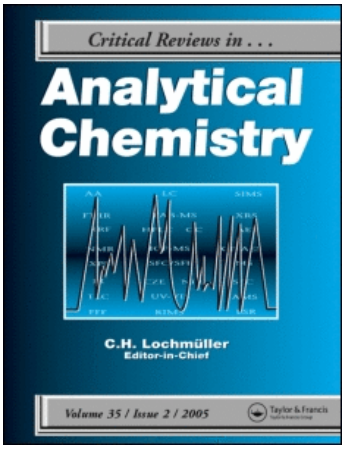

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An Overview of Analytical Chemistry of Phenolic Compounds in Foods

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ABSTRACT: A review is presented with references to the most important work dealing with the polyphenolic compounds taking account different aspects related with analytical chemistry of these compounds such as distribution, preparation, and/or treatment of sample and analytical techniques applied to their determination in foods. Contributions from 1985 to date are discussed.

KEY WORDS: hydroxybenzoic and hydroxycinnamic acids, flavonoids, foods, chromatography, capillary electrophoresis, spectrophotometry.

Table of Contents

* Author whom correspondence should be addressed.

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I. INTRODUCTION

The term phenolics or polyphenols can be redefined, in a precise way and from the point of chemical view as naturally occurring organic species that possess at least one aromatic ring with one or more hydroxyl groups attached to it. Nonetheless, these functional groups may be substitued for esters, methyl-ester, glycosides, etc. The polyphenol compounds are widely distributed in the plant kingdom, principally in the form of byproducts generated from plant metabolism.

The main reason for investigation of polyphenolic compounds and in particular flavonoids stems from their biological importance as secondary plant metablites, their ecological role an antioxidant capacity, physiological effects, employment as markers in taxonomic studies, and their properties related to food quality.

The polyphenol compounds are widely distributed in the plant kingdom, principally in the form of byproducts generated from plant metabolism. Thus, they may accumulate as end-products of two distinct biochemical pathways: the shkimate pathway, which gives rise to phenylpropanoids and cumarins, or the acetate path, which yields the simplest phenones and several quinones. Furthermore, they may be generated through an intermediate metabolic path that begets flavonoids, which happen to be the most important and numerous group of the polyphenol compounds. In view of the above-mentioned characterization, the introduced Figure 1 may be considered as the diagrammatic representation of the metabolic routes relative to polyphenol compounds generated by plant metabolism.

The chemical nature of polyphenol compounds is very heterogeneous and embodies an ample assortment or structural arrays of polyphenol families that can range from free to conjugated structures (free structure linked to other (s) substance of a kind not related to polyphenols).[1-11] Quite frequently it implies the participation of a variety of natural sugars, chemically bounded by virtue of their molecular size to one or several polyphenol functional groups. The monosaccharides such as glucose, galactose, arabinose, rhamnose, xylose, manose, and glucuronic as well as galacturonic acids are found to be linked this way too. As a rule, the flavonoids are polyphenol compounds that reflect a greater number of this structural design. The hydroxycinamic acid also constitutes a representative example of structures that are associated with other substances, not necessarily polyphenol in nature.

All of these aspects justify the intense interest in polyphenolic compounds that has been manifested over several decades and accounts for the many reviews and monographs devoted to various aspects of these compounds.[1-7, 31]

The present review, in principle shows the classification of the polyphenolic compounds, taking account of different aspects related with these compounds. On the other hand, it contains a study of the distribution and concentration in which the polyphenolics are found. Furthermore, the review examines the methods used for the preparation and/or treatment of sample as well as the analytical techniques to which the samples were subjected to carry out the determination of the phenolic compounds in foods. The results have been tabulated and considerated in their totality together with the experimental conditions of different techniques to facilitate comparison.

II. CLASSIFICATION OF POLYPHENOLIC COMPOUNDS

The polyphenol compounds constitute a miscellaneous group of organic structures, and because of this it is necessary to establish an adequate classification that could serve as a valid reference for current and future investigation. Table 1 shows the most general classification of the principal structures or classes of polyphenol compounds taking into account the main carbon skeleton that constitutes the fundamental axis for structural differentiation. As can be observed, the term flavonoid includes other types of polyphenol compounds, basically comprised of species such as anthocyanins, flavanones, flavanols, flavones,

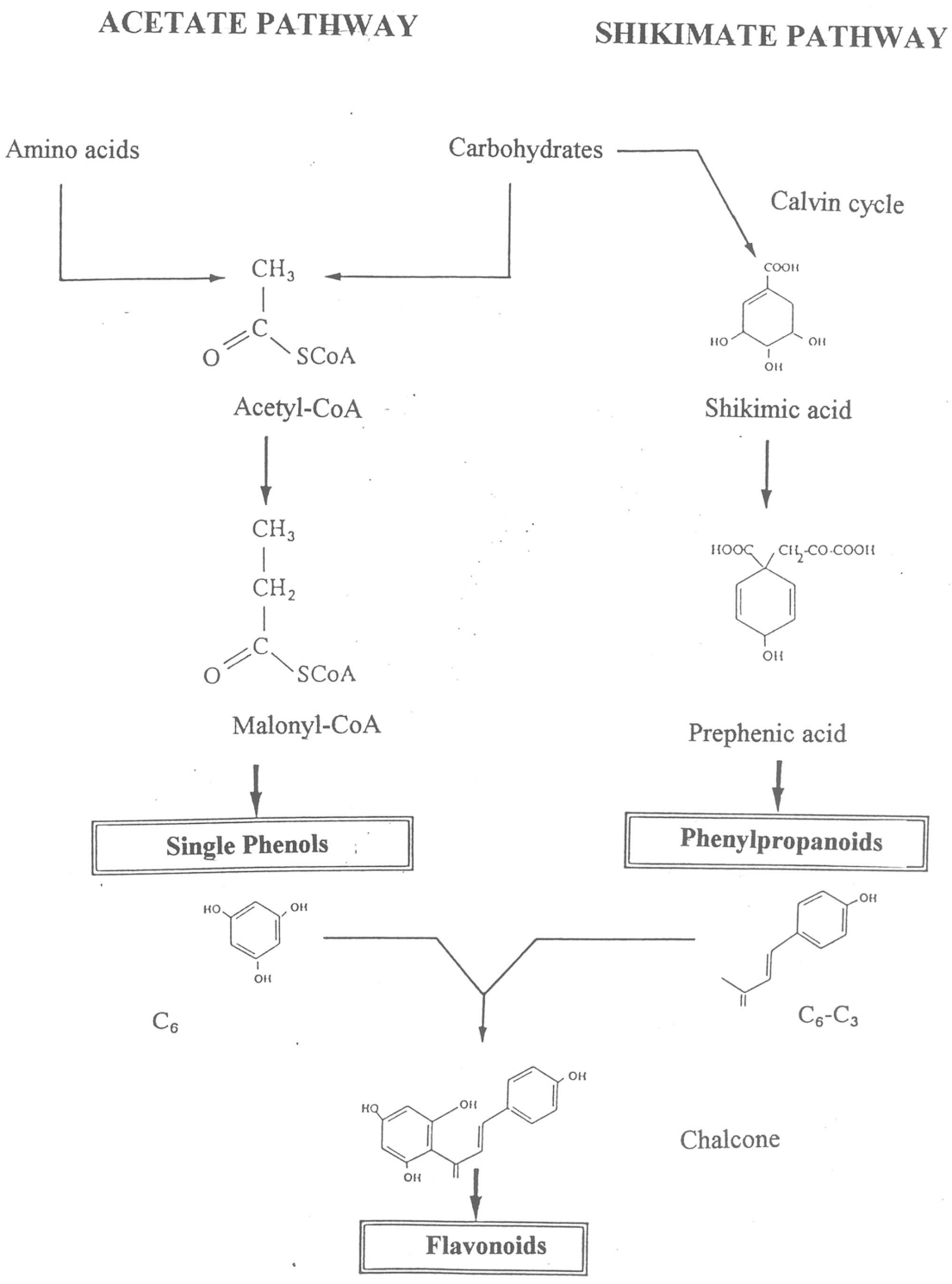


FIGURE 1. Scheme of the biosynthetic pathways of the polyphenolic compounds.

TABLE 1
Phenolic Classes (Harborne, 1989)

Structure	Phenolic class
C_6	Phenols
C_6-C_1	Hydroxybenzoic acids
C_6-C_2	Acetophenones and phenylacetic acids
C_6-C_3	Cinnamic acids coumarins, isocumarins and cromones
C_6-C_4	Naftoquinones
C_6-C_1-C_6	Benzofenones, xantones
C_6-C_2-C_6	Stilbenos, antraquinones
C_6-C_3-C_6	Flavonoids:flavanones,flavonols, anthocyanidins, chalcones, flavanols [1], aurones, flavones e isoflavones[2]
$(C_6$-$C_3)_2$	Lignans
$(C_6$-C_3-$C_6)_2$	Bioflavonoides, biflavanes
$(C_6$-$C_3)_n$	Lignins
$(C_6$-C_3-$C_6)_n$	Proanthocyanidins[3]

[1] Mainly flavan-3-ol structures.
[2] Isoflavonoids.
[3] Condensed tannins.

flavonols, and isoflavonoids.[5] All these substances are highly important from a nutritional point of view. An alternative classification of polyphenol compounds with respect to that shown in Table 1 was proposed by Harborne[5] in function of their place and distribution in the plant kingdom. From what is known about these compounds we can make a clear distinction between those that are universally distributed and those whose presence is rather sporadic. Thus, for example, it can be seen that hydroxybenzoic and hydroxycinimic acids as well as flavonoids are universally distributed in foods of vegetable origin. On the contrary, the isoflavonoids constitute a discreet group whose presence is confined to the family of leguminous plants.

If we take into account the heterogeneousness and extensive distribution of the polyphenol compounds in the plant kingdom, it can be easily deduced that this type of substance forms an integral part of our daily diet through the ingestion of foodstuffs that are extracted from vegetables. In virtue of this observation, we could suggest a more useful and simpler classification that would

include the principal polyphenol groups or families present in these types of nutrients.

The ensuing classification can be established on the basis of molecular size of the mentioned compounds, that is, on the basis of low, median, and high molecular weight. Table 2 depicts this type of classification. In the same table, one can find the name of the corresponding structure and the nearest polyphenolic class, taking into account the molecular weight of the compound in question. As can be observed, hydroxybenzoic and hydroxycinamic acids are included within the low molecular-weight group of polyphenols; the flavonoids are representative of intermediate molecular weight compounds, whereas tannins are an example of polyphenols with high molecular weight. On the other hand, it merits recalling that the flavonoid denomination comprehends all structures that tend to be dominant in the vegetal diet such as antocianidins, flavonols, flavan-3-ol, flavanones, and flavones, each of them pertaining to a particular family with well-defined properties. Likewise, it is important to indicate that high-molecular-weight polyphenols are concurrently classified into two large groups: hydrolyzable tannins and condensed tannins, according to whether the constituent monomer is an acid or a flavan-3-ol, respectively. In this review the discussion is centered on all the aspects relative to polyphenolic compounds, based on the classification introduced in Table 2. In continuation, the most general aspects of each cited polyphenolic families are briefly discussed.

A. Phenols and Hydroxybenzoic Acids

They are the simplest structures within the congregation of polyphenolic compounds, which as indicated include C6 and C6-C2 structures, respectively. Despite their structural simplicity, the resulting analites have been cited on many occasions in studies dealing with plant taxonomy, closely reflecting the degree and the nature of the debuting structures with given properties of the plant. Likewise, hydroquinone structures are the most representative phenols in terms of prevalence, variety, and frequency of occurrence. Similarly, a member of this family — arbutin — constitutes a typical compound of scientific interest. With respect to the acid structures, it merits to emphasize the importance of vanillic and gallic acids as the most representative and widely distributed polyphenolic structures. Despite its pronounced structural complexity, elagic acid may be considered as yet another representative member of this polyphenolic family that with gallic acid make up for a monomer base akin to hydrolyzable tannins. Finally, it has to be added that this type of compound is responsible for a determined biological function being accountable for vital plant activities such as the seed's germination and the proper growth of the same. The most representative structures of hydroxybenzoic acids are shown in Figure 2A.

B. Hydroxycinnamic Acids

Strictly speaking, the hydroxycinimic acids constitute the most widely distributed group of compounds otherwise known as phenylpropanoids. Among them are four basic structures that exist in their free natural state corresponding to coumaric, caffeic, ferulic, and sinapic acids. Similar to other polyphenolic compounds, a majority of these structures are found in the vegetable kingdom chemically associated to other types of compounds. A clear example of universal importance constitutes the esterification of caffeic acid with quinic acid to form a structure widely distributed in comestibles under the generic name of chlorogenic acid.

On the other hand, many determined biological functions are intimately related to the presence of these compounds in the plants and comprehend antibiotic properties as well as those relative to the inhibition of growth and germination. The most important structures analogous to hydroxycinamic acids are listed in Figure 2B.

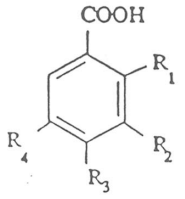
C. Flavonoids

Figure 3 represents the principal structures from this flavonoid group most extensively distributed in foods: anthocyianins, flavanols, flavanones, flavonols, flavones, isoflavonoids, and chalcones. In an effort to clarify structural no-

TABLE 2
Clasification of the Main Phenolic Class as a Function of Molecular Weight

Molecular weight	Structure	Phenolic class	
Low[1]	C_6-C_1	Hydroxybenzoic acids	
	C_6-C_3	Hydroxycinnamic acids	
Intermediate[1]	C_6-C_3-C_6	Flavonoids	Anthocyanidins Flavonols Flavanols (flavan-3-ol) Flavanonas Flavonas Isoflavonas Chalconas
High [2]	$(C_6$-$C_1)_n$	Hydrolyzable tannins Condensed tannins	
	$(C_6$-C_3-$C_6)_n$		

[1]Easy extractable phenolics or soluble phenolic fraction.
[2]Non extractable phenolics or no soluble phenolic fraction.

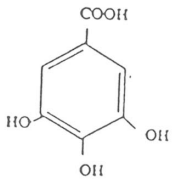


$R_1=R_2=R_4=H$; $R_3=OH$ ⇒ p-Hyddroxybenzoic acid

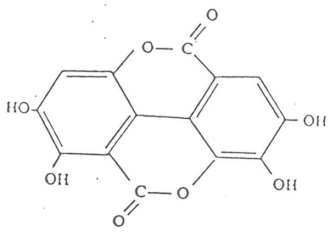
$R_1=OH$; $R_2=R_3=R_4=H$ ⇒ Salicylic acid

$R_1=R_4=H$; $R_2=OCH_3$; $R_3=OH$ ⇒ Vanillic acid

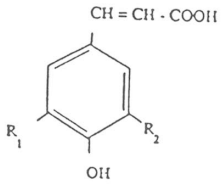
$R_1=R_4=H$; $R_2=R_3=OH$ ⇒ Protocatechuic acid



Gallic acid



Ellagic acid



$R_1=R_2=H$ ⇒ p-Coumaric acid

$R_1=OH$; $R_2=H$ ⇒ Caffeic acid

$R_1=OCH_3$; $R_2=H$ ⇒ Ferulic acid

$R_1=R_2=OCH_3$ ⇒ Sinapic acid

FIGURE 2. Representative structures of (A) Hydroxybenzoic acids and (B) Hydroxycinnamic acids.

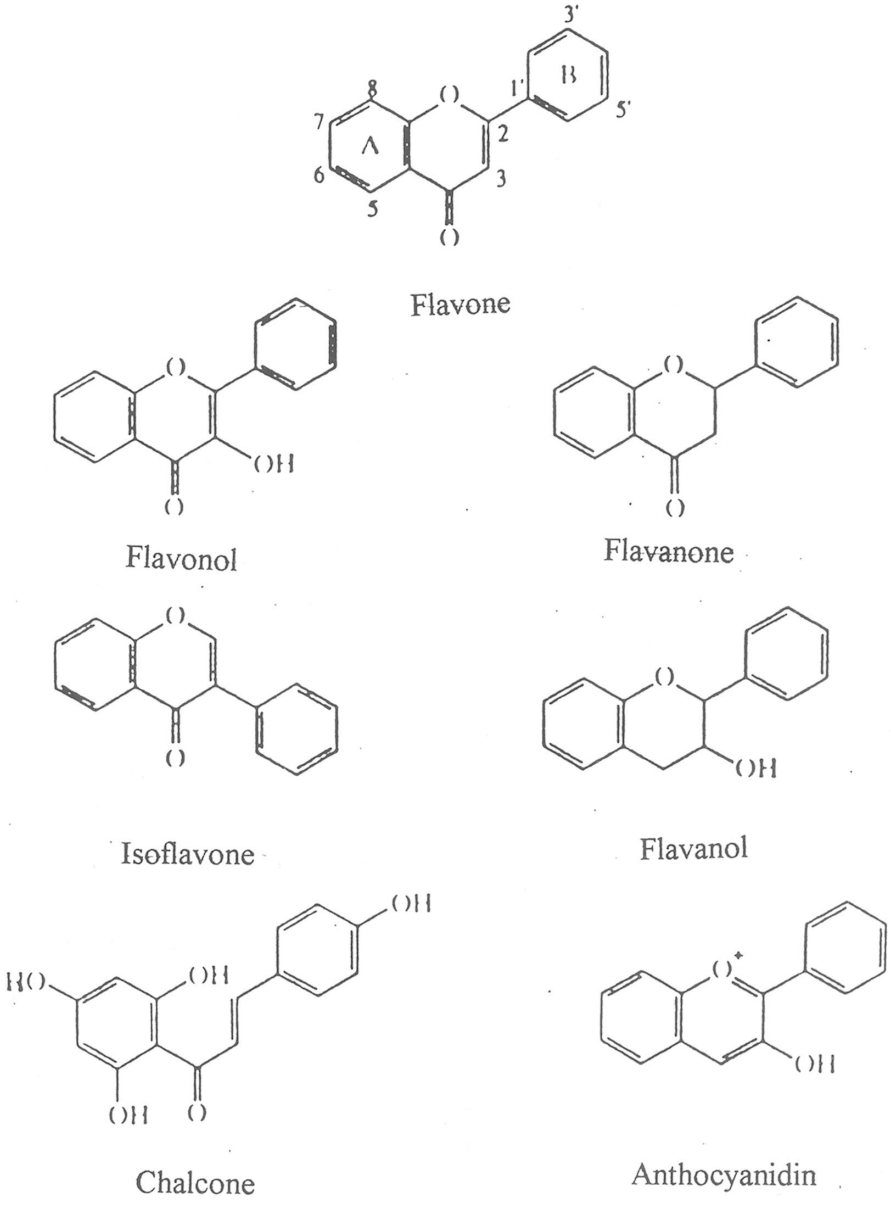


FIGURE 3. Major structures of flavonoids.

menclature employed in this type of compound, we have conveniently introduced in the upper part of the said figure branched rings corresponding to the structure, supplemented by the numbering of carbon atoms. Before initiating discussion on general description of each flavonoid structure, it is appropriate to indicate that the term "aglycone" represents a flavonoid not linked to any other chemical substance, independently of the type of flavonoid in consideration. The term "glycoside", or more generally "glycosilated structure," employed to indicate structures linked to any participating sugar.

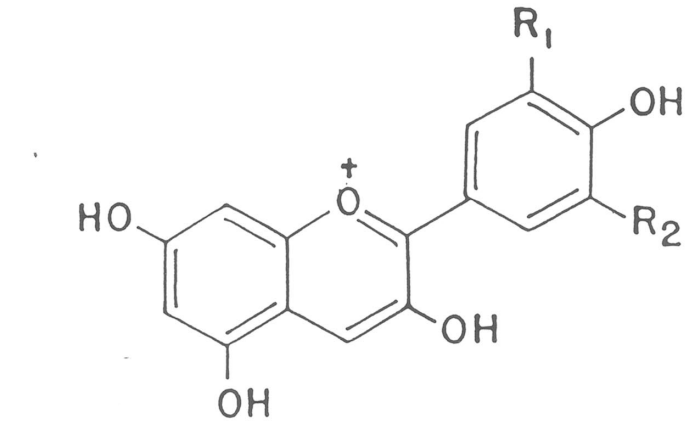
D. Anthocyanidins

The anthocyianidins are pigments that confer colors to the fruits,[12,13] despite the fact that in some of these fruits (orange and tomato) the mentioned color is due to carotenoids. The non-glycosilated anthocyianidins (aglycones) can be found as cations in the acid medium in a form of different isomers. The pigmentation properties of anthocyianidins have been exploited by the food industry as possible additives in juices and jams.[14] The ensuing stability of anthocyianidins depends in great measure on the species in which it is found, on pH (due to their acid-base properties), and on physical factors such as the light absorbance or susceptibility to oxidation. The spelled out characteristics impede proper purification of this type of structure and consequently reduce the commercial availability, which in turn reflects in the diminished presence in the food industry as additives and dyes. With respect to the most widely distributed anthocyianidin structures in the plant kingdom, the following six species are responsible for most of the pigmentation in the fruits: cianydin, normally found in its free molecular state (not glycosilated), is perhaps the most common,[15,16] followed by delphinidin, peonidin, pelargonidin, petunidin, and malvidin. The anthocyanidins generally debut as glycosides of the previously cited aglycons, which also account for the typical polyphenolic fruit structure.[17-21] It is possible to detect quantitative differences of their distribution based on the degree of fruit ripeness as well as on the climatic conditions under which they were grown, with the light intensity and the environmental temperature as the most decisive factors. One of the principal characteristics exhibited by fruit anthocianidins derives from the fact that in the immense majority of cases these compounds tend to be monoglycosilated. The glycosilation of the anthocianidins is always accomplished at position 3 through an oxygen bridge (*o*-glycosidic bond) with glucose, arabinose, and galactose. Cyanidine glycosilated with glucose as a participating sugar is the most abundant structure in the fruits, although other glycosilated structures may also be found in the total anticianidinic composition of this type of sample. On the other hand, acetylated antocianidins (chemically bonded to an acid) are also randomly distributed in fruits, especially in grapes, where the composition of these substances is quite complex due to the presence of monoglycosilated structures originating from five different aglycons. At the same time, they can debut acetylated with acetic or coumaric acids.[22-27] Figure 4A lists the most representative antocinidin structures that abound in foods of vegetable origins.

