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Determination of caffeoylquinic acids in feed and related products by focused ultrasound solid–liquid extraction and ultra-high performance liquid chromatography–mass spectrometry

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ABSTRACT

A method to determine caffeoylquinic acids (CQAs) in three sources (herbal extract, feed additive and finished feed) using for the first time focused ultrasound solid–liquid extraction (FUSLE) followed by ultra-high performance liquid chromatography (UPLC) coupled to quadrupole-time of flight mass spectrometry is presented. Pressurized liquid extraction (PLE) was also tested as extraction technique but it was discarded because cynarin was not stable under temperature values used in PLE. The separation of the CQAs isomers was carried out in only seven minutes. FUSLE variables such as extraction solvent, power and time were optimized by a central composite design. Under optimal conditions, FUSLE extraction was performed with 8 mL of an 83:17 methanol–water mixture for 30 s at a power of 60%. Only two extraction steps were found necessary to recover analytes quantitatively. Sensitivity, linearity, accuracy and precision were established. Matrix effect was studied for each type of sample. It was not detected for mono-CQAs, whereas the cynarin signal was strongly decreased due to ionization suppression in presence of matrix components; so the quantification by standard addition was mandatory for the determination of di-caffeoylquinic acids. Finally, the method was applied to the analysis of herbal extracts, feed additives and finished feed. In all samples, chlorogenic acid was the predominant CQA, followed by criptochlorogenic acid, neochlorogenic acid and cynarin. The method allows an efficient determination of chlorogenic acid with good recovery rates. Therefore, it may be used for screening of raw material and for process and quality control in feed manufacture.

1. Introduction

Caffeoylquinic acids are esters of the caffeic acid and the quinic acid, that are naturally present in several plants, such as artichoke, coffee, and burdock among others, and have been shown to possess a multitude of preservative and pharmacological activities, such as antioxidant, antiviral, antibacterial, anti-inflammatory, reduction of the relative risk of cardiovascular disease and diabetes type 2, antispasmodic activities, inhibition of the mutagenicity of carcinogenic compounds, etc. [1–4].

Chlorogenic acid ((1S,3R,4R,5R)-3-((E)-3-(3,4-dihydroxyphenyl)acryloyloxy)-1,4,5-trihydroxycyclohexanecarboxylic acid, also known as 3-O-caffeoylquinic acid) (CA) and cynarin (1R,3R,4S,5R)-1,3-bis((E)-3-(3,4-dihydroxyphenyl)acryloyloxy)-4,5-dihydroxycyclohexanecarboxylic acid, also known as 1,3-di-O-caffeoylquinic acid) (CY) have been reported as the most abundant mono- and di-CQAs, respectively, in commercial and laboratory extracts from artichoke leaves [5,6]. However, the content of (1S,3R,4R,5R)-1,3-bis((E)-3-(3,4-dihydroxyphenyl)acryloyloxy)-4,5-dihydroxycyclohexanecarboxylic acid (a diastereoisomer of CY also known as 1,5-di-O-caffeoylquinic acid in literature, in this work named CYD from cynarin diastereomer) was found higher than that of CY in artichoke [7,8]. This compound represents the major di-CQA in artichoke heads and pomace, whereas in the juice, CY was predominant, due to the isomerization during processing [8]. Moreover, mono-CQAs can also undergo isomerization. It has been reported that in phosphate buffer (pH 7.4), plasma and urine, CA first isomerizes to cryptochlorogenic acid ((1R,3R,4S,5R)-4-((E)-3-(3,4-dihydroxyphenyl)acryloyloxy)-1,3,5-trihydroxycyclohexanecarboxylic acid, also known as 4-caffeoylquinic acid) (CCA) and then to neochlorogenic acid ((1R,3R,4S,5R)-3-((E)-3-(3,4-dihydroxyphenyl)acryloyloxy)-1,4,5-trihydroxycyclohexanecarboxylic acid) (NCA) by intramolecular acyl migration [9]. It has been also reported that diCQAs could

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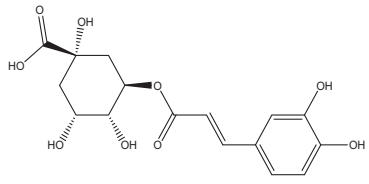
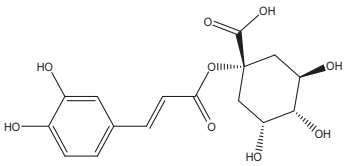
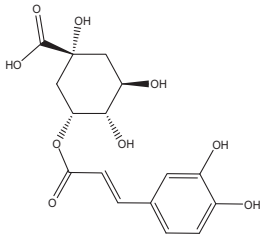


