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Chemometric-Assisted Optimization of RP-HPLC Method for Determination of Some Bioflavonoids in *Brassica oleracea* Species and Their Antioxidative Activity

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Abstract In the present work, the rapid RP-HPLC method with UV (DAD) detection for simultaneous quantification of bioflavonoids: quercetin, apigenin, catechin, epicatechin, kaempferol, and luteolin in *Brassica oleracea* species samples (cauliflower, broccoli, and Brussels sprouts) was developed with the aid of LC-Simulator (ACD Labs® suite) software. A series of extracts obtained with different extraction method were evaluated for antioxidant activity. The optimal conditions for separation and quantification were established after nine scouting runs entered to LC-Simulator software. The optimized separation was achieved on Hypersil GOLD aQ column with isocratic elution and mobile phase composition A:2 % acetic acid in water and B:acetonitrile in 91:9 (*v*/*v* %) ratio. The R_s values were in the range from 2.6 to 8.00, indicating good selectivity of the method. The obtained results generally show good agreement with published data. Low detection limits (0.02–0.055 μg/mL) were obtained with acceptable recoveries (90–109 %). Total time of analysis was less than 11 min; therefore, the proposed method represents significant improvement over existing methods. Extracts from *Brassica* vegetables, obtained using different extraction procedures, were studied for their radical scavenging effects. Scavenging of DPPH showed different kinetics at the beginning of the assay period and after 15 min from the initialization of reaction. Different kinetics suggested the presence of polymerized and/or less active antioxidants with different scavenging mechanisms for particular polyphenolic compounds.

Keywords Computer optimization · Bioflavonoid · *Brassica* vegetables · Standard addition · Antioxidative activity

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Introduction

Flavonoids are a large class of phenolic compounds which are subclassified as flavones, flavonols, isoflavones, flavanones and catechins, chalcones, and anthocyanidins depending on phenyl substituent in the C_2 or C_3 position in benzo-γ-pyrone nucleus. Interest in the bioflavonoids is related to their diversity, biological significance as secondary plant metabolites and ecological role (Harborne 1994), use as chemotaxonomic markers (Kamiya et al. 1979), impact on fruit quality (Rouseff 1980), physiological effects (Forkmann et al. 1992; Herrmann 1970; Cody et al. 1988), and industrial applications (Ortuno et al. 1995).

The flavonoids are potent antioxidants, free radical scavengers (Sato et al. 1996), and metal chelators; they inhibit lipid peroxidation (Cook and Samman 1996) and exhibit various physiological activities (Middleton 1976; Leibovitz and Mueller 1993; Dakora 1995; Raghavan et al. 1995; Das et al. 1994), including anti-inflammatory (Thompson 1993), anti-allergic, anticarcinogenic, antihypertensive, and antiarthritic activities (Read 1995). Structures of investigated bioflavonoids are presented in Fig. 1.

In the present paper, we aimed to develop the method for simultaneous determination of several bioflavonoids in species *Cruciferae brassica*. *Brassica* vegetables have been found to be a significant host of antioxidant phytochemicals