Food Chemistry 174 (2015) 104–109

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Quantification of total pigments in citrus essential oils by thermal wave resonant cavity photopyroelectric spectroscopy

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ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form 26 September 2014
Accepted 6 November 2014
Available online 14 November 2014

Keywords:
Carotenoid content
Chlorophyll content
Optical absorption coefficient
Citrus essential oils

ABSTRACT

A general theory of thermal wave resonant cavity photopyroelectric spectroscopy (TWRC-PPE) was recently proposed by Balderas-López (2012) for the thermo-optical characterisation of substances in a condensed phase. This theory is used to quantify the total carotenoids and chlorophylls in several folded and un-folded citrus essential oils to demonstrate the viability of using this technique as an alternative analytical method for the quantification of total pigments in citrus oils. An analysis of variance (ANOVA) reveals significant differences ($p < 0.05$) among the means of optical absorption coefficient data for the folding degree and fruit type in citrus oils. The experimental results show that TWRC-PPE spectroscopy can be used to quantify concentrations up to five times higher of total carotenoids and chlorophylls in citrus oils than UV–Vis spectroscopy without sample preparation or dilution. The optical limits of this technique and possible interference are also described.

1. Introduction

Carotenoids and chlorophylls are among the most abundant organic pigments present in numerous fruits and vegetables. Carotenoids are tetraterpenes that are highly important for human health because of their potential role in preventing cancer and cardiovascular diseases; this potential role is related to their antioxidant and immunological activities (Rao & Rao, 2007). In addition, chlorophylls are magnesium-containing chlorins that have recently been shown to protect against cancer (Dashwood, 1997).

Citrus species are well known to possess a wide-ranging carotenoid pattern and are considered to be the most complex natural source of this compound type (Dugo & Giuffrida, 2011). Chlorophylls are related to the photosynthesis present in citrus species during growth and maturation; their amount decreases in citrus fruits with ripening, and the amount of carotenoids increases (Ladanyia, 2008). The highest amount of carotenoids and chlorophylls in citrus species is present in the pericarp of citrus peels from which citrus essential oils are obtained (Di Giacomo & Di Giacomo, 2002).

Essential oils are major by-products of citrus juice processing and important flavouring ingredients in food and beverage products, with the annual consumption of citrus essential oils estimated to be 56,200 tonnes per year over the last decade (Schmidt, 2010). A prize was once paid for oils with a high carotenoid content because they can be simultaneously used as flavouring and natural colour enhancers (Crandall, Kesterson, & Dennis, 1983).

Citrus essential oils are primarily folded (concentrated) by using high vacuum fractional distillation, and monoterpenes, primarily D-limonene (representing an average of more than 90% of the content of citrus essential oils), are partly removed by this process, depending on the folding degree (Stuart, Lopes, & Oliveira, 2001). The primary folding degrees that are commercially available are 2- to 5-fold for lime, lemon and mandarin oils and 2- to 10-fold for grapefruit and orange oils. Folded oils are less prone to oxidation, their solubility in water increases, and they have high organoleptic qualities; the amounts of carotenoids and chlorophylls increase with the folding process, which increases the colour of the oils (Clark & Chamblee, 1992; Vora, Matthews, Crandall, & Cook, 1983).

Conventional UV–Vis spectroscopy is a widely used analytical technique for quantifying natural pigments in several food products, including citrus essential oils (Crandall et al., 1983; John

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http://dx.doi.org/10.1016/j.foodchem.2014.11.039
0308-8146/

Scott, 2011); however, the quantification of pigments by this technique commonly requires sample preparation or dilutions (Crandall et al., 1983; Lichtenthaler & Buschmann, 2011) due to it can measure solutions with optical absorption coefficients of up to 7 cm^{-1} (equivalent to an absorbance of approximately 3 a.u.), the dispersed light is a strong limitation and there is no criterion for verification in advance for the validity of the Beer's-Law absorption model.

In principle, thermal wave resonant cavity photopyroelectric (TWRC-PPE) spectroscopy has been proposed to measure the thermo-optical properties of substances in the condensed phase (Balderas-López, 2011). In contrast with conventional UV–Vis, the TWRC-PPE technique can measure solutions with optical absorption coefficient values of approximately 28 cm^{-1} (equivalent to an absorbance of approximately 12 a.u.). Dispersed light is a minor limitation and can provide an experimental criterion for validating Beer–Lambert's model (Balderas-López, 2012); therefore, sample preparation or dilutions are required less often for measurements, reducing the time and costs of the measurement. This study compares the optical characterisation and corresponding quantification of a mixture of pigments with a high optical absorption in a liquid solution, as in the case of citrus essential oils, when applying the Beer–Lambert law for light absorption using the TWRC-PPE spectroscopy and the conventional UV–Vis spectroscopy. The optical limits of the TWRC-PPE spectroscopy and possible interferences are also described.

2. Materials and methods

2.1. Materials

A variety of USP-grade essential oil samples were selected as follows: Mexican orange (*Citrus sinensis*), bitter orange (*Citrus aurantium*), Persian lime (*Citrus latifolia*), Mexican lime (*Citrus aurantifolia*), California lemon (*Citrus limon*), pink grapefruit (*Citrus paradise*), white grapefruit (*Citrus x paradise*), mandarin (*Citrus reticulata blanco* var. *mandarin*), green mandarin (*Citrus reticulate*), and orange essence (Química Aromática S.A., Acolman, Mexico). A selection of USP-grade samples of folded citrus essential oils that were produced by high vacuum fractional distillation were also studied herein as follows: 2-, 3-, and 4-fold green mandarin, 2-, 4-, and 5-fold Persian lime, and 2-, 4-, 5-, and 10-fold Mexican orange (Química Aromática S.A., Acolman, Mexico); the choice of different citrus species and different folding degrees is expected to cover a wide range of carotenoid and chlorophyll concentrations in