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Analysis of a series of chlorogenic acid isomers using differential ion mobility and tandem mass spectrometry

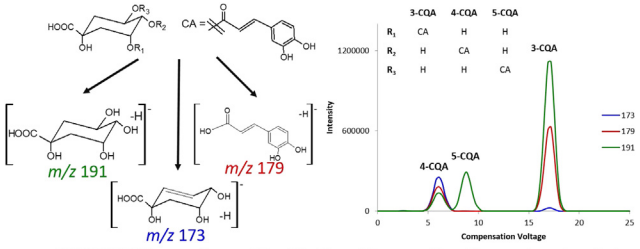
Jamie L. Willems [a], Mona M. Khamis [b], Waleed Mohammed Saeid [b], Randy W. Purves [c], George Katselis [d], Nicholas H. Low [a], Anas El-Aneed [b, *]

[a] Department of Food and Bioproduct Sciences, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8, Canada
[b] Drug Design & Discovery Group, College of Pharmacy and Nutrition, University of Saskatchewan, 107 Wiggins Road, Saskatoon, SK, S7N 5E5, Canada
[c] Crop Development Centre, College of Agriculture and Bioresources, 51 Campus Drive, Saskatoon, S7N 5A8, Canada
[d] Canadian Centre for Health and Safety in Agriculture (CCHSA), and Department of Medicine, University of Saskatchewan, 104 Clinic Place, Saskatoon, SK, S7N 2Z4, Canada

HIGHLIGHTS

- MS/MS behavior of three the most common mono-caffeoylquinic acids were determined.
- Similarities and differences between the dissociation pathways in the positive and negative ionization are illustrated.
- The MS/MS in the negative ion mode differentiated the three isomers based on product ion intensities.
- ESI-FAIMS-MS/MS successfully separated the three isomers in juice samples with a run time of less than 1 min.

GRAPHICAL ABSTRACT



FAIMS-MS/MS separation and identification of three positional isomers based on their unique MS/MS behavior in the negative ion mode

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ABSTRACT

Chlorogenic acids are among the most abundant phenolics found in the human diet. Of these, the mono-caffeoylquinic acids are the predominant phenolics found in fruits, such as apples and pears, and products derived from them. In this research, a comprehensive study of the electrospray ionization (ESI) tandem mass spectrometric (MS/MS) dissociation behavior of the three most common mono-caffeoylquinic acids, namely 5-*O*-caffeoylquinic acid (5-CQA), 3-*O*-caffeoylquinic acid (3-CQA) and 4-*O*-caffeoylquinic acid (4-CQA), were determined using both positive and negative ionization. All proposed structures of the observed product ions were confirmed with second-generation MS^3 experiments. Similarities and differences between the dissociation pathways in the positive and negative ion modes are discussed, confirming the proposed structures and the established MS/MS fingerprints. MS/MS dissociation was primarily driven via the cleavage of the ester bond linking the quinic acid moiety to the caffeic acid moiety within tested molecules. Despite being structural isomers with the same *m*/*z* values and dissociation behaviors, the MS/MS data in the negative ion mode was able to differentiate the three isomers based on ion intensity for the major product ions, observed at *m*/*z* 191, 179 and 173. This differentiation was consistent among various MS instruments. In addition, ESI coupled with high-field asymmetric waveform ion mobility spectrometry-mass spectrometry (ESI-FAIMS-MS) was employed for the separation of these compounds for the first time. By combining MS/MS data and differential ion

* Corresponding author.
E-mail address: anas.el-aneed@usask.ca (A. El-Aneed).

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mobility, a method for the separation and identification of mono-caffeoylquinic in apple/pear juice samples was developed with a run time of less than 1 min. It is envisaged that this methodology could be used to identify pure juices based on their chlorogenic acid profile (i.e., metabolomics), and could also be used to detect juice-to-juice adulteration (e.g., apple juice addition to pear juice).

1. Introduction

The term phenolics refers to a class of plant metabolites, which are defined by the presence of at least one phenol functional group [1–3]. Phenolics are ubiquitous in nature and have been reported to have a number of positive functions in plants that include, but are not limited to: protection against pathogens, parasites, predators, ultraviolet radiation and oxidants; cell signalling; attraction of pollinators and seed dispersing animals; and cell wall strengthening [1,4–6]. In addition, phenolics contribute to the sensory properties of food; for example, phenolics affect the bitterness, astringency and colour of fruit juices [2,4,7].

