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Simultaneous kinetic spectrometric determination of three flavonoid antioxidants in fruit with the aid of chemometrics

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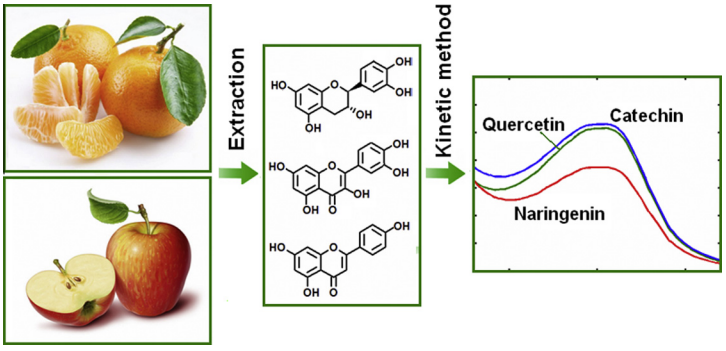
HIGHLIGHTS

- Quantifying three anti-carcinogenic flavonoids, catechin, quercetin and naringenin.
- Yellow product was obtained from reduction of Cu(II) to Cu(I) by the flavonoids.
- Chemometrics was used to process overlapped kinetic spectra.
- Simultaneous assay of three anti-carcinogenic flavonoids in fruit samples.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple, inexpensive and sensitive kinetic spectrophotometric method was developed for the simultaneous determination of three anti-carcinogenic flavonoids: catechin, quercetin and naringenin, in fruit samples. A yellow chelate product was produced in the presence neocuproine and Cu(I) – a reduction product of the reaction between the flavonoids with Cu(II), and this enabled the quantitative measurements with UV–vis spectrophotometry. The overlapping spectra obtained, were resolved with chemometrics calibration models, and the best performing method was the fast independent component analysis (fast-ICA/PCR (Principal component regression)); the limits of detection were 0.075, 0.057 and 0.063 mg L^{-1} for catechin, quercetin and naringenin, respectively. The novel method was found to outperform significantly the common HPLC procedure.

Introduction

Flavonoids are various phenolic compounds, which constitute one of the largest groups of secondary metabolites in fruit, vegetables and nuts; in general, they are considered to be beneficial as anti-cancer, -virus, -inflammatory, and -allergy compounds [1,2]. The basic structure of a flavonoid contains a phenol ring in the 2-phenylbenzopyrone structure [3]. Such compounds are of particular interest to food and analytical chemists as well as nutritionists, especially, with respect to the anti-oxidant reactivity against free radicals; a key role of these anti-oxidants is their ability to prevent oxidative stress in metabolic cell processes, e.g. the total amount of flavonoids is an important index, which reflects the quality and medicinal value of Traditional Chinese Medicines (TCMs) [4,5].

Chemical diversity of flavonoids from plants or vegetables, complicates the individual separation and determination of the antioxidants, although such methods have been reported, e.g. (i) a

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spectrofluorometric method for the determination of catechin, quercetin and naringenin in the concentration ranges of 0.2–6.0 × 10^{-5} M, 0.05–1.0 × 10^{-5} M, and 0.4–6.0 × 10^{-5} M, respectively [6]; (ii) a capillary electrophoresis method applied for the analysis of quercetin (concentration range – 0.4–16.0 mg L^{-1} and detection limit (LOD) – 0.03 mg L^{-1}) [7]; (iii) gas chromatography analysis of catechin and quercetin (LODs – 0.106 mg L^{-1} and 0.395 mg L^{-1}, respectively) [8]; (iv) solid-phase/high performance liquid chromatography (HPLC) for the determination of catechin and quercetin in rice (LODs – 0.85 μg g^{-1} and 1.10 μg g^{-1}, respectively) [9]; and (v) UV–vis absorbance spectroscopy was another technique used for the analysis of quercetin (range – 1.0–6.0 × 10^{-6} M; LOD – 0.25 × 10^{-6} M) [10]. However, all of these methods, especially those involving chromatography, are generally time-consuming, require special reagents, and in some cases require quite expensive instrumentation. Therefore, novel, relatively simple and cheap analytical methods for the simulataneous determination of flavonoids such as catechin, quercetin and naringenin (Scheme 1), would be especially useful [5]. The development of such simpler methods is often facilitated with the use of mathematical modeling. Thus, in the first instance the differential kinetic method based on the differences of reaction rates, especially in cases where an analyte reacts with a common reagent [11], is generally considered as an alternative approach for developing suitable models for the simultaneous determination of several analytes [12]. If the kinetic spectral data shows significant overlapping, then chemometrics methods of data analysis can be applied to resolve the overlapping signals. Typical methods for such work include partial least squares (PLS), principal component regression (PCR) and artificial neural network (ANN) [13], and suitable prediction models may be built; these do not require prior knowledge of the reaction order and rate, and can be used for prediction of unknown samples. If significant background or noise is present, data pretreatment methods can be applied to minimize or smooth such signals.