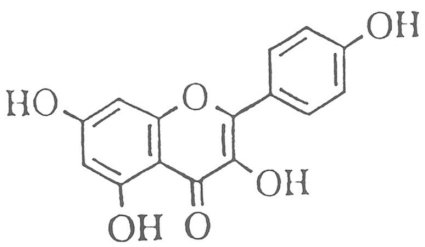
E. Flavonols

This type of flavonoid structures is vastly distributed in the plant kingdom, forming an integral part of our daily diet. Likewise, due to their anticarcinogenic properties which are explained later, the interest in these compounds is increasing steadily and occupies a relevant position on the list of currently undertaken investigations.[28-30]

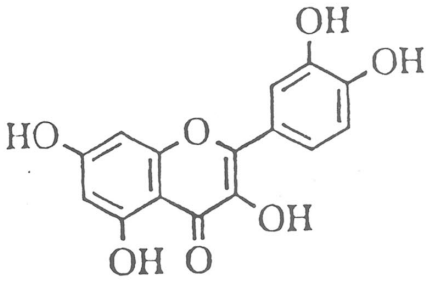
The structural composition of the flavonols depends to a large extent on the environmental factors. Over 200 aglycons have been described so far, among them quercetin, kaempferol, myricetin, and isorhamnetin as the most extensively distributed species.[31] In many instances flavonols appear as glycosilated species, where as a rule a *o*-glycosidic bond is formed through hydroxyl group at the position 3. Hence, monoglycosilated structures debut as dominant in the following order[32,33] 3-glucosides, 3-galactosides, 3-rhamnosides and 3-glucuronides, provided that glucose, galactose, rhamnose, or glucuronic acid



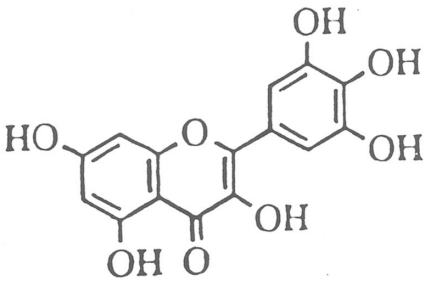
Anthocyanidin structure



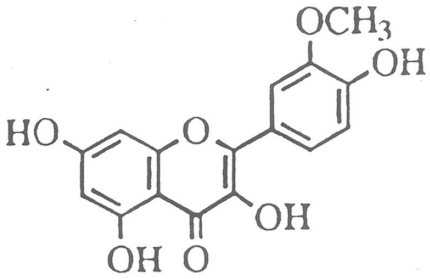
Kaempferol



Quercetin



Myricetin



Isorhamnetin

FIGURE 4. (A) Common structure of anthocyanidins. Delphinidin: R_1=R_2=OH; Petunidin: R_1=OCH_3, R_2=OH; Malvidin: R_1=R_2=OCH_3; Cyanidin: R_1=OH, R_2=H and R_1=OCH_3, R_2=H (B) Representative structures of flavonols.

are attached to these structures. The complete classification of mentioned structures demands the knowledge of the nature of a or b aglycon sugar bonds as well as the optical configuration of the involved sugar (dextro or levo). As a rule, D-configured sugars, normally glucose, galactose, xylose, and glucoronic acid, participate in the β bonds, while the α bonds are made of L-sugars such as arabinose and rhamnose. An example of this arrangement can be appreciated in the composition of flavonols from apples,[34-36] which are constituted by quercetine glycosides, such as α-L-arabinofuranoside, β-D-galactopyranoside, β-D-glucopyranoside, α-L-rhamnopyranoside, and β-D-xylopyranoside. Both flavonols and flavones (which are described later) have been an object of bibliographical reviews and investigations that fully document their composition and properties.[37,38]

Based on the bibliographical information, it can be established that glycosilated structures of quercetin and kaempferol are the most abundant in plant kingdom. Subsequently, rutin, quercetin-3-rutinoside, and kaempferol-3-rutinoside constitute the most representative structures of this type of compound. The Figure 4B lists the principal flavonoles that are found in foods of vegetable origin.

F. Flavanols (flavan-3-ols)

The flavan-3-ol structures constitute one of the most commonly distributed flavonoid families in nature.[31,39] Within their structural framework, it is convenient to distinguish between the monomer units corresponding to (+) catechin and (-) epicatechin structures and oligomer structures of the same, known as procyanins. When it comes to the latter, the most relevant structures correspond to the so-called B1, B2, B3, B4 procyanins constituted by dimeric associations of (+) catechin and (-) epicatechin. Also, the polymeric structure formed by the previously mentioned monomers compose the base of tannins designated as condensed. From the structural point of view, one of the most outstanding characteristic of flavanols is that generally they are distributed in the plant kingdom as aglycones; that is to say, they lack glycosilated forms. Lastly, let us indicate that this structural arrangement is one of the most common in the plant kingdom. Consequently, Figure 5 enumerates in detail monomeric structures of (+) catechin and (-) epicatechin, as well as the structures of the principal oligomeric procynidins such as B1, B2, B3, and B4.

G. Flavanones

The flavanones constitute a minority group within the flavonoids, and as such are distributed in smaller quantities, except for the citric fruits, a group of fruits in which they constitute a polyphenolic majority. With respect to aglycons, the most frequent type corresponds to hesperetin, naringenin, and narirutin, whose structures are shown in Figure 6A. While in the citrus fruits flavanones are found fundamentally as aglycones, in other plants the glycosilated structures constitute the prevailing species. The glycosylation takes place at the position 7 and involves, as a rule, monosaccharide and disaccharide sugars composed of glucose and rhamnose, where the existing 1–6 and 1–2 associations have resulted useful in establishing differences among various citric fruit families from different crops.[40-42]

H. Flavones and isoflavonoids

The flavones constitute the least representative polyphenolic group in foods. The most widely distributed aglycons correspond to apigenin and luteolin, whose structures are shown in Figure 6B. Like flavonoids, flavones can simply appear as glycosylated structures. With respect to isoflavonoids, it is necessary to indicate that they constitute a polyphenolic minority group in foods on equal terms with flavanones and flavones. The isoflavonoids are characteristic of the leguminous plants and are found broadly distributed in similar foodstuffs.[43, 44] Like the immense majority of these types of polyphenolic compounds, these structures also appear glycosylated. Figure 6C shows four of the most representative types of aglycons frequently distinguished in polyphenolic compounds.

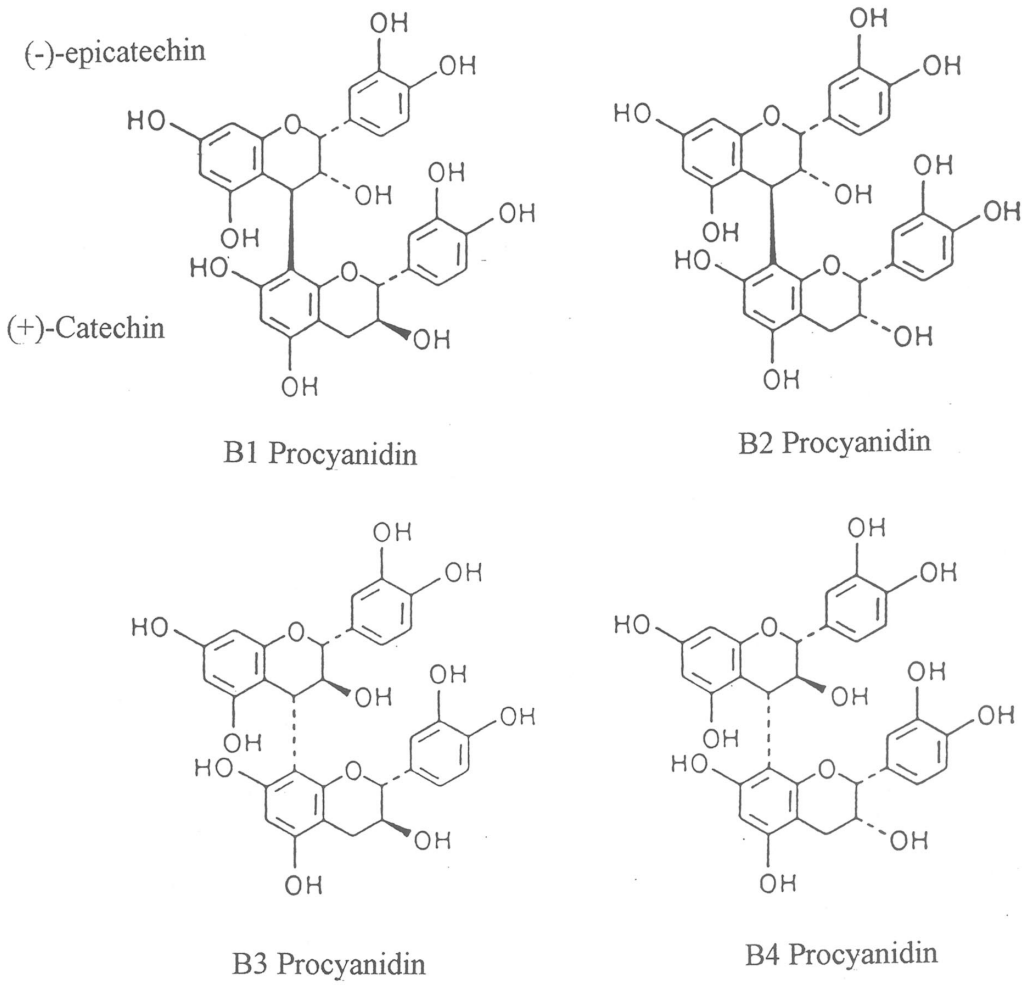
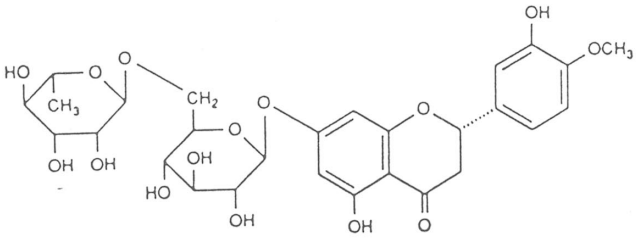
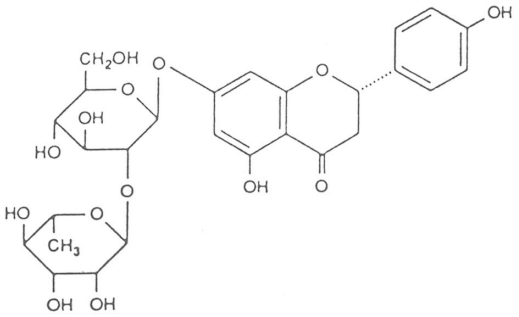


FIGURE 5. Representative structures of flavanols (Flavan-3-ols).

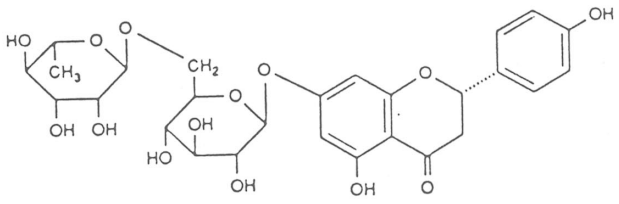
A



Hesperidin



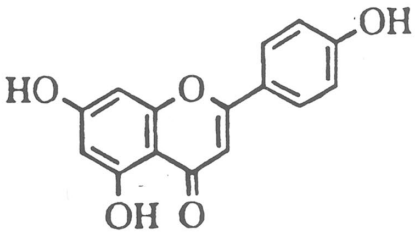
Naringin



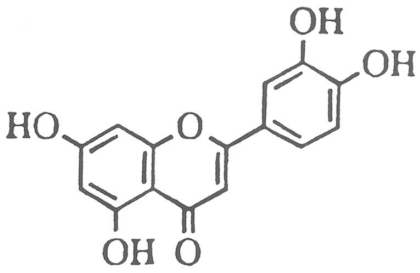
Narirutin

FIGURE 6. Representative structures of (A) flavanones; (B) flavones; (C) Isoflavonoids.

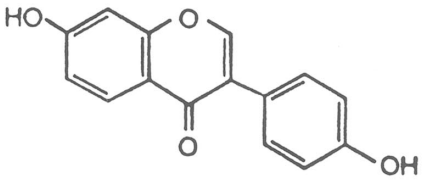
B



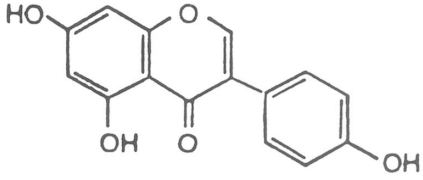
Apigenin



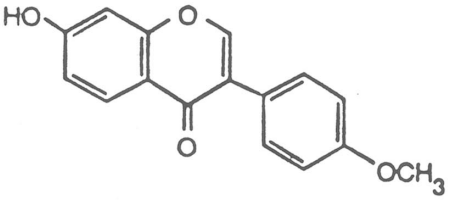
Luteolin



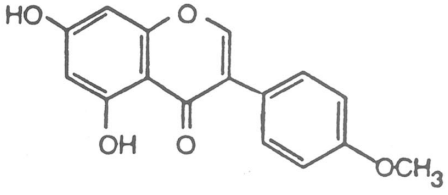
Daidzein



Genistein



Formononetin



Biocanin-A

I. Tannins

In general, the term tannin refers to a fraction of polyphenolic compounds with given properties, whose fundamental characteristic is high molecular weight. These structures possess a high association capacity with other essential biological polymers such as proteins and carbohydrates. Based on this property, they have been traditionally assigned an antinutrient quality.

In the plant kingdom, tannins are usually found in two large metabolic modalities: the hydrolyzable and condensed tannins. Hydrolyzable tannins are simpler structures built from units of free or esterified gallic acid, also known as gallotannins. The condensed tannins, commonly called proantocyianins, are natural polymers composed of flavan-3-ol units. The most recurrent of these structures are proantocyanins that are based on (+) catechin and (-) epicatechin, which in turn form structural units. One can clearly appreciate in Figure 7 how tannin acid structures and polymeric proantocyianins constitute the most representative example of each tannin group.

III. PROPERTIES OF POLYPHENOLIC COMPOUNDS

Polyphenolic compounds are a complex group of substances widely distributed in plants, and they are a common component of the human diet. These compounds, in particular flavonoids stems, have been asigned such diverse biological properties as their ecological role,[10] antioxidant, antiinflamatory, antiallergic, and anticarcinogenic activity,[8] physiological effects,[45,46] employment as markers in taxonomic studies,[47] and their properties related to the food quality.[48] In continuation we shall describe briefly the most relevant attributes assigned to polyphenolic compounds.

With respect to their role as plant metabolites, it needs to be mentioned that polyphenolic compounds participate in plant metabolism, being responsible for their growth and exhibiting determined interactions with other live organisms. Among other functions we may cite safeguarding plants against infections and aggression by other microorganisms. Overwhelming evidence suggests that these compounds are very important to the life cycles of plants. Likewise, the polyphenolic compounds can serve as screens against UV radiation, a crucial function backed by the increase of their levels when plants are subjected to high doses of radiation.[49-55]

On the other hand, the polyphenolic compounds are potent antioxidants, interceptors of free radicals, metal chelation agents, and inhibitors of lipid peroxidation. At the same time, these compounds exhibit determined properties related to the prevention of oxidation, inflammation, allergies, and cancer.[56, 57] Correspondingly, the flavonoids reduce arterial pressure and regulate cardiac rhythm. This effect can be easily explained by flavonoids high antioxidant activity that protect the internal lipid-protein lining of the arteries. Consequently, they avert the onset of arteriosclerosis and facilitate the relaxation of cardiac muscle, thus lowering cardiac output and with that a drop of arterial pressure. In a related topic, certain investigations suggest the direct role of the free radicals in triggering cancer with subsequent trials demonstrating the ability of flavonoids to trap these radicals and therefore prevent the advancement of the neoplasm. The biological properties of quercetin, by and large, the most exhaustively studied flavonoid, vouch decidedly for each previously cited assertion.[58-66]

With respect to further physiological properties of polyphenolic compounds, it needs to be emphasized that the presence of hydroxy groups permits polyphenols to associate with proteins and carbohydrates, thus reducing the availability of the macro nutrients. On the other hand, the solubility of these compounds is going to predetermine conclusively their physiological effects. The soluble polyphenols are absorbed and metabolized in the gastrointestinal tract, whereas the insoluble ones are simply expelled with the feces.[67]

In the course of discussion concerning polyphenolic taxonomic properties, it merits mentioning that their idiosyncratic traits constitute one of the most important aspects related to identification of organic composites. This argument derives from the great variety and complexity assumed by their filial structures in plant kingdom, where it is possible to establish determined relationships be-

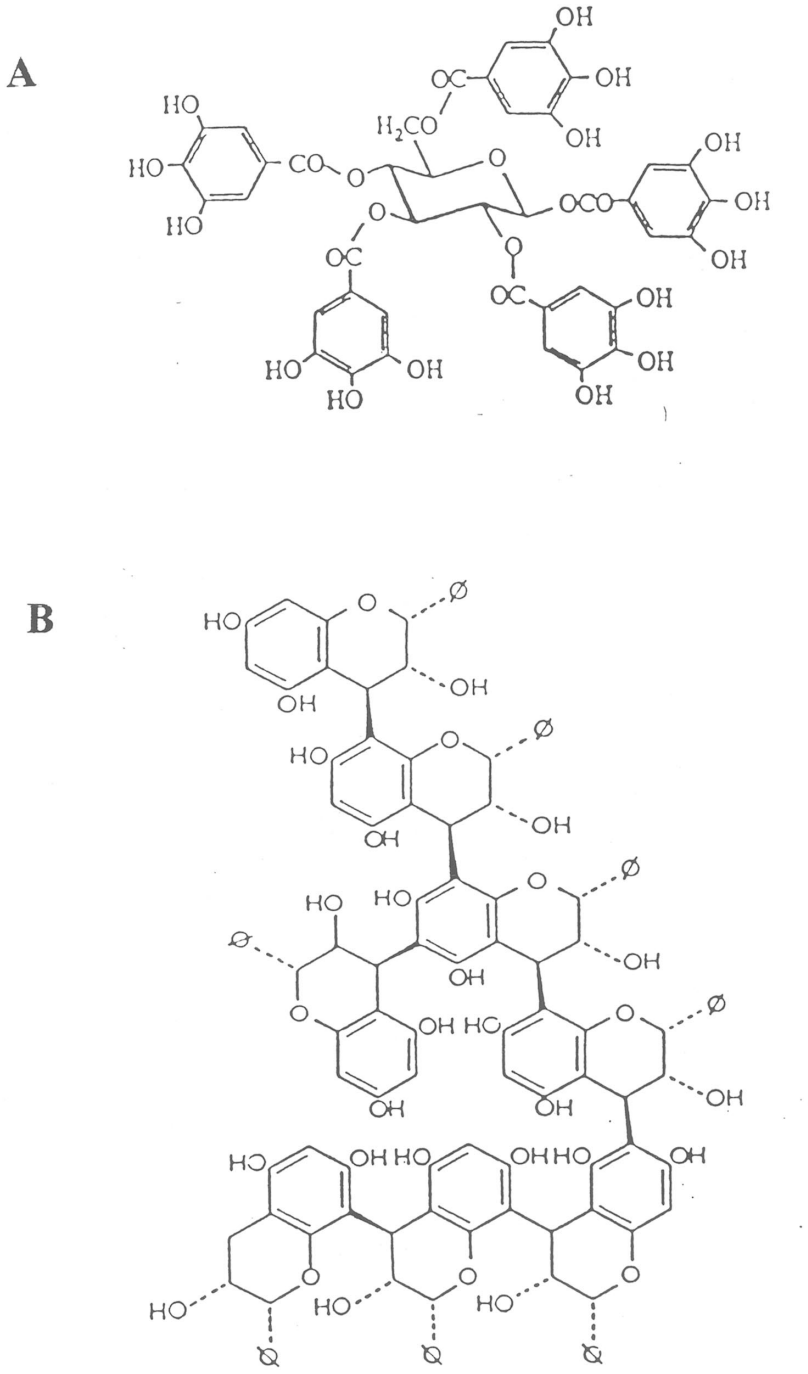


FIGURE 7. Representative structures of (A) hydrolysable tannins (tannic acid); (B) condensed tannins (proanthocyanidin).

tween the parts of the plant and/or the entire plant and the specific polyphenolic composition.[22,68-72] Thus, for example, the numerous varieties of Vitis vinifera (grapes) are classified into two large groups in the function of the presence and/or absence of methylated antocianidines.[73] Other thoroughly studied case concerns citrus fruits that have been classified according to the differences in their flavanone composition.[74] This valuable property of the polyphenolic compounds as taxonomic markers of given fruit matrixes has accounted for their importance as signets that vouch for fruit's quality. In this sense, the precise composition of flavanons has allowed to distinguishing between pure orange juices and those adulterated with grapefruit.

Finally, let us indicate that flavonoids can contribute to the quality of the fruit in many ways, such as color and the aroma of the same.[75-78] Thus, in fruits such as apples the flavonoids contribute to the texture as well as to the formation of the brown color that manifests itself after these fruits are diced or stored for a long time. The latter fact is the result of the enzymatic oxidation catalyzed by polyphenolic compounds that eventually turn into quinones, a polymerized structure responsible for the brown color. This modification implies not only economic disadvantages but transcends in an alteration of the color, texture, and nutritional value of the fruit. Additionally, the flavonoids participate in the formation of given sediments that yield undesirable suspensions in wines and juices. The formation of these solids consists fundamentally on associations between proteins and catechins.

