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Chlorogenic acids – their properties, occurrence and analysis

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Chlorogenic acids (CQAs), the esters of caffeic acid and quinic acid, are biologically important phenolic compounds present in many plant species. Nowadays much is known from their pro-health properties, including anti-cancer activity. Yet, the supposition that they may be helpful in fighting obesity and modify glucose-6-phosphatase involved in glucose metabolism have led to a revival of research on CQAs properties and their natural occurrence. Much attention is also paid to the proper analysis of CQAs content in plants and plant products due to the fact that the main CQAs representative in nature i.e. 5-*O*-caffeoylquinic acid (5-CQA) is commonly employed as a quality marker in the control of various natural products.

The common procedures used so far for CQAs determination in plants involve conventional long lasting solvent extraction realized at higher temperatures prior to chromatographic analysis. The drawbacks associated to the conventional extraction techniques have led to the search for new alternative extraction processes that additionally could improve extracts quality. The latter is particularly important as regards CQAs applications, and the fact that these compounds easily transform/degrade to others. According to reports, the conventional heating of 5-CQA in the presence of water causes its isomerization and transformation. The reports prove that 5-CQA not only isomerizes to 3- and 4-*O*-caffeoylquinic acid, but

also undergoes other transformations such as esterification and reactions with water. Hence, in the attempt to improve the process of chlorogenic acids extraction and to make it more effective and environmentally friendly, innovative so-called "enhanced" techniques of extraction have been recently developed and applied. To guarantee the proper analysis of compounds with very similar properties forming the chlorogenic acids family and their numerous transformation/degradation products, hyphenated techniques, in particular LC-MS, are currently being used.

The purpose of this review is to summary and overview of the sources, properties, and methods which have been developed to improve the extraction and analysis of chlorogenic acids in plants and plant products, with particular reference to 5-CQA and 1,3-di-CQA as these compounds of the CQAs family with many beneficial effects on human health.

1. INTRODUCTION

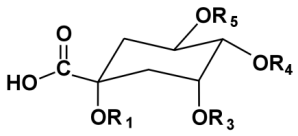
Chlorogenic acids (CQAs) are naturally occurring phenolic compounds found in all higher plants [1, 2]. Due to their various biological properties, such as antispasmodic [3], antioxidant [4], inhibition of the HIV-1 integrase [5, 6] and inhibition of the mutagenicity of carcinogenic compounds [3], they are very important plant secondary metabolites. In the light of recently published results [7–12], they are also supposed to be helpful in fighting obesity and modify glucose-6-phosphatase involved in glucose metabolism. These suppositions, although unproven, were sufficient to a revival of research on CQAs properties and their natural occurrence, the more that new applications of CQAs are suggested in pharmaceuticals, foodstuffs, food additives and cosmetics.

From the chemical point of view, CQAs constitute a large family of esters formed between quinic acid and one or more *trans*-hydroxycinnamic acid derivatives, most commonly caffeic, *p*-coumaric, and ferulic acid [2]. Taking into account the identity, number and position of the acyl residues, in this group of compounds may be distinguished:

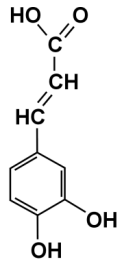
- mono-esters of caffeic, p-coumaric and ferulic acid: caffeoylquinic acids (CQAs), *p*-coumaroylquinic acids (pCoQAs) and feruloylquinic acids (FQAs), respectively,
- di-esters, tri-esters and the single tetra-ester of caffeic acid: diCQAs, triCQAs and tetraCQA, respectively,

– mixed di-esters of caffeic and ferulic acid or caffeic and sinapic acid: caffeoylferuloylquinic acids (CFQAs) and caffeoylsinapoylquinic acids (CSiQAs);
– mixed esters involving various permutations of one or three caffeic acid residues with one or two dibasic aliphatic acid residues *e.g*. glutaric, oxalic, succinic.

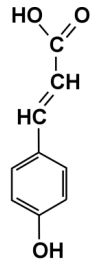
Fig. 1 presents the general structures of mono- and dicaffeoylquinic, p-coumaroylquinic, and feruloylquinic acids together with structures of the most characteristic representatives of mono- and dicaffeoylquinic acids for plants *i.e.* 5-*O*-caffeoylquinic acid and 1,3-di-*O*-caffeoylquinic acid.



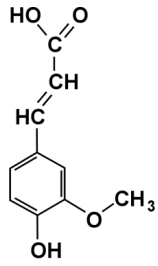
Quinic acid



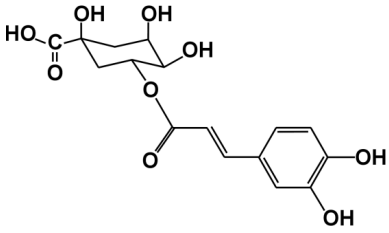
Caffeic acid



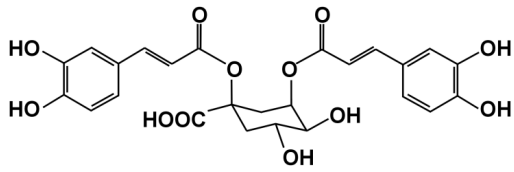
***P*-coumaric acid**



Ferulic acid



5-*O*-caffeoylquinic acid



1,3-di-*O*-caffeoylquinic acid

No.	Name and	Abbreviation	R_1	R_3	R_4	R_5
1	1-*O*-caffeoylquinic acid	1-CQA	C	H	H	H
2	3-*O*-caffeoylquinic acid (neochlorogenic acid)	3-CQA	H	C	H	H
3	5-*O*-caffeoylquinic acid (chlorogenic acid)	5-CQA	H	H	H	C
4	4-*O*-caffeoylquinic acid (cryptochlorogenic acid)	4-CQA	H	H	C	H
5	1,3-di-*O*-caffeoylquinic acid	1,3-diCQA	C	C	H	H

cd. Fig. 1.

No.	Name and	Abbreviation	R_1	R_3	R_4	R_5
6	1,4-di-*O*-caffeoylquinic acid	1,4-diCQA	C	H	C	H
7	1,5-di-*O*-caffeoylquinic acid	1,5-diCQA	C	H	H	C
8	3,4-di-*O*-caffeoylquinic acid	3,4-diCQA	H	C	C	H
9	3,5-di-*O*-caffeoylquinic acid	3,5-diCQA	H	C	H	C
10	4,5-di-*O*-caffeoylquinic acid	4,5-diCQA	H	H	C	C
11	1-*O*-*p*-coumaroylquinic acid	1-*p*CoQA	*p*Co	H	H	H
12	3-*O*-*p*-coumaroylquinic acid	3-*p*CoQA	H	*p*Co	H	H
13	5-*O*-*p*-coumaroylquinic acid	5-*p*CoQA	H	H	H	*p*Co
14	4-*O*-*p*-coumaroylquinic acid	4-*p*CoQA	H	H	*pCo*	H
15	1,3-di-*O*-*p*-coumaroylquinic acid	1,3-di*p*CoQA	*pCo*	*p*Co	H	H
16	1,4-di-*O*-*p*-coumaroylquinic acid	1,4-di*p*CoQA	*p*Co	H	*p*Co	H
17	1,5-di-*O*-*p*-coumaroylquinic acid	1,5-di*p*CoQA	*p*Co	H	H	*p*Co
18	3,4-di-*O*-*p*-coumaroylquinic acid	3,4-di*p*CoQA	H	*p*Co	*p*Co	H
19	3,5-di-*O*-*p*-coumaroylquinic acid	3,5-di*p*CoQA	H	*p*Co	H	*p*Co
20	4,5-di-*O*-*p*-coumaroylquinic acid	4,5-di*p*CoQA	H	H	*p*Co	*p*Co
21	1-*O*-feruloylquinic acid	1-FQA	F	H	H	H
22	3-*O*-feruloylquinic acid	3-FQA	H	F	H	H
23	5-*O*-feruloylquinic acid	5-FQA	H	H	H	F
24	4-*O*-feruloylquinic acid	4-FQA	H	H	F	H
25	1,3-di-*O*-feruloylquinic acid	1,3-diFQA	F	F	H	H
26	1,4-di-*O*-feruloylquinic acid	1,4-diFQA	F	H	F	H
27	1,5 -di-*O*-feruloylquinic acid	1,5-diFQA	F	H	H	F
28	3,4-di-*O*-feruloylquinic acid	3,4-diFQA	H	F	F	H
29	3,5-di-*O*-feruloylquinic acid	3,5-diFQA	H	F	H	H
30	4,5-di-*O*-feruloylquinic acid	4,5-diFQA	H	H	F	F

