Industrial Crops & Products 124 (2018) 626–632

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Prediction of thymoquinone content in black seed oil using multivariate analysis: An efficient model for its quality assessment

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ARTICLE INFO

Keywords:
Black seed oil
Nigella sativa
Thymoquinone
Quality control
Multivariate analysis

ABSTRACT

Nigella sativa L. seeds are a rich source of diverse phytochemicals, well recognized for their medicinal properties. The seed oil (Black Seed Oil) is the more widely used therapeutic form and is readily adulterated. In this study, 30 commercial oil samples collected from different regions were assessed regarding their thymoquinone concentration using High Performance Liquid Chromatography. A discrepancy was shown in thymoquinone content ranging from 0.010 in one Saudi sample to 13.296 mg/g oil in one Egyptian sample. Ultra-violet spectroscopy was conducted on the methanolic extracts and multivariate analyses were applied to the collective data with Principal component Analysis (PCA) showing clear segregation of samples with high thymoquinone concentration positively clustered along PC1 (explaining 94% of the variance). This model allowed for the prediction of thymoquinone at correlation coefficients of 0.997 and 0.984 for the calibration and test set respectively verifying the robustness of the method. Lipid profiling of the oil samples was also conducted for quality verification. The model developed offers a simple, efficient and reliable method for the quality assessment of Black Seed Oil.

1. Introduction

One of the highly acclaimed nutraceuticals today is Black Seed oil (BSO), produced from the seeds of *Nigella sativa* L. (Family Ranunculaceae), the more popular species of the genus *Nigella* (Peter, 2012). Both seeds and seed oil of *Nigella sativa* are used in various traditional systems of medicine and reports for their practice in folk medicines include the treatment of many aliments (Ahmad et al., 2013; Hosseinzadeh et al., 2013). *N. sativa* seeds are frequently used in the Middle East as a spice and for decorating baked goods. Both seeds and oil show a wide range of therapeutic properties including antioxidant, antimicrobial, anti-inflammatory, anti-ulcer, anti-asthmatic, anti-hypertensive, immunomodulatory and cytotoxic effects as well as other remedial effects described in literature (Adam et al., 2016; Bourgou et al., 2012; Gholamnezhad et al., 2015; Heshmati et al., 2015; Hobbenaghi et al., 2014; Jrah Harzallah et al., 2011; Majdalawieh and Fayyad, 2015; Mahmmoud and Christensen, 2011; Ramadan et al., 2012; Rifat uz et al., 2013).

N. sativa seeds are a rich source of diverse classes of phytochemicals including fixed and volatile oils, proteins, flavonoids, glycosides, alkaloids, saponins, mucilage, tannins, resins, bitter principles, minerals, and vitamins (Farag et al., 2014). BSO constitutes about 25–50% of the total seed components and comprises different saturated fatty acids (mostly palmitic and stearic acids) as well as unsaturated fatty acids (oleic and linoleic acids) attributing to the oil's widely recognized nutritional value (Cheikh-Rouhou et al., 2007; Lutterodt et al., 2010; Nickavar et al., 2003; Piras et al., 2013). Thymoquinone (TQ) (Fig. 1), a lipid soluble C10 terpene present in its crystalline triclinic form, is considered the most relevant bioactive component in *N. sativa* seeds and in addition to its biological role, it also serves as an analytical marker for BSO (Farag et al., 2017; Gali-Muhtasib et al., 2006; Velho-Pereira et al., 2011).

BSO is well known as the more therapeutically utilized form of *N. sativa* seeds, and recently acknowledged as a top selling food supplement, which due to its high demand, is readily adulterated and blended with various less expensive vegetable oils. In an effort to establish a reliable and efficient method for the quality assessment of BSO, different analytical methods have been reported for its analysis and quantitation of constituents including fatty acids, triacylglycerol and/or other major bioactive compounds. Some methods depend on the identification and quantification of TQ including: high performance - thin layer chromatography (HP-TLC), (Velho-Pereira et al., 2011), high performance liquid chromatography (HPLC), (Lutterodt et al., 2010), gas chromatography coupled with mass spectroscopy (GC–MS), (Farag et al., 2017; Jrah Harzallah et al., 2011; Kiralan, 2012; Viuda-Martos et al., 2011), stable isotope dilution (SID) (GC–MS) technique (Johnson-

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https://doi.org/10.1016/j.indcrop.2018.08.037
Received 7 February 2018; Received in revised form 13 August 2018; Accepted 13 August 2018

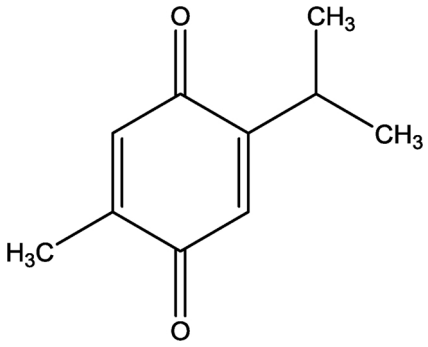

Fig. 1. Chemical structure of thymoquinone.

