



ELSEVIER

Contents lists available at ScienceDirect

Talanta

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

Comparative study of the effect of auxiliary energies on the extraction of Citrus fruit components



Carlos A. Ledesma-Escobar^{a,b,1}, Feliciano Priego-Capote^{a,b,*},
María Dolores Luque de Castro^{a,b,*}

^a Department of Analytical Chemistry, Annex C-3, Campus of Rabanales, University of Córdoba, Córdoba, Spain

^b University of Córdoba Agroalimentary Excellence Campus, ceiA3, and Institute of Biomedical Research Maimónides (IMBIC), Reina Sofía Hospital, University of Córdoba, E-14071 Córdoba, Spain

ARTICLE INFO

Article history:

Received 22 April 2015

Received in revised form

29 June 2015

Accepted 4 July 2015

Available online 6 July 2015

Keywords:

Desirability function approach

Auxiliary energies

Extraction

Metabolites

Citrus

ABSTRACT

A comparative study of methods for ultrasound-assisted extraction (USAE), microwave-assisted extraction (MAE) and superheated liquid extraction (SHLE) of compounds from citrus has been performed. The suited conditions for each method were evaluated to maximize the concentration of 10 representative compounds (sugars, carboxylic acids, phenolic acids and flavonoids) by a desirability function approach based on the chromatographic peaks obtained by LC–DAD. Extracts obtained under the suited conditions were analyzed by LC–QTOF MS/MS. The ANOVA on the molecular entities showed 232 significant entities ($p < 0.01$), and pairwise comparison revealed that USAE and MAE methods are the most similar (50 different entities), and USAE and SHLE the most dissimilar (224 different entities). A discrimination test by PCA showed a clear discrimination among the extraction methods, explaining 78.51% of the total variability. Similarities in the abundance of the monitored compounds was tested by ANOVA showing that the extraction of carboxylic acids (malic and citric acids) was equal by all methods; while for each of the other eight compounds, at least one extraction method is different from the others. Under the evaluated conditions the SHLE method is the less favorable to extract metabolites from citrus, being the best the USAE method.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Citrus is the most abundant crop of fruit trees in the world, with an annual production of approximately 115.5 million tons cultivated in more than 100 countries all over the world, mainly in tropical and subtropical areas [1]. These fruits are recognized for the refreshing scent of their essential oils, their high content of vitamin C and other bioactive compounds like carotenoids, limonoids, phenols and vitamin B complex, which play a key role as nutraceuticals [2]. Citrus components have shown to be very useful both for the maintenance of human health and in industrial

Abbreviations used: LC, liquid chromatography; DAD, diode array detector; QTOF, quadrupole–time of flight detector; MS, mass spectrometry; ESI, electrospray ionization source; RT, retention time; SHLE, superheated liquid extraction; MAE, microwave-assisted extraction; USAE, ultrasound-assisted extraction; SFE, supercritical fluid extraction; SE, shaking method; PCA, principal component analysis

* Corresponding authors at: Department of Analytical Chemistry, Annex C-3, Campus of Rabanales, University of Córdoba, Córdoba, Spain. Fax: +34957218615.

E-mail address: qa1lucam@uco.es (M.D. Luque de Castro).

¹ Present address: Chemical and Biochemical Engineering Department, Technological Institute of Veracruz, Av. Miguel Angel de Quevedo 2779, Veracruz, Ver. 91897, Mexico.

<http://dx.doi.org/10.1016/j.talanta.2015.07.011>

0039-9140/© 2015 Elsevier B.V. All rights reserved.

applications, mainly in food, cosmetics and pharmaceuticals industries [3]. In recent years, most studies on bioactive compounds in fruits have focused on specific classes or families of compounds. Special attention has been paid to phenols, and, more specifically, to flavonoids. A number of studies has shown the great antioxidant, radical scavenging [4] and anti-inflammatory properties of these phytochemicals [5], and also many epidemiological and intervention studies have associated the consumption of these compounds with lower risks of different types of cancer and cardiovascular diseases [6]. The interest in these compounds has also promoted research on the extraction, identification, purification and bioactive properties of them, which have resulted in multiple discrepancies, most of them owing to differences in the extraction methods [7]. The extraction step is crucial to obtain the expected results, as its suitability strongly influences the quality of the final extract. For this reason, solvents and extraction techniques must be carefully chosen, taking into account the characteristics of both the sample matrix and the target compounds. Common solid–liquid extraction (properly known as leaching or lixiviation) methods applied to citrus have been based on maceration–stirring, Soxhlet extraction, supercritical fluid extraction (SFE), superheated liquid extraction (SHLE), microwave-assisted extraction (MAE) and