IV. DISTRIBUTION AND CONCENTRATION OF POLYPHENOLIC COMPOUNDS IN FOODS

As was already mentioned, the polyphenolic compounds are characteristic of many plants, and they are found practically in all the foods of vegetable origin, by such constituting an integral part of our diet. However, it is not easy to establish a polyphenolic distribution if we take into account that the quantity of phenols depends on the site of their ultimate accumulation in the fruit as well as on the type of the fruit we wish to study. This variability in terms of distribution and concentration is essentially due to many factors such as climatic, genetic, and cultivation treatment.[79,80] Likewise, the foods of vegetable origin that have had undergone a certain technological treatment or more specifically food processing, the qualitative and quantitative variability is intimately related to the nature of the mentioned process.

On the other hand, it was possible to establish determined appraisements or draw general conclusions by determining phenolic distribution in the given plant and investigating debuting structures. In this sense, certain families of flavonoids such as flavonols, flavanols, or their monomers (flavan-3-ol) and polymers (proantocyanins), as well as antocyanins, may be quantitatively predominant in one type of foods, while flavones, flavanones, and chalcones may be quite specific to another type.

Inasmuch as that due to previously exposed considerations, the consent arose to verify the presence of polyphenolic compounds in foods and, should it be affirmative, calculate quantitative levels of existing structures. Based on these deliberations, the available bibliographical information has been construed, taking into account the different types of foods such as fruits, vegetables, and legumes. Likewise, it was seen convenient to stack wine with grapes given the close relationship existing between both products and the nature of the most relevant articles published in this regard. Finally, the distribution of polyphenolic compounds in tea samples has been contrived, taking into account the available bibliographical information. In continuation, we discuss the distribution of polyphenolic compounds in each of the previously mentioned food groups.

A. Grapes (*Vitis vinifera*) and Wines

The principal families of polyphenolic structures found in the grapes as well as in the wines are constituted by phenolic acids and their corresponding esters such as[27,81-87] flavanoles, catechins, proantocyianins,[27,81-84,86-89] plus flavonoles and their glycosilated structures.[27,81,83,84,86,87,90] The acids fraction is constituted by hydroxybenzoic as well as hydroxycinamic acids.

In reference to the former, let us emphasize that the principal representative structures are gallic and vanillic[83-85] acids. The family of hydroxycinimic acids is represented by caffeic, coumaric, caftaric, and coutaric acids.[83-85] Likewise, the *p*-hydroxybenzoic and ferulic acids constitute the most visible hydroxybenzoic and hydroxycinamic group of structures, respectively. The latter also form a part of the total acid fraction.

Within the flavan-3-ol group, (-) epicatechin[84] and especially (+) catechin as well as their corresponding B1, B2, B3 procyanins are the most widely available structures. Finally, the relative ratio of flavonoles and their corresponding glycosides constitute a valuable contrivance for the determination of quality and age of wine while helping establish their heterogeneity. Quercetin and their glycosides are the most abundant flavonoles, with myricetin present in smaller concentration and kaempferol practically absent in red wine, while quercertin and their glycosides had proved to be characteristic of white wine. Table 3 illustrates the polyphenolic composition and concentration found in grapes and wine samples. The most relevant information with respect to the distribution of polyphenolic compounds in different parts of grapes (skin and seed) as well as in grape juice allows establishing a relationship between the presence of polyphenolic compounds and the parts of the grapes where such structures are found.[82] In effect, the acids are proper of skins and juices, being the caffeic and coumaric acids esterified with tartaric acid as the most characteristic of the juice. Furthermore, this composition happens to reflect an incomparably complex matrix. The seeds contain gallic acid and flavan-3-ol structures, consequently constituting the simplest part of the grape with clear allusion to polyphenolic composition. This fact is supported by the additional studies carried out on grape seeds where (+) catechin, (-) epicatechin structures and their respective proantocyanins have been ostensibly identified.[88]

Spanos and Wrostald[27] have studied polyphenolic composition of Thompson grapes in detail as well as the influence of different parameters on the same, such as the presence of SO_2, storage, and the type of processing. Thus, in the presence of SO_2, the most frequently found polyphenolic compounds were caffeic and caftaric as representative of hydroxycinnamic structures with gallic as hydroxybenzoic acids, (+) catechin and B1 procyanin representing flavanols and rutin glycoside of quercetin. Similarly, other bibliographical studies and those reflected in Table 3 demonstrate the presence of all cited polyphenolic structures preferentially found in grape samples.[81]

On the other hand, wine is one of the most complex mediums for the determination of polyphenolic compounds, and, strictly speaking, their matrices differ accordingly depending on the grapes employed in their elaboration, also given the fact that the wine is a product of a complex fermenting process called vinification**.** It is well known that polyphenolic compounds contribute to the aroma and they can be indicative of the grape variety, the sum of growing conditions, and fermentation treatments.[84] Subsequently, the standard concentration of several polyphenolic compounds has been employed in the determination of wine variety, its age, and ultimately the quality of the same.[86] The previously commented properties of wine with well-established benefits to health in general motivates the growing interest in the study of polyphenolic compounds concerning these types of samples.

The origins of most relevant bibliographical studies dealing with wine samples date back to 1986, supplemented by an almost ceaseless flow of additional information that continues to be supplemented until now.[84-87, 91] The studies that surged in the literature over this period have been grouped in Table 3, where the characteristics of polyphenolic structure and the corresponding host, concentration, and type of wine are conveniently summarized. As can be deduced from the mentioned table, the most current and relevant wine bibliography faithfully confirms the presence of all previously cited polyphenolic structures.

Revilla et al.[90] identified and quantified quercetin, myricetin, kaempferol, and isorhamnetin in different samples of white and red wines varieties, successfully describing a relationship between the presence of a given flavonol and the color it reveals in the studied wine. In this sense, the quercetin was the only identifiable flavonol in white wines that has proven that there no other

TABLE 3
Distribution and Concentration of Phenolic Comopounds in Grapes and Wines
Grapes (Vitis vinifera)

Sample	Structure	Analytes	Concentration	Ref
Cencibel (Peel)	Hydroxybenzoics Hydroxycinnamics Flavanols	Gallic acid Caffeic acid Coumaric acid	NP	82
Cencibel (Seeeds)	Hydroxybenzoics Flavanols	Gallic acid Catechin Epicatechin Procyanidins		
Juice	Hydroxybenzoics Hydroxycinnamics	Gallic acid Caffeic acid		
Seeds (Chardonay)	Flavanols	(+)Catechin (-)Epicatechin Procyanidin B1 Procyanidin B2 Procyanidin B3 Procyanidin B4 Procyanidin C1	3012 mg/kg 4175 mg/kg 708 mg/kg 767 mg/kg 679 mg/kg 592 mg/kg 979 mg/kg	88
Grapes Thompson	Total phenols	Quercetin	260 mg/L	222
Grapes Flame			850 mg/L	
Grapes negras			920 mg/L	
Grapes Thompson (processed juice)	Hydroxybenzoics	Gallic acid Hydroxybenzoic (der.)	0.7 mg/L 2.4 mg/L	27
	Hydroxycinnamics	Caffeic acid Ferulic acid Coumaric acid	0.4-2.3 mg/L 0.2 mg/L 0.3 mg/L ND	
	Flavanols	Rutin Quercetin glycoside	5.7 mg/L 3.5 mg/L	

TABLE 3 (continued)

Sample	Structure	Analytes	Concentration	Ref
Grapes	Hydroxybenzoics	Gallic acid Protocatechuic acid	NP	81
	Flavanols	Procyanidin B3 Catechin	NP	
	Flavonols	Quercetin Myricetin Isoquercitrin Rutin	NP	

Wines

Sample	Structure	Analytes	Concentration	Ref
Red and white wines	Flavonols	Quercetin Myricetin Kaempferol Isorhamnetin	10-2000 µg/L 500-10720 µg/L 50-200 µg/L 75-170 µg/L	90
Sherry	Hydroxybenzoics	Gallic acid Protocatechuic	7.7-18.0 mg/L 4.8-10.0 mg/L	85
	Hydroxycinnamics	Caftaric acid Cutaric acid Caffeic acid Ferulic acid	4.4-19.2 mg/L 3.0-6.0 mg/L 4.5-12.8 mg/L 4.0-5.3 mg/L	
	Flavonoids	Catechin	3.77-16.5 mg/L	
White wines	Hydroxybenzoics	Gallic acid Protocatechuic	2.60 mg/L	140
	Hydroxycinnamics	Caftaric	46.76 mg/L	
	Flavonoids	(+)Catechin (-)Epicatechin	7.91 mg/L	
Wines (varieties, origins)	Total phenols	Gallic acid	260-2860 mg/L	146

TABLE 3 (continued)

Sample	Structure	Analytes	Concentration	Ref
Cabernet	Total phenols	Quercetin	1800 mg/L	222
Petite			3200 mg/L	
Orvieto	Hydroxybenzoics	Gallic acid	52-480 mg/L	83
Gewürt		Protocatechuic	14-61 mg/L	
Barbera		Vanillic acid	4-133 mg/L	
Barolo				
	Hidroxycinnamics	Caffeic acid	2-46 mg/L	
		Chlorogenic acid	1-4 mg/L	
		Ferulic acid	1-7 mg/L	
	Flavanols	(+)Catechin	7-48 mg/L	
	Flavonols	Quercitrin	3-6 mg/L	
		Rutin	17 mg/L	
	Tannins	Tannic acid	4000-34000 mg/L	

N.P. Not published

flavonols present in this kind of wine. On the contrary, the red wines were rich in myricetin, nonetheless they contained other flavonole structures as well. Guillen et al.[85] have found distinct levels of phenolic acids such as hydroxibenzoic and hydroxicinnamic, complemented by neutral phenols in different samples of Spanish wines. The caffeic, caftaric, feruilic, and cutiric acids that represented hydroxycinnamic structures and (+) catechin that stood for flavan-3-ol structures were the most dominant polyphenolic compounds in these types of samples.

On the other hand, the available literature speaks of comparative studies between juice and wine samples taken from the above-mentioned grape samples.[84] The corresponding results from these types of studies have revealed that both matrices share a similar polyphenolic composition, except for tyrosol, which was present in wines but absent in the respective juices. This anomaly might be a consequence of the fermentation process of grapes during wine elaboration. Also, in both studied matrices, hydroxicinnamic acids turned out to be a majority, followed by flavonoids and hydroxibenzoic acids, mainly gallic and protocatechuic. However, quantitatively speaking, the vinification process is responsible for a considerable loss of hydroxycinnamic acids (20%) and a net gain of flavonoid fraction, while the levels of hydroxibenzoic acids were maintained at constant rates.[84]

Nonetheless, at the quantitative level, it was possible to establish that the polyphenolic content in red wines (740 to 2900 g/l) is greater than in white wine (260 to 700 mg/l) or rose wine for that matter, according to a Sato et al.[91] study carried out on thirty one different wine samples. However, in these studies, the quantitative levels corresponding to polyphenolic compounds were earmarked as "total polyphenols" ,and that is why the differences among the levels could not be attributed to a given polyphenolic compound.

B. Fruits

The fruits constitute, without doubt, one of the principal sources of polyphenolic compounds.

Whether fresh or processed as juices, nectars, or marmalades, the fruits form an integral part of our diet, bestowing on it crucial vitamins, oligoelements, and fiber as a part of daily nutritional requirements.[92] In this sense, fruits such as apples make up for over 8% of the total food intake in the Dutch diet.[31] Also, 30% of the consumed polyphenols originate from processed fruits converted to juices. The benefits, potentiated by the specific polyphenolic properties, add to the quality of the fruit features such an aroma, color, texture, and constitutes a principal reason for resurgence of numerous studies carried out on these types of samples, retroactive to the last 10 years. In continuation, we detail the polyphenolic composition of different types of fruits as well as the concentration levels akin to each compound as it debuts in that class of nutrients. We also cite bibliographical references analogous to each type of the fruit. In the first we try to describe fruits from each family integrating different species as in example of apples, as it a most commonly represented and consumed fruit variety. The information obtained from pears samples is discussed simultaneously with that concerning apples, given that they share qualitative similarities, particularly when it comes to structural polyphenolic configuration. The polyphenolic composition of other types of fruits, also denominated bay fruits, or rosaceae, which registers such varieties as bilberries, strawberries and raspberries are described jointly in the last section of this article.

1. Citrus Fruits

Citrus fruits constitute a very important dietary apportionment that make up for almost a total of fruits consumed in an occidental diet. When we refer to these fruits, we usually speak about oranges (*Citrus sinensis* and *Citrus aurantium*), lemons (*Citrus limon*), and to lesser extend tangerines (*Citrus reticulata*), grapefruits (*Citrus paradise*), and limes (*Citrus limmeta*). Despite the existing great diversity and variety in this family of fruits and an ample range of crops, these fruits are normally characterized by the presence of flavanons that claim a status of dominant polyphenolic structures (almost exclusive to this type of species). Surprisingly, these polyphenolic compounds have never been seen so abundant in other types of fruits, being practically absent in related species. Nevertheless, flavanols and flavonols can be present in citric fruits in the form of polyphenolic structures, even though such a presence is rather unlikely.[93] That is why the participation of flavonoids in citrus fruits may be limited to three structural groups: flavanons, flavonols, and flavanols, and the flavanons can exist in a methoxylated or glycosylated form. Table 4 loosely enumerates the most relevant information deduced from the available bibliography concerning their distribution, and should it be necessary the concentration of polyphenolic compounds normally found in this type of fruits.

Based on the available bibliographical information, it can be assessed that citrus juices were studied quite extensively by a number of scientists.[94-101] The recently published papers have proven beyond doubt the presence of flavanones in different parts of the fruit as well as in the whole fresh fruits. The most dominant polyphenolic compounds are methoxylated and glycosylated flavones.[80,102-105] The presence of these structures accompanied by hydroxicinamic acids have been also observed in the samples of jam,[106,107] in juices, and in commercialized nectars.[108]

The first important studies concerning orange and tangerine juices were carried out by Sandra et al.[95] Consequently, it was shown that all of the studied samples had the same chromatographic profile, which was that of flavanones. In the studies related to this type of samples,[94-96,100] the evidence demonstrated that nobiletin (methoxylated flavanone) was the prevailing polyphenolic structure, independent of the investigated orange variety. On the other hand, the citrus fruit flavanones can exist in a glycosylated form, as mentioned, where naringin, hesperidin, and narirutin seem to be the most abundant species. Thus, naringin and neohesperidin were detected by Cancalon and Brian[98] in grapefruit juices. Nonetheless, they were missing in orange juices. A recently published study[101] on the quantitative determination of predominant flavonoids in concentrated orange and grapefruit juices has also established that the principal difference between both citrus species consists in the presence of naringin in grapefruit.

TABLE 4
Distribution and concentration of Phenolic Compounds in Citrus Sampes

Sample	Structure	Analytes	Concentration	Ref
Orange and mandarin juices	Methoxylated flavones	Nobiletin	3.1-11.9 mg/L 17.0 mg/L	95
Orange juice	Methoxylated flavones Flavanones glycosides	Sinistein Nobiletin Narirutin Hesperidin	Not published	100
Processed orange juices	Methoxylated flavones	Nobiletin Sinensetin	Not published	96
Orange juice	Methoxylated flavones	Nobiletin Sinensetin	4.2-7.0 mg/L	94
Concentrates juices (orange and grapefruit)	Flavanones	(Orange)Narirutin 24-30 mg/100g Hesperidin:120-150 mg/100g (Grapefruit)Narirutin 62-68 mg/100g Naringin: 200 mg/100g		101
Orange juice	Flavanones glycosides	Narirutn Hesperidin	18.5-120.7 mg/L 129.5-364.9 mg/L	97
Orange juice	Methoxylated flavones	Sinistein Nobiletin	Not published	99
Mandarin juice	Flavones Flavanones	Naringin Neohesperidin	Not published	102
Commercial orange juices	Cinnamic acids	Ferulic acid: 0.54 mg/L Chlorogenic: 2.0 mg/L		108
	Flavonols	Rutin: 1.22 mg/L		
	Flavone glycoside	Apigenin-7-O-glucoside: 7.82 mg/L		
Orange juice	Cinnamic acids	Caffeic acid Coumaric acid Ferulic acid Sinapic acid	Not published	109

TABLE 4 (continued)

Sample	Structure	Analytes	Concentration	Ref
Commercial orange jams	Flavanones	Neoeriocitrn: Naringin: Neohesperidin:	50-80 mg/kg 80-115 mg/kg 60-95 mg/kg	107
Citrus jams (orange, mandarin, lemmon and grapefruit)	(Lemmon)Erocitrin (Orange) Hesperidin/Neohesperidin (Grapefruit) Naringin (Mandarin) Hesperidin		Not published	106
Fresh oranges (epicarp, mesocarp and endocarp) and juices	Flavonols Flavones Flavone glycosides	Kaempferol, rutin Apigenin, luteolin Hesperidin	Epicarp: 1.8-10.9 mg/g Mesocarp: 14.4-27.0 mg/g Endocarp: 0.3-0.9 mg/g	80
Mandarin and Grapefruit	Flavanona	Hesperidin	Not published	104
Orange Mandarin Grapefruit	Flavanones Flavones Coumarins	Not published	Not published	103
Lemmon	Hydroxycinnamics Flavonols Flavanones	Chlorogenic acid: 5.8 mg/L Rutin: 24.5 mg/L Hesperidin 1890 mg/L		83
Orange, Lemmon Grapefruit	Flavonols	Quercetin Myricetin	3.4-7.4 mg/L <0.5 mg/L	65
Orange (varieties)	Flavone glycosides	Hesperidin: 130-220 mg/L Narirutin : 13-30 mg/L (between varieties)		104
Grapefruit (varieties)	Flavanones	Narirutin Naringin Neohesperidin	12-1188 mg/100g 13000 mg/100g 14-274 mg/100g	104

Furthermore, these authors have obtained relatively high values of hesperidin and narirutin in orange, whereas narirutin and naringin levels were higher in grapefruit, respectively, as can be seen in Table 4.

Ooghe and et al.[97,99] have studied exhaustively the composition of flavonoids (glycosylated and methoxylated flavanones) in different varieties of orange with the purpose of establishing differences among them, and to determine the real polyphenolic composition in related samples. Based on this research, the authors suggested possible contamination profiles as applied to polyphenols of glycosylated flavanones variety in oranges samples, because naringin was missing (Table 4). With the purpose of establishing a rigorous polyphenolic composition and to detect potential contaminants of orange juice, Castle et al. studied methoxylated flavanones,[102] noting that processing techniques did not affect the composition of the mentioned compounds. In the same study the presence of naringine in oranges that belonged to *Citrus aurantium* species was also documented.

Fernandez de Simon et al.[108] have identified and quantified different polyphenolic structures in commercialized orange juices such as hydroxicinamic acids with ferulic, caffeic, and chlorogenic species and flavonoles such as rutin and glycosylated structures of apigenin and luteolin. The hydroxycinamic acids in orange juices were also observed by Rouseff et al.[109]

The studies on the distribution of polyphenolic compounds in jams from citrus fruits[106, 107] have documented the presence of flavonoids in these products where their concentration ranged between 60 and 115 mg/kg.

On the other hand, it is possible to find studies in the current bibliography dealing with the identification and/or quantification of polyphenolic compounds in fresh citrus fruits samples.[80,102-104] Thus, for example, Nogata et al.[80] have carried out identification and quantification of 25 flavonoids in two species of citrus fruits (*Citrus unshiu* and *Citrus grandis*) and established the presence of the same in different matrices that compose the fruit: epicarpium, mesocarpium, and endocarpium, as well as in the corresponding juice samples.[69] In both species, the highest concentration levels of flavonoids were found in the mesocarpium, while flavonoles and their glycosilates were located in the epicarpium.

Recently, the influence of the variety, the ripeness, and the geographic location on the quantitative levels of the predominant flavanones (narirutin, naringine, and neohesperidin) have been studied exhaustively in *Citrus grandis* and *Citrus paradise* fruits that represent grapefruit varieties. As seen in Table 4, naringin was the most prevailing flavanone in both varieties, although *Citrus paradise* contained higher amounts of flavanones than *Citrus grandis.* As far as ripeness is concerned, it was demonstrated that its degree meant a lesser quantity of polyphenolic structures in both varieties. The quantitative levels of each favanon originating from different varieties proved to be very variable, due fundamentally to climatic contrasts and the growth rate which are the very same variables that were previously shown symptomatic to this type of structures.

From the available information concerning citrus fruits, it can be established that naringin is the most representative flavanone in the grapefruit, neohesperidin in the orange, and eriocitrine is typical to the lemon. It is also important to indicate that naringin appears in some orange varieties as well, henceforth, its exclusion from these types of fruits cannot be fully validated.[31]

2. Apples (Malus domestica)

The apples, as previously mentioned, contribute a high percentage of polyphenols within a global estimation of these compounds in daily diet. Thus, in the Dutch diet apples, red wine, tea, and onions are the nutrients that contain the greatest quantities of polyphenolic compounds.[31] Furthermore, if we take into account that the color as well as the aroma are properties related to the quality of the fruit, which in turn is linked to the type of polyphenolic compounds, it is not surprising that polyphenols from apples are the most extensively studied species.

Although the first relevant studies concerning apples can be traced back to the late 1980s, nevertheless, the serious investigation concerning their polyphenolic composition in view of different varieties did not surge until 1990, and that is how it

stands today. Like with other foods, the effort of determining perfect storage technique or an ideal preservation method had tremendously benefited the knowledge about these compounds. With respect to the polyphenolic structures present in apples, it can be said that these fruits (especially the skin) constitute one of the most complex matrices, featuring distinct structures made of different polyphenolic species. The polyphenolic composition of apples could be established in a general way, but still quite rigorously, on the basis of hydroxybenzoic[108,110,111] and hydroxycinamic[36] acids,[108,110-117] including flavonoids. The latter fraction is constituted by flavan-3-ol structures such as (+) catechin, (-) epicatechin, and main oligomeric procianidines: B1, B2, B3, and B4,[36,111,115-117,119] chalcones: xyloglucosilated phloretin and phlorizin[35,36,105,108,111,115-117] and by flavonoles with their corresponding glycosides.[35,36,105, 108,115-117]

From the bulk of the results that appear in the literature it can be deduced that the glycosidic composition of this type of sample consists solely glycosides of quercetin: quercetin —3-*O*-galactoside(hiperin), 3-*O*-glucoside (isor-quecetrin), 3-*O*-xyloside (reinoutrin), 3-*O*-arabinoside (avicularin), and 3-*O*-rhamnoside (quercitrin). Likewise, Oleskec et al.[114] identified a new polyphenolic compound whose structure is derived from the phloretin found in the skins of Rhode Island apples. This new compound, present with the already identified phlorizin, is integrated into the total fraction of chalcones akin to **the** phenolic family and was soon denominated phloretin xyloglucoside because of the participating sugar.