Q- quinic acid, C-caffeic acid, *p*Co- *p*-coumaric acid, F- ferulic acid

Fig. 1. Structures of mono- and dicaffeoylquinic, p-coumaroylquinic, and feru-lylquinic acids

One of significant features of CQAs is their chemical instability and formation/degradation to other compounds, including chlorogenic acids representatives [13–16]. According to report [15], the conventional heating of 5-*O*-caffeoylquinic acid (5-CQA) in the presence of water causes its isomerization and transformation. In [16] it was proved that 5-CQA not only isomerizes to 3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid, but also undergoes other transformations such as esterification and reaction with water *i.e.* hydrolysis and/or addition of water molecule to the double bond. These processes take place not only in CQAs solutions but also during their isolation from plant materials and they could lead to obtain extracts with lower content of biologically active phenolic compounds.

Recently, there has been an increased interest in natural products rich in chlorogenic acids. Consequently, more efficient extraction methods, leading to obtain extracts with enhanced CQAs content and reduced content of their degradation products, are being sought. Unfortunately, the elimination/limitation of CQAs degradation/transformation is not the only problem in the CQAs determination in plants or plant products. The number of compounds needed to be identified during a single analysis is steadily increasing, through the number of commercially available standards is limited to just a few. This fact, in the face of the large number of CQAs with very similar properties and their tendency for easy formation of transformation/degradation products during their extraction, causes that CQAs analysis is not easy. Recently, more and more often it is realized by means of hyphenated techniques, in particularly using LC-MS^n.

The aim of this review is to summarize and give an overview of the sources, properties, and methods that have been developed to improve the extraction and analysis of chlorogenic acids in plants and plant products, with special attention to 5-*O*-caffeoylquinic acid and 1,3-di-*O*-caffeoylquinic acid as these compounds of the CQAs family with many beneficial effects on human health.

2. CQAS OCCURRENCE

CQAs belong to very common plant constituents. They are present, for example, in apples, stone fruits (peaches, nectarines, plums, lychees, mangoes, cherries), berry fruits (blueberries, blackcurrants, blackberries, bilberry), brassica vegetables (kale, cabbage and Brussels sprouts),

apiaceae (celery, carrots, caraway and coriander), and others miscellaneous vegetables like corn salad, anise stars and potato [1, 2, 17–30]. Nevertheless, despite their high prevalence, they are believed to be the most characteristic constituents of plants from *Asteraceae* family, such as artichoke (*Cynara scolymus*), black salsify (*Scorzonera hispanica* L.), purple coneflower (*Echinacea purpurea* L. *Moench*), common yarrow (*Achillea millefolium* L.), milk thistle (*Silybum marianum* L. *Gaertner*), coltsfoot (*Tussilago farfara* L.), tansy (*Tanacetum vulgare* L.), chamomile (*Matricaria chamomilla* L.) [1, 2, 31–44]. Yet, even if these compounds are the most characteristic for the plant family, the CQAs content is very differentiated. According to Lattanzio et al. [31–44], the total content of CQAs in artichoke ranges from 8 % on dry matter basis in young artichoke tissues to less of 1 % in senescent ones. Of the total content of the CGAs in this plant, 5-CQA is the most represented single component (39 % of all CQAs in the artichoke), followed by 1,5-diCQA (21 %) and 3,4-diCQA (11 %). The content of 1,3-diCQA is 1.5 % of all CQAs. For contrast, black salsify provide 180 mg/kg 5-CQA with little or none content of the other CQA derivatives [35].

CQAs are present in all parts of plants (seeds, roots, tubers, leaves and flowers) as well as in different products prepared from them, in particularly in beverages, such as coffee, green and black tea, yerba mate, juice, infusion and even wine. It should be emphasized, however, the CQAs qualitative and quantitative composition is various and dependent not only on plant parts but also on the plant physiological stage, the conditions of storage and plants processing, especially when the processing is accompanied by heat and/or enzymatic treatment.

Whole apples have been reported to contain 62–358 mg/kg CQAs [19–21]. They are found at similar concentrations in the apple's isolated flesh and skin (mean values 30–60 mg/kg) but are absent in the seeds and pomace [21–23]. With the change of apples variety changes not only the total CQAs content but also the CQAs composition. Yet, regardless of the apples variety, 5-CQA is the main constituent. Variability of quantitative and qualitative composition of CQAs is also characteristic of juices and ciders. In commercially available juices, the CQAs content can be from undetectable level up to 208 mg/dm^3 5-CQA alone [19], and in ciders in the range of 11–480 mg//dm^3 or even higher when these are produced from the fruit of *Sorbus domestica*, known from a high 5-CQA content (1500 mg/kg) [24].

The mean CQAs content in stone fruits is in the range 150–600 mg/ kg. Compared with apples, in the fresh stone fruits 3-CQA

is more prominent relative to 5-CQA. However, in peach and apricot juice the 5-CQA level is about 3–4 times greater than 3-CQA, with the total CQAs content reaches 9 and 4 mg/dm^3, respectively [1].

The CQAs content in potato tubers have been reported in the range 500–1200 mg/kg dry basis [25–26]. They are concentrated in the peel where they may reach level of 2–5 g/kg or even higher [27]. Of the CQAs, the di-CQAs are present in a little amounts while the mono-CQAs amounts are greater. After harvesting period, the content of CQAs rises slowly, particularly during tubers storage in the light or during wound healing.

One of the richest dietary sources of CQAs are coffee beans. Depending on the species, green coffee beans contain 6–12 % CQAs on the dry weight of the coffee beans. During roasting there is a progressive degradation and transformation of CQAs. It is estimated that 8–10 % CQAs are lost for every 1 % of the dry weight of the total CQAs amount in the coffee beans. Clifford and Walker evaluated [28] that a 200 cm^3 cup of roast and ground coffee might supply from 20 mg 5-CQA (weak brew, very dark roast) up to 675 mg 5-CQA (strong brew, very pale roast robusta). Soluble coffee powder (2 g per cup) has been reported to consist of 50–150 mg of 5-CQA [1].

Yerba mate, a traditional South American beverage prepared from the leaves of *Ilex paraguariensis* A. St.-Hil., is rich in mono- and diCQAs. There have been few investigations of the brew composition but green mate material bought and brewed in Europe provided 107–133 mg CQAs per approx. 200 cm^3 of which diCQA represented one third. In contrast, roasted mate provided 16–41 mg CQAs per 200 cm^3 [37]. Mazzafera [38] reported that when authentic material is brewed in the traditional manner (50–60 g leaves per liter boiling water) 480–520 mg of 5-CQA is extracted.

In view of the above presented examples showing the CQAs content in plants, it can be stated that among all CQAs the most ubiquitous compound, that is present in larger quantities, is 5-CQA. For these reasons 5-CQA is commonly employed as a quality marker in the control of various natural products and it belongs, undoubtedly, to the most frequently studied representatives of the chlorogenic acids family. The other reasons for researchers interest in 5-CQA include: its important role in the plant's response to stress, is an important biosynthetic intermediate, for example, in lignin biosynthesis, and exhibiting antiviral, antibacterial, antifungal and anticarcinogenic effects combined with relatively low toxicity and side effects [5, 8, 10, 11, 29]. The other compound that focus

the researchers attention, due to exhibited biological properties, is 1,3-diCQA. This compound, in contrast to 5-CQA, is mainly characteristic for the *Asteraceae* family, especially for artichoke. Yet, despite a rare occurrence and a low content in plants, 1,3-diCQA is the most well-known and frequently investigated derivative of dicaffeoylquinic acids [31]. 1,5-diCQA and 3,4-diCQA, though they occur in larger quantities in many plants, they are not so intensively investigated by researchers [31–35].

