and size homogeneity [174].

The addition of small quantities of VG in ionotropic gelation matrix modified encapsulation matrix and wall material properties, improving lycopene preservation in calcium-alginate bead, by modifying beads microstructure, pores size and transport mechanism [175].

The potentiality of polyelectrolyte beads containing exudated *P. alba* gum is currently assessed in real food matrices. The impact of PAEG on *in vitro* lipid digestibility of polyelectrolyte vehiculization systems for high nutritional value oils was recently explored, and

preliminary results showed that it does contribute to modulate the enzyme accessibility to lipid substrate (*unpublished data*). Promising results allow considering *P. alba* gum as a non-conventional polyelectrolyte in the design of vehiculization systems to protect and guarantee the bioavailability of functional lipids for food and pharmaceutical applications. The exploitation of *P. alba* exudate gum suggests a potential beneficial effect in local economies stemmed from the use of an available resource currently untapped.

2.4 Multivariate Analysis for Phytopharmaceuticals Development

The screening and detection of potential phytopharmaceutical sources from different plants or by-products from agroindustrial processing require the analysis of a great number of data and variables. Besides, most bioactive extracts are a complex mixture of compounds and a typical strategy is to determine few specific compounds, used as markers, to classify different types of samples. However, another possibility is to use a technique that provides a fingerprint related to a great number of compounds.

Multivariate analysis (MVA) includes several mathematical tools applied to data provided by techniques and/or equipment in multiple dimensions or variables per sample. The main areas in which MVA is useful to nutraceutical production are: a) Quality control of raw materials: including bioactive extraction optimization, bioactive quantification and botanical/geographical discrimination, b) monitoring the changes in bioactive profiles during processing, c) Detailed metabolite analysis of samples, also known as metabolomics.

MVA methods can be supervised or unsupervised and both are useful to classify or discriminate samples according their similarities. Supervised methods use information of the class of each sample analyzed to classify them into groups. On the other hand, in unsupervised methods (such as principal component analysis, PCA, and cluster analysis, CA) class information is not used and samples discrimination is performed according to similarities [176].

Many nutraceutical components are a complex mixture of compounds and a typical strategy is to determine few specific compounds, used as markers, to classify different types of samples. However, another possibility is to use a technique that provides a fingerprint related to a great number of compounds. This approach was used by Chasset *et al.* [177] with propolis from different regions of France, applying PCA on the reverse phase high performance thin layer chromatography (RP-HPTLC) outcomes and direct analysis in real time mass spectroscopy (DART-MS). PCA shows that with both techniques together allowed to improve discrimination of the samples into three types of propolis (orange, blue and intermediate). Thus, the loadings from PCA were associated to three markers compounds (galangin, chrysin and pinocembrin) [177]. Additionally, Ciccoritti *et al.* [178] used bioactive profiles of different cultivars of wheat as MVA inputs. Total polyphenols content (TPC), antiradical capacity (DPPH), total alkylresorcinols (colorimetric determination) and quantification of alkylresorcinols by GC-FID in combination with PCA were useful to discriminate different wheat cultivars according to their botanical origin [178].

PCA analysis is useful to visualize samples similarities in scatter plots of the scores. However, many authors also consider as necessary to use additional methods to confirm the pattern observed in PCA-plots. The combination of a chromatographic fingerprint with

PCA and CA is a powerful tool for assessing herbal products quality, or for selecting the best cultivars, on the basis of the preferred properties. For example, HPLC-DAD in combination with PCA and CA was used for quantification of several bioactive compounds (cinnamic acids, flavonols, monoterpenes, benzoic acids, chatechins, organic acids and vitamins) from the different cultivars of raspberry's buds [179].

Many studies include supervised methods applied to discriminate samples from different regions or different botanical origin. Partial least squares discriminant analysis (PLS-DA) is the most used supervised method. PLS-DA is a variation of PLS regression analysis, which is a supervised method related to PCA. For example, UV-spectroscopy and ultra-fast liquid chromatography (UFLC) were used as PLS-DA input to discriminate five samples from two different parts of mushrooms [181].