Table 5 indicates the distribution of polyphenolic compounds in apples according to the studies undertaken in the last 10 years. The bibliographic results have been listed in relation to the main polyphenolic structures that constitute this type of fruit: hydroxicinamic acids, flavanols, chalcones, and flavonols. Also, the same Table 5 lists the variety of studied apples, the used matrix (skin, pulp, or juice), the identified polyphenolic components, and in specific cases the resulting concentration of the same. Let us cite the study carried out by Spanos and Wrolstad[36] in apple juice from the Granny Smith variety as a representative example of different polyphenolic families found in apples. These authors suggest the presence of numerous hydroxicinamic structures differentiated into free acids (chlorogenic, caffeic, and cumaric) as well as esterified components of the same. Monomeric flavan-3-ol structures such as (+) catechin and (-) epicatechin plus B1, B2, B3, B4 procianidines constituting total flavanol fraction. With respect to the most hydrophobic flavonoids, such as chalcones and flavonols, these authors suggested the presence of two dihydrochalcones (phloretin xyloglucoside and phlorizin) as well as glycosides of quercetin galactoside, glucoside, xyloside, arabinoside, and rhamnoside.

The published works dealing with the comparative studies and assessment of differences between the skins and flesh from distinct varieties of apples[115,116] have jointly confirmed the presence of flavonoles and their glycosides in the skins, while in the pulp this compound was detected only in trace amounts. This fact can be justified by the premise that the synthesis of flavonoles and their glycosides depends on the light.[120] Chlorogenic acid, phlorizin, and catechins are characteristic of skin and pulp, a part of the fruit where chlorogenic acids levels turned out to be as high as 400 mg/kg of fresh fruit. Therefore, the skins reveal a more complex polyphenolic content and, as a rule, higher than the one found in pulps of both matrices. MacRae et al.[115] proved that the highest levels of chlorogenic acid corresponded to the Jereymac variety, while the lowest could be found in the red apple variety. However, the levels of phlorizin were just the opposite when compared with chlorogenic acid levels. Flavonoles were the predominant poliphenolic species in skins, which demonstrated that glycosylated structure of quercetin, also known as rutin, was used as a marker for differentiating between Jerseymac and Spartan varieties.

Perez-Ilzarbe et al.[116] have also shown notable presence of chlorogenic acid in apple pulp, underlining its high levels of up to 320 mg/kg of fresh fruit the in Reineta variety. The flavan-3-ol structures were differentiated into (+) catechin, (-) epicatechin B2 procyanidin, structures that also reflected high levels in skins of red and Reineta apples. In this case, B2 procianidine turned out to be the prevailing procyanidine.[36,117] Also, the juices

TABLE 5
Distribution and Concentration of Phenolic Compounds in Apples
Hydroxycinnamic structures

Matrix	Phenolic compound	Variety	Concentration						Ref.
Pulp	Chlorogenic, caffeic and coumaric acids *p*-Cumarolquinic acid	Granny-Smith McIntosh	Not published						36
Pulp	Chlorogenic, Coumaric, Ferulic and Sinapic acids	Golden Delicious	Coumaric: 25 mg/kg; Ferulic: <0.5 mg/kg; Sinapic: 9 mg/kg; Chlorogenic: 81 mg/kg						110
Peel Pulp	Chlorogenic acid	Red Delicious Spartan Cortland Jerseymac McInthosh Golden Delicious Granvestein Northern Spy	Peel (mg/kg) 91 126 149 121 123 20 61 97		Pulp (mg/kg) 97 242 89 426 268 172 292 319				115
Peel Pulp Juice	Chlorogenic acid Coumarolquinic acid Ferulic glucoside acid Coumaric glucoside acid	Red Delicious Reineta Golden Delicious Green Doncella Granny-Smith	Peell (mg/kg) 42.3 319.8 62.8 83.1 64.8		Pulp (mg/kg) 30.6 204.1 71.7 42.1 0.90		Juice (mg/L) 0.92 6.88 0.09 0.19 9.07		116
Pulp Commercial juice	**(1)** Coumaric acid **(2)** Coumaric acid (der.) **(3)** Chlorogenic acid	Bedan Kermerrien Petit Jaune Judor	**(1)** (μM) 141.5 181.3 33.5 73.9		**(2)** (μM) 26.2 19.6 6.9 6.3		**(3)** (μM) 139.4 702.8 98.5 20.6		111

TABLE 5 (continued)
Distribution and Concentration of Phenolic Compounds in Apples
Flavan-3-ol-structures

Matrix	Phenolic compound	Variety	Concentration						Ref.
Pulp	(+) Catechin (-) Epicatechin	Granny-Smith McIntosh	Not published						36
Pulp	(+) Catechin (-) Epicatechin	Golden Delicious	(+) Catechin :10 mg/kg (-) Epicatechin: 78 mg/kg						110
Peel Pulp	Catechins	Red Delicious Spartan Cortland Jerseymac McInthosh Golden Delicious Granvestein Northern Spy	Peel (mg/kg) 1431 677 549 929 554 590 455 800		Pulp (mg/kg) 186 167 129 300 238 130 143 275				115
Peel Pulp Juices	(+) Catechin (-) Epicatechin Procyanidin B2	Red Delicious Reineta Golden Delicious Green Doncella Granny-Smith	Peel (mg/kg) 5.4 54.7 0.15 33.6 34.1		Pulp (mg/kg) 7.2 22.3 3.9 83.4 21.3		Juice (mg/L) 0.1 0.1 0.3 0.2 1.1		116
Pulp Commercial juice	**(1)**(+) Catechin **(2)**(-) Epicatechin **(3)**Procyanidin tetramer **(4)**Procyanidin Trimer C1	Bedan Kermerrien Petit Jaune Judor	**(1)** 11.5 23.6 2.4 1.5	**(2)** 11.2 137.5 2.6 1.3	**(3)** 11.8 80.0 1.2 0.6	**(4)** (μM) 2.9 19.2 2.0 2.4			111

TABLE 5 (continued)
Distribution and Concentration of Phenolic Compounds in Apples
Estructuras flavan-3-ol

Matrix	Phenolic compound	Variety	Concentration					Ref.
Whole apple (peel and pulp)	**(1)**(+) Catechin **(2)**(-) Epicatechin **(3)**Procyanidin B1 **(4)**Procyanidin B2 **(5)**Procyanidin C1 +tetramer	 Nuestra Señora San Pedro San Juan	**(1)** (mg/kg) 69.5 46.2 36.0	**(2)** (mg/kg) 225.6 193.8 159.7	**(3)** (mg/kg) 22.2 27.5 10.8	**(4)** (mg/kg) 138.6 129.0 90.0	**(5)** (mg/kg) 89.7 119.8 66.5	117

Chalcones structures

Matrix	Phenolic compound	Variety	Concentration						Ref.
Pulp	Phloretin xyloglucoside Phlorizdin	Granny-Smith McIntosh	Not published						36
Peel Pulp	Phlorizdin	Red Delicious Spartan Cortland Jerseymac McInthosh Golden Delicious Granvestein Northern Spy	Peel (mg/kg)	331 203 123 146 145 212 242 87	Pulp (mg/kg)	25 21 11 16 16 17 14 18			115
Peel Pulp Zumo	Phlorizdin	Red Delicious Reineta Golden Delicious Green Doncella Granny-Smith	Peell (mg/kg)	333.6 521.2 89.2 654.5 6.2	Pulp (mg/kg)	9.9 15.3 7.7 16.4 3.0	Juice (mg/L)	1.3 4.5 0.2 0.1 3.1	116

TABLE 5 (continued)
Distribution and Concentration of Phenolic Compounds in Apples
Chalcones structures

Matrix	Phenolic compound	Variety	Concentration		Ref.
Whole apple (peel and pulp)			**(1)** (mg/kg)	**(2)** (mg/kg)	117
	(1) Phloretin xyloglucoside	Nuestra Señora	14.2	82.8	
	(2) Phlorizdin	San Pedro	38.0	93.1	
		San Juan	27.4	196.1	

Flavonol structures

Matrix	Phenolic compound	Variety	Concentration					Ref.
Pulp	Quercetin-galactoside Quercetin-xylose Quercetin-arabinoside Quercetin-ramnoside	Granny-Smith McIntosh	Not published					36
Peel			**(1)**	**(2)**	**(3)**	**(4)**	**(5)** (mg/kg)	115
	(1) Quercetin-ramnoside	Red Delicious	104	117	210	604	546	
	(2) Rutin	Spartan	71	57	244	783	662	
	(3) Quercetin-xyloside	Cortland	80	159	192	735	589	
	(4) Quercetin-glucoside	Jerseymac	128	185	119	554	556	
	(5) Quecetin-galactoside+ arabinoside	McInthosh	361	117	254	696	777	
		Golden Delicious	369	110	195	869	539	
		Granvestein	440	139	145	594	508	
		Northern Spy	661	169	297	924	801	

TABLE 5 (continued)
Distribution and Concentration of Phenolic Compounds in Apples
Flavonol structures

Matrix	Phenolic compound	Variety	Concentration				Ref.
Peel			Peel **(1)**	**(2)**	**(3)**	**(4)** (mg/kg)	116
Pulp	**(1)** Hiperin+isoquercetrin	Red Delicious	91.3	0.4	21.1	69.7	
Juices	**(2)** Rutin	Reineta	78.1	1.1	21.3	57.9	
	(3) Avicularin	Golden Delicious	427.3	3.3	29.1	123.6	
	(4) Quercitrin	Verde Doncella	613.6	15.0	47.7	131.2	
		Granny-Smith	953.7	19.2	105.1	347.5	
			Pulps **(1)**	**(2)**	**(3)**	**(4)** (mg/kg)	
		Red Delicious	0.1	trazas	0.1	0.1	
		Reineta	0.2	0.1	0.1	0.1	
		Golden Delicious	0.3	0.1	0.1	0.1	
		Verde Doncella	1.7	trazas	0.3	0.6	
		Granny-Smith	0.3	0.1	0.2	0.9	
			Juices **(1)**	**(2)**	**(3)**	**(4)** (mg/L)	
		Red Delicious	0.1	0.1	0.1	0.1	
		Reineta	0.2	0.1	0.1	0.5	
		Golden Delicious	0.1	traces	0.1	0.2	
		Green Doncella	0.1	0.1	0.1	0.1	
		Granny-Smith	1.0	0.1	0.3	1.2	
Whole apple	**(1)** Rutin		**(1)** (mg/kg)	**(2)**	**(3)**	**(4)**	117
(peel and pulp)	**(2)** Isoquercetrin+hyperin	Nuestra Señora	11.7	8.1	traces	16.7	
	(3) Avicularin	San Pedro	7.0	4.8	traces	5.8	
	(4) Quercitrin	San Juan	12.5	3.4	traces	33.6	

obtained from the same varieties by manual extraction demonstrated lower quantitative levels of polyphenols than those corresponding to pulps. Finally, let us indicate that Perez-Ilzarbe et al. found the same qualitative composition in all the studied varieties and established polyphenolic differences corresponding to each variety.

On the other hand, we must call attention to the presence of protocatechuic acid as an hydroxybenzoic acid only in determined commercial apple juices[108] and in French varieties of fresh apples.[111] Finally, it is worth mentioning that the study carried out by Suarez-Valles et al.[117] suggested the presence of flavonoles in Spanish varieties that amounted to catechin, epicatechin, B1, B2 procyanins and C1, glycosides of quercetin (rutin, isoquercitrin, avicularin, and quercitrin) plus phlorizin as the only chalcone structure, observing the absence of xyloglucoside phloretin and chlorogenic acid in clear contradiction with the preceding projects.

With respect to samples corresponding to apple products such as commercialized juices and jams,[107,108,121] it calls attention the presence of hydroxycinamic acids such as caffeic and chlorogenic and hydroxybenzoic acids derivatives such as *p*-hydroxybenzoic acid aldehyde in the juices. Likewise, flavan-3-ol structures such as (+) catechin and (-) epicatechin, or flavonols such as quercetin, kaempferol, and phlorizin were quantified in the same samples, while the quantitative levels of the latter compound were very high (in the order of 30 mg/L). The characterization of jam samples in apples showed a simpler polyphenolic composition and practically confined to typical chalcones such as phloretin xyloglucoside and phlorizin.[107]

3. Pears (Pyrus communis)

Pears constitute a popular addition to our diet, although to a lesser extent than apples. They are nonetheless consumed in large quantities both in Western and especially in the Mediterranean regions, normally fresh or as their derivatives such as juices, whose samples contribute a portentous amount of total ingested polyphenols. The study of polyphenolic compounds contained in pears had not been initiated until the year 1980;[112,122] however, a complete characterization is still in the making. This fact is backed by the Olesckec[33] study, which tried to identify pears polyphenols almost 10 years after they published results of the study carried out on apple skin glycosides.[114] This argument could also prove why quantitative data relative to these compounds in pear samples are more limited, especially when it comes to fresh fruits or fruit parts.

The polyphenolic composition of pears could be established on the basis of structural design outlined in continuation. On the one hand, simple polyphenolic structures among which it is necessary to emphasize the presence of arbutin (hydroquinone)[32,107,120,123,124] and hydroxycinamic acids with chlorogenic cumaric (esterified with quinic acid) as the prevailing acids.[32,33,108,112,120,125-128] On the other hand, one can find flavonoids structures composed of flavan-3-ol structures and flavonols with their structurally related glycosides. Thus, (+) catechin and (-) epicatechin were identified by different authors.[32,33,108,112,120,127,129,130] Nonetheless, the current knowledge concerning configurations relative to procyanin structures in pear samples is rather scarce. The flavonoles composition in pear samples includes quercetin, kaempferol, and isorhamnetin glycosides.[32,33,108,122,131]

The results concerning the distribution and concentration of polyphenolic compounds in pears and reported by different authors have been logged in Table 6, where polyphenolic structure as well as the corresponding compound from each fruit variety were also indicated. Spanos and Wrosltald[32] have investigated the composition as well as the quantitative levels of polyphenolic compounds in pear juices corresponding to Comice, Anjou, and Bartlett varieties. Notwithstanding, the same authors have also studied the influence of SO_2 addition, the ripeness, the processing, and storage on these types of fruits in corresponding samples. In the first place, it was observed that arbutin was the simplest polyphenolic structure accompanied by the assortment of hydroxicinamic structures where chlorogenic, caffeic, and cumaric acids could be computed as free hydroxicinamic acids. The cumarolquinic acid and other structures denominated "oxidated cinnamics" also contributed to the total fraction of hydroxycinnamic acids.

TABLE 6
Distribution and Concentration of Phenolic Compounds in Pears

Matrix/Variety	Phenolic Structure	Phenolic compound	Concentration	Ref.
Whole fresh pear and juice	Hydroquinones	Arbutin	Not published	32
Comice, DÁnjou, Bartlett	Hydroxycinnamic acids	Chlorogenic acid Caffeic acid p-Coumaric acid		
	Flavan-3-ol	(+) Catechin (-) Epicatechin		
	Flavonols	Procyanidins B1, B2, B3, B4 Procyanidin C1 and tretramer		
Fresh pears Bartlett, Bon Cheretien, Packingham, Bartlett, Bosc, D´Anjou	Flavonol glycosides	Quercetin-3-glucoside Isorhamnetin-3-glucoside Isorhamnetin-3-rutinoside Isorhamnetin-3-galactoside Isorhamnetin-7-xiloside	Not published	122

TABLE 6 (continued)

Matrix/Variety	Phenolic Structure	Phenolic compound	Concentration	Ref.
Fresh pears Guyot	Hydroxycinnamic acids	Caffeico- quinic acid ester	Not published	33
		Coumaric-quinic acid ester		
	Flavonol glycosides	Coumárico-malic acid ester		
		Quercetin-3-rutinoside		
		Quercetin-3-glucoside		
		Isorhamnetin-3-rutinoside		
		Isorhamnetin-3-galactoramnoside		
Fresh pear Conference	Hydroxycinnamic acids	Caffeic acid	Not published	112
	Flavan-3-ol	(+) Catechin		
		(-) Epicatechin		
Commercial pear juice	Hydroxybenzoic acids	Vanillic derivatives	0.02-0.12 mg/L	108
	Hydroxycinnamic acids	Coumaric acid	0.08-0.12 mg/L	
		Caffeic acid	0.01-0.07 mg/L	
		Chlorogenic acid	1.14-7.88 mg/L	
	Flavan-3-ols	(+) Catechin	Traces	
		(-) Epicatechin	0.40 mg/L	
	Flavonols	Quercetin	0.03-0.14 mg/L	
		Quercetin -3-rutinoside	0.01-0.14 mg/L	
		Quercetin -3-galactoside	0.15-2.46 mg/L	
		Isorhamnetin glicosides	0.03-4.00 mg/L	

Matrix/Variety	Phenolic Structure	Phenolic compound	Concentration	Ref.
Pear jam	Hydroquinones	Arbutin	6.53-113.33 mg/kg	107
	Hydroxycinnamic acids	Chorogenic acid	Not published	
Whole fresh pears (onference Beauré Hardy Comice)	Flavonol aglycones	Quercetin Kaempferol	3.3-10.0 mg/kg <2 mg/kg	142

The (+) catechin, (-) epicatechin, as well as B1, B2, B3, and B4 procyanins were detected only in the course of their treatment with SO_2 and consequently quantified under these specific circumstances. Two quercetin glycosides (rutin and quercetin-3-*O*-galactoside) were also identified, accompanied by three isorhamnetin glycosides whose characterization was impossible to complete thoroughly. This glycosidic fraction, previously investigated by additional authors, especially Duggan,[122] has been believed to contain quercetin-3-*O*-glucoside and 3-*O*-rutinoside, 3-*O*-galactoside, which in fact are isorhamnetin glycosides. The studies undertaken by Spanos and Wrolstad[32] indicated a great influence exerted by varieties and degree of ripeness on the quantitative levels of polyphenols in pears. Thus, prolonged storage was responsible for the partial loss of all polyphenolic structures and the total loss of procyanins. The pear glycosidic composition to which we have referred previously has been investigated recently by Oleszek et al.,[33] who identified four hydroxycinamic structures (two esters of caffeic acid with quinic acid and two esters of cumaric acid with quinic-malic acids) plus up to eight glycosylated flavonols. The quercetin glycosides were composed of rutinosides, glucoside and malonilglucoside, and glycosylated structures, with isorhamnetin as a principal structure were rutinoside, galactorhamnoside, glucoside, malonilglucoside, and malonilgalactoside.

In accordance with previous commentaries and also as indicated in Table 6, the quantitative data concerning polyphenolic compounds in pears are rather scarce. In this sense, the available bibliography deals principally with the quantification of hydroxycinamic acids such as chlorogenic, caffeic, and cumaric proper of commercial pear juices. In all these structures, the lion's share belongs to chlorogenic acid, with concentration values comprised between 1 and 8 mg/L. With respect to flavanol structures, it must be emphasized that (-) epicatechin is the only flavan-3-ol monomer that has been quantified in these types of samples. The fraction of flavonoles in these samples is confined to the glycosilated structures of quercetin and isorhamnetin, which basically constitutes all the existing flavonoids found in the mentioned samples.[108] Likewise, we must indicate when it comes to the determination of polyphenolic compounds in pear jam samples. The current bibliography limits polyphenolic composition to arbutin with concentration values comprised between 7 and 115 mg /kg of sample.[107]

4. Bay Fruits

From the bibliographical point of view, it was not until 1990 and especially in the last 4 years that these types of fruits (generally less consumed) such as strawberries, raspberry, bilberries, and blackberries became an object of relevant investigations concerning polyphenolic compounds.[132-139] The principal reason for this surge of interest stemmed from the need to identify antocianidines, the structures that are in abundance in these types of fruits, being responsible for their blue, red, and purple colors, which was brought to light at the beginning of this article. It was also mentioned that due to this natural feature (the presence of antocyanins in these types of fruits) it attracted the attention of scientists, who started to recognize them as a potential source of food additives with a tremendous commercial viability.[133]

However, the qualitative and quantitative studies of other polyphenolic structures normally found in these types of fruits are still very limited, and the most relevant bibliography does not speak of significant progress until 1993, with the publication of monographic study carried out by Rommel and Spanos on raspberries, an undertaking that constituted a most comprehensive guide to polyphenolic compounds that are present in these types of fruits.[134-136] Table 7 lists the distribution as well as the content of the polyphenolic compounds as copied from the literature found in this type of fruits. Based on the results comprised in Table 7, it can be shown that polyphenolic compounds in these fruits are basically composed of hydroxybenzoic[134,135] and hydroxycinamic acids[136] accompanied by flavonoids, mainly flavan-3-ol,[136] flavonoles,[135] and antocianidines.[132-138] By and large, the three most frequently occurring hydroxybenzoic acids were gallic, *p*-hydroxybenzoic, and protocatequic subsequently identified in fresh raspberries,[130] where the *p*-hydroxybenzoic acid was the most predomi-

TABLE 7
Distribution and Concentration of Phenolic Compounds in Bay Fruits

Sample	Phenolic structure	Phenolic compound	Concentration		Ref.
Fresh raspberries	Hydroxybenzoic acids	*p*-Hydroxybenzoic acid	32-59 ppm		130
		Protocacheuic acid	Not published		
		Gallic acid	Not published		
	Hydroxycinnamic acids	Coumaric acid	trace amounts-14 ppm		
		Ferulic acid	(Total concentration in each structures)		
		Caffeic acid			
	Flavan-3-ol	(+)Catechin	11-33 ppm (including different varieties)		
		(-) Epicatechin	7-112 ppm		
		Gallocatechins	trace amounts-7.4 ppm		
Fresh raspberries and juices	Hydroxybenzoic acids	Ellagic acid	Domestic juices (ppm)	Commercial juices (ppm)	134 135
	Flavonols	Quercetin-3-Glucoronide	28.2	22.4-80.4	
		Total Quercetin	54.4	9.7-88.5	
		Total Kaempferol	118	31.2-211	
			3.5	2.0-6.0	

TABLE 7 (continued)

Sample	Phenolic structure	Phenolic compound	Concentration	Ref.
Raspberries Blueberries Cherries Blackberries Grapes Strawberries	Anthocyanidins	Delfinin Cyanidin Petunidin Pelargonidin Peonidin Malvidin	Not published	132 133
Blueberries	Hydroxycinnamic acids	Chlorogenic acid	Not published	137
	Anthocyanidins	Cyanidin-3-glucoside	110-260 mg/100g frescos (Total anthocyanidin content)	
Fresh cherries	Hydroxycinnamic acids	Neochlorogenic acid Coumaric-quinic acid ester	20-130 mg/100 g 25-130 mg/100 g	138
	Anthocyanidin glycosides	Cyanidin-3-glucoside Cyanidin-3-rutinoside	trace amounts-43 mg/100 g 15-250 mg/100 g	
		Peonidin-3-glucoside Peonidin-3-rutinoside	0.3-1.1 mg/100 g 2-20 mg/100 g	
		Pelargonidin-3-rutinoside	0.3-4 mg/100 g	
		Anthocyanidins	80-300 mg/100 g (brown cherry) 2-40 mg/100 g (white cherry) (Total anthocyanidin content)	

nant phenolic structure in a concentration that ranged between 30 and 60 ppm. Nonetheless, flavan-3-ol structures, such as the (+) catechin, (-) epicatechin, and galocatechin were also quantified in different varieties of strawberries, blackberries, and raspberries.[130] As a rule, these studies demonstrated that the dominant structures were (-) epicatechin, followed by (+) catechin and galocatechin, while the latter structure was found only in trace amounts. In another study, carried out by Romel and Wrosltald, which involved quantification of polyphenolic compounds contained in a commercial raspberry juice, the authors described each of the mentioned structures as they appeared in a given variety.[134-136] After thorough analysis, raspberry juices yielded average values of total elagic acid concentration that ranged between 22 and 80 ppm, where each structural form of elagic acid contributed to the total with a relaive concentration in the order of 7 ppm. On the contrary, the concentration levels corresponding to total elagic acid in the manually extracted juices were lower, with concentration values of 30 ppm. Therefore, the increase of the ellagic acid content was attributed to the nature of specific technological processes that the mentioned fruits might have been subjected to during their transformation.