In the context of the above discussion, a kinetic method has been previously developed for the simultaneous determination of four different flavor compounds [14]; a first derivative data pretreatment was followed by partial least squares modeling (1st der-PLS) for calibration building and prediction; this approach produced somewhat improved results as compared to the more common standardized data pretreatment followed by PLS modeling. Monakhova et al. [15] used the advanced independent component analysis algorithm (ICA) to resolve the overlapping spectra from mixed analytes found in samples such as food additives, drugs and energy drinks; the result was comparable or even better than two other chemometrics methods, MCR-ALS (multivariate curve resolution-alternating least squares) and SIMPLISMA (simple-to-use-interactive self-modeling mixture analysis).

The fast independent component analysis (fast-ICA) is a well-known linear modeling method [16,17]; it is known for its computational complexity and accuracy of prediction [18–20]. The basic concept of this method is to use the neural network learning rule, convert it into a fixed-point iteration, and find the maximum of the non-Gaussianity as a measure of statistical independence [21,22]. This algorithm is not dependent on any user-defined parameters, and is simple to converge to obtain the most accurate solution allowed by the data. It has been used in different areas, such as for: the interpretation of analytical responses from medicinal samples [23], image processing [24], monitoring of statistical results and resolution of overlapping spectra [25].

The aims of this work were: (1) to develop a UV–vis kinetic spectrophotometric method for the simultaneous analysis of flavonoids in fruit as represented by the compounds: catechin, quercetin and naringenin; (2) to investigate several chemometrics methods, including the ICA data pretreatment algorithm, for the construction of the best performing calibration and prediction model, and (3) to compare the performance of the selected chemometrics prediction model for the prediction of flavonoids with results obtained from HPLC analysis.

Data modelling theory

Independent component analysis (ICA) and its algorithms

In general, the ICA model can be represented as:

$$\boldsymbol{A} = \boldsymbol{XS} \tag{1}$$

where $\boldsymbol{A}$ is an $n \times m$ observed signal matrix in which n represents measured signals and m the variables, i.e. in this work – a UV–vis absorbance matrix, $\boldsymbol{X}$, is an $n \times d$ matrix, with d relative concentration values based on the number of overlapping spectral profiles in the complex spectrum, and $\boldsymbol{S}$ is a $d \times m$ matrix denoting the source signals (m), i.e. the independent components (ICs) [19]. If $n > d$, the dimensions of the observed data matrix can be reduced to the same as that of the m source signals with the use of the singular value decomposition (SVD) or pseudo-inversion methods.

The goal of ICA is to find the maximum likelihood of the matrix, $\boldsymbol{X}$, i.e. defined as $\boldsymbol{W} = \boldsymbol{X}^{-1}$, so that:

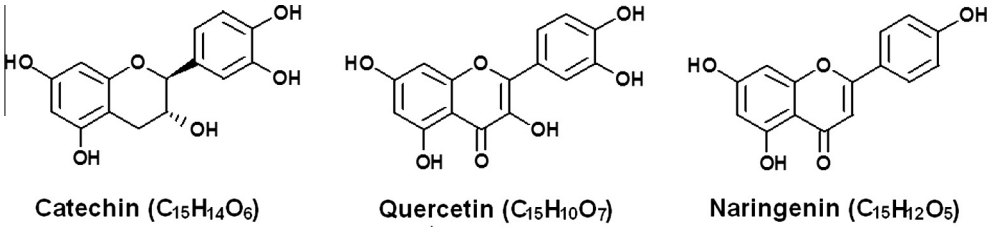
$$\widehat{\boldsymbol{S}} = \boldsymbol{WA} = \boldsymbol{X}^{-1}\boldsymbol{XS} = \boldsymbol{S} \tag{2}$$

Statistically, variables x_1 and x_2 are said to be independent if neither one or the other has any information about each other. Technically, x_1 and x_2 are independent if their joint probability density, $p(x_1, x_2)$, is the factorial of their marginal probability density $p(x_1)$ and $p(x_2)$:

$$p(x_1, x_2) = p(x_1)p(x_2) \tag{3}$$

Therefore, ICA modelling facilitates the extraction of source-spectral profiles of components embedded in the measured data, which is non-Gaussian [26].

Many ICA algorithms are available for processing analytical chemistry data; they include fast-ICA, joint approximate diagonalization of eigen-matrices (JADE), infomax ICA, mean-field ICA (MF-ICA) and kernel ICA (KICA). The fast-ICA algorithm was chosen for this work because it was demonstrated to be robust and fast to compute [26].



Scheme 1. Chemical structures of the three flavonoids.