Another supervised method considered to classify similar samples is linear discriminant analysis (LDA). LDA searches for directions with maximum separation among the classes. LDA was used by Valdés *et al.* [181] to classify seven cultivars of almonds analyzing their skin, which is a by-product from almond manufacture. TPC, antioxidant activity and flavonoid content were used as input variables for LDA and a good classification was achieved using two factors [181].

In addition, MVA could be applied for monitoring a process involving a change into the bioactive compounds profile. For example, FT-NIR coupled to PCA and LDA was used as a characterization method for fortified fermented milk with two sources of antioxidants (grape and olive pomaces by-products). PCA was used to eliminate outliers from the original data set and LDA was used as a classification tool and a variable selection method [182]. In another example, pork sausages were enriched with two concentrations of polyphenols and physiochemically and microbiologically monitored during 14 days of storage. In this case PCA and PLS-DA determined matches among samples [183].

Spectroscopy could be typically coupled with MVA to monitoring different processes. For example, in commercial production of wines the use of the correct yeast strain is critical. Moore *et al.* [184], studied the composition of 40 yeast strains inoculated into grape must and analyzed them using FT-MIR using PCA, OPLS-DA. PCA-plot allowed discrimination of strains from laboratory and industrial strains. By OPLS-DA a better classification of the samples into the two target groups could be performed, caused by the modifications induced to cell wall structure in the selection processes of yeast domestication [184].

The authenticity of a product or an ingredient is of outmost relevance in several industries in order to cover legal aspects and favor economic performance. PLS-DA was applied to FT-MIR spectroscopy to classify samples of adulterated rosehip oil with others lowly economical edible oils (soybean, corn and sunflower). The results show an excellent separation of the samples, including adulterations with a proportion of 5% of non-rosehip oils [185].

An emerging area of interest for analysis of the bioactive compounds could be done using metabolome studies [186]. Metabolomics comprises the quantitative determination of intracellular metabolites, for which after the separative steps mass spectrometry (MS) and/or nuclear magnetic resonance spectroscopy (NMR) [187] are frequently employed. Considering the complexity of MS and NMR data, multivariate analysis is commonly used to recognize compositional differences among samples [188]. Bhatia *et al.* [189], analyzed the metabolic profile from five different extracts of fruit, leaves, latex, stem and roots of *Commifora wightii* by means of GC-MS and NMR. 118 chemically diverse metabolites were identified and the outcomes were used in PCA and PLS-DA to discriminate and classify, respectively,

the extracts from those several parts of the plant. PCA and loading analysis showed that the cluster separation could be attributed to the several different bioactive compounds [189].

Table 2.4 summarizes the multivariate methods described before.

The use of MVA is an increasing and is a useful strategy used by many authors to discriminate raw materials and products and their profile change during processes or storage of bioactive compounds. There are many choices for discrimination or classification problems, and emergent or new methods also could be performed. There are some important

Table 2.4 Use of multivariate analysis as a tool to discriminate bioactive compounds profiles for different fields.

Area of interest	Product	Analytical technique	Multivariate method		Refs.
			Unsup.	Sup.	
Geographical discrimination	Propolis	RP-HPTLC DART-MS	PCA		[177]
Botanical discrimination	Wheat grains	GC-FID TPC DPPH	PCA		[178]
Botanical discrimination	Raspeberry buds	HPLC-DAD	PCA, CA		[179]
Geographical discrimination	Mushrooms	UV–spectroscopy UFLC	HCA	PLS-DA	[180]
Botanical discrimination By-product exploitation	Almonds skin	TPC HPLC-MS		LDA	[181]
Monitoring a food process By-product exploitation	Fermented milk (fortified with olive and grape by-products)	FT-NIR	PCA	LDA	[182]
Monitoring a food process By-product exploitation	Sausage fortification with olive by-product	GC–MS		PLS-DA	[183]
Monitoring changes in yeasts during wine fermentation	Yeasts from wine fermentation	FT-MIR	PCA	PLS-DA OPLS-DA	[184]
Food fraud detection	Rosehip oil	FT-MIR		PLS-DA	[185]
Discrimination of different parts of a plants	*Commifora wightii*	GC–MS NMR	PCA	PLS-DA	[189]

considerations before and after the use of a multivariate method. An appropriate selection of MVA method is crucial and this is not trivial. If the user needs only a similarity grouping, unsupervised methods are a good choice, because their simplicity and capacity to reduce the dimensionality of the data. In that case, PCA and CA are mainly the most popular methods used. In the other hand, supervised methods require a major number of samples. There is an advantage of using supervised methods over unsupervised ones, which is, the firsts use the information of the class for each sample, and in consequence would be a better separation of the data set. The second consideration is to verify that the number of components or functions chosen to group or classify the samples accounts for a large amount of data total variance. The lower the total variance accumulated, the poorest the resolution of the grouping or classification. Finally, it is useful to search for original variables correlations and the relation of these original variables with latent variables or functions.