On the other hand, the quercetin-3-glucoronic glycoside was the most frequently occurring polyphenolic compound in both the manually and industrially extracted juices, with values of 54 and 90 ppm, respectively. Likewise, up to 36 glycosilated structures originating from quercetin and kaempferol were identified in trace amounts. The total quantitative values of quercetin and kaempferol obtained from both freshly squeezed or purchased juice were in the order of 118 and 4 ppm and 211 and 6 ppm, respectively. Consequently, the quercetin was identified as the notably dominant flavonol in all the studied samples.

On the other hand, it is worth recalling that these types of fruits are characterized by a high antocyanins content and that these structures have been studied in depth during recent years by different authors. Thus, Hong and Wrolstad have described different antocyanin structures belonging to six aglycones (delphinidine, cyanidin, petunidin, pelargonidin, peonidin and malvidin), all present in strawberries, raspberries, blackberries, bilberries, and raisins.[132,133] Thereafter, Gao and Mazza[137,138] carried out the identification and quantification of 25 antocyanins in bilberries originating from different crops. The studied total antocyanin content varied in these samples in an interval of 110 and 260 mg/100 g of fresh fruit. Likewise, in other studies conducted by the same authors but concerning cherries from 11 different crops yielded total antocyanins content comprised between 80 and 300 mg/100 mg for fresh dark cherry and between 2 and 40 mg/100 mg for fresh pale cherry.[138]

C.Vegetables

The term vegetable entails all types of foods classified as produce and vegetables. Together with the fruits, minus legumes, they constitute, by and large, the most abundant dietary stores of polyphenols. Some of these vegetables, such as the onions (*Allium cepa*), attain a notable perceptual weight with respect to the global intake of polyphenols. To this effect, for example, onions constitute 10% of the polyphenols consumed by the Dutch[31] and together with the tomatoes constitute the most consumed vegetables in the Mediterranean area.[92] However, the analytical studies geared at the characterization of polyphenolic compounds in these vegetables are initiated, chronologically speaking, way after those that involved other foods, more precisely, around 1992.

Qualitatively speaking, polyphenolic allotment in vegetables can be arranged to represent two important structural families: hydroxycinamic acids[140] and flavonoids. Within this last group, it merits to emphasize that the most notorious are the flavonoles[65,141-146] and to the lesser extent the flavones,[65,141,142] as well as the glycosides of both phenolic structures. Likewise, the recent studies helped identify and quantify antocyanins structures in red onion, besides flavanones.[143] For the sake of clarifying the principal aspects of bibliographical characterization, Table 8 comprehensively lists phenolic structures and compounds as well as quantitative levels corresponding to each of the species found in foods of this kind. Thus, for example, Winter and Herrmann[140] extensively studied hydroxycinamic fraction in different veg-

TABLE 8
Distribution and Concentration of Phenolic Compounds in Vegetables

Sample	Phenolic structure	Phenolic compound	Concentration (mg/kg fresh mass)					Ref.
			(1)Tomatoes	(2) Peppers	(3) Spinach			140
(1)Tomatoes	Hydroxycinnamic acids	Chlorogenic acid	12-71	traces-632	traces-5			
(2) Pepper		Caffeic-quinic acid ester	traces-11	traces-11	No determinated			
(3) Spinach		Caffeic acid-1-glucoside						
		Coumaric acid-1-glucoside	traces	traces	No determinated			
		Ferulic acid-1-glucoside	traces-19	No determinated	traces-21			
		Caffeic acid-4-glucoside	traces	traces-11	traces-88			
		Coumaric acid-O-glucoside	5-39	No determinated	No determinated			
		Ferulic acid-O-glucoside	traces-68	No determinated	No determinated			
			traces-15	No determinated	No determinated			
		Coumaric-tartaric acid ester	No determinated	No determinated	189-230			
			(1)Onion	(2)Leek	(3)Lettuce	(4)Endives	(5)Celery	141
(1)Onion	Flavonols	Quercetin	5076	<20	319	<10	<10	
(2)Leek		Kaempferol	<20	295	<20	271	<20	
(3)Lettuce		Myricetin	<10	<10	<10	<10	<10	
(4)Endives								
(5)Celery	Flavones	Luteolin	<10	<10	<10	<10	358	
		Apigenin	<40	<10	<40	<40	1787	

Sample	Phenolic structure	Phenolic compound	Concentration (mg/kg fresh mass)				Ref.
			(1)	**(2)**	**(3)**	**(4)**	145
(1) Tomato	Flavonol glycosides	Quercetin	2-203	185-634	11-911	n.d.	
(2) Onion							
(3) Lettuce	Flavone glycosides	Luteolin	n.d.	n.d.	n.d.	40	
(4) Endives		Apigenin	n.d.	n.d.	n.d.	191	
			(1)		**(2)**		144
(1) White onion	Flavonol glycosides	Quercetin-3,4′-diglucoside	504-638		812-1000		
(2) Red onion		Quercetin-4′-monoglucoside	360-653		428-900		
	Flavonol aglycones	Quercetin	29		41		
Red onion	Anthocyanidin glycosides	Cyanidin-3-glucoside	19.4				143
	Flavonols glycosides	Cyanidin-3-arabinoside	11.0				
		Cyanidin-3-malonilglucoside	129.0				
		Cyanidin-3-malonilarabinoside	74.0				
		Anthocyanidins	233.0 (Total anthocyanidin content)				
		Quercetin-3,4′-diglucoside	569.6				
		Quercetin-7,4′-diglucoside	4.6				
		Quercetin-3-glucoside	Traces				
		Dihydroquercetin-3-glucoside	26.8				
		Isorhamnetin-4′-glucoside	341.8				
		Flavonols	950.0 (Total flavonol content)				

etables such as tomatoes, peppers, spinach, beets, beans, lettuce, and ruff. These studies have shown that hydroxycinamic structures are generally made of caffeic and cumaric acid esters. Likewise, the glycosilated structures, derivatives of the previously mentioned species, have been identified in tomato samples, but with lower distribution than the corresponding esterified forms.[147-149]

The tomatoes were studied in both forms of development, that is, as a mature fruit and underdeveloped, as a rule confirming a decrease of the glycosilated polyphenolic concentration in the mature samples, while the esterified structures were not affected by the degree of maturity/ripeness. The most dominant structures in tomatoes proved to be caffeic and cumaric acids, both glycosilated with glucose (39 and 68 mg/kg, respectively) and chlorogenic acid that resembled esterified structures of caffeic acid with quinic acids (60 mg/kg). The peppers yielded a very different polyphenolic profile, for they contained higher levels of chlorogenic acid, in the order of 600 mg/kg per sample. Another group of studied vegetables was constituted by spinach and beets, characterized by a notable presence of ferulic acid glycosilated with glucose, where their values ranged from trace aggregates to 88 mg/kg of fresh vegetable. In a similar way, this type of vegetable harbored an ester of cumaric acid with tartaric acid that was quantified as a dominant structure yielding concentrations comprised between 189 and 230 mg/kg, relative to each variety examined. Finally, in regard to hydroxycinamic structures, other vegetables such as beans, bush beans, and peas reflected polyphenolic traces in the leaves and the pods of the plants. Furthermore, it was proven that other hydroxycinamic species such as esterified structures of caffeic and cumaric acids with malic acid participated in their qualitative composition.

The main architect of study on flavonoid fraction in vegetables was Hertog et al., in 1992 and 1993.[65,141,142] The principal objective of these authors consisted of the exact quantification of flavonols present in vegetable samples, given the prodigious anti-cancer property attributed to this type of polyphenolic compound to which we referred at the beginning of this article.

Many flavonoles, such as quercetin, kaempferol, miricetin, and flavones, represented by apigenin and luteolin have been quantified in different vegetables, where flavonoles concentration was always greater than flavones.[65,141,142,145] In view of the results listed in the bibliography, it was possible to establish differences among studied vegetables on the basis of polyphenolic compounds distribution and content. Thus, quercetin proved to be the most characteristic and predominant flavonole in onions with concentrations comprised between 200 mg/kg and 600 mg/kg, according to the class of onion; in essence, red and white onions, respectively.[145] Other authors have reported quercetin levels in onion samples reaching 5076 mg/kg.[141] Kaempferol was the most predominant flavonol in lettuce (295 mg/kg of fresh vegetable) and in celery (271 mg/kg).[141] The polyphenolic characterization carried out in tomatoes manifested the presence of quercetin in a concentrations that oscillated between 2 and 200 mg/kg. The same type of studies conducted on different vegetables akin to Dutch diet have proven that quercetin and kaempferol constituted the bulk of polyphenolic compounds in this type of foods.[142]

On the other hand, it should be emphasized that after boiling or frying onions and tomatoes, both contained less quercetin than in the natural state. Accordingly, other studies that dealt with the influence of storage and common cooking processes on samples of different onion varieties were published recently.[144] In these studies, the collaborating authors investigated the influence of storage conditions and ordinary cooking techniques on this type of food and how these affected polyphenolic distribution. Thus, while the storage proved to cause only small quantitative changes, the boiling and frying processes triggered a loss of up to 25% of total quercetin glycoside content. This fact clearly indicates the influence of different food treatments on the polyphenolic quantitative levels. Consequently, if one wishes to estimate the exact polyphenolic fraction that forms an integral part of the diet, it is necessary to take into account the freshness of the sample or whether it has been processed prior to quantification.

Finally, let us indicate that despite the fact that the antocyanins are not the dominant polyphenolic structures in vegetables, this type of polyphenol has also been studied in red onion samples.[143]

Subsequently, four cianidin glycosides (the most prevalent antocyanin in plant kingdom) linked to glucose, arabinoside, malonilglucose, and malonilarabinoside, respectively, were studied, where the total antocyaninic concentration reached 233 mg/kg. In parallel, four glycosilated structures of quercetin bound to glucose and isorhamnetin glycoside linked to glucose as a participating sugar were also identified. The total quantification of this fraction led to concentrations in the order of 950 mg/kg of fresh onion. From the quantitative point of view, it can be deduced that flavonoles and especially quercetin are once more the prevailing polyphenolic structures in this type of sample.

D. Legumes

The legumes constitute one of the principal energy reserves found in foods. However, it is well known that their potential use as source of macro nutrients is limited by the high content of condensed tannins. These polyphenolic compounds interact with the dietary proteins as well as with the digestive enzymes, subsequently reducing the nutritional value of these nutrients after ingestion and thus make them essentially unavailable for energetic conversion. This group of polyphenolic compounds can also be divided into two subgroups according to the previously mentioned classification: hydrolyzable and condensed. At the same time, they can be classified as constituents of a heterogeneous and complex family within the family of polyphenolic compounds. In view of this assumption, legumes are generally considered as representative species of condensed tannins. The peculiarity of these compounds accounts for the fact that investigation geared at the study of the representative species was also amplified to the study of polyphenolic compounds[150-154] in general.

On the other hand, various bibliographical studies have brought forth the proof of the presence of isoflavonoids in this type of food samples.[44,155-159] According to what was previously mentioned, the isoflavonoids constitute a group of polyphenols akin to the legume family, and this in turn can be extended to include the flavonoids species.[5] Because of this, it can be affirmed that polyphenolic structures or families that form part of the total polyphenolic fraction in this type of sample are fundamentally isoflavonoids and condensed tannins that act as the most representative structures with intermediate and high molecular weight, respectively.

Table 9 lists distribution and when necessary the concentration of polyphenolic compounds in legume samples following close examination of recent bibliography. In continuation, we shall comment briefly on the most relevant results presented in that table.

As can be observed, the tannins have been quantified in different varieties of beans (*Phaseolus vulgaris)*.[150,152,154] The incentive from most of these studies can be attributed to the antinutritional behavior that of polyphenolic compounds exhibit in this type of food. One of the first studies listed in the bibliography, namely, the one conducted by Cansfield[154] dealt with quantitative and qualitative differences concerning proantocyanins (condensed tannins) occurring in colored beans harvested from different cultivation areas. As a matter of fact, the analysis of white beans samples did not indicate the presence of previously mentioned structures. The quantitative levels of proantocyanins in colored beans harvested from different geographical areas varied between 2 and 6% (w/w). Bos and Jetten[161] carried out the quantitative determination of total tannins in different varieties of colored and noncolored beans, more precisely in the skins and in the cotyledons as well as in the whole beans. In all the studied cases, the skins that surrounded the seeds reflected greater polyphenolic concentration than cotyledons, with levels in the order of 0.6% (p/p) for cotyledons and up to 7% (p/p) for skins. The quantified tannin levels in whole beans reflected the average of values secured from all studied parts of the plants.

By and large, the legume polyphenolic fraction may be constituted by tannins as well as low-molecular-weight polyphenolic compounds, according to previously mentioned results. In consequence, a study concerning both fractions has been carried out in white and black beans,[150] with black beans exhibiting greater composition of tannins and high-molecular-weight polyphe-

TABLE 9
Distribution and Concentration of Phenolic Compounds in Legumes

Legume sample	Phenolic structure	Phenolic compound	Concentration (mg/kg)	Ref.
Beans	Proanthcyanidins	Proanthcyanidins of different molecular weight	2-6% (w/w)	154
White and black beans	Proanthcyanidins	Proanthcyanidins	Not published	151 152
Whole, peel and seed beans	Condensed tannins	Condensed tannins	Seeds : 0.61% (w/w) Peels : 7.00% (w/w) Whole beans: 2.00% (w/w)	161
White and black beans	Condensed tannins Phenolic structures of low molecular weigth	Condensed tannins Phenolic structures of low molecular weigth	White beans : 0.9 mg/kg Black beans : 4.5 mg/kg	150
Soy beans	Isoflavonoids	Daidzein Genistein	Not published	157
Peas	Isoflavonoids	Anisole	Not published	158
Lupin	Isoflavonoids	Genistein	Not published	156
Dried and processed beans	Isoflavonoids	Genistein Daidzein Cumestrol Formononetin	3-1400 mg/kg (between all analyzed legeumes) 70-1000 mg/kg 36-5611 mg/kg 2-460 mg/kg	44

Legume sample	Phenolic structure	Phenolic compound	Concentration (mg/kg)	Ref.
Legume roots	Isoflavonoids Flavanone	Genistein Daidzein Cumestrol Naringenin	Not published	161

nols than white beans. The quantitative levels of total polyphenols obtained in that type of samples were 0.9 and 4.5 mg/kg for white and black beans, respectively.

On the other hand, and paraphrasing previous assertion, isoflavonoids constitute another aggregate of polyphenolic structures that make their presence in legumes. The ever-increasing interest in these polyphenolic compounds is largely due to their antineoplasm properties, which they share with flavonoles compounds.[162,163] The determination of isoflavonoids in legumes has been undertaken by different authors and largely in bean samples.[44,156-159] The most representative isoflavonoids found in beans are daidzein, genistein, and cumestrol, all labeled as the most prevailing species accompanied by formononetin and biochanin-A in lesser quantities. Kitada et al.[157] studied the presence of some of these isoflavonoids in soybeans, given their importance as constituents of baby formulas, finding genistein and daidzein to be the most dominant isoflavonoids. The study on isoflavonoids has been also extended to pea samples, where other isoflavonoids structures such as anisole and kevitone were the most prevailing species.[158] The most important work on legume isoflavonoids was conducted by Frank et al.,[44] also considered as the monumental quantitative data contribution to the investigation of polyphenolic compounds present in this type of sample. In these studies, the authors quantified many isoflavonoid structures, such as daidzein, genistein, cumestrol, formononetin, and biochanin-A that existed either in free form or glycosilated. The study was conducted on wide variety of legumes samples through hydrolysis treatment. From the bulk of results offered by Frank et al.,[44] we ought to emphasize high concentrations of daidzein and genistein in all soybeans and black beans. Nonetheless, other legumes such as Brussels cabbages showed high levels of cumestrol and formononetin. Therefore, the encountered quantitative variance permits to establish differences among the studied groups of legumes (similarly to other types of samples), taking as a reference the concentration akin to different isoflavonoids. Finally, let us indicate that more recent studies based on the investigation concerning the presence of flavonoids in *Phaseolus vulgaris* seeds are in agreement with the previously exposed results, albeit, suggesting an addition of naringenine o the previoulsy mentioned produce.[159]

E. Tea

Tea is one of the most popular drinks in the world, one that can be consumed simply as brewed (green tea) or fermented (black or brown tea). Therefore, the reason for the investigation of this product can be justified by virtue of importance given to the rest of the polyphenolic compounds in foods, basically prompted by the need for establishing a relationship between the quality of the tea with some other group of produce and to the study of the effects that these polyphenolic compounds can exert on human health. Aside from other substances found in tea such as alkaloids, amino acids, and vitamins, polyphenolic compounds are perhaps the most relevant constituents of all, both in terms of occurrence and concentration.[164] As was already indicated, tea like wine, apples, and onions, is the principal source of polyphenols in the daily diet.[31] Based on this premise, we describe briefly in the following paragraph the polyphenolic composition relative to this type of produce.

The polyphenolic composition of tea is complex, because practically all the polyphenolic structures found there contribute to the total fraction of the spoken of compound, that is to say, hydroxybenzoic and hydroxycinamic acids plus flavonoid structures such as flavanols, flavonols, and flavones.[164-166]

The principal polyphenolic compound found in tea samples concerning hydroxybenzoic acid species is gallic acid, which can debut in free form or esterified with quinic acid. The latter, also known as teogalin, is an ester proper of tea and therefore very characteristic of the tea varieties. When it comes to that species, quantitative values consulted in the bibliography reveal concentrations levels between 0.4 and 1.6 g/kg and 0.9 and 5.5 g/kg, according the studied tea variety.[167,168] Inasmuch as that in the group representing hydroxycinamic acids, the prevailing polyphenol compounds are those already cited and as a matter of fact widely distributed in foods: chlorogenic acid and the esters of the *p*-cumaric acid. The concentrations of these polyphenolic compounds in black tea vary between 100 to 200 mg/100 g, contributing an important share to the total acid fraction.[169]

With respect to the flavonoids composition, we ought to mention in the first place that the constituent majority is vested on catequines, which make up 30% of the total. To such an extent that the quality of tea depends on the quantitative levels of these structures, and this in turn confers on catechins a rather special role. Thus, the tea of greater quality proceeds from the youngest plants, because these accumulate the greatest levels of catechins. Also, the elaboration process of teas is utterly important, because flavanols exhibit a high sensibility to oxidation mediated by the polyphenoloxidases, and consequently it can be established that green tea as a rule contains greater concentrations of catechins than the black or brown tea lost during the fermentation treatment. Taking into account what was exposed previously, it is hard to argue why the investigation of flavanols, and catequines in particular should not become one of the main objectives of current scientific research.[164] The principal flavanols that constitute the total catechin fraction in tea are (+) catechin, (-) epicatechin, and galates of both monomers.[164,166]

On the other hand, flavonols are structures responsible for particular physiological effects as well as the color of the tea. The principal structures involved in the polyphenolic composition are present in their free form only at trace levels, whereas the glycosilated structures constitute the prevailing fraction. Subsequently, within this group it was possible to identify myricetin, quercetin, and kaempferol glycosides.[170-172] Subsequent studies realized by Bokuchava and Ulyanova[173] helped to quantify the mentioned structures, where myricetin levels of up to 2 mg/g were found in different classes of tea. Finally, with respect to flavones, it needs to be underlined that these polyphenolic structures are represented rather poorly in this type of sample, with apigenin as the prevalent flavone. By and large, these type of compounds are generally glycosilated through a carbon atom (C-glycoside), a fact that hinders their hydrolysis and, consequently, their complete identification. This is the reason why the mentioned polyphenolic compounds have been studied to the lesser extent in samples of tea.[174,175]

After comprehensible introspection of bibliographical results, it can be established that when it comes to concentration and distribution of polyphenolic compounds in foods, they reflect a markedly heterogeneous character. In effect, it can be concluded that hydroxybenzoic and hydroxycinamic acids are found widely distributed, although the flavonoids and in particular catechins plus flavonols are the most abundant species. Other flavonoid families, such as flavanones and isoflavonoids, are more specific to given produce like citrus fruits and vegetables, respectively. Drinks such as wine and tea and fruits like pears and apples are representative examples of comestibles that contain all polyphenolic structures in their composition.

