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Analytical Methods

Characteristic fingerprint based on gingerol derivative analysis for discrimination of ginger (*Zingiber officinale*) according to geographical origin using HPLC-DAD combined with chemometrics

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ABSTRACT

Chromatographic fingerprints of gingers from five different ginger-producing countries (China, India, Malaysia, Thailand and Vietnam) were newly established to discriminate the origin of ginger. The pungent bioactive principles of ginger, gingerols and six other gingerol-related compounds were determined and identified. Their variations in HPLC profiles create the characteristic pattern of each origin by employing similarity analysis, hierarchical cluster analysis (HCA), principal component analysis (PCA) and linear discriminant analysis (LDA). As results, the ginger profiles tended to be grouped and separated on the basis of the geographical closeness of the countries of origin. An effective mathematical model with high predictive ability was obtained and chemical markers for each origin were also identified as the characteristic active compounds to differentiate the ginger origin. The proposed method is useful for quality control of ginger in case of origin labelling and to assess food authenticity issues.

1. Introduction

Recently, food authenticity and quality control issues have raised worldwide attention in the food manufacturing sector. There is a growing concern about the qualification of food with a clear regional identity due to the expansion and diversity of foods in international trade. The origins of food are nowadays essential for import and export trading in order to ensure the traceability for consumers, traders or even food producers. Because foods from different countries of origin have distinct qualities and different prices, information about a food's origin is necessary to verify its specifications and to guarantee its quality and efficacy (Dennis, 1998; Primrose, Woolfe, & Rollinson, 2010). To protect consumer rights and prevent deceptive practices, fingerprinting analysis has been conducted in many up-to-date studies and has become one of the most powerful systemic approaches. For the purpose of determining authenticity, it is effectively applied to discriminate and establish the characteristic profiles of ordinary foods or foods with complex compositions. In addition, fingerprint methods have been introduced as a recommended strategy for quality evaluation and control of traditional Chinese medicine by the World Health Organization (WHO) and the State Food and Drug Administration (SFDA) of China (Gong, Liang, Xie, & Chau, 2003).

Several analytical methods have been constructed with the use of chemometrics to create the pattern of food compositions in the form of a mathematical model. The application of chemometric techniques can greatly improve the quality of the fingerprint obtained from complex chromatographic or spectroscopic profiles. Pattern recognition by means of multivariate statistical analysis can be divided into two categories: unsupervised and supervised. Unsupervised pattern recognition is utilized for data visualisation by observing the relationship between samples and variables with no predetermined class. It is generally used as a primary step in order to monitor group of samples. These kinds of techniques are called exploratory analysis methods, and include principal component analysis (PCA), hierarchical cluster analysis (HCA), and self-organising maps (SOMs) (Brereton, 2009; Lloyd, Brereton, & Duncan, 2008; Wold, 1995). On the other hand, supervised pattern recognition is a statistical method applied for data classification by attempting to create a model to predict the class of an unknown sample; techniques include linear discriminant analysis (LDA), soft independent modeling of class analogy (SIMCA), K-nearest neighbor (KNN), and partial least squares discriminant analysis (PLSDA). Different classifiers can be set to assess and validate the performance, accuracy and precision of the model.

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Worldwide, many traditional Asian herbs – in the form of raw material, ingredients in processed food, food supplements or traditional medicines – are increasingly being consumed for their health-promoting effects. In fact, herbal properties are related to their chemical ingredients, and the therapeutic effect of those herbs derives from the synergic effect of their active components. The fingerprints of herbs are normally established from those active compounds. The variation of the compositions might depend on varieties, climate, agricultural areas, harvesting seasons, storage conditions and manufacturing processes. Geographical origin is considered to be one of the critical parameters affecting the variations (Drašar & Moravcova, 2004).

Ginger, *Zingiber officinale* Roscoe, is one of the most important and well-known traditional herbs, as a valued spice and a classical medicine in Asia for centuries. It has been recognized for its characteristic aroma and pungency. Ginger is believed to have originated in Southeast Asia, and is grown only under cultivation (Ravindran et al., 1994). It is a seasonal crop which is cultivated only once a year in tropical and subtropical regions such as China, India and Southeast Asia, which are major producers of ginger (Ravindran & Babu, 2005). Nowadays, ginger has beneficial uses in both traditional and modern medicine for the treatment of nausea, vomiting, motion sickness, diarrhea, and digestive and respiratory disorders. Furthermore, ginger also possesses numerous significant pharmacological properties such as anti-inflammatory, antimicrobial, anticarcinogenic, analgesic and antioxidant activities (Ali, Blunden, Tanira, & Nemmar, 2008; Butt & Sultan, 2011). Due to its health benefits, ginger is being exported around the world and extensively consumed in fresh form; it is also used in many kinds of food additives, dietary supplements, and traditional medicines. The U.S. Food and Drug Administration (FDA) regards ginger as "generally recognised as safe" (GRAS) as a food additive (Kubra & Rao, 2011). The biological activities of ginger arise from its active chemical components, which are gingerols, the pungent principles of ginger and gingerol-related compounds (Cheng, Liu, Peng, Qi, & Li, 2011; Jiang, Sólyom, Timmermann, & Gang, 2005; Shao, Lv, Parks, Wu, Ho, & Sang, 2010).