ultrasound-assisted extraction (USAE); being the last two the most widely reported [8]. The major part of the proposed methods have focused on a specific family or group of compounds, mainly on phenols and their bioactive properties. In this sense, USAE has shown to be efficient for extraction of phenols from citrus, faster than maceration, especially at low temperatures and short operational times [9]. Similarly, MAE has shown to be a fast alternative to extract phenolic compounds without degradation [10]; however, other metabolites like ascorbic acid or carotenoids have been affected by microwave irradiation or high temperature, resulting in degradation [11]. Also, the stability of phenolic compounds in the presence of superheated extractants has been tested, and efficiencies higher than 85% at 150 °C and 90% at 100 °C have been obtained [12], thus demonstrating that, despite SHLE is a good alternative to slow extraction methods for these compounds, above 100 °C degradation occurs, an effect more significant as the target compounds are more thermolabile [13]. In spite of the large number of studies on extraction of bioactive compounds from citrus, few of them have compared different extraction methods. The discrepancies among the scant studies on this subject strongly depend on the metabolites under study; studies that are also characterized by the lack of optimization of each method prior to comparison [14]. As an example of this behavior, Orió et al. reported in 2012 a comparative study among MAE, USAE, SFE and maceration for the extraction of alkaloids from *Mitragyna speciosa*, using four extractants (methanol, ethanol, 1:1 methanol–water, and 5:95 ethanol–water at pH 3), without any optimization step; actually, the selection of the operating conditions for each extraction method was unclear. They concluded that the best method for extraction of alkaloids is MAE with 1:1 methanol–water as extractant. Also, in a previous study by Piñeiro et al. in 2004, on comparison of SHLE and USAE for extraction of catechins, the authors selected the SHLE working conditions based only on a kinetics study, while those of the USAE method were selected after testing only two temperatures and 10 min as extraction time [15].

In an attempt to solve the existing discrepancies, the goal of the present research was to test the effect of the different auxiliary energies (ultrasound, microwaves and temperature+pressure), on the extraction of compounds of interest from citrus. Ten compounds representative from the different chemical families in citrus fruits such as sugars, flavonoids, carboxylic acids and phenolic acids were selected for monitoring the extraction process. For this purpose, the variables affecting the method based on the use of each type of energy were studied at different levels to look for the conditions that maximize the relative abundance of targeted compounds based on a desirability criterium. Extracts obtained under the best working conditions in each method were compared to establish similarities/dissimilarities between them. Lemon (*Citrus limon*) was selected as model sample for the different studies.

2. Materials and methods

2.1. Sample

Five kilogram of edible lemons (*C. limon*) were purchased in a local market in Córdoba, Spain (January, 2014). The fruits were washed, cut in slices, lyophilized to constant weight and finally grinded (particle diameter ≤ 0.5 mm). The powder was stored in the dark at -20 °C until use.

2.2. Reagents

All solvents were LC grade or higher when required. n-Hexane, ethanol and formic acid were from Scharlab (Barcelona, Spain);

acetonitrile and methanol from Fluka (Buchs, Switzerland). Deionized water (18 M Ω cm) from a Millipore Milli-Q water purification system (Bedford, MA, USA) was used to prepare the mobile chromatographic phases and extractant mixtures.

2.3. Apparatus and instruments

The reference extracts were obtained by shaking using a Vibromatic reciprocating shaker (Selecta, Barcelona, Spain). Ultrasound was applied by a Branson 450 digital sonifier (20 kHz, 450 W) equipped with a cylindrical titanium-alloy probe (12.70 mm diameter). Microwave assistance was provided by a focused microwave digester (200 W) Microdigest 301 (Prolabo, Paris, France). Superheated liquid extractions were carried out by a laboratory-made dynamic extractor consisting of the following units: (a) an extractant supplier, (b) a high pressure pump (Shimadzu LD-AC10), which propels the extractant through the system, (c) a switching valve placed next to the pump to develop static extractions if required, (d) a stainless-steel cylindrical extraction chamber (550 \times 10 mm inner diameter and 4.3 ml internal volume), where the sample is placed (this chamber is closed at both ends with screws whose caps contain cotton-made filters to ensure the sample is not carried away by the extractant), (e) a restriction valve to maintain the desired pressure in the system, (f) a cooler made of a stainless-steel tube (1 m length and 0.4 mm inner diameter) and refrigerated with water, and (g) a gas chromatograph oven (Konix, Cromatix KNK-2000), where the extraction chamber is placed and heated.

The analytical equipment consisted of an LC-DAD—Agilent 1100 coupled to a diode array detector G1315A—and an LC-QTOF MS/MS—Agilent 1200 series coupled to an electrospray ionization source and a quadrupole–time of flight detector Agilent Q-TOF 6540.

2.4. Extraction

The lemon samples (1 g dry weight each) were extracted in 20 ml of ethanol–water mixtures by the different methods (USAE, MAE, SHLE and shaking) the parameters of which were optimized by multivariate methods using as response the LC-DAD chromatograms according to the experimental design detailed below. The extracts were obtained under the suited working conditions for each method and their global profiles provided by LC-QTOF MS/MS were compared.

The experimental factors analyzed for each auxiliary energy employed (USAE, MAE and SHLE methods) are described in Table 1. Shaking extraction was used as reference method to establish the improvement provided by the different auxiliary energies. A kinetics study was developed with each method to know if a plateau of efficiency is obtained for a given time, thus demonstrating the extraction equilibrium. Monitoring between 10 and 120 min at 10 min intervals provided the kinetics profile.

2.5. LC-DAD analysis

Chromatographic separation was performed by using an Inertsil ODS-2 C18 analytical column (250 \times 4.6 mm i.d. 5 μ m particle) from Análisis Vínicos (Tomelloso, Ciudad Real, Spain). The injection volume was 20 μ l, and the mobile phase was composed by deionized water (A) and acetonitrile (B) at a constant flow rate of 1 ml min $^{-1}$. The gradient was as follows: 4% to 10% B in 5 min; change from 10% to 25% B in 30 min; from 25% to 100% B in 15 min and constant 100% B for 5 min. After analysis, the column was equilibrated to the initial conditions within 5 min. The selected wavelengths for monitoring were 210, 254 and 320 nm.

Download English Version:

<https://daneshyari.com/en/article/7678725>

Download Persian Version:

<https://daneshyari.com/article/7678725>

[Daneshyari.com](https://daneshyari.com)