On the other hand, when studying polyphenolic composition corresponding to different species, qualitative differences in their composition can be significant, as a rule, which allows establishing interesting differences among the same. Nonetheless, after examination of different varieties, akin to the same vegetable species, the principal differences in the polyphenolic composition can be established only in quantitative terms. An example of both situations is embodied in citric fruits and apple polyphenols. That is why naringin can be considered a representative flavonoid of the *Citrus paradise* species (grapefruit), because it is absent in *Citrus sinensis* (orange). On the contrary, the examination of polyphenolic composition in the different varieties of *Malus domestica* (apple) proves similarity in phenolic composition with differences at quantitative levels only, and with respect to each studied variety.

On the other hand, following the bibliography to the letter, it may be shown that the most important difference between fruits and vegetables can be established on the basis of glycosidic composition akin to flavones, as they are much more abundant in vegetables than in fruits.

With respect to the different parts of the plant, where polyphenolic compounds can be located with ease, it is accurate to indicate that, despite being widely distributed in all the plant parts, the cortical layer (skins and pods) are rich in flavonol structure, while catechins can also be found in deeper layers such as seeds.

Finally, it is important to indicate that the presence of polyphenolic structures in a given food matrix and their corresponding quantitative

levels are highly dependent on different parameters such as the type and origin of the cultivation, the environmental growth conditions, germination and/or ripeness, and the kind of processing treatment. This fact underlines the difficulty of carrying out quantitative determination needed to secure exact polyphenolic levels for dietary requirements. Considering the importance of such undertaking, current bibliographical reviewers have appealed to the scientific community involved in the investigation of polyphenols to conduct rigorous studies that would shed more light on polyphenolic levels, fractions, and the composition of these compounds in our diet.

V. SAMPLE PREPARATION METHODS

The preparation of samples for analytical studies that would determine the composition of polyphenolic compounds in vegetable matrices consists of various stages, basically conditioned by the matrix and the polyphenolic nature of the sample as well as on the method employed in the derivation. This process may be quite heterogeneous, comprised of numerous phases or reduced to a basic minimum. Nonetheless, the last phase (final objective) of this process consists preparation of the sample extract uniformly enriched in all the components of interest and free of subjacent impurities that might accompany the matrix.

It must be stressed that like in any analytical study, sampling is the most decisive step, because this procedure determines the final result. In the determination of polyphenolic compounds in comestibles, the chosen sample has to be the most representative as possible if we wish to calculate with utmost precision quantitative levels of structures that form a part of the sample. As was mentioned already, the quantitative polyphenolic composition depends on many variables and therefore it is difficult to count on an ideal result.

On the other hand, the polyphenolic compounds, save for very concrete cases, where the determination of the same is carried out directly in the liquid matrix, need to be properly extracted from the vegetable matrix. Thus, the extraction must be done with the most adequate solvent and under ideally predetermined analytical conditions of temperature and pH, following, for example, the conventional methods of solid-liquid extraction. On numerous occasions, the polyphenolic compounds are found in liquid matrices, such as would be the case of wine and fruit juices, where the above-mentioned process is also crucial and similar to classic liquid-solid extraction, or if need arise by means of solid phase extraction, avoiding the use of solvents. In the end, the effort amounts to lowering costs and reducing sampling time during the already spoken of conventional extraction. In any case, the extraction stage is extremely important, as its outcome will depend the release of analytes proper of vegetable matrix into the medium, and this in turn will allow the quantitative determination of the extract.

In special instances, the hydrolysis stage, generally in acid medium, can be carried out simultaneously or after the extraction stage, sensibly reducing a number of polyphenolic structures that form a part of the final extract. This is due to the fact that in the course of hydrolysis glycoside bonds rupture and all the glycosilated structures become simplified to aglycones from where they derive in the first place.

Once the polyphenolic mixture is chased into solution, it may happen that due to their structural complexity, other stages posterior to fractionating must be employed, let it be for the purpose of separating polyphenolic compounds into different fractions or simply to eliminate impurities or unnecessary substances (clean up).

In the following paragraphs we describe the preparation and/or sample processing relevant to different kinds of foodstuffs based on the bibliographical antecedents and in close relation to these types of samples

A. Grapes and Wines

It is easy to deduce that despite intimate the relationship that exists between both samples, their treatment is utterly different due to the agglomeration state of each species.

Grapes have to be classified in accordance to determined conditions that need to be respected exactly (cultivation, harvesting time, commercial origin, variety, degree of maturity, etc.). A poste-

riori, they ought to be stored under a temperature that oscillates between 24 and 30°C untill the time of fractionation.[27,88,176] Table 10 lists conditions usurped from bibliography for the extraction of polyphenols from grapes and wines. Likewise, the same table indicates polyphenolic structures extracted from each species.

As can be observed, the extraction of different polyphenolic structures present in this type of sample has been carried out using distinct organic solvents as extracting agents. Thus, a mixture of methanol-water and final extraction with acetone have been used to isolate catechins from grape seeds.[88] The latter proved to be the most adequate for the extraction of both high-molecular-weight polyphenolic compounds and condensed tannins.[176] Similarly, the ethanol/water mixture allowed the extraction of all polyphenolic structures assembled in grape skins and seeds.[89] In the liquid samples such as the grape juices and wine, the most frequently employed extracting agent is ethyl acetate and/or diethyl-ether.[82] The isolation of catechins, whether monomers ((+) catechin and (-) epicatechin) or their protocyanins is done by passing the extract through a Sephadex column to avoid interference with other structures derived from cinamic acids.[27]

With respect to wine samples, it should be mentioned that the extraction process has been carried out relying on the conventional liquid-liquid extraction,[90, 177,178] whereas in the solid phase C18 cartridges were employed as absorbents.[84,85] While in the first instance scarcely polar solvents were used, in the solid phase extraction methanol was employed for the elution of polyphenols fixed to the absorbent Aside from the traditional use of ethyl acetate and/or diethyl ether for the extraction, other authors have done fractionated extraction under different pH, such as pH = 7 for neutral polyphenols and pH = 2 for acid-like.

On the other hand, a novel study that was published in 1990 proposed eradication of wine sampling by traditional treatment and its substitution by direct injection in HPLC systems.[81] To this effect, the most recent and relevant bibliography[86,87,179] also speaks in favor of using direct injection for the determination of polyphenolic compounds in wine samples, given a tremendous evolution of HPLC, on which we dwell later. The recommendation for substituting traditional wine sample treatment is based fundamentally on three considerations: the foremost certifies that some polyphenolic compounds can easily undergo oxidation if the extraction is prolonged, especially in the alkaline medium, even though the extraction is conducted in an inert atmosphere; the other refers to the possible rapture of glycosidic bonds; and the last one alleges the possibility of isomerization in the process, especially when it comes to hydroxycinamic derivatives, with the subsequent loss of these structures.

Finally, let us mention other processes employed in extraction such as solvent recycling often employed to increase efficiency of the extraction[90,176] and preconcentration by means of rotatory evaporator under low temperature and pressure conditions in order to avoid decomposition of existing analytes.

B. Fruits

1. Citrus

The preparation of citric sample, like in grapes and wines, depends on whether we deal with juices or fresh fruit, but fundamentally it is linked to the polyphenolic nature of these specimens. It was already mentioned that, in general, the most representative polyphenolic citric family is comprised of flavanones. Within this group, completely methoxylated flavanones and flavanone glycosides were eventually differentiated. These two structural types exhibit very distinct polarities, thus, while the former possess highly pronounced hydrophobic character, the latter are much more acute in that sense. In great measure, this finding implies conducting fractionated and if possible more selective extractions in order to simplify posterior analysis.

The analysis must be done on fruits harvested recently and if possible from different cultivation areas.[80,103,106] When the analysis is conducted on samples from concentrated juices, some authors include the date of harvest[94,106] and when it is done on juices, jams, or commercially purchased fruits the investigators resort to the identification of shipments by special codes.[97,99,100,106,107]

TABLE 10
Sample Preparation in Phenolic Analysis
Grapes

Analytes	Extraction	Other treatments	Remarks	Ref
Tannins	Ultrasonic system Solvent: acetone Mutiple extraction (3) Time: 16+4+4 h.	Isolation on Sephadex column Preconcentration in rotatory evaporator (T<40ºC)		176
Acids Flavonols	None	Filtration Preconcentration	No sample treatment Direct injection onto HPLC system	27
Procyanidins	None	Isolation on Sephadex column Preconcentration in rotatory evaporator	Characterization of procyanidin fraction B1, B2, B3, B4 y B5	
Flavan-3-ol monomers (Catechin and epicatechin) and procyanidins	Extraction with different solvents **(1)** MeOH 80 % (v/v) (4 h) **(2)** MeOH 50 % (v/v) (4 h) **(3)** Acetone 75 % (v/v) (1 h)	Flavonoid fraction isolation C18 cartridge pH=7 (flavonoids) pH=2 (phenolic acids)	Disttribution of phenolics in peel and seed grapes.	88
Total phenolics	Solvent: EtOH/ H_2O/HCl pH=3	Amberlite AX2 clean-up Polar compounds clean-up	Direct spectrophotemetric total phenolics	89
Acids Flavonols	Solvent: Dyethyl-eter pH=3.5	No published	None	82

TABLE 10 (continued)
Sample Preparation in Phenolic Analysis
Wines

Analytes	Extraction	Other treatments	Remarks	Ref
Total phenolics	System: Magnetic extraction Solvent: BuOH T=25°C	Rotatory evaporator preconcentration (T<35°C)	None	177
Flavanols	Solvent: Dyethil-eter Mutiple extractions	Rotatory evaporator preconcentration (T<35°C)	None	90
Hydroxycinnamic and hydroxybenzoic acids	System:Continuos rotation 0.8 r.p.m. (3h). Solvent: Dyethil-eter	Rotatory evaporator preconcentration (T<35°C)	None	178
Acids Flavonoids	Solid-liquid extraction System: (C18) cartrigde Eluents: MeOH and MeOH (20%) pH=9	Elution of flavonoid and phenolic acids fractions MeOH (20%): eluent for acids MeOH: eluent for flavonoids Preconcentration (T<35°C)	Solid-phase extraction	84 85
Total phenolics	None	Filtration	Direct spectrophotometric determination or direct injection in HPLC systems.	85 87 179

The juice concentrates, once diluted and centrifuged, are stored at – 20°C, until the time when the proper isolation of corresponding polyphenols is going to take place.[94,97,107] In some cases, NaOH is added to completely dissolve minute solid particles that may persist after filtration and centrifugation stages.[94,109] Fruit and/or jam samples are dried at 50°C then powdered before submitting them to any other analytical process.[80,102,103,106]

The isolation of polyphenolic compounds from citric fruits has been accomplished by subjecting samples to an extraction routine. The most relevant characteristics of the extraction processes obtained from available bibliography for each type of studied sample are summarized in Table 11. Likewise, it was deemed necessary to include structures extracted in each case as well as characteristics of other relevant treatments.

As it can be deduced from that table, the apolar solvents, such as benzene, hexane, or dimethylformamide, have been employed repetitively in liquid-liquid extractions for complete isolation of methoxylated flavones from both orange juice samples[94,96,99,100] and fresh fruit alike.[103,104] After inspection of the same table, it can be seen that like in grape samples certain authors have resorted to liquid-solid extraction, employing octadecylsilice cartridges for absorption and methanol for elution.[80,95]

The principal advantage of using liquid-solid extraction (clean-up) when compared with conventional liquid-liquid extraction rests in the elimination of benzene as a toxic solvent and a faster speed of the process to avoid conventional centrifugation stages, necessary for the elimination of emulsions created during powerful agitation and spinning. For the extraction employed to identify different polyphenolic structures, the listed studies indicate the use of various solvents. A clear example of this is the work realized by Perfetti et al.[100] on orange juices, who resorted to hexane for the isolation of caretonoids and methylene chloride for the extraction of methoxylated flavones, by which glycosilated flavanones were adequately differentiated in the aqueous fraction. Another employed alternatives according to the bibliography is the use of organic solvent mixtures as the unique extracting agents.[80] In effect, in these studies MeOH/DMSO mixtures were employed to conduct the extraction of freeze-dried orange samples corresponding to different parts of the fruit, combined to solid phase extraction of polyphenolic compounds in juices obtained from the same fruits. Other studies have insinuated the conventional extraction based on multiple recycling of the solvents[101] applied to samples from concentrates of oranges and grapefruits. The suggested method secures the maximum yield of predominant polyphenolic compounds found in these types of samples.

With respect to detailed treatments, in addition to extraction carried out in citric samples, it needs to be stressed that majority of authors suggest a final preconcentration, generally realized in rotavapor at temperatures inferior to 40°C and under reduced pressure. Likewise, let us indicate that in this type of sample the fractionation stages described earlier for sampling total polyphenolic fraction obtained during extraction process were not carried out.

a. Apples and Pears

Even though both fruits were described separately in the preceding chapters, because of the polyphenolic differences found in both, in this chapter it was believed convenient to deal with them as one due to the similarity in sample treatment. Hence, both types of fruits were treated in a similar manner and that is why the gathered information is also listed as unique. The weighed considerations involved in the sampling are similar to those already discussed for citric samples, such as degree of maturity, harvest date, studied varieties, etc. It must be remember that the number of studied varieties corresponding to these samples is much bigger. To this effect, we should be aware (as it was explained beforehand) that in general polyphenolic composition proved to be independent of the investigated variety. This fact justifies the employment of similar sample treatment, independently of specimen variety in consideration.

Table 12 lists all data quoted in the bibliography with respect to investigated polyphenolic structures, solvents, and laboratory conditions employed in the extraction process as well as

TABLE 11
Sample Preparation in Citrus Samples
Juices

Analytes	Extraction	Other treatments	Remarks	Ref
Methoxylated flavones	System: Shaker Solvent: Bencene Mutiple extractions (3)	Not published	None	94
Methoxylated flavones	System: Water bath Solvent: Dimethylformamide Time: 10 min.	Clean-up (C18 cartrigde) Preconcentration (T<40°C)		96
Methoxylated flavones Flavones glycosides	Solvent: Metilen chlorhidre	Preconcentration (T<40°C)		100
Flavones Flavanones	Solvent: Metilen chlorhidre	Preconcentration (T<40°C)	Mandarin oils	208
Methoxylated flavones	System: Shaker Solvent: Bencene	Preconcentration (T<40°C)		99
Flavanones	System: Shaker Solvent: Dimethylsulfoxide Mutiple extractions (5) T=25°C T=55°C	None	Extraction efficiencies optimization	101
Hydroxycinnamics	Solvent: Ethylacetate Mutiple extractions (2)	Preconcentration (T<35°C)	Hydroxycinnamic extraction optimization	109

TABLE 11 (coninuted)
Sample Preparation in Citrus Samples
Juices

Analytes	Extraction	Other treatments	Remarks	Ref
Methoxylated flavones	Liquid-solid extraction System: C18 cartrigdes Eluent: Acetonitrile/water	Preconcentration (T<40°C)	Solid-phase extraction	95
Flavonoids	Liquid-solid extraction System: C18 cartrigdes Eluent: Methanol	None	Solid-phase flavonoid extraction	80

Fruits and jams

Analytes	Extraction	Other treatments	Remarks	Ref
Flavones Flavanones	Solvents: Methanol, piridin, dimethylformamide, dimethylsulfoxide, dioxane, water	Not published	Solvent extraction optimization	102
Flavonoids	Systema: Shaker Solvent: Methanol/dimethylsulfoxide (1:1) T=25°C Extraction time=10 min.	Clean-up C18 cartrigdes	Flavonoid quantitation	104
Flavanones	Solvent: Dimethylsulfoxide	None	Different fruits (orange and grapefruit)	103

TABLE 11 (coninuted)
Sample Preparation in Citrus Samples
Fruits and jams

Analytes	Extraction	Other treatments	Remarks	Ref
Flavonoids	Systems: Ultrasonic Solvents: Methanol/water (peels) Butanol (juices)	Preconcentración a vacío	Extracción en diferente matrices de fruta (piel y zumo) y en mermeladas	106

TABLE 12
Sample Preparation in Apple and Pear Fruits

Analytes	Extraction	Other treatments	Remarks	Ref
Flavonol glycosides	Solvent: Methanol	Preconcentration (T<40°C)	Fresh apple peels extraction (Spartan variety)	35
Flavonol glycosides Chalcones	Solvent: Aqueous methanol (85% (v/v)) Extraction number (2)	Hexane extraction: lipids and carotenoids (clean-up) Clean-up sugars (C18 cartrigdes) Preconcentration (T<40°C)	Fresh apple peels extraction (Rhode-Island variety)	114
Hydroxybenzoic and hydroxycinnamic acids (+)Catechin	System:High speed shaker Solvent: Aqueous methanol (80%) Extraction time: 2 min.	Clean-up lipids Preconcentration (T<40°C)	Fresh apple pulps extraction (Golden variety)	110
Hydroxycinnamic acids Flavan-3-ols Flavonols Chalcones	Solvent:Ethylacetate Extraction number (2)	Procyanidins isolation onto Sephadex column Preconcentration (T<35°C)	Processing studies Fresh whole apples extraction (Granny-Smith variety)	36
Hydroxycinnamic acids Catechins Flavonols Chalcones	System: Homogenizer Solvent: Methanol	Preconcentration (T<35°C)	Fresh apple peels and pulps extraction (varieties: Gravenstein, McInthos, Cortland, Spartan, Golden and Starcky)	115

Analytes	Extraction	Other treatments	Remarks	Ref
Hydroxycinnamic acids Flavan-3-ols Flavonols Chalcones	System: Homogenizer Solvent: Methanol Ethylacetate reextraction	Preconcentration (T<40°C)	Fresh apple peels and pulps extraction (varieties: Golden, Reineta, Starcky and Granny-Smith)	116
Hydroxycinnamic acids Flavan-3-ols Flavonols Chalcones	Solvent: Ethylacetate Extraction pH=7 (Flavonoid extraction) pH=2 (Acid extraction)	Preconcentration (T<40°C)	Fresh apple pulps extraction (french varieties)	111
Hydroxycinnamic acids Flavan-3-ols Flavonols Chalcones	Solvent: Ethylacetate Extraction (pH=7) T=30°C Extraction time: 5 min.	Preconcentration (T<40°C)	Fresh whole apples extraction (Spanish varieties)	117
Flavonol glycosides	System: Homogenizer Solvent: Methanol	Ethylacetate partition Acid hydrolysis of glycosides (Chlorhidre acid 0.1M, 100°C, 1 hr) Preconcentration (T<40°C)	Fresh peel pear (Packingham variety)	122
Flavonol glycosides	Solvent: Methanol Extraction time: 5 min.	Hexane extraction of lipids Isolation catechin and flavonol fractions (C18) Preconcentration (T<40°C)	Glycosidic extraction and characterization in peel pear (Guyot variety)	33

TABLE 12 (continued)

Analytes	Extraction	Other treatments	Remarks	Ref
Hydroxycinnamic acids	System: Homogenizer Solvent:Hot water (100 °C, pH=3)	Enzymatic hydrolysis od esterified structures Hydrolyzed isolation onto Polyamide column (Eluent: methanol)	Fresh apples and pears extraction	122
Catechins	System: Homogenizer Solvent: Methanol	Hydrolyzed isolation onto Polyamide column (Eluent: methanol)		
Hydroxycinnamic acids Flavan-3-ols Flavonols Chalcones	Solvent: Diethyleter Extraction number (2) Ethylacetate reextraction	Preconcentration (T<35°C)	Extraction in pear and apple commercial juices	108
Arbutin Chalcones	System: Shaker Extraction in two steps First: Methanol (80%) T=25°C, 18 h. Second: Butanol	Clean-up of pectins and sugars in butanol extracts onto Amberlite XAD column Preconcentration (T<35°C)	Apple and pear jams extraction	107
Total phenolics	None	None	Spectrophotometric determination in apple juices	121

other hydrolysis treatments and/or fractionating methods carried out in this type of fruit whenever necessary. After examining information contained in Table 12, it is possible to detect anew the dominance of conventional extraction methods in both modalities: liquid-liquid and solid-liquid, depending on the type of fruit matrix, that is, fruit juice (extracted manually or commercially) or the entire fruit (fresh or processed), respectively. With respect to the employed extracting agent it can be established that the methanol and/or methanol-water mixtures have been the most frequently used extracting agents,[33,35,110,112,115,116,122] followed by ethyl acetate.[32,36,108,111,116,117] Most of the cited studies employed one or the other as the unique extracting agent. The ethyl acetate was the most widely used extracting agent in the first studies realized on apple skins. This solvent allows for an adequate extraction of glycosilated flavonoles and even to carry out pseudo-selective extraction, enhancing these substances into enriched fraction.[122] With respect to these agents, published reports by Spanos and Wrolstad[32,36] confirmed that ethyl acetate has been used as the sole extracting agent. Nevertheless, the most recent studies have shown that this solvent, despite being adequate for the extraction of flavanols and chalcones is not the most suitable for the extraction of glycoside fraction as applied to conventional liquid-liquid extraction in apple juices.[117]

On the other hand, the same solvent (methanol), whether pure or mixed with determined quantities of water, has proven to be one of the most adequate solvents for the extraction of all the polyphenolic compounds present in pears and apples. The presence of water can partially improve the extraction of determined polyphenolic compounds that are somewhat more polar, such as arbutin, typical of pears or hydroxybenzoic acids, as well as determined glycosides. In effect, this solvent has the allowed extraction of hydroxycinamic acids, chalcones, and flavonoles contained in different samples of fresh apples that represented distinct varieties[115,116] as well as the extraction of catechins and flavonoles harbored in different pear skins akin to distinct varieties.[33]

Ethyl acetate has been used in the extraction of all polyphenolic structures contained in French apple varieties.[111] Notwithstanding, in jam samples extraction of other treatment combinations were employed that included the incorporation of different solvents such as butanol.[107]

On the other hand, some extraction systems included classic homogenizers.[110,112,116,122] Furthermore, in a certain number of conducted studies the investigators paid attention to the influence of variables such as temperature and solvent recycling that were included in global estimation yield.[32,36,108,116] By and large, the extraction temperature is hardly ever given, which by default rules out other temperatures for the said process, normally carried out under ambient temperature.