Ajinwo and Li, 2014) and liquid chromatography coupled with electron spray ionization and tandem of flight mass spectrometer (LC-ESI-TOF) (Avula et al., 2010; Farag et al., 2014). Other techniques rely on the oil's lipid profile where thin layer chromatography (TLC), (Zaoui et al., 2002), GC–MS (Bassim Atta, 2003; Cheikh-Rouhou et al., 2007; Kökdil et al., 2006; Lutterodt et al., 2010, Piras2013) and HPLC (Piras et al., 2013) were the main investigative techniques used for the identification of both saturated and unsaturated fatty acids.

Nowadays, the use of chromatographic and/or spectroscopic techniques coupled with multivariate analyses present a better alternative for products with multiple bioactive ingredients. This allows for the accountability of various variables or in this case chemical markers rather than a single constituent (Gad et al., 2013a). The application of fourier transform infrared (FTIR) spectroscopy coupled with multivariate analysis could detect the adulteration of BSO by the selection of specific discriminating frequency regions (Nurrulhidayah et al., 2011; Rohman and Ariani, 2013). Using the metabolomics approach, the complete profiling of *Nigella* seeds extract by implementing ultra performance liquid chromatography coupled with photo-diode array detector and time-of-flight mass spectrometry UPLC-PDA-MS and GC–MS with multivariate analysis was utilized to classify 6 *Nigella* species (Farag et al., 2014). In a more recent study, using the nuclear magnetic resonance (NMR-metabolomics) approach, successfully segregated *N. sativa* seeds according to their geographical origins (Maulidiani et al., 2015). Yet, most of these methods call for expensive instrumentation, multi-steps of sample preparation resulting in markedly extended analysis time in addition to external calibrations.

In this study, a simple, inexpensive and efficient model is presented as a reliable method for the quality assessment of BSO. The model was developed by building a correlation between the spectroscopic analysis of BSO and its content of TQ. The collective data obtained from the quantification of TQ in a total of 30 commercial oil samples was correlated to their ultra-violet (UV) spectroscopic data resulting in the establishing of a model that relied on the latter data only to authenticate the oils. The fatty acid profiles of the oils were also assessed using GC–MS as additional verification to the oil quality. The significance of this model is that it provides an alternative to expensive and time-consuming chromatographic methods. UV spectroscopy coupled with multivariate analysis has been successfully used previously for the quality control of other natural products (Gad et al., 2013b; Roshan et al., 2013). To the best of the authors' knowledge, this is the first report of applying this model to the quality assessment of BSO and may be similarly developed for other fixed oil quality assessments.

2. Materials and methods

2.1. Materials and reagents

A total of 30 samples of BSOs were collected from local markets in Egypt, Saudi Arabia, England and the USA. All samples were labelled as "cold pressed" on the product labels. The origins of the oils according to their labels were as follows: 19 Egyptian (Egy1-19), 3 Saudi (Sau1-3), 5 Ethiopian (Eth1-5), 1 Pakistani (Pak), 1 Syrian (Syr) and 1 American sample (US) (Table 1). All samples were stored in airtight containers at room temperature away from light until the analysis. TQ was purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). All solvents were of HPLC grade purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

Table 1
Collected *Nigella sativa* Oil (BSO) samples code and their origin.

Sample code	Market Source	Origin
Egy1	Egypt	Egypt
Egy2		
Egy3		
Egy4		
Egy5		
Egy6		
Egy7		
Egy8		
Egy9		
Egy10		
Egy11		
Egy12		
Egy13		
Egy14		
Egy15		
Egy19		
Egy16		
Egy17		
Egy18		
Egy19		
Eth1	Saudi Arabia	Ethiopia
Eth2		
Eth3		
Eth4		
Eth5		
Sau1	Egypt	Saudi Arabia
Sau2	Saudi Arabia	
Sau3		
Syr	Saudi Arabia	Syria
Pak	England	Pakistan
US	US	Atlanta, USA

2.2. HPLC analysis and TQ quantification

One gram of the BSO was successively extracted three times with methanol (3 mL). The three methanol extracts were combined and kept in the dark at −4 °C. HPLC analysis was conducted to determine the content of TQ in BSO using HPLC Agilent 1200 series Quaternary Pump with Diode Array Detector (Agilent Technologies, Santa Clara, CA, USA). All separations were carried out on 175 a Waters Symmetry C18 column (3.9 × 150 mm, 5 µm). The mobile phase consisted of methanol/water/2-propanol at a ratio of 45:50:5 (v/v/v) (isocratic elution). Flow rate was 1.5 mL/min. UV monitoring was performed at 254 nm for all components of interest as described by Lutterodt et al. (2010). Authentic TQ was used as a standard for the calculation of TQ content in all samples.

2.3. UV spectroscopy

All samples were analyzed by UV spectroscopy (200–400 nm) using a UV-1601 PC, UV–vis spectrophotometer Q3 (Shimadzu, Japan). The UV spectrum was recorded for each samples in three replicates and the average was used for the multivariate data analysis.

2.4. Fatty acid composition GC–MS analysis

Fatty acid methyl esters (FAMEs) were prepared from BSO samples. One mg of oil was reacted with NaOH-MeOH (0.1 mol/L) for 5 min,