Due to their structural diversity, phenolics are typically subdivided into a number of structurally related classes. One class is the chlorogenic acids, which are composed of quinic acid linked to *trans*-cinnamic acids, such as caffeic acid, via an ester bond [8–10]. The most common chlorogenic acid is 5-*O*-caffeoylquinic acid (5-CQA; chlorogenic acid) [8,9,11]. However, many other structural isomers are present in plants, such as 3-*O*-caffeoylquinic acid (3-CQA; neochlorogenic acid) and 4-*O*-caffeoylquinic acid (4-CQA; cryptochlorogenic acid) (Fig. 1) [10]. Along with these, diCQAs, triCQAs and a tetraCQA have been reported in literature [8].

Chlorogenic acids are one of the most abundant classes of phenolics in the human diet [9,12], and have been purported to have numerous health benefits. For instance, it was demonstrated that they possess antioxidant [13–16], anti-inflammatory [17] and anti-HIV properties [18] as well as the ability to inhibit carcinogenesis [12,19]. In addition, chlorogenic acids have been reported to play an important role in food quality. In coffee, high levels of mono-caffeoylquinic acids are indicators of lower coffee quality while higher levels of di-caffeoylquinic acids were positively correlated with higher quality. [20]· [21] Chlorogenic acids are also prominent in fruit juices [22]. For example, 5-CQA is reported as the major phenolic found in both apple and pear juices [23–25]. However, the exact structural identity of the various chlorogenic isomers in these juices has only been partially determined [24,25]. In addition, chlorogenic acids are thermally labile, as such they can undergo structural changes under common food processing conditions such as canning, roasting and pasteurization [26]. Therefore, the ability to differentiate between various chlorogenic acids (Fig. 1) and an understanding of their breakdown products is important for assessing food quality and in estimating the health benefits of foods rich in these compounds.

Various analytical techniques have been developed for the separation and detection of chlorogenic acids, where the most widely employed method is reversed phase high performance liquid chromatography (RP-HPLC), typically coupled with photodiode array (PDA) detection [3,27]. Another analytical method that has been used to confirm structural information about chlorogenic acids in a variety of food products, including apples, coffee beans and dried plums is HPLC coupled to mass spectrometry (MS) [28–30]. MS is a superior platform for the analysis of phenolics due to its high sensitivity and selectivity. It allows for the identification and quantification of minor chlorogenic acids in a complex phenolic mixture [27].

To fully utilize MS, an understanding of the analyte dissociation patterns during tandem (MS/MS) and multi-stage (MS^n) mass spectrometric analysis is required. The establishment of MS/MS fingerprints can be used for structural elucidations as well as for identifying unique product ions that can be used for targeted multiple reaction monitoring (MRM) quantitative analysis [31,32]. The application of MS/MS analysis to a selection of chlorogenic acids and other closely related compounds employing ion trap mass spectrometers have been reported in the literature [9,28,33–36]. However, the reported work was primarily focused on the negative ion mode in which only few product ions were structurally identified. In addition, some of the reported work proposed the formation of multisite-radical product ions [33,35], which is not probable during collision induced dissociation (CID)-MS/MS conditions. As such, there is an absence of universal MS/MS

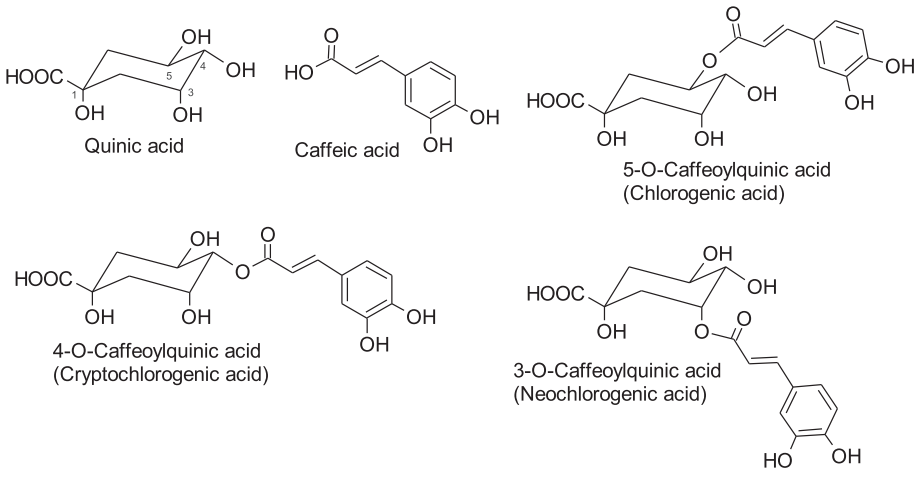

Fig. 1. Structures and nomenclature of the common mono-caffeoylquinic acids and their building blocks, quinic acid and caffeic acid (trivial names in brackets).