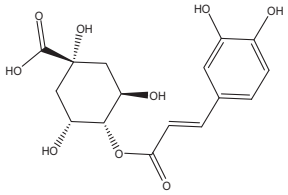
CA
(1S,3R,4R,5R)-3-((E)-3-(3,4-dihydroxyphenyl)acryloyloxy)-1,4,5-trihydroxycyclohexanecarboxylic acid



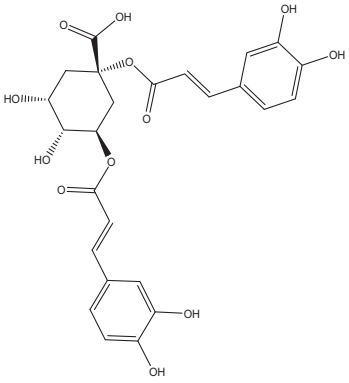
1-CQA
(1S,3R,4S,5R,E)-1-(3-(3,4-dihydroxyphenyl)acryloyloxy)-3,4,5-trihydroxycyclohexanecarboxylic acid



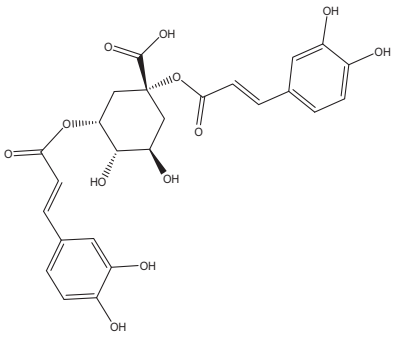
NCA
(1R,3R,4S,5R)-3-((E)-3-(3,4-dihydroxyphenyl)acryloyloxy)-1,4,5-trihydroxycyclohexanecarboxylic acid



CCA
(1R,3R,4S,5R)-4-((E)-3-(3,4-dihydroxyphenyl)acryloyloxy)-1,3,5-trihydroxycyclohexanecarboxylic acid



CY
(1R,3R,4S,5R)-1,3-bis((E)-3-(3,4-dihydroxyphenyl)acryloyloxy)-4,5-dihydroxycyclohexanecarboxylic acid



CYD
(1S,3R,4R,5R)-1,3-bis((E)-3-(3,4-dihydroxyphenyl)acryloyloxy)-4,5-dihydroxycyclohexanecarboxylic acid

Fig. 1. Chemical structure of the target analytes.

isomerize to each other and degradate to mono-CQAs, caffeic acid and compounds with the formulae $C_{15}H_{14}O_{16}$ [10]. Chemical structures of the mentioned compounds are shown in Fig. 1.

The interest in using natural products with therapeutic properties as additives has increased in recent years. Artichoke (*Cynara scolymus* L.), an edible vegetable from the Mediterranean area, is a good source of CQAs with high bioavailability, showing pharmaceutical properties such as antioxidant and antimicrobial activity [4,11]. In addition, it acts also as a hypolipidemic agent through inhibition of hepatic cholesterol biosynthesis and reduction of blood cholesterol [12]. By-products of artichoke processing, which may represent up to 50–60% of the fresh weight [13], are of interest to recover CQAs that can be used in animal feedstuff for their health-promoting properties.

Commercial products containing CQAs from artichoke and other plant extracts can differ for the methodologies of preparation as for the different content in polyphenolic compounds. The determination of CQAs is usually performed by reverse-phase liquid chromatography with UV detection and/or mass spectrometry [5–10,14–20]. In most cases, the extraction of CQAs from the freeze-dried vegetable is carried out with 50–80% methanol or ethanol–water mixtures [6–8,14,15,18,20] at temperatures between room temperature and 50 °C for times ranging from 30 min to 2 h. The extraction can also be assisted by ultrasounds [14]. In other works [16,17], a two-step procedure using first a 70:15:15 acetone:ethanol:methanol and then ethyl acetate, at 4 °C and for 1 h each, has been reported.

This is the first time that focused ultrasound solid–liquid extraction (FUSLE) is proposed for the recovery of CQAs from artichoke extract and feedstuff. FUSLE is a simple, fast and low-cost extraction technique [21]. It is based on the cavitation phenomenon and it is performed by the direct immersion of the ultrasound emitting microtip in the extraction mixture (solid sample and liquid solvent). FUSLE is faster and more efficient than the conventional