3. THE BEGINNINGS OF CQAS CHEMISTRY

The first report on chlorogenic acids, concerning the properties of chlorogenic acid alone, most likely appeared in 1837 [35]. The first documented reference comes from 1844. In this year, Rochleder [35] noticed that caffeine in green coffee beans could combine with an acid that could be precipitated with lead salts. The free acid, generated by the treatment of precipitate with sulfuric acid, gave a yellow color on the addition of ammonia. Two years later the same researcher proposed an empirical formula of $C_{16}H_9O_8$ for the free acid and reported that the yellow ammonical solution become green on exposure to oxygen [35]. (That is why the compound despite absence of chlorine is called "chloro".) Later that year, Payen [35] announced the isolation of a crystalline potassium caffeine chloroginate that formed about 3.5 % of green coffee beans and proposed an empirical formula of $C_{14}H_8O_7$. Gorter [35] reported a melting point at 206–207°C for pure white crystals of chlorogenic acid, and proposed an empirical formula of $C_{32}H_{38}O_{19}$. Alkaline hydrolysis at low temperatures yielded caffeic acid (CA) and quinic acid (QA) in equimolar quantities. To make this observation compatible with the proposed empirical formula, Gorter suggested that CA associate with QA to produce hemi-CQA, two molecules of which condensed to give chlorogenic acid. In 1920, Freudenberg reported that the enzyme tannase hydrolyze chlorogenic acid to equimolar quantities of CA acid and QA. In 1932, Fischer and Dangschat deduced that chlorogenic acid is 3-caffeoylquinic acid. (According to a pre-IUPAC nomenclature for cyclitols, the positional number of carbon atoms in the quinic acid ring were assigned in an anticlockwise manner.) Under current IUPAC recommendations (positional number assigned to the carbon atoms of the quinic acid ring in clockwise sense), 3-caffeoylquinic acid is now designated 5-*O*-caffeoylquinic acid (5-CQA, see Fig. 1) [35].

The first report on dicaffeoylquinic acid derivatives was prepared by Panizzi and Scarpati [45] and concerned the isolation of cynarin from artichoke leaves. In these report, cynarin was characterized as a yellowish-colored crystalline substance exhibited left-handed rotatory power and a weak acid reaction, that is slightly stable in air alkaline solution and change the color to green in the presence of ferric chloride. The cynarin structure was initially described as 1,4-*O*-dicaffeoylquinic acid [45]. Later, the compound was identified as 1,5-*O*-dicaffeoylquinic acid by the same researchers. Now, according to IUPAC recommend-dation, cynarin is called 1,3-*O*-dicaffeoylquinic acid (see Fig. 1).

4. CQAS PROPERTIES

Compounds belonging to the chlorogenic acid family exhibit very differentiated physicochemical properties dependent on the identity, number and position of the acyl residues esterified with the quinic acid as well as on the functional groups present on the aromatic moiety of the acyl residues.

The monoCQAs, in general, and the less polar diCQAs, in particular, are more soluble in the lower alcohols or alcohol–water mixtures. They are insoluble in benzene, chloroform, and petroleum ether. The diCQAs, in addition to the lower alcohols, are well dissolved in ethyl acetate, butyl acetate, and acetone. Taking into account the position of acyl residue in the quinic acid, water solubility declines in the sequence: 1- > 3- > 4- > 5. For diCQAs, the order can be presented as follows: 1,3- > 1,4- > 1,5- > 3,4- > 3,5- > 4,5-. In general, those CQAs with a greater number of free equatorial hydroxyl groups in the quinic acid residue are more water soluble than those with a greater number of free axial hydroxyl groups [46]. As to the influence of functional groups present on the aromatic moiety of the acyl residues, a hydroxyl group raises the polarity whereas a methoxy group reduces it. Concerning the effect of the identity of hydroxycinnamic acid derivatives, water solubility declines in the following order: 5-CQA > 5-CoQA > 5-FQA [35].

In the case of CQAs, one of the characteristic processes widely characterized in literature and responsible for modifying the structure of CQAs is acyl migration [47 ,48]. This process describes the migration of cinnamoyl moieties from one quinic acid alcohol group to another. It is responsible for regioisomerization of appropriate CQAs, thus it constitutes a special case of transesterification reaction. The migration

process is temperature and pH dependent with increased transesterification taking place at basic pH [15, 47]. Moreover, the process can be inter- and/or intramolecular. According to [47], under aqueous basic conditions the intramolecular acyl migration in monoCQAs is fully reversible. Through intramolecular acyl migration in monoCQAs, for example, 5-CQA first isomerizes to 4-CQA (also known as crypto-chlorogenic acid), then to 3-CQA (neochlorogenic acid), and finally to 1-CQA [49]. It should be added, that among the all 5-CQA isomers the presence of 3- and 4-CQA was confirmed in plants, the 1-CQA isomer is known only from theoretical considerations [2]. Similarly to the monoCQAs, the diCQAs could also undergo the intramolecular acyl migration. Yet, the monoCQAs are generally much more stable than the diCQAs under the same conditions. Taking into account the diCQAs stability, in [50] it was reported that 4,5-diCQa is much more stable than 3,4-diCQA and 3,5-diCQA. Citing the authors of this report, this might be due to the fact that diCQAs are more stable when the ester bond link to the quinic acid exist as an equatorial bond rather than axial one. Only one ester bond of 3,4-diCQA and 3,5-diCQA exist as an equatorial bond while all ester bonds of 4,5-diCQA exist as equatorial bonds.

Apart from the acyl migration process, researchers reported another chemical changes for CQAs, such as hydrolysis of the ester bond, decarboxylation of cinnamoyl moieties, epimerization at the quinic acid, dehydration, lactones formation, and reaction with water or methanol [2, 15, 35]. It should be added that the above mentioned processes can compete with each other, as in the case of hydrolysis and acyl migration competition under aqueous basic conditions [47].

CQAs are highly susceptible to the influence of temperature. As temperature increase, they are not only more easily subjected to intramolecular isomerization and transesterification but also to degradation. The diCQAs degrade to the corresponding monoCQAs, and then to caffeic and quinic acid. The amount of each formed component depends on the heating time and temperature [3, 14, 16]. According to [50], total of eight degradation products of 3,4-diCQA, six degradation products of 3,5-diCQA, four degradation products of 1,3-diCQA, and three degradation products of 4,5-diCQA were identified in the diCQAs solutions. Only two degradation products of 3-CQA, three degradation products of 5-CQA, and four degradation products of 4-CQA were detected and identified in the monoCQAs solutions [50].

The study of the stability of CQAs in two solvents (methanol and aqueous methanol solution, 50% v/v) kept in brown glass bottles at 4°C

showed slow degradation of the CQAs. Meanwhile, noticeable decreases in concentrations of CQAs were observed when kept in transparent glass at room temperature. CQAs degraded easily in methanol. According to the authors it was probably because increasing the concentrations of the respective adducts or esters of methanol [50].

CQAs are photosensitive and when they are exposed in ultraviolet or visibly light undergo *trans-cis* isomerization [43]. Isomerization kinetics is affected by irradiation time, wavelength and temperature. Yet, for samples stored at room temperature in methanol, significant degradation of monoCQAs was observed after 7 days their exposition to the light [50]. This may suggest that single factor, such as temperature, light or solvent has little effect on the stabilities of monoCQAs. Under dual factors of light and temperature, both mono- and diCQAs decompose easily [15, 47, 50].

5. PRO-HEALTH BIOLOGICAL ACTIVITY OF CQAS

There are several advantageous health properties associated to the family of CQAs, such as hepatoprotective and choleretic activities, antioxidant, antiviral, antibacterial, anti-cancer and anti-inflammantory properties, modulation of gene expression of antioxidant enzymes, reduction of the risk of cardiovascular disease *via* suppressing P-selectin expression on platelets, and reduction of the relative risk of diabetes type 2 and Alzhaimer's disease [5–13, 51] (Fig. 2).

