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Classification and authentication of Iranian walnuts according to their geographical origin based on gas chromatographic fatty acid fingerprint analysis using pattern recognition methods

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ABSTRACT

Recently, food authenticity has raised worldwide attention in food manufacturing and a growing concern about food qualification, based on a clear regional identity, is noticed. Therefore, the development of suitable methodologies allowing the characterization of different products, based on their geographical origin, is of great importance. In this study, the potential of gas chromatographic fatty acid fingerprints in combination with multivariate data analysis was examined to classify walnuts from different regions in Iran according to their geographical origins. Walnut samples were collected during the harvesting period 2013–2014 from six regions in Iran. Chromatographic fingerprints of the walnut oil were employed to discriminate the walnut origin. Principal component analysis-Linear discriminant analysis (PCA-LDA) results showed that the six regions of geographical origin can be identified based on the fatty acid fingerprints. Almost all samples were correctly classified by the PCA-LDA model using cross validation (99.2%). The average percent correct classification for the prediction set was 98.3%, indicating the satisfactory performance of the model. A high percentage of correct classifications for the training data demonstrates the strong relationship between the fatty acid profile and the origin, while a high percentage for the prediction set shows the ability to indicate the origin of an unknown sample based on its fatty acid chromatographic data.

1. Introduction

Walnuts form a significant source of nutrients and can also be identified as a profitable agricultural product. The walnut kernel is a high-quality source of fatty acids and tocopherols (vitamin-E homologues) that mainly contributes to reducing blood cholesterol levels leading to a reduced risk of coronary heart diseases [1,2]. The walnut kernel generally contains about 60% oil by weight; however this may range from 52% to 70%, depending on the cultivar, availability of water and geographic location [3,4]. The major constituents of the oil are triglycerides. The triglyceride composition of the walnut oil contains high levels of monounsaturated and polyunsaturated fatty acids, mainly including linoleic (57–62%), oleic (12–20%), linolenic (11–16%) and palmitic (6–8%) acids [4].

Currently, the three most important varieties of walnut that commonly employed are *Juglans regia* L., *Juglans cinerea* L. and *Juglans nigra* L. The Persian walnut *(Jug lans regia L.)*, which is widely cultivated around the world, has the highest quality among the walnut varieties. It has a sweet taste with a relatively large kernel and has a thin shell, which makes it easy to crack [5]. The Persian walnut, originating from Central Asia, mainly grows in mild and dry environments with low rainfall, such as the Middle East and Mediterranean climates. Iran is the third largest walnut producer worldwide with annual production of 150,000 tons (11% of the world's total walnut production) [6]. Walnut orchards are spread all around the country, since most regions fulfill the requirements of growing this native tree.

Considering geographic specifications of quality, research efforts have been focused on the classification of food products according to

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their geographical origins. Recently performed researches are based on the analysis of certain chemicals, such as the fatty acids [7], aroma components and multi-element compositions [8–11]. This can be achieved using a wide range of instrumental techniques, such as gas chromatography (GC) [12], gas chromatography with mass spectrometry (GC–MS) [13], liquid chromatography with mass spectrometry (LC–MS) [14] and Nuclear Magnetic Resonance (NMR) [15].

Fingerprint analysis has become one of the most powerful systematic approaches to determine authenticity. A fingerprint is a characteristic profile of a sample, which can be established through common techniques, such as chromatography and spectroscopy. In this regard, chromatographic fingerprints are appropriate records [16,17], reflecting the chemical composition, for quality control, discrimination or classification of numerous food products [18–23].

A chromatographic fingerprint is highly complex multivariate data consequently small differences between similar chromatograms might visually be ignored. Therefore, chemical pattern recognition methods, such as principal component analysis (PCA) and linear discriminant analysis (LDA) could be considered as reasonable methods to classify the samples. Chromatographic fingerprinting combined with chemometric pattern recognition methods has widely been used in food product analysis [21,24–27].

In recent years, fatty acid profiling has become a promising approach to distinguish fat-rich foods by geographical origin or botanical identity [28–30]. However, classification of walnuts based on gas chromatographic fatty acid contents has not been reported yet in the literature.