2.5 Conclusions

The characterization of natural ingredients including biocompounds eco-friendly extracted from undervaluated non-traditional sources will be an attractive topic in medicinal, food and even in cosmetic industries.

Many nontraditional unexploited sources of bioactive compounds, such as native botanicals, by products and wastes from agroindustrial production have been presented. However, some aspects should be overcome before they could be effectively employed for nutraceutical, pharmaceutical or cosmetic applications, which comprise:

a) The implementation of adequate extraction techniques, which not only reduce time and save operational costs, but also allow to assess complete bioactive release from the vegetable matrices. Research has to be done also for the transference of these technologies from laboratory to industrial scale.
b) The standardization of analytical procedures for evaluating the pursued functions, with adequate interlaboratory comparisons. At this point, it is important to clearly indicate the expression of bioactives concentrations. We suggest to do that on dry basis of the original material.
c) The availability of conservation techniques for bioactives and adequate coating materials, which are important strategies to increase the possibilities of the industrial application of natural components, allowing the modification of release kinetics.
d) The use of multivariate methods, which allow interpreting data represented in the multiple variables that include analytical results and samples composition and conditions.

Other important issues for nutraceuticals or pharmaceuticals development, are the definition of effective dose or maximum daily allowance for a given bioactive and the effective absorption and distribution of the bioactives in the body, which requires the performance of digestibility tests.

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Abbreviations

CYTED	Iberoamerican Program of Science and Technology for Development
GG	guar gum
ECG	Espina corona seed gum
VG	vinal seed gum
PAEG	*Prosopis alba* exudate gum
ANMAT	Argentine National Administration of Drugs, Food and Medical Technology
OE	Oleuropein
OLE	olive leaves extracts
LPH	lactase florocin hydrolase
HT	hydroxytyrosol
UAE	ultrasound assited extraction
EAE	enzymes assisted extraction
MAE	microwaves assisted extraction
DMAE	Dynamic microwave assisted extraction
EUMAE	Enzyme-based ultrasonic/microwave-assisted extraction
CDs	cyclodextrins
DPPH	diphenyl picryl hydrazyl
ABTS	2,2-azinobis 3-ethyl-benzothiazoline-6-sulfonic
TP	total phenols
AA	antioxidant activity
K	Kardinal
GG	Gran Gala
KR	King's Ransom
CC	Cristóbal Colón
FRAP	ferrric reducing
CUPRAC	cupric reducing antioxidant capacity
ORAC	oxygen radical absorbance capacity
TLC	Thin layer chromatography
RR	*Rapistrum rugosum*
SA	*Sinapis arvensis*
HRMS	high resolution mass spectroscopy
BSA	bovine serum albumin
PBS	phosphate buffered saline solution
AGEs	advanced glycosilation end products
CEL	Nε-carboxyethyllysine

CML	Nε-carboxymethyl-lysine
HPLC	hight performance liquid chromatography (DAD: is with diode array detection)
FPI	Flaxseed protein
FG	flaxseed gum
GE	Gelatine
SA	sodium alginate
GDL	glucono-β-lactone
MVA	Multivariate analysis
PCA	principal component analysis
CA	cluster analysis
RP-HPTLC	reverse phase high performance thin layer chromatography
DART-MS	direct analysis in real time mass spectroscopy
GC-FID	gas chromatography with flame induction detector
PLS-DA	partial least squares discriminant analysis.
UFLC	ultra-fast liquid chromatography
UV	ultra violet-spectroscopy
LDA	linear discriminant analysis
FT-NIR, FT-MIR	Fourier Transformed Near Infrared and Medium Infrared
MS	mass spectrometry
NMR	nuclear magnetic resonance spectroscopy

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