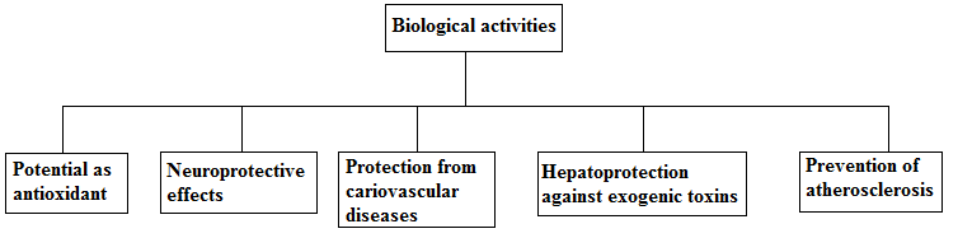


Fig. 2. Overview of chlorogenic acids biological activities.

Some of these properties are already well known and tested, others are not yet proven and are now under close scrutiny. This is, for example, the case of the supposition that CQAs may be helpful in fighting obesity and modulate the glucose-6-phosphatase involved in glucose metabolism [12, 13], and that they are a novel class of antiviral compounds, namely

inhibitors of integrase, being potent inhibitors of HIV-1 replication, both *in vitro* and *in vivo* [5, 6, 52].

Finally, it should be emphasized that despite the abundance of data demonstrating CQAs biological activities, some of them are still controversial. This is the case with their antioxidant properties. CQAs have been reported to inhibit the formation of reactive oxygen species and act as their scavengers, thus they may play beneficial role in the prevention of oxidative and age-related diseases [4, 5, 7]. According to new results however the issue, whether these compounds are potent anti-oxidants or pro-oxidants, it remains debatable. This is a consequence of the fact that CQAs properties switch from anti- to pro-oxidant activity, depending on their concentration, the presence of free transition metal ions, or on their redox status [35[35]].

In the view of the CQAs biological properties and their high susceptibility to the negative impact of surroundings (solvent type, pH, temperature and even light) it is obvious why so many efforts have been undertaken to modify the way in which the compounds are isolated. Searching for new and alternative methodologies of CQAs extraction from plants is additionally supported by fact, that some of those compounds can be found in nature at very low concentrations. Thus, to obtain their sufficient amounts very effective isolation methods are needed. All the more that their structural diversity and complexity make chemical synthesis rather unprofitable.

6. ISOLATION OF CAFFEOYLQUINIC ACIDS

The common procedures used so far for determination of compounds in plants and related products involve two-step processes. In the first one, the compounds are liberated from pre-prepared sample matrix. In the second, they are analysed using, more frequently, chromatographic techniques. Depending on the sample matrix type, different procedures are applied for sample pre-treatment. Solid samples, such as plants and solid food stuffs, are usually homogenized, what may be preceded by lyophilisation or freezing with liquid nitrogen. Liquid samples, such as beverages, are usually first filtered and/or centrifuged, and then they are subjected either direct analysis or, what is more common to enhance the determination selectivity, they are subjected further steps of preparation.

To fully isolate the compounds released from matrices and/or to concentrate them a broad range of sample preparation techniques

(*e.g.* distillation, sublimation, extraction to gas, liquid or solid phase) are currently being used. Most approaches to plant components analysis involve the application of liquid extraction methods, such as extraction in the Soxhlet apparatus, under reflux and maceration. These are so-called conventional liquid extraction techniques. Their extraction ability is based on the extracting power of different solvents and the application of heat and/or mixing. The conventional techniques are known from many disadvantages, such as time-consuming, relatively high solvent consumption, often unsatisfactory reproducibility and poor extraction of polar substances. Despite these drawbacks, however, the techniques are still commonly applied, mainly due to the simplicity of their use. To present properly the techniques used for CQAs extraction, it should be added, that the Soxhlet extraction is used less often. Nevertheless, this technique is still considered as one of the reference method to compare isolation efficiency of newly developed methodology.

In an attempt to reduce or eliminate the drawbacks of the conventional liquid extraction methods and to improve the extraction process innovative extraction methods such as microwave-, ultrasound-, and pressure-assisted extraction have been developed and introduced. These techniques, additionally, could improve extracts quality what is important taking into account the CQAs applicability as pro-health additives to foods and supplements.

6.1. Ultrasound Assisted Solvent Extraction

The mechanism of ultrasounds in liquids relies on the mechanical effect caused by the implosion of cavitational bubbles. The implosion of cavitation bubbles generates macro-turbulence, high-velocity inter-particle collisions and perturbation in micro-porous particles of the natural materials which accelerates diffusion and enhances the mass transfer phenomena [53]. The increasing interests on applying ultrasounds to plants and plant products extraction lies in its advantage on reducing extraction time and increasing yield of heat-sensitive compounds at lower processing temperatures [54]. This is because the technique provides the greater opportunity of enhancing its extraction ability. Apart from the careful choice of an appropriate solvent characteristic for the conventional techniques, the process can be further optimized and this is an important part of the ultrasound-assisted solvent extraction (UASE) process. During the optimization, ultrasounds frequency, their amplitude, number of the applied extraction cycles, irradiation time and nominal output power are taken into consideration. Another advantageous feature of UASE, that is

equally important, is the cost of equipment necessary for the technique. The cost is low, as the process is most often performed in ultrasonic baths being in each laboratory. Yet, the process can be realized in the different way, applying an ultrasonic disintegrator. In this case, the ultrasound emitting tip is directly immersed in the extraction mixture, and the process is called focused ultrasound assisted solvent extraction (FUASE) for distinguishing [55]. FUASE, compared to UASE, by some researchers is considered to be even more reproducible and faster, mainly due to a few times greater ultrasonic power [56].

Li et al. [57] investigated an ultrasound-assisted method for the extraction of 5-CQA from fresh leaves of du-zhong (*Eucommia ulmoides* Oliv). During experiments, the influence of four extraction variables (solvent type, its volume and concentration and extraction time) on the yield of 5-CQA was examined. As solvents, water, methanol, ethanol and isopropanol were tested. From the obtained results it was concluded that the mean extraction efficiency of 5-CQA decreases in the order: methanol > ethanol > water > isopropanol. It was found that the aqueous methanolic mixture (70 % methanol in water, v/v) gives the highest extraction yield. An increase of extraction efficiency was also observed with the increase of the ratio of solvent volume to sample (the ratio of 20 was optimal) and the number of extraction cycles. It was proved that, in comparison to the conventional extraction techniques. triplicate extraction of 5-CQA for 30 min using each time fresh extractant reveals the highest 5-CQA yield. The optimized UASE process, by the same researchers, was applied for extraction of 5-CQA from fresh and dried bark of *E. ulmodies* and four other traditional Chinese medicines. It was turned out that independently of the plant matrix type the isolation efficiency of UASE was very high, and the obtained results highly reproducible.

6.2. Microwave Assisted Solvent Extraction

The microwave assisted solvent extraction (MASE) is considered as a novel method for isolation of CQAs from plants and related products using microwave heating. The principle of microwave heating, conversely to conventional heating, is based on direct absorption of microwave energy and its conversion into thermal energy, when the microwaves are passed through the medium [58, 59]. The conversion of electromagnetic energy to heat occurs *via* two mechanisms, specifically, by dipole rotation and ionic conduction, *i.e.* by means of reversal of dipoles and displacement of charged ions present in the sample as well as in the solvent. Usually, these two mechanisms occur simultaneously, which

effectively convert microwave energy into thermal one. Dipole rotation happens when the dipolar molecules attempt to follow the electric field in the same alignment. The induced oscillation leads to collisions between dipoles and surrounding molecules, and thus creates heat. Ionic conduction refers to the migration of ions under the influence of the electric field produced by microwaves. In this case the heat is generated due to the resistance of the medium to flow ions [58]. It should be added that in both mechanisms the generated thermal energy is immediately redistributed in the medium, which leads to homogeneous heating of the medium.

The MASE effectivity may be affected by a large variety of factors, such as frequency and power of microwaves, duration of microwaves irradiation, moisture content and particle size of plant samples, type and concentration of solvent, ratio of solid to liquid, extraction temperature and number of extraction cycles. Of these factors, solvent is regarded as one of the most important parameters, which affects the solubility of compounds and the absorption of microwaves energy. The higher the dielectric constant and dielectric loss, the higher is the solvent capacity to absorb microwave energy and the faster solvent heating. It should be added, however, that both polar and non-polar solvents can be used in MASE. Moreover, by combining solvents differing in their dielectric constant, the extractant properties and its selectivity can be modified. Nevertheless, the amount of water content in the extracted material and/or in solvent significantly influences the extraction yield [60].