With respect to the application of fractionation stages, which serve for the isolation of determined structural groups that, as mentioned, allow for the most adequate characterization, let us emphasize that their employment has been of particular interest to the analytical chemistry. For in some cases, polyamide columns,[112] Sephadex LH-20,[32,36] XAD amberlite resins,[107] and C18 cartridges[33] were used in treatment of this type of sample. While the C18 cartridges have been used for the elimination of sugars only, especially in samples containing high amounts of this element like jams, the employment of Sephadex LH-20 columns as a stationary phase has allowed for an adequate isolation of B1, B2, B3, B4, and C1 procianidines present in apples and pears, thus avoiding the interference with hydroxycinamic structures akin to this type of samples.[32,36]

Finally, let us indicate that all sample treatments relative to apples and pears, as a rule, include a preconcentration stage at the end of the process, which, like in previous cases, is carried out in vacuum and under low-temperature conditions (T 40°C) in order to prevent analytes decomposition before their determination.

b. Bay Fruits

This type of fruit, as previously mentioned, has been studied essentially to determine the antocyaninic content. The most important difference in sample treatment of this fruit type is restricted to the process of extraction, and in particular to the solvent used in the said process. As

far as this kind of sample is concerned and judging from the bibliographical results, the extraction of the antocyanins is carried out with the help of methanol as an extracting agent plus some modifying acid (formic and hydrochloric).[132,134-138] On the other hand, besides the information on the type of solvent, the bibliography also recommends 15°C temperature and complete darkness as ideal experimental conditions of the extraction process. This *a priori* condition is justified by the fact that polyphenolic compounds exhibit great sensibility to the light and high temperature because they induce phenolic decomposition, an interference mentioned already in previous paragraphs.

C. Vegetables

The sampling of vegetables includes aspects related to time and state of maturity,[141,142] let it be in fresh produce samples[140-142,144,145] or their processed counterparts.[142,145] By and large, the chosen vegetable samples are normally freeze dried, then ground and stored at –20°C until extraction and hydrolysis.[140-142,144,145] Table 13 contains the most current and relevant bibliographical information concerning sample preparation during extraction and hydrolysis of polyphenolic compounds in this type of specimen. With respect to the extraction process, emphasis is placed on the issue of general consent among the researchers to use a mixture of methanol/water as an extracting agent, where the water percentages vary between 50 and 80% (v/v).[65,140-142,144,145] Similar to previously discussed cases, this water percentage in the methanol mixture allows an increase in the solubility of glycosilated structures to the detriment of free state flavonoid structures. Likewise, as seen in Table 13, these mixtures not only allow the extraction of flavonols and flavones but also the extraction of hydroxycinamic structures.[140] With respect to the employed extraction systems let us emphasize that once more conventional systems such as countercurrent[65,141,142] and mechanical extraction are deemed as the most appropriate.[140,144,145] Like in previous cases, the solvent recycling during the extraction process has been taken into account and properly discussed.[140,144]

When it comes to sample treatment procedure, hydrolysis of glycosides is without doubt one of the most important stages. That is why the Hertog et al. studies were deemed as the most relevant to the cause, because they offer a formula that optimizes the hydrolysis conditions for the complete characterization of flavonols present in vegetables.[65,141,142] The mentioned process is realized simultaneously with extraction after employing a mixture MeOH (65%) and HCl 1.2 *M* as hydrolyzing and extracting solution. Subsequently, the same authors calibrated time and temperature of the process, as well as the nature and concentration of the acid in function of sample's glycosidic composition.

Finally, let us indicate that like in other sample types, the last stage, known as pre concentration is realized under low-pressure conditions to avoid rise of temperature beyond 30°C and thus secure proper analytical yields.[140,144]

D. Legumes

According to what was stated before, this type of comestible rightfully considered a source of extractable low-molecular-weight polyphenols has been studied to a lesser extent than those corresponding to fruits and vegetables. The legumes exhibit a high content of condensed tannins, which confer on this type of produce antinutritional properties. On the other hand, isoflavonoids are the other predominating polyphenolic fraction in this type of sample. The legume's varieties as well as methods of cultivation are important in selecting the most appropriate sample for an in-depth analysis.[44,150,154,58] Likewise, in some cases, legume samples have been subjected to other treatments, such as grinding,[44,150,157] protein liberation,[151] or the elimination of lipid components.[157]

Table 14 lists the information obtained from literature relative to the isolation processes of polyphenolic compounds contained in legumes. Readings from the mentioned table indicate that condensed tannins or proantocyanins are generally extracted with mixtures of acetone and water.[151,152] Pure water has been also employed as an extracting agent in this type of compound.[154] The authors justify the use of this solvent as an extracting agent

TABLE 13
Sample Preparatoin in Vegetable Samples

Analytes	Extraction	Other treatments	Remarks	Ref.
Hydroxycinnamic acids	System: Homogenizer Solvent: Aqueous methanol (80%) Extraction number (2) T=25°C Extraction time: 30 min.	Separation of esterified and glycosidic hydroxycinnamic structures onto Polyamide column Eluents: Esterified structures: methanol/formic acid) Glycosides: methanol Preconcentration (T<40°C)	Fresh vegetable extraction	140
Flavonols Flavones	System: Ultrasonic Solvent: Aqueous methanol (65%)	Flavonols and flavones glicosides hydrolysis Chlorhidre acid 1.2M, T=90°C Flavonol glycosides hydrolysis: 2 h. Flavone glycosides hydrolysis : 4 h.	Extraction and hydrolysis optimization in fresh vegetables.	65 141 142
Flavonol glycosides	System: Homogenizer Solvent: Aqueous methanol (70%) Mutiple extractions (3) (1200 r.p.m., 1 min.)	Preconcentration (T<40°C)	Fresh onion varieties extraction	144
Flavonol and flavone glycosides	System:Ultrasonic Solvent: Aqueous methanol (50%)	Flavonol and flavone hydrolysis: Chlorhidre acid 1.2M, T=90°C Flavonol glycosides hydrolysis: 2 h. Flavone glycosides hydrolysis: 4 h.	Extraction and hydrolysis in fresh vegetables	145

TABLE 14
Sample Preparation in Legume Samples

Analytes	Extraction	Other treatments	Remarks	Ref
Proanthocyanidins (Condensed tannins)	Solvent: Water Mutiple extractions (11)	Protein purification Separation of phenolic compounds onto Sephadex LH-20 column in two fractions: Flavonoids eluent: Aqueous ethanol (95%) Condensed tannins eluent: Acetone	Extraction and purification of condensed tannins in dried legumes	154
Proanthocyanidins (Condensed tannins)	Solvent: Acetone/water (70/30)	Tannin purification onto Sephadex LH-20 column	Proanthocyanidins extraction in legumes	151
Proanthocyanidins (Condensed tannins)	System: Shaker Solvent: Acetone/water (80/20) Extraction time: 15 min.	Separation of phenolic compounds onto Sephadex LH-20 column in two fractions: Flavonoids eluent: Aqueous ethanol (95%) Condensed tannins eluent: Acetone (70%)	Extraction and purification of condensed tannins in black beans	152
Proanthocyanidins (Condensed tannins)	Solvent: Methanol (1% in chlorhidre media) T=25°C Extraction time: 24 h.	Separation of phenolic compounds onto Sephadex LH-20 column in two fractions: Flavonoids eluent: Aqueous ethanol (95%) Condensed tannins eluent: Acetone (50%)	Extraction and purification of condensed tannins in white and black beans	150
Isoflavonoids	Solvent: Aqueous methanol (80%) T=80°C Extraction time: 4 h.	Separation of phenolic acids in C18 cartrigdes	Isoflavonoids extraction in soy beans	157
Isoflavonoids	Solvent: Aqueous methanol (85%)	None	Isoflavonoids extraction in peas	158

Analytes	Extraction	Other treatments	Remarks	Ref
Isoflavonoids	System: Ultrasonic Solvent: Aqueous ethanol (96%) Antioxidant (BHT, 1%)	Isoflavonoid glycosides acid hydrolysis Chlorhidre acid 2 M T=100°C Extraction time: 1 h.	Extraction and hydrolysis of isoflavonoids in a wide of legumes (24)	44

because of the fact that condensed tannins bound to protein structures could otherwise become a part of the final extract, given that these associations simulate extract polarities. Nonetheless, this peculiarity helps to distinguish tannic fraction associated with the proteins. In all the cases, the extraction of condensed tannins found in legumes is conditioned by the subsequent stage of purification using Sephadex LH-20 column. The objective of this purification stage or fractionation consists of separating proantocyanins fraction from the rest of polyphenolic compounds that ultimately form the part of total composition in the original extract. In all the cases, EtOH at 95% has allowed the elimination of low-molecular-weight polyphenolic compounds, while acetone and water mixtures or pure acetone have been used to elute a second fraction composed by proantocyianidines.[150-152,154]

On the other hand, when it comes to isoflavonoids, the extraction methods include the employment of MeOH and EtOH, generally aqueous and accompanied by a certain quantity of acid.[44,150,157,158] As a rule, the extracts that contain isoflavonoids, do not need treatments beyond fractionation or separation, except for the example brought forth in study realized by Kitada et al.[157] In this case it was necessary to continue with the treatment in order to eliminate polyphenolic fraction constituted by acids before carrying out electrochemical derivations used for its subsequent characterization. It needs to be emphasized that the acid hydrolysis of glycosilated isoflavonoids in different legumes was also conducted by Frank et al.[44] These authors carried out extraction simultaneously with hydrolysis in a way realized by Hertog et al. on vegetable samples, as discussed in the previous examples. These unrelated events manifest indiscriminately similar behavior of glycosilated structures, whether they are related to flavones, flavonols, or isoflavonoids. Also, similar to previously described cases, the sample treatment implied working under conditions that helped prevent oxidation of polyphenolic compounds, in a manner explained by Frank et al. who employed BHT as an antioxidant.[44]

E. Tea

Finally, it was deemed convenient for the sake of clarification to briefly comment on some aspects concerning sample treatment of this type of produce. As in some other cases, conventional extraction techniques were of great use for tea analysis in both solid-liquid or liquid-liquid modalities. To carry out the extraction of different polyphenolic compounds found in teas, distinct solvents such as boiling water, acetone, and methanol were employed.[180] In general, the analysis of flavanols was accomplished posterior to fractionation and isolation in Sephadex LH-20 in order to purify the mentioned fraction.[181] Likewise, the hydrolysis treatments were also relied on to determine glycosides of flavonoles and flavones.

On the other hand, like in wine samples, some authors defend simplified sample treatment,[182,183] by employing the direct injection of liquid tea samples into the HPLC systems, which are filtered prior to that procedure.[184] Based on all previously exposed information, it can be deduced that the preparation of a sample for the determination of polyphenolic compounds in teas comprises a number of heterogeneous stages. Generally, the necessary stages would vary in function of the sample type, the nature of the involved polyphenolic compound, and the analytical technique to be used for this purpose.

In the global concept of sample treatment, extraction is given the priority status, except in very special cases, like wine, for example, which can be analyzed without previous treatments. The selection of an adequate extraction agent has transcendental connotations for the outcome of the analysis and that is why it should be realized in the function of the sought objective. This objective should clearly state what kind of polyphenolic structures we wish to extract. Therefore, solvent mixtures composed of alcohol and water at different percentages are suitable for the extraction of polyphenolic compounds with complex matrices. The employment of fractional extractions are not favored because of the possible losses and enrichments in time-consuming preparations. Likewise, it needs to be emphasized that extraction temperatures above 25°C are not recommendable because, this could trigger decomposition of determined polyphenolic matrix.

Additionally, isolation by means of gel (Sephadex) and C18 cartridges chromatography may be useful in the characterization of complex

samples of polyphenolic nature and to which different structural groups of the same invariably contribute. The mentioned techniques are particularly useful for the isolation of catechins and their proantocyanins from the rest of polyphenolic compounds. Likewise, hydrolysis, generally in acid medium, can be employed after the extraction or purification stage to determine flavonol glycosides present in vegetables and legume isoflavonoids. These processes allow for the simplification of polyphenolic composition by reducing glycosilated structures to one or two polyphenolic species. Finally, let us indicate that save for few very concrete cases, the extraction methods were not systematically investigated.

VI. ANALYTICAL TECHNIQUES USED IN THE DETERMINATION OF POLYPHENOLIC COMPOUNDS FROM FOODS

In this section we describe the most representative analytical methods mentioned in the bibliography for the separation and or quantification of polyphenolic compounds found in foods. In the first place, we discuss chromatographic techniques such as fine layer chromatography, gas, and in particular high-performance liquid chromatography. Within this category of chromatographic techniques special attention is given to detection systems, due to their tremendous importance in characterization of polyphenolic compounds. In continuation we briefly comment on studies carried out with the help of capillary electrophoresis. Finally, a summary of available bibliographical studies that rely on the formation of colored compounds for the spectrophotometric determination on line of total polyphenols contained in comestibles given in Chromatography Methods.

A. Thin Layer Chromatography (TLC)

Before the onset of chromatography, the analysis of polyphenolic compounds was an extremely tedious task and perhaps the most difficult endeavor for those responsible for analytical determination. The birth of paper chromatography revolutionized the analysis of organic substances, and during the 1950s and 1960s paper chromatography was widely used for the determination of polyphenolic compounds, especially when applied for flavonoids determination.[31] In no time this type of chromatography was substituted by thin layer chromatography (TLC). It was considered a very simple and cheap technique that offered great versatility with respect to simultaneous qualitative analysis of polyphenolic compounds in distinct samples through the employment of adequate absorbents and specific reagents.[185-187] The choice of stationary phase as well as an adequate solvent depends on the studied polyphenolic structures. Consequently, the most hydrophilic flavonoids were separated with TLC by employing stationary phases such as polyamide[188] and microcrystaline cellulose.[33] On the other hand, a classical stationary phase made of silicone gel has been used widely to separate more apolar flavonoids such as flavons and isoflavonoids. As a most representative example, we may cite the separation of methoxylated flavones and glycosilated flavones in orange juices, employing the aforementioned absorbents.[189,190] Likewise, this technique has numerous applications in the analysis of antocyanins as confirmed by many bibliographical pilot studies.[191]

The detection, as is well known, is carried out by close inspection of migratory spot under the ultraviolet light. Furthermore, in the current chemical arsenal we dispose of an array of specific reagents that can be applied to each compound, previously separated on the plate.[108,192] Therefore, for the sake of an example we may cite aluminum chloride, boron hydride, sodium,[190] and vanillin[193] as the most common reagents employed in TLC. Inasmuch as that, based on the ensuing reaction and in virtue of the generated color, it is possible to accomplish identification of determined compounds, or at least the involved species of polyphenolic family. Thus, for example, while flavonoles and flavanones do not react with vanillin and HCl in the methanol medium, these reagents nonetheless are capable of reducing flavanones giving off a red or violet color that intensifies throughout reaction, allowing the identification of individual species from a complex polyphenolic environment.[194] Other, more recent

studies have shown the advantage of employing diphenyl dichloride as an reagent because it generates different fluorescence in its reaction with flavones and flavonols.[195] On the other hand, CCF has been employed in its dual modality with the purpose of solving complex mixtures, especially when it comes to separation of quasiidentical pairs of polyphenolic compounds[108,196] and also when the separation is conducted under high-pressure conditions, providing excellent resolutions.[197]

B. Gas Chromatography

One of the principal objections proffered against this kind of chromatography had to do with difficulty by which it quantifies flavonoids. In its beginnings, gas chromatography (GC) was used in an attempt to facilitate the determination of polyphenolic compounds. However, due to the fact that it lacks high volatility, it was necessary to resort to the derivation stage, which in practice resulted in being too complicated for any useful application in the characterization of this type of substance. A very representative example of its application may be found in the determination of flavonoids contained in citric fruits, which after recovery in a polyamide column were derived into esterified structures to be characterized by CG.[198] The CG has been also employed in the determination of flavones found in orange skin oil by using open tubular capillary columns.[199] On the other hand, CG coupled with mass spectrometer (MS) has been employed in the determination of previously derived and hydrolyzed citric juice flavanones.[200] The GC-MS combination has been also used for the analysis of fruit flavones, flavonols, flavanones, and chalcones without the obligatory derivation step.

C. High-Performmance Liquid Chromatography (HPLC)

High-performance liquid chromatography is, without doubt, the most useful analytical technique for characterization of polyphenolic compounds. The foregoing affirmation is fully justified in view of great volume of published studies made available in the last decade. Similarly, if we to compare the review studies headed by Marknam in 1982[201] with the one organized by Robards and Antolovich[31] in 1997, the significance of HPLC clearly outweighs that of CCF. In effect, the role played by the former in the identification as well as in quantification of polyphenolic structures is yet to be surpassed. For example, suffice it to mention that the separation of numerous antocyanin structures takes 20 min under isocratic regime, or that we may use direct injection to analyze wine samples with the convenience of carrying out the separation of polyphenolic compounds by means of elution gradient in the mobile phase.[179,186,187]

The studies pertaining to this technique and carried out between 1982 to 1998 have been accumulated in Table 15. Subsequently, this table indicates the type of studied sample and polyphenolic structures akin to each sample as well as the proper characteristics of the employed technique such as the type and dimensions of the column, the elution method, and characteristics of its principal variables. The mentioned table also contains the detection system applied to each case. In continuation, we delve on to the most representative aspects and deal with the peculiarities of greater interest, following data offered in the bibliography.

With respect to chromatographic methods, let us mention the so-called normal phase that has been employed in the separation of flavonoids found in orange juice and mature tomato seeds.[202,203] These structures, known to be made of flavones, flavanones, and flavonols and their relative glycosides were separated in polyamide column and eluted with methanol in order to isolate them from other extremely polar compounds. Subsequently, the mentioned structures were separated under an isocratic regime through the employment of LiChrosorbSi60 column. The normal phase was also used in the identification of orange and grapefruit flavonoids.[105] When this type of stationary phase is employed, the most polar compounds become irreversibly trapped in the interior of the column and, consequently, gradually alter subsequent separations.[204] It is mainly because of this why practically all the published articles suggest using apolar stationary phases for

TABLE 15
Detemination of Phenolics in Foods by High-Performance Liquid Chromatography

Grapes and wines and their related samples

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Red and white wines	Flavonols (4)	Novapak C18 (8X100mm)	Isocratic H_2O/MeOH/ HAc	UV, 365 nm.	Quantitation	90
Wines	Acids Flavonols Catechins Cinnamics (13)	Superspher RP-18 250 mm	Ternary gradient A/HAc B/HAc C/HAc/Agua/ AcN	UV, (DAD) 260,280, 328.355 y 370 nm	Direct injection	81
Grapes (juices)	Acids Catechins Flavonols (18)	Supelcosil LC-18 (4.6X250 mm, 5µm)	Lineal gradient A/Fosfato ácido de potasio B/MeOH	UV (DAD) 280, 320 nm.	Sample filtration and injection	27
Grapes (seeds)	Catechins and proanthocyani-dins (7)	Altech (2.1X100mm, 5 µm)	Lineal gradient A/HAc 10% B/ Agua	UV (DAD) 280 nm	Identification purposes	88
Wines	Anthocyanidin	Lichrospher RP-18	Lineal gradient A/Ac. Fórmico B/Fórmico/Agua/MeOH	Vis. 520 nm		89
Wines	Acids Catechins Flavonols (16)	HR 80 (4.6X80 mm, 3µm)	Gradient A/SDS, phosphate, Nitrilacetic, MeOH 50% B/SDS, phosphate, Nitrilacetic MeOH 50%	Array electrodes		83
Wines	Acids Catechins Proanthocyani-dins Flavonols (19)	Nucleosil 120 C18 (4X250mm, 5µm)	Lineal gradient A/HAC (pH 2.65) B/AcN/HAc	UV(DAD) 280, 320, 365 nm	Quantitative determination	84

TABLE 15 (continued)

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Wines	Acids Catechins Proanthocyani-dins Flavonols(24)	Supersheer RP 18 (4X250mm)	Ternary gradient A/HAc (5%) B/Agua C/AcN	UV (DAD) 280, 320,365 nm	Direct injection	86 87 179
Wines	Acids Catechins(22)	LKB Spherisorb (4X100, 3μm) LKB Spherisorb (4X250, 5μm) LiChrospher (4X125, 5μm) LiChrospher (4X250, 5μm) Novapak (3.9X150, 4μm)	Linear gradient A/MeOH/ HAc B/MeOH	DAD 280, 320 nm	Different columns and gradients	178

Citrus: Fruits, juices and related foods

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Orange (juice)	Methoxylated Flavonas (5) Flavanonas glycosides (3)	Zorbax C18 ODS (4.6X250mm)	Lineal gradient A/HAc (1%)/MeOH (5%) B/HAc (1%)/MeOH (5%)/AcN	UV 280 nm.		100
Orange (juice)	Methoxylated Flavonas (5)	Hypersil ODS (4.6X250 mm, 5μm)	Isocratic AcN/H_2O	Fluorescence λ(exc) 330 nm λ (emi.) 430 nm	Quantitative determination	96

TABLE 15 (continued)