In this study, the potential of gas chromatographic fatty acid fingerprints in combination with multivariate data analysis was examined to classify walnuts from different regions in Iran according to their geographical origins. To the best of our knowledge, no reports have been published on the classification of Iranian walnuts according to their geographical origin. In addition, no reports either were found on the application of gas chromatographic fatty acid fingerprinting for the authentication of walnuts based on their geographical origin. However, the walnut quality of different regions in Iran is different and Iranian people know about this quality differences. This work is a beginning of traceability and authentication of this special product. Therefore, the main aim of this study was to establish a reliable model using pattern recognition methods and GC fingerprints for the classification and authentication of the Iranian walnuts. For this purpose, the chromatographic fatty acid profiles of walnut samples, produced in six Iranian regions were considered. Unsupervised (PCA) and supervised (SIMCA and PCA-LDA) pattern recognition techniques were both applied to effectively analyze the obtained data. The predictive ability of a model has been evaluated by the statistical parameters of a prediction set.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, sulfuric acid (98%) and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). Light petroleum ether (bp 40–60 °C, analytical grade) was supplied from Daejung (Shiheung, Gyeonggi-Do, Republic of Korea).

2.2. Sample collection

Walnut samples were collected from six geographical regions in Iran. The exact location of these regions, including Bavanat (Bav), Maragheh (Mar), Ramsar (Ram), Tuyserkan (Tuy), Saman (Sam) and Alamout (Ala), has been shown on the map (Fig. 1).

Six orchards were selected in each region for sampling purposes during the harvesting period 2013–2014 (October 2013). An amount of 1 kg walnuts was purchased from each orchard. The further sampling

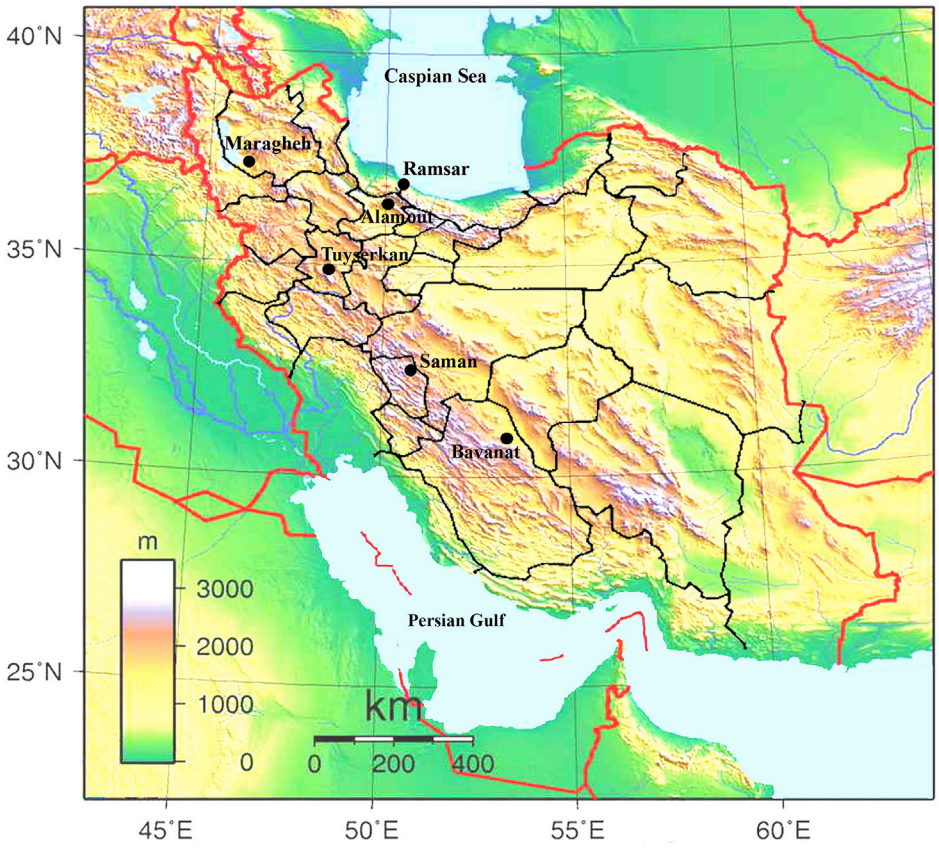

Fig. 1. Map of Iran: illustration of the six geographical origins of the walnut samples.