In [61] the MASE applicability to CQAs extraction from green coffee beans was described. During these investigations, the influence of four extraction variables (solvent type, extraction time, extraction temperature and microwave power) on extraction efficiency of 5-CQA was tested. Among the examined solvents (ethanol, methanol and water), water proved to be the best extractant giving the highest yield of 5-CQA. It is due to the fact that the dielectric constant and polarity of water is higher than the alcohols. It was observed that extending the extraction time from 2 to 5 minutes resulted in the increased extraction efficiency. However, a further extension of the time resulted in a reduction of the 5-CQA yield. Taking into account the effect of extraction temperature on the total CQAs yield, it was shown that the highest yield was obtained at the temperature of 50°C. Finally, testing the effect of microwave power it was noticed that the total CQAs yield was increased with the increase in microwave power up to 800 W. Comparing the MASE results with those of the conventional extraction techniques,

the authors concluded that the optimized MASE procedure equally effective isolates 5-CQA as the conventional techniques. The authors at the same time stressed that MASE reduced both time and solvent consumption. Therefore, MASE can be recognized as more environmentally friendly process providing high quality extracts rich in CQAs.

According to some researchers, the performance of MASE is highly influenced by the ratio of solid to liquid. A larger solvent volume can generally dissolve compounds of interest more effectively and lead to a higher extraction yield. As shown in [62] the extraction yield of 5-CQA from *Eucommia ulmodies* increased with the improvement of the solvent volume (*i.e.* the reduction of the ratio of solid to liquid). The 5-CQA yield increased steadily when the plant mass was kept constant and the liquid volume was raised. That is to say, extraction yield of plant constituents is most likely depending on how ratio of solid to liquid is regulated. Therefore, in MASE the plant sample extraction is typically conducted in multiple steps, using the consecutive extraction cycles with fresh portion of the extractant. This practice helps to improve the extraction yield and prevents prolonged heating of the sample in the same solvent volume. In addition, the use of fresh portions of extractant prevents the saturation of the solvent with analytes, increasing their mass transfer and extraction kinetics [63]. In other words, MASE can extract compounds more rapidly, thus the heat-sensitive compounds are shortly exposed to potentially harmful conditions and in consequence their yields can be higher in comparison to those obtained by the conventional extraction methods.

6.3. Pressurized Liquid Extraction

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), pressurized solvent extraction (PSE), enhanced solvent extraction (ESE), pressurized hot water extraction (PHWE), subcritical water extraction or superheated water extraction (SWE), has gained popularity due to its lower solvent consumption and significantly shorter extraction times required for complete isolation of bioactive compounds from plants. In this technique samples are enclosed in stainless steel extraction cells and extracted with solvents at high pressure and temperatures (50–200°C). The use of elevated pressures and temperatures enhances the extraction yield compared to the conventional extraction methods by increasing the solubility and mass transfer effects and, also decreasing the viscosity and surface tension of solvents. PLE has proven to be an equivalent or superior alternative for the extraction of different analytes from various matrices [64–66]. In comparison to the

conventional techniques PLE was found to dramatically decrease time consumption and solvent use and, due to this fact the technique is frequently described as a green extraction technique [67]. For isolation of polar compounds PLE is also considered as a potential alternative technique to supercritical fluid extraction [59].

Alonso-Salces et al. [68] optimized PLE for determination of phenolic compounds in Golden Delicious apple peel and pulp taking into account the effect of experimental variables, such as solvent composition, extraction temperature, static extraction time and pressure. According to presented results, the extraction yield increased with the percentage of methanol in the extraction mixture. The most powerful extraction mixture was 60 % solution of methanol in water. As to the effect of extraction temperature, it was observed that up to 60°C the extraction yield first slightly increases and then falls down with a further increase in the temperature. Besides, it was found that the temperature increase leads to obtain cloudy extracts. Therefore, even if the extraction at 60°C provided the highest phenolics yield, 40°C was chosen as the optimum temperature. Studying the static extraction time (5, 10 and 15 min), it was observed that the amounts of extracted analytes were practically constant during these periods of time. When the extraction pressure was examined (1000, 1250 and 1500 p.s.i.), any significant influence on the efficiency of the process was detected. The same effect has been reported by others authors [69], because of the fact that the purpose of pressurizing the extraction cell is to prevent the solvent from boiling at the extraction temperature and to ensure that the solvent remains in intimate contact with the sample. In addition to the PLE parameters such as solvent type, temperature and static extraction time, Luthria [70] showed that particle size, flush volume and solid-to-liquid ratio also influence the extraction yield of phenolics from parsley (*Petroselinum crispum*) flakes. Erdogan et al. [71] 5-CQA recovered from various parts of *Anatolia propolis* using PLE at 40°C, 1500 psi for 15 min.

7. CHLOROGENIC ACIDS ANALYSIS

There are many analytical methods to identify and quantify chlorogenic acids in natural samples [3, 4, 14–17, 19, 26, 30, 32, 33, 36, 37, 39–50, 55, 57, 62, 66, 68–108]. According to our knowledge, however, there is no official and universally accepted methods of CQAs analysis. Therefore many laboratories develop and validate their own

analysis methods. All the more that depending on the analytical technique chosen different approaches to sample preparation may be considered and, as it was stated before, nowadays there is a tendency to replace the conventional extraction techniques by newer and more effective processes. It should be also remembered that even when using the same analytical technique, different equipment and different its settings can be selected. The fact that CQAs constitute a large family of compounds with various properties present in natural samples on different concentration levels additionally makes their analysis more difficult. Hence, frequently, in the course of single run, only a few CQAs representatives with very similar properties are analyzed, for example monoCQAs or diCQAs, and even an individual compound *i.e.* 5-CQA is subjected to analysis. Yet, due to the application of modern highly selective, sensitive and accurate analytical techniques this trend begins to slowly change in direction of analysis large numbers of CQAs in one single run. Examples of currently applied methods of CQAs analysis are summarized in Table 1.

According to the literature study [3, 4, 14–17, 19, 20–26, 30, 32, 33, 36, 37, 39–50, 55, 57, 61, 66, 68–108] and data presented in Table 1, it can be concluded that liquid chromatography (LC) and capillary electrophoresis (CE) with different detection systems are the most commonly applied.

7.1. Electrophoretic analysis

Guan et al. [88] developed a method based on capillary electrophoresis with electrochemical detection (CE-ED) to determine 5-CQA in sweet potato. In this method the laboratory-built CE-ED system was used. A +30 kV high-voltage power supply provided a separation voltage between the ends of the capillary. The inlet end of the capillary was held at a positive potential, and the outlet end was maintained at ground. All samples were injected electrokinetically, applying 18 kV for 6 s. A three-electrode cell system consisting of a carbon-disk working electrode, a platinum auxiliary electrode, and a SCE (saturated calomel electrode) reference electrode was used in combination with ampere-metric detector. By the migration time of the analyte as compared with electropherogram of the standard solution, the active ingredient in sweet potato samples can be identified and quantitatively determined. The results obtained by Guan

Table 1. Conditions used for isolation and determination of chlorogenic acids in different matrices.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Du-zhong (*Eucommia ulmodies* Oliv.)	UASE with aqueous methanol solution	RP-HPLC-UV, 240 nm; Dalin Chromatographic C18 (5 µm, 250 mm × 4.6 mm); mobile phase: methanol/water/acetic acid (19:81:1,5, (v/v)); flow rate: 1cm^3/min	Chlorogenic acid Mean recovery 99,9 % Good reproducibility above 90 %	[57]
Green coffee beans	MASE with different solvents: water, methanol, ethanol	UV spectrophotometric method λ = 325 nm	Chlorogenic acid yield 8.4 ± 0.28 %	[61]
Du-zhong (*Eucommia ulmodies* Oliv.)	MASE with aqueous methanol solution	RP-HPLC-UV, 240 nm; Spherigel C18(5 µm, 250 mm × 4.6 mm); mobile phase: methanol-water-acetic acid (20:80:1 v/v) flow rate: 1 cm^3/min	Chlorogenic acid	[62]
Apple peel and pulp	Pressurized liquid extraction with aqueous methanol solution	RP-HPLC-DAD, 320 nm; Novak-Pak C18 (4 µm, 300 mm × 3.9 mm) mobile phase: water-acetic acid (90:10 v/v) (A): methanol (B); gradient from 0 % B with 10 min, reach to 15 % at 40 min, isocratic 15 % at 60 min,	Chlorogenic acid Peel 410 µg/g , LOD = 0.3 µg/g Pulp 540 µg/g, LOD = 0.09 µg/g *p*-Coumaric acid derivatives Peel 17 µg/g, LOD = 0.2 µg/g	[68]