In 2011, a comprehensive review of current developments was reported for ginger (Kubra & Rao, 2011). Chromatographic techniques are typically utilized as a straightforward method to determine the chemical composition of ginger and various ginger products with the use of different detection methods, i.e. LC-DAD (Bailey-Shaw et al., 2008; Pawar, Pai, Nimbalkar, & Dixit, 2011; Salmon, Bailey-Shaw, Hibbert, Green, Smith, & Williams, 2012), LC-MS (He, Bernart, Lian, & Lin, 1998; Jiang et al., 2005; Lee, Khoo, Halstead, Huynh, & Bensoussan, 2007; Tao, Li, Liang, & Van Breemen, 2009), LC-NMR (Saha, Smith, Lenz, & Wilson, 2003), and GC–MS (Gong, Fung, & Liang, 2004; Singh, Kapoor, Singh, de Heluani, de Lampasona, & Catalan, 2008). Since the gingerols and shogaols are only available commercial standards of ginger compositions, the analysis of pungent constituents in most studies has focused on the determination of gingerols and shogaols. Among those reports, the effects of various varieties (Pawar et al., 2011; Salmon et al., 2012) and maturity stages (Bailey-Shaw et al., 2008) on the contents of [6]-, [8]- and [10]-gingerol were investigated. Even though the physical appearance of ginger might sometimes be used to roughly discriminate among different varieties or ages of ginger, it is not suitable for accurate origin labeling.

To the best of the authors' knowledge, there are no previous reports on the development of a fingerprint study of ginger profiles to distinguish their geographical origins in various ginger-producing countries. The aim of this work was to establish a combination of a basic conventional analytical method (HPLC-DAD) and novel pattern recognition methods for the fingerprinting study of ginger from different countries of origin.

Since gingerols and gingerol derivatives are well-known and responsible for a pungent bioactive principle of ginger, the chemical profiles of gingerol derivatives from ginger samples of five ginger-producing countries (80 samples in total) were defined to generate chromatographic fingerprints. Chemometrics tools of unsupervised (similarity analysis, HCA, PCA) and supervised (LDA) pattern recognition were both applied to effectively handle all of the data. The developed method provided an efficient analytical procedure, and the qualified model which was statistically validated. In addition, chemical markers for each origin were obtained and established as the characteristic compounds to allow easier discrimination of the sources of ginger.

2. Experimental

2.1. Plant materials and reagents

Eighty samples of fresh ginger (*Z. officinale*) from China (18 samples), India (18 samples), Malaysia (8 samples), Thailand (18 samples) and Vietnam (18 samples) were provided and authenticated by the Horticulture Research Institute (HRI), Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand, and by Gold Ginger Thai Co. (Petchaboon, Thailand). All samples were randomized by origin and collected in spring, 2012. The raw herbs were labeled according to their sources and then kept in a refrigerator at 4 °C until analysis.

Standards of [6]-gingerol, [8]-gingerol, and [10]-gingerol were obtained from ChromaDex (Irvine, CA, USA). HPLC-grade acetonitrile was purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA). High purity water for HPLC analysis was prepared using a Milli-Q water purification system from Millipore (Bedford, MA, USA). Analytical grade methanol from Merck (Darmstadt, Germany) was used for sample preparation. Standard solutions (1000 mg L^{-1}) of [6]-gingerol, [8]-gingerol, and [10]-gingerol for HPLC and LC–MS/MS identification were prepared individually by dissolving each compound in methanol; and then were stored at 0 °C in a refrigerator until use.

2.2. Instrumentation and chromatographic condition

HPLC fingerprint analysis was performed on an Agilent 1100 series HPLC-DAD system (Agilent Technologies, Santa Clara, CA, USA) equipped with a G1322A vacuum degasser, a G1312A binary pump, a G1313A autosampler, a G1316A column compartment, and a G1315A diode array detector. All separations were carried out on a Waters Symmetry C18 column (3.9 × 150 mm, 5 μm). A binary gradient elution system composed of water (A) and acetonitrile (B) was applied as follows: 0.0–2.0 min, 10–55% B; 2.0–8.5 min, 55% B; 8.6–12.5 min, 65% B; 12.6–19.0 min, 100% B. Each run was followed by equilibration time of 10 min. The injection volume was 3 μL per sample, the flow rate was 1 ml min^{-1} and the column temperature was maintained at 27 °C. The DAD detector was set at 230 nm for acquiring chromatograms.

All chromatographic peaks were identified and confirmed using LC–MS/MS experiment using an HPLC system coupled to a Xevo™ TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA, USA). MS spectra were recorded in the range of m/z 100–1000 using electrospray ionisation (ESI) as the ionisation source in positive/negative ion-switching mode. The mass spectrometer settings used were: capillary voltage 3 kV, source temperature 150 °C, desolvation temperature 500 °C, cone gas (nitrogen) flow 150 L h^{-1}, desolvation gas (nitrogen) flow 800 L h^{-1}, and collision gas (argon) flow 0.15 ml min^{-1}. Instrument control and data acquisition and evaluation were performed with the Micromass MassLynx 4.1 software package provided by Waters.