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Orange Mandarin (juices)	Methoxylated Flavonas (9)	Hypersil ODS (2.1X200 mm, 5μm)	Linear gradient A/H_2O/THF B/AcN/THF	UV (DAD) 320, 335, 345 nm	Quantitative determination	95
Orange (juices)	Cinnamic acids	Adsorbosphere C18 (4.6X250 mm, 5μm)	Isocratics Agua/AcN/ MeOH/THF	UV (DAD) 300nm	Optimization mobile phases Elution purity	109
Orange, mandarin lemmon and pummelo	Flavonoids (6)	LiCrochart RP-18 (4X125 mm, 5μm)	Gradiente lineal A/Fórmico B/MeOH	UV (DAD) 280 nm	Fresh fruits and jams analysis	106
Orange	Flavanonas Flavonas (12)	Bondapak C18 (4X250 mm, 5μm)	Isocratic/ Gradiente (various)	UV (DAD), 280 nm.	Identification purposes	102
Orange (juices)	Methoxylated Flavonas (6)	Novapak (3.9X150 mm, 4μm)	Gradiente lineal/no lineal A/THF/Agua B/AcN	UV (DAD) 340nm	Simultaneous juice analysis	99
Orange (juices)	Flavanonas glycosides (3)	Novapak (3.9X150 mm, 4μm)	Linear gradient A/phosphate B/AcN	UV (DAD) 280 nm		97
Citrus	Flavonoids (25)	LiChrospher (4X250 mm, 5μm)	Linear gradient A/phosphoric B/MeOH	UV (DAD) 285 nm	Quantitative determination in the different parts of the fruit	80
Orange and pummelo (juices)	Prominent flavonoids (3)	Alltima C18 (4.6X250mm, 5μm)	Isocratic (various) Agua/THF Agua/AcN/OrO H/fórmico	UV (DAD) 283 nm	Extraction efficiencies Elution purity	101
Pummelo varieties	Flavanonas (3)	Bondapak C18 (4X250 mm, 5μm)	Isocratic H_2O/MeOH/ AcN	UV (DAD) 280 nm	Quantitative determination in varieties	103

TABLE 15 (continued)

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Mandarin	Flavanonas (3)	Bondapak C18 (4X250 mm, 5μm)	Isocratic H_2O/MeOH/ AcN/HAc	UV (DAD) 280 nm		104

Rosaceae fruits

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Blueberries Strawberry Raspberries	Anthocyanidin	Suplecosil ODS (5X250 mm, 5μm)	Isocratic A/HAc 15% B/AcN	Vis (DAD) 520 nm	Characterizati on studies	132 133
Strawberry Blueberry juice	Ellagic acid Flavonols Flavan-3-ol Hydroxybenzoi c and cinnamic acids	Spherisorb ODS (4.6X250 mm, 5μm)	Gradiente lineal A/HAc B/AcN	UV (DAD) 260,280,325,3 60 nm		134 135 136
Fresh Blueberries	Anthocyanidin	SuperBac Pep-S (4X250 mm, 5μm)	Gradiente lineal A/Fórmico B/MeOH	Vis. (DAD) 525 nm	Quantitation and distribution	137
Fresh cherries	Anthocyanidin and other phenolics	SuperBac Pep-S (4X250 mm, 5μm)	Lineal gradient A/Formic acid B/MeOH	UV-Vis. (DAD) 280 y 525 nm	Quantitation and distribution	138

Fresh and processed vegetables

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Fresh vegetables	Cinnamic acids (4)	Ultrasphere ODS (4.6X250 mm)	Linear gradient A/MeOH 10%/HAc B/MeOH	UV (DAD) 320 nm	Identification and distribution	140

TABLE 15 (continued)

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Fresh vegetables	Flavonols and Flavonas (aglycones 4)	Novapak C18 (3.9X150 mm, 4μm)	Isocratic (2) I/AcN/ phosphate II/MeOH/ phosphate	UV (DAD) 370 nm	Hydrolysis optimization. Quantitation as aglycones	65 141 142
Fresh onions	Flavonol glycosides	ODS Dynamax (4.6X250 mm, 8μm)	Lineal gradient A/THF/H_2O B/AcN	UV (DAD) 270 nm	Poccesing and storage influence	144
Tomatoes onions lettucce apio	Flavonoids	Symmetry C18 (3.9X150 mm, 5μm)	Gradient A/TFA (pH 2.5) B/AcN	UV (DAD) 365 nm	Quantitative determination	145

Dried and processed legumes

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Soy legumes	Isoflavonoids	LiChrosorb RP-8 (4X250 mm, 5μm)	Isocratic AcN/phosphate (pH 2.0)	UV, 260 nm Amperometric detection E=0.90 V vs Ag/AgCl (ER)	Array detection	157
Peas	Isoflavonoids	Polyol RP-18 (4.6X250 mm, 5μm)	Linear gradient A/HAc B/MeOH	UV 280 nm.		158
Legumbres (secas y procesadas)	Isoflavonoids (aglycones) (5)	Novapak C18 (3.9X150 mm, 4μm)	Linear gradient A/HAc B/AcN	UV (DAD), 260, 342 nm Fluorescence λ(exc) 340 nm λ (emi) 418 nm	Hydrolysis of glycosides and quantitation	44

TABLE 15 (continued)

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Legume	Flavonoids	ODS Hypersil (4X250 mm, 5µm)	Lineal gradient A/HAc B/AcN	UV (DAD), 260, 275, 290, 345, 365 nm.	Quantitation	159

Apples and pears

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Fresh apples	Procyanidins (5)	Normal phase Absorption Zipax PAM Pellamidon LiChrosorb60 ETH Permaphase CoPellPAC (4.6X500mm)	Isocratic HAc/MeOH	UV, 280 nm	Procyanidin chromatography. Stationary and Separation optimization	118
Fresh apples	Procyanidins (7)	Reversed phase ODS Permaphase Phenyl Sil-X Phorether Sil-X C18 Bondapak SAS Hyperspheres (4.6X120mm) nmPG Cp CPG	Isocratic MeOH/H_2O/ $HClO_4$			118

TABLE 15 (continued)

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Fresh apples	Procyanidins (3)	Exclusion chromatography Poragel 60 (6nm) Poragel 100 (10 nm) Poragel 200 (20nm) Cpg-1075 (7.5nm)	Isocratic THF/H_2O			118
Apple juices	Cinnamics Catechins Procyanidins (2) Chalcones	Spherisorb 5 Hexyl (5X100mm)	Ternary gradient A/ H_2O (pH 2.5) B/MeOH C/H_2O (pH 7.0)	UV, 280 nm		113
Fresh apples (peels)	Quercetin glycosides (4)	PR C18	Linear gradient A/TFA (0.1%) B/THF	UV, 270 nm	Glycosides identification	35
Fresh apples (peels)	Quercetin glycosides and chalcones	RP C18 (8X100mm)	Isocratic AcN/HAc	UV (DAD) 350 nm	Glycosides identification. Phloretin xyloglucoside identification	114
Fresh apples (pulps)	Acids and catechins	Econosil C18 (4.6X250 mm, 5μm)	Lineal gradient A/HAC (5%) B/AcN	UV, 254 nm	Elution purity	110
Fresh apples and pears	Cinnamics Catechins and procyanidins. Chalcones and flavonols	Supelcosil LC-18 (4.6X250 mm, 5μm)	Linear gradient A/Phosphate B/MeOH	DAD 280, 320 nm.	Proccesing and storage influence. Procyanidins isolation in Sephadex	32 36

TABLE 15 (continued)

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Fresh apples (peel and pulp).	Acids Catechins chalcones and flavonols (5)	NovaPak C18	Gradient A/TFA B/THF	UV(DAD) 270nm	Distribution of phenolics in the fruit	115
Apple varieties (Peels and pulps)	Cinnamics Catechins and flavonols (12)	NovaPak C18 (3.9X300 mm)	Linear gradient A/HAC B/HAc/AcN	UV (DAD) 280, 340 nm	Quantitation and distribution	116
Fresh apples (pulp varieties)	Acids and flavonoids (15)	ODS Spherisorb C18 (4.6X250 mm, 5μm)	Linear gradient A/HCL B/MeOH	UV, 220, 255, 275, 290. 320 nm.	Quantitative determination	111
Apples and pears (jams)	Acids and flavonoids	Lichrochart 100 RP-18 (4X125 mm, 5μm)	Linear gradient A/Formic B/MeOH	UV (DAD) 280, 350 nm	Quantitative determination	107
Apples and pears (commercia–l juices)	Acids and catechins (19)	Novapak C18 (3.9X300 mm)	Linear gradient A/HAc (2%) B/MeOH/AcN	UV (DAD), 280, 340 nm	Quantitative determination	108
Apples and pears (commercia–l juices)	Flavonols (aglycones)	Novapak C18 (3.9X150 mm)	Isocratic H_2O/MeOH/ AcN	UV (DAD), 254, 365 nm	Quantitative determination	108
Apples and pears (commercia–l juices)	Flavonols (glycosides)	Novapak C18 (3.9X150 mm)	Isocratic THF/H_2O/HAc	UV (DAD), 254, 365 nm	Quantitative determination	108
Fresh apple varieties	Catechins Chalcones Flavonols	Spheriorb ODS (4.6X250 mm, 3μm) NovaPak C18 (3.9X300 mm, 4μm)	Linear gradient A/Phosphoric (pH 2.8) B/MeOH	UV (DAD), 220, 260, 275, 350 nm.	Quantitativede termination and bibliographic comparation	117

TABLE 15 (continued)

Sample	Analytes	Column	Elution	Detection	Comments	Ref.
Fresh pears varieties	Isorhamnetin and quercetin glycosides	Adsorbosphere C18 (4.6X150 mm)	Linear gradient A/Phosphoric (pH 2.6) B/AcN/MeOH/ H_2O	UV (DAD) 370 nm	Glycosides identification	33
Apples	Procyanidins	NovaPak C18 (3.9X100 mm, 4μm)	Lineal gradient A/HAc 2% B/AcN	UV (DAD) 280 nm	Characterization	119
Tea and derivatives						
Sample	**Analytes**	**Column**	**Elution**	**Detection**	**Comments**	**Ref.**
Green tea	Flavanols (5)	Bondapak C18 (4X300 mm, 10μm)	Isocratic HAc/MeOH/DMF/ H_2O	UV, 280 nm		164
Green tea and other varieties	Flavonols	Ultron-NC18 (4.6X150 mm)	Linear gradient A/Phosphoric B/AcN/DMF	UV, 280nm		164
Tea varieties	Flavonol glycosides (14)	ODS-Hypersyl	Isocratic I/HAC/AcN II/HAc/dioxan/ MeOH	UV 354nm		164
Liquor tea	Acids Flavanols Flavonols	ODS-Hypersyl (4.6X250 mm, 5μm)	Linear gradient A/HAc (2%) B/AcN	UV-VIs (DAD) 280, 380,460, 510 nm	Characterization by DAD	205

the separation of polyphenolic compounds, therefore adopting, and practically unanimously, partition chromatography in an inverse phase as matter of fact (Table 15). The most common stationary phases are prepared with chemically modified silicone containing hydrocarbon chains, where the denominated C8 have been used to the lesser extend than C18. On the other hand, the employed elution modality, whether isocratic or gradient, depends on the polyphenolic composition present in the samples. The isocratic elution has been employed in those samples whose polyphenolic composition is constituted by the same group or structural family (Tble 15). Therefore, by this method it is possible to conduct the determination of flavonols (quercetin, miricetin and kaempferol) in wine samples,[90] methoxylated flavones,[96,102-104] cinamic acids (caffeic, chlorogenic, ferulic, and cumaric),[109] and flavonoids (naringin, hesperidin, and neohesperidin)[101] in citric fruit samples. Another example that illustrates this point is the separation of antocyanins from different *rosaceae* fruits such as strawberries and raspberries by means of acetonytrile and acetic acid at 5% as a mobile phase.[132,133] The isocratic elution has been also employed in certain vegetables and legumes.[141,142,157] Thus, Hertog et al. relied on this type of elution for identification and subsequent quantification of principal aglycones proper of flavonoles (quercetin and kaempferol) and flavones (apigenin and luteolin) in different vegetables comprising the Dutch diet. Likewise, isoflavonoids have been separated by isocratic elution from soybean samples, more precisely in a C8 column with the help of acetonitrile and a phosphate (pH 2.0) as a mobile phase.[157]

On the other hand, it is necessary to indicate that the majority of published chromatography studies certify the use of elution in mobile gradient phase. This fact should not surprise anybody because we are dealing with complex samples that contain polyphenolic compounds that show a different retention pattern. As a matter of fact, it is worth mentioning the chromatographic separation of cinamic acids, flavanols, chalcones, and apple skin flavonols,[36,116,117] plus flavones, flavanones, and citric fruit flavonols from citric fruits.[80] Also, it must be mentioned that majority of chromatography experts have employed linear gradient under constant flow. When it comes to temperature used in the separation, it can be said that in general it must never be too high. Hence, for the analysis of wine and citric fruit samples some authors recommend 40°C,[80,83,84,95,97,99] although as a rule, and as seen in Table 15, most of the separations were carried out under ambient temperature. On the other hand, the published studies speak of using methanol, acetonitrile, and, to the lesser extent, tetrahydrofurane (THF) as participating solvents in the mobile phase as well as of incorporating into the medium small quantities of weak acids such as formic, acetic, or phosphoric. Subsequently, under the mentioned conditions it was possible to solve many complex samples originated from wines, citric fruits, rosaceae, and apples. In effect, a method of elution with binary mobile gradient phase and constituted by AcH at 5% as low-grade eluting solvent in a mixture constituted by aqueous acetonitrile, in the presence of the same acid modifier allowed obtaining numerous peaks in wine samples.[86] The said method required a time gradient of 150 min due to a large number of polyphenolic compounds present in the sample. However, in order to obtain a complete resolution, the gradient method was carried out by means of a ternary solvent mixture, where the third solvent reaction also consisted on a dissolution of lighter acetic acid (1%). Such would be the case of studies carried on bilberries sample, where 25 antocyianins were separated in less than 40 min by means of a gradient elution consisting of methanol and formic acid in a SuperPac column.[137] To this effect, Nogata et al.[80] established a standard gradient schedule using H_3PO_4 0.1 *M* and MeOH as a solvent with high eluting power to carry out the separation of 25 flavonoids in a C18 column in a record time of 90 min.

With respect to detection system used in high-performance liquid chromatography suitable for the derivation of polyphenolic compounds, it needs to be emphasized that the UV-VIS detection is undoubtedly the most common. The fluorescence and the electrochemical detection systems have been used to the lesser extent. The veracity of this statement is reflected in Table 15, which also lists the analytical conditions required for detection gathered

from available bibliography. Thus, it can be observed that the immense majority of published studies rely on the detection of polyphenolic compounds at column's exit by taking advantage of radiation absorption by these compounds in the UV-VIS region of electromagnetic spectrum. The most frequently used wavelength has been 280 nm,[27,35,80,81,4-90,100-104,108,111,116-119,134-136,138,164,205] because at that wavelength it is known to absorb all the polyphenolic compounds.[5] Another employed wavelength, although to the lesser extent, has been 254 nm.[108,110,111] Generally speaking, both wavelengths exhibit similar analytic sensibility; however, the 280 nm wavelength is used more frequently as the basis of absorption in the mobile phase, especially when acetic acid is employed as an acid modifier. Nonetheless, some bibliographical studies recommend employing different wavelengths with the purpose of achieving maximum sensibility, and if possible an adequate selectivity depending on the type of sample and its polyphenolic composition. Following this philosophy, it was possible to detect cinamic acids and their hydroxylated derivatives at 325 nm,[84,86,87,95,109,111,134-136,140,179] flavonol glycosides at 350 nm,[81,107,108,116] and aglycones at 370 nm.[86,87,90,134-136,141,142,179] Nonetheless, neither hydroxybenzoic acids nor flavan-3-ol exhibit absorption at the previously mentioned wavelengths, and, consequently, they do not offer interference in the chromatogram. An excellent example of the same argument is seen in the employment of visible region wavelength of the spectrum for the identification of antocyanins. These structures possess an intense absorption band sensitivity, generally above 500 nm, at which no other polyphenolic structures absorb. This phenomenon allows detection of the mentioned structures in complex samples without the interference of subjacent polyphenolic species.[89,137,138]

On the other hand, the spectroscopic molecular UV-VIS absorption amounts to one of the most powerful identification tools currently employed in the detection of polyphenolic compounds when these are combined with chromatographic techniques. The usefulness of this technique has been manifested by the incorporation of array diode detectors (DAD). It is well known that this type of detector offers certain advantages with respect to the detection, for they secure chromatograms at any wavelength, accompanied by the absorption spectrum of each eluted band. The absorption spectrum can be combined with retention parameters for the possible identification of an unknown compound and also to measure purity of the elution band in question. This finding has gained in the last years enormous publicity due to its practical application in the analysis of polyphenolic compounds, especially thanks to its usefulness in chromatographic techniques when applied to quantitative sample analysis.[101] The incorporation of this type of detector has led to the publication of a series of relevant articles that deal with the different possibilities of this type of detection in complex tea and wine samples, respectively.[86,205] The polyphenolic characterization of wine samples by direct injection into HPLC, without subjacent treatment of the same, first suggested by Roggero was possible thanks to the incorporation of diode detectors.[86] Other researchers have studied different parameters that can be evaluated by computer software that yielded useful information concerning identification of polyphenolic compounds.[206,207] Among these studies one can find a detailed description concerning the isolation of determined procianidines structures quantified by absorption bands and by other parameters secured by derivative spectroscopy. Finally, it needs to be indicated that according to the bibliographical information, the serial diode detectors have been essential in the characterization of polyphenolic compounds from all types of foods, not only in drinks like tea or wine, but also in the detection of polyphenolic compounds in fruits and vegetables.[208-213,214-217]

The fluorescence has been also employed for this purpose, although to the lesser extent than UV-VIS detection with hope of improving sensitivity as well as selectivity after the identification of the polyphenolic compound.[44,96,218] It is quite relevant to emphasize that one of the first investigations carried out with HPLC and related to the study of nonvolatile orange and tangerine oils fractions suggests the fluorimetric detection in conjunction with conventional UV-IVS detection.[218] Thereafter, and also in citric fruits samples, fluorescence detection was used to identify five principal methoxylated flavones in orange juices.[96] Similarly, after the determination of isoflavonoids in a large number of legume samples, Frank et

al.[44] detected cumestrol by relying on this technique, given the superior fluorescent character of this compound.

With respect to the latter, it is worth mentioning that electrochemical detectors were also employed in the characterization of polyphenolic compounds such as isoflavonoids found in soybean[157] or other polyphenols proper of wine and orange samples.[83] In the first case, the elution was conducted under an isocratic regime, while in the second the elution was carried out under gradient routine. When it comes to the second case, 16 electrodes connected in series at different potentials were subsequently employed.

Finally, let us indicate that HPLC coupled to mass spectrometer has allowed the resolution of many complex mixtures of polyphenolic compounds.[219,220,221]

D. Capillary Electrophoresis

From a technical point of view, the determination of polyphenolic compounds stored in vegetables and produce does not seem to benefit from the use of this technique, although some articles dealing with this subject can be retrieved easily from the bibliography. The model separation involving this method was applied during the isolation of polyphenols from orange juice, using sodium borate buffer at 35 m*M* with 5% of AcN and 21 kV voltage as electrode potential.[222] The developed method allowed the determination of flavonoids in alkaline medium and the elimination of carotenoids by electrically induced osmotic flow.

The behavior of flavonoid migration in micelle electrokinetic chromatography has been studied to a lesser extent.[223] Some factors, such as applied voltage, capillary temperature, the concentration and nature of the electrolyte (that is to say, complex or simple buffers), the concentration and nature of surfactant agent responsible for micelles and organic modifiers have demonstrated an influence on resolution and the selectivity of the separation.[223,224] The addition of organic modifiers such as methanol alters the interaction between analytes and miceller phase. Therefore, it was possible to observe that the presence of this organic solvent triggers decomposition of peaks corresponding to the most hydrophobic flavones. Nonetheless, this phenomenon can be avoided if acetonitril is used as an organic solvent.

However, many scientists maintain that both capillary electrophoresis and HPLC are indispensable analytical techniques, because in many cases they complement each other, especially when it comes to secure general information about the presence of polyphenolic compounds in certain foods.[225,226,227,228] Even though analytical glitches may complicate HPLC, this can be resolved through the employment of electrophoresis techniques.[31]

E. UV-VIS Spectrophotometry

The spectrophotometric methods are not new to the field of analytical chemistry, as they are often used to determine what in scientific terms is known as total polyphenols. The following chemical mixture, Folin-Ciocalteu became the most frequently prescribed reagent[229,230] for the formation of colored compounds, crucial in polyphenolic determination. Basically, this method consists of generating a certain color through the addition of the mentioned reagent into alkaline medium replete with a liquefied sample. In most cases, the transformation is accomplished in the presence of anhydrous sodium carbonate (75 g/L) with the subsequent spectrophotometric evaluation at 750 nm. Swain and Goldstein[231] have reviewed different spectrophotometric methods currently available in the bibliography, and based on the evidence they have strongly recommended Folin-Ciocalteu as the most suitable reagent for spectrophotometric estimation of total polyphenols. However, vanillin method seems to be more adequate for isolation of catechins when these are suspected to constitute prevailing polyphenolic structures in a given sample. Nonetheless, it needs to be stressed that Folin-Ciocalteu reagent, which was and still is used with relative frequency, also reacts with other polyphenolic structures[232] and, consequently, these detractors ought to be eliminated in a stage previous to detection, or well calculated "a posteriori" as the weight exerted on total polyphenolic fraction. As a matter of fact, other reagents such as Prussian blue

have been also employed for that purpose, albeit less frequently.

It is well known that spectrophotometric methods generally yield a gross estimation of the polyphenolic content. Consequently, these methods were employed in the rough analysis of polyphenolic compounds found in wines,[91,233,234,235] legumes,[150] and apple juice.[121] Notwithstanding, they may be useful in batch analysis or individual separation through continuous flow. Applying this criterion Carmona et al.[150] successfully determined total polyphenolic configuration of tannins and nontannins in samples from common white and black beans varieties. In these experiments extracts of ground beans mixed with MeOH and HCl at 1% were separated into two fractions: tannins and nontannins, after passing the substrate through Sephedex LH-20 column.

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