Cont Table 1.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Apple peel and pulp		up to 100 % at 70 min, isocratic 100 % at 75 min, 0 % at 85 min flow rate: 0.8 cm^3/min	Pulp 46 µg/g, LOD = 0.06 µg/g	
Anatolia propolis	PLE with aqueous ethanol solution and/or aqueous acetone	RP-HPLC-DAD, 280 nm; ACE 5 C18 (4 µm, 250 mm × 4.6 mm); mobile phase: water-acetic acid (73:3) (A): acetic acid-acetonitrile-water (3:25:72) (B); gradient 0–40 min, from 100% A to 30% A, with flow rate 1 cm^3/min; 40–45 min, from 30% A, to 20% A, with flow rate 1 cm^3/min; 45–55 min, from 20% A to 15% A, with flow rate 1.2 cm^3/min; 55–57 min, from 15% A to 10% A, with flow rate 1.2 cm^3/min; and 57–75 min 10% A, with flow rate 1.2 cm^3/min	Chlorogenic acid Caffeic acid *p*-Coumaric acid	[71]
Blueberries	Maceration with acidified aqueous methanol solution	RP-HPLC-DAD (separation conditions not available)	Chlorogenic acid	[75]

Cont Table 1.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Cider apples	Hammer mill, pressed, clarified	RP-HPLC-DAD, 280 nm; ODS-2 Spherisorb (3 µm, 250 mm × 4 mm); mobile phase: 2 % acetic acid (A): methanol (B); gradient from 0 % B (0 min) to 45 % B (55 min); flow rate 0.7 cm^3/min	Chlorogenic acid	[76]
Apple and pear	UASE with methanol at ambient temperature	RP-HPLC-DAD, 280 nm; Nucleosil 120 C18 (5 µm, 250 mm × 4.6 mm); mobile phase I: phosphoric acid 0.01 M (A):ACN (B) gradient from 2 to 15 % B at 10 min and 35% B at 35 min; flow rate: 2 cm^3/min mobile phase II: phosphoric acid 0.01 M (A):methanol gradient 5% B to reach 50% at 10 min, 100% at 25 min flow rate 1 cm^3/min	Chlorogenic acid Caffeic acid	[77]
Apple	Maceration with ethanol, at presence of metabisulfite	RP-HPLC, 280 nm, 320 nm; Rosil C18 (3 µm, 150 mm × 4.6 mm)	Chlorogenic acid	[78]

Cont Table 1.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Apple musts and ciders	SPE C18	RP-HPLC-DAD; 280 nm, 320 nm, 360 nm; Spherisorb Hexyl (5 µm, 250 mm × 4.6 mm); mobile phase: phosphoric acid (A): methanol (B) gradient from 2% B to reach 11 % at 15 min, 18.5 % at 20 min, 20.3 % at 23 min, 21.5 % at 25 min, 39.5 % at 55 min	Must: chlorogenic acid, cinnamic ester recovery range 100–110 % Cider: hydroxycinnamic acids recovery range 84–107 %	[79]
Pear	Maceration with aqueous ethanol solution	RP-HPLC-DAD, 325 nm, 280 nm, 360 nm; Adsorbosphere C18 (3µm, 150 mm × 4.6 mm); mobile phase: water with H_3PO_4 (A): acetonitrile-methanol-water 1:3:1 (B); gradient from 5 % reach to 12 % at 5 min, 12% at 10 min, 50 % at 44 min, 50 % at 70 min; flow rate: 0.8 cm^3/min	Hydroxycinnamic acids	[80]

Cont Table 1.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Sweet cherry	Maceration with acidified aqueous methanol, and with methanol-formic acid-water mixture	RP-HPLC, 280 nm, 525 nm; SuperPac Prp-S (5 µm, 250 mm × 4 mm); mobile phase: formic acid in water (5:95 v/v) (A): methanol (B); gradient from 30 % B reach to 38% at 8 min, 40% at 8.5 min, 46 % at 20 min, 60% at 30 min, 85 % form 30.5 min to 34.5 min and 30 % at 35 min; flow rate: 1 cm^3/min	Neochlorogenic acid 3-*p*-coumaroylquinic acid	[81]
Kiwifruit juice	SPE C18	RP-HPLC-DAD, 280 nm, 320 nm, 360 nm; Spherisorb C18 (5 µm, 250 mm × 4.6 mm); mobile phase: water (A): acetonitrile (B); gradient from 3 % B reach to 40 % B at 44 min; flow rate: 1 cm^3/min	Chlorogenic acid, Chlorogenic acid derivatives	[82]
Cranberry juice	SPE C18 and acid-catalysed hydrolysis	RP-HPLC-UV, 280 nm, 360 nm; mobile phase: water-acetic acid (97:3 v/v) (A): methanol (B); gradient from 0 % B reach to 10 % at 10 min, 70 % at 40 min, 0 % at 47 min	Caffeic acid Chlorogenic acid	[84]

Cont Table 1.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Leaves of yacon (*Smallanthus sonchifolius*, Asteraceae)	Soxhlet extraction with methanol and ethyl acetate; Reflux extraction with water; Maceration with boiling water	RP-HPLC-ECD, amperometric detection range: +550 mV to +1200 mV versus SCE; Separon SGX C18 (5 µm, 250 mm × 4 mm); mobile pase: KH_2PO_4 (A): acetonitrile (B) in ratio 90/10 or 80/20, v/v flow rate: 1 cm^3/min	Chlorogenic acid Linearity 0.96–24.0 mg/dm^3 LOD = 0.96 mg/dm^3 Caffeic acid Linearity 1.0–10.0 mg/dm^3 LOD = 1 mg/dm^3 Ferulic acid Linearity 0.2–10.7 mg/dm^3 LOD = 0.2 mg/dm^3	[85]
Hypericum perforatum	Reflux with methanol	RP-HPLC-thermospray (TSP) – triple quadrupole mass spectrometry, full scan spectra from 200 to 700 m/z in positive ion mode; RP-HPLC-electrospray (ESI)-ion trap mass spectrometer, full scan spectra from 200 to 700 m/z in the negative ion mode; RP-HPLC-PDA; TP 54 RP-18 (5 µm, 250 mm × 4.6 mm); mobile phase: water-phosphoric acid (99.7:0.3, v/v) (A): acetonitrile (B): methanol (C); flow rate: 1 cm^3/min	Chlorogenic acid Linearity 0.8–1.2 mg/dm^3	[86]

Cont Table 1.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Eleutherococcus senticosus	Reflux with methanol	RP-HPLC-UV, 254 nm, PDA, 254 nm and 280 nm, fluorescence: ex: 230 nm, em: 350 nm I chromatographic system: ODS Hypersil (5µm, 200 mm × 4.6 mm); mobile phase: methanol:water:acetic acid (23:77:1 v/v/v); flow rate: 1 cm^3/min; II chromatographic system: Symmetry C18 (5µm, 250 mm × 4.6 mm); mobile phase: methanol:0.001 M phosphoric acid (23:77 v/v); flow rate: 1 cm^3/min	Chlorogenic acid PDA detector: LOD = 227 ng/dm^3 LOQ = 757 ng/dm^3 FL detector LOD = 4167 ng/dm^3 LOQ = 12627 ng/dm^3 Caffeic acid PDA detector: LOD = 73 ng/dm^3 LOQ = 221 ng/dm^3 l FL detector LOD = 1463 ng/dm^3 l LOQ = 4433 ng/dm^3 l ferulic acid PDA detector: LOD = 107 ng/dm^3 LOQ = 324 ng/dm^3 l FL detector LOD = 1429 ng/dm^3 LOQ = 4330 ng/dm^3 l	[87]

Cont Table 1.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Sweet popato (*Ipomoea batatas* L.)	UASE with anhydrous ethanol and water (4:1)	Capillary Electrophoresis with Electrochemical Detection (CE-ED) inlet end – positive potential fused-silica capillary (75 cm × 25 µm) sample injection at 18 kV for 6 s	Chlorogenic acid linearity $2.0 \cdot 10^{-6}$ to $1.0 \cdot 10^{-4}$ g/dm^3 LOD = $2.88 \cdot 10^{-7}$ g/dm^3	[88]
Tobacco	Maceration with methanol and reflux with a boiling water	Capillary electrophoresis with indirect chemiluminescence system; quartz capillary (50 cm × 75 µm); flow rate: 5.5 mm^3/min	Chlorogenic acid LOD = $2{,}2 \cdot 10^{-5}$ mol/dm^3	[89]
Honeysuckle products	UASE with aqueous methanol solution	Microemulsion electrokinetic chromatography with PDA detector, 328 nm; uncoated fused silica capillary (75 µm × 50.2 cm); conditioning by flushing with methanol for 10 min, 0.5 mol/l HCl for 10 min, 0.5 mol/l NaOH for 10 min, water for 15 min and microemulsion for 15 min.	3-CQA, 4-CQA, 5-CQA, 1,3-diCQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA LOD = 2.0 µg/cm^3 LOQ = 6.8 µg/cm^3	[90]
Coffee	Maceration with a mixture of methanol and deionized water	Micellar electrokinetic chromatography with DAD detector, 325 nm; fused-silica capillary (75 µm × 30 cm); conditioning by flushing with deionized	5-CQA, 4-CQA, 3-CQA, caffeic acid, ferulic acid LOD = 0.98 µg/cm^3 LOQ = 4 µg/cm^3	[91]

Cont Table 1.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Coffee		water for 5 min, 1 M NaOH for 30 min, deionized water for 10 min; hydrodynamically injection for 3 s at 25 mbar		
Lonicerae Japonicae Flos	Maceration with water	HPLC-DAD Near-infrared spectroscopy	Chlorogenic acid	[96]
Dandelion *(Taraxacum officinale)*	Maceration with aqueous methanol solution	RP-UPLC-MS/MS; MRM mode UPLC HSS T3 (1.8 µm, 2.1 mm × 100 m); mobile phase: water-0.5 % formic acid (A): acetonitrile-0.5 % formic acid (B); gradient increased to 72:28 (A:B) by 16 min with a ramp to 2:98 by 17 min and held for 1 min	3-CQA, 4-CQA, 5-CQA, 1,3-diCQA, 1,5-diCQA	[98]
Gardeniae fructus	Soxhlet extraction with aqueous methanol solution	LC-DAD-MS^4, 254 nm, 320 nm; ion trap mass spectrometer in negative mode; Column (5 µm, 150 mm × 3 mm); mobile phase: water-0.5 %formic acid (A):methanol (B) gradient from 10 % B to 70 % in 60 min,	3-CQA, 4-CQA, %-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA	[102]

Cont Table 1.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Gardeniae fructus		10 min isocratic, return to 10 % B at 90 min and 10 min isocratic to re-equilibrate flow rate: 0.5 cm^3/min		
Food samples	FUSLE with aqueous methanol solution	UPLC-ESI-QTOF; ESI in negative mode; Selective ion monitoring(SIM) Acquity BEH C18 (1.7 µm, 2.1 mm × 50 mm); mobile phase: water-0.1 % formic acid (A): acetonitrile-0.1 % formic acid (B); gradient from 3 % B for 1.5 min, increased to 8% B in 2.5 min, 25 % B in 1.5 min, to 40 % B in 0.4 min and to 100 % B in 0.5 min; flow rate: 0.5 cm^3/min	Chlorogenic acid 1-CQA 1,3-diCQA	[104]
Green coffee bean	Extract of green coffee beans soluble in water	HPTLC,direct densitometry scanning, 330 nm glass plates Kieselgel 60 F 254; mobile phase: ethyl acetate-dichloromethan-formic acid-acetic acid-water (100/25/10/10/11, v/v/v/v/v);	Chlorogenic acid HPTLC LOD = 80 ng/band LOQ = 250 ng/band HPLC LOD = 10 µg/cm^3 LOQ = 30 µg/cm^3	[108]

Cont Table 1.

Sample	Sample preparation	Analytical method	Analytes	Ref.
Green coffee bean		RP-HPLC-UV, 330 nm; Luna C18 (5 µm, 4.6 mm × 150 mm); mobile phase: water-0.03 % TFA (A): CAN (B); linear gradient from 10 % B to 100 % B; flow rate: 1 cm^3/min		

et al. [88] indicate that CE-ED is accurate, sensitive, and useful method for the quantitation of 5-CQA in sweet potato.

Jiang et al. [89] employed an improved capillary electrophoresis procedure for the determination of chlorogenic acid in tobacco samples. In the procedure, the analyte zone, after being separated by capillary electrophoresis, it was determined by indirect chemiluminescence of luminol–potassium hexacyanoferrate. In this system, luminal was added into running buffer solution and introduced at the head of separation quartz capillary, and potassium hexacyanoferrate was introduced at the end of capillary. 5-CQA in the samples was determined quantitatively by the calibration of standard addition method and the correction of matrix background signal. According to the authors, the proposed capillary electrophoresis-chemiluminescence system can avoid the electrolysis of chemiluminescence reagent, retain the stability of chemiluminescence baseline and prolong the working time of running and electrode buffer solutions.

A special mode of capillary electrophoresis employing a micro-emulsion as carrier electrolyte, so-called microemulsion electrokinetic chromatography (MEEKC) method, in which analytes may partition between the aqueous phase of microemulsion and its oil droplets acting as a pseudostationary phase, was employed by Tang et al. [90] for the simultaneous determination of seven CQAs acids in honeysuckle – related products. The proposed MEEKC procedure with PDA detection at 328 nm, with the addition of tartrate for improving separation efficiency, was sufficient for separation and quantification of three monoCQAs and of four diCQAs within 22 min. Citing the authors of the report, the method proposed by them can be extensively used to determine individual CQA isomers in honeysuckle or its preparations, and can provide more references for the quality evaluation and control of CQA related products.

Another modification of capillary electrophoresis i.e. micellar electrokinetic chromatography (MEKC), in which samples are separated by differential partitioning between micelles (pseudostationary phase) and a surrounding aqueous buffer solution acting as mobile phase, was proposed by Risso et al. [91] for separation of CQAs in coffee. The electrophoretic conditions consisted of an sodium dodecyl sulphate (SDS) (70 mM)-phosphate (17.6 mM)-methanol (5%, v/v) buffer system, pH = 2.5, 22.1°C, –17 kV and detection at 235 nm. The separations were carried out on a fused-silica capillary with effective and total length of 22.5 cm and 30 cm, respectively. The method was tested both with green

and roasted coffee beans, and in both cases it was proved to be effective and selective. According to the authors of the paper, the method can be used to monitor the presence of mono-CQA isomers in natural as well as processed vegetables.

7.1. Chromatographic analysis

Numerous papers have been published in qualitative and quantitative analysis of CQAs in plants by liquid chromatography (LC) coupled with spectroscopic detection techniques, such as ultraviolet (UV), fluorescence (FL) nuclear magnetic resonance (NMR) and mass spectroscopy (MS), and even near-infrared (NIR) spectroscopy [56, 61,62, 84, 86, 87, 89, 92–99]. It is worth to add that nowadays the coupling of separation and detection techniques can involve more than one separation or detection techniques, e.g. LC-UV-MS, LC-MS-MS, LC-NMR-MS, and the like [14, 16, 47, 99–101, 102]. For example, a simple LC-DAD/ESI-MS^n method has been used by Gouveia and Castilho [103] for the screening of various phenolic compounds, including CQAs, in *Helichrysum devium* extracts. On the other hand, where CQAs are present on low concentration levels and their precise analysis is essential, on-line coupling with the CQAs extraction can be incorporated to build in a more integrated and sophisticated system *e.g.* FUSLE-LC-MS [104].

High-performance liquid chromatography (HPLC) is still the most widely used analytical separation technique. Though, the more advanced form of LC i.e. ultra-performance liquid chromatography (UPLC) becomes increasingly popular in the determination of plant extracts compounds, due to the improved resolution, shorter retention times and higher sensitivity [55, 104–106]. In general, LC separations of CQAs are performed by reversed phase system. The commonly applied stationary phases are based on C18 [3, 16, 17, 26, 30, 36, 40, 44, 46–48, 50, 55, 57, 68, 71, 76–78, 80, 86, 105]. The mobile phase consists of mixtures of methanol-water [14, 47–50, 57, 62, 68, 76, 79, 81, 84, 87], and acetonitrile-water [15–17, 30, 36, 40, 41, 44, 46, 55, 71, 77, 82, 98, 104]. Detectors used to monitor CQAs separations are diode array detectors (DAD) or photodiode array detectors (PDA). It should be remembered, however, that only a few CQAs standards are available commercially, and the precise analysis of the individual CQAs by means of conventional LC-DAD(PDA) systems is not easy. Hence, nowadays the use of MS and tandem MS is preferred by most laboratories because of their higher selectivity and sensitivity. In order to improve the ionization capacity of CQAs in MS, formic acid at a concentration level of 0.1% is frequently

added to the mobile phase [14–16, 30, 36, 40, 41, 47–50, 55, 81, 98, 102, 104, 108]. Ionization is usually performed by electrospray ionization (ESI) working in negative mode [14–16, 36, 40, 41, 46, 49, 50, 55, 86, 98, 99, 100, 102, 104, 105]. The most common MS analysers in LC-based methods for CQAs analysis in plants are ion trap (IP), triple quadrupole (TQD) and time of flight (TOF) [14–17, 36, 40, 41, 47–49, 55, 86, 99, 104, 105, 107]. Tandem MS gives the possibility of measuring in selected reaction monitoring (SRM), which is a very selective acquisition mode. Typically 2 or 3 SRM transitions are selected for target analysis of CQAs: one for quantification and an additional one for confirmation purposes.

Urakova et al. [108] developed and compared two LC methods, high-performance thin-layer chromatography (HPTLC) and HPLC, for separation and quantitative determination of 5-CQA in green coffee bean extracts. HPTLC analysis were performed on glass plates Kieselgel 60 F 254 (Merck) using densitometric determination at 330 nm. HPLC separations were achieved using a Luna C18 column. Data showed the absence of statistically significant differences for HPTLC and HPLC results. Thus, according to the authors, the HPTLC-densitometric method could be used for the quantitative determination of 5-CQA in extracts as an alternative to HPLC in rapid screening or in routine analysis.

The quantification of a series of related caffeoylquinic acid derivatives was carried out using UPLC-MS-MS [107]. In this method a liquid chromatograph was coupled to TQD mass spectrometer *via* ESI as the ion source working in negative mode. Detection of CQAs was conducted in multiple reaction monitoring (MRM) mode by analysis two transition ions per compound. In the report it was proved that this hyphenated technique offers a number of distinct advantages over conventional HPLC systems for the separations and identification of isomeric compounds. Additionally TQD, applied in the system, allows for more definite identification and indeed quantification of individual compounds based on their molecular mass and MS/MS fragmentation.

Clifford M. et al. [102] investigated qualitatively the chlorogenic acids of *Gardeniae Fructus* by liquid chromatography/multi-stage mass spectrometry (LC/MS^4). Using this method it has been possible to discriminate between individual isomers of mono-, di- and triCQAs without the need to isolate the pure compounds. In the applied system, a LC chromatograph with DAD was interfaced with an ion-trap mass spectrometer fitted with an ESI source operating in full scan. MS^2, MS^3 and MS^4 fragment-targeted experiments were performed to focus only on compounds producing a precursor ion at *m/z* 335, 397, 497, 511, 543,

559, 573, 659, and 673. In the report, twenty-nine chlorogenic acids were detected and twenty-five characterized to regioisomer level on the basis of their fragmentation, twenty-four for the first time from this source. Fourteen of these twenty-nine chlorogenic acids have not previously been reported from any source.

Tena et al. [104], to determine CQAs in feed and related products, used for the first time focused ultrasound solid-liquid extraction (FULSE) followed by UPLC-PDA coupled to quadrupole-time of flight (Q-TOF) mass spectrometry. As before, electrospray ionization was operated in negative mode. Wavelength values of 329 and 325 nm were selected to record the UV chromatograms of 5-CQA and 1,3-diCQA, respectively. According to the presented results, the method allows an efficient determination of CQAs with good recovery rates. Therefore, citing the authors, the method may be used for screening of raw material and for process and quality control in feed manufacture.

Xiao et al. [96] developed a rapid quantitative analytical method for the determination of three components (secologanic acid, chlorogenic acid and galuteolin) in the *Lonicerae Japonicae Flos* extracts using near-infrared (NIR) spectroscopy. The concentrations of analytes were determined by using HPLC-DAD as the reference method. A gradient elution HPLC method was established. The NIR spectra of the samples were obtained at 1-nm intervals over the spectral region from 1000 to 2500 nm using a SupNIR-4510 instrument. Each spectrum was obtained by averaging 16 scans. The useful bands of NIR ranged from 1000 to 2500 nm, which correspond to the first or second overtone. The intensity of NIR absorption decreases as the overtone increases. NIR spectroscopy can be used in manufacturing industry because of the stable, feasible and ascendant model established.

Finally, the less frequently applied chromatographic techniques must be mentioned. One of them is centrifugal partition chromatography (CPC). This technique, like other countercurrent chromatography techniques, is based on the phenomenon of liquid-liquid partitioning between two immiscible liquid phases that stay at equilibrium. But the significant difference between this technique and others is the retention mechanism of stationary phase. In the case of CPC, this mechanism is based on hydrostatic force, formed by the centrifugal field in the rotor in one-axis centrifuge. CPC was applied by Kim et al. [109] for preparative isolation of 5-CQA from highbush blueberry leaves (*Vaccinium corymbosum L.*). A water fraction containing a high concentration of 5-CQA (14.5 % of dry weight extract) was obtained by defatting a crude methanol extract

from blueberry leaves. To isolate 5-CQA from this water fraction a two-phase solvent system of ethyl acetate–ethanol–water at a volume ratio 4:1:5 (v/v/v) was applied. HPLC, UV, ESI/MS and NMR spectra were used to confirm the structure of 5-CQA in the CPC fraction. The HPLC chromatogram showed that the fractions collected by CPC contained 5-CQA acid with 96% purity based on peak area percentage. The total amount of chlorogenic acid isolated from 0.5 g of a water fraction was 52.9 mg, corresponding to 10.6% of the water fraction.

CONCLUSIONS

The objective of this review is to give an overview of the occurrence, properties, and methods that have been developed to improve the extraction and analysis of chlorogenic acids in plants and related products, with special attention to 5-O-caffeoylquinic acid and 1,3-di-O-caffeoylquinic acid as these compounds of the chlorogenic acids family with many beneficial health effects. It is because of the potential biological activity of these compounds, recently, there has been an increased interest in natural products rich in chlorogenic acids. Consequently, more efficient extraction methods, leading to obtain extracts with enhanced CQAs content and reduced content of their degradation products, are being sought. On the other hand, taking into account the application of CQAs as pharmaceutical and food additives and their properties, more efficient and reliable analytical methods are constantly sought after.

In the last few years, a number of improvements in the strategy of extraction and analysis of CQAs in different natural samples have been achieved. The application of newer, faster, more efficient and sophisticated extraction techniques, such as UASE, MASE or PLE, has significantly improved the extraction yield of CGAs with a significant reduction of organic solvents consumption. The overall quality of the extracts has also been improved. In addition, this has resulted in a reduction of the manipulation errors, and in consequence it has led to improved reproducibility and repeatability of the analytical methods.

There is no official and universally accepted methods of CQAs analysis in plants and related products. The large number of compounds belonging to CQAs family and the fact that these methods need to be applicable to many various plants make this wish hard to accomplish. Nevertheless, nowadays two analytical techniques commonly applied for

the analytical separation of CQAs can be distinguished. It is capillary electrophoresis and liquid chromatography. The latter, especially when is coupled to a tandem MS/MS, it can be even considered as the technique that occupies the leading position for the analysis of compounds belonging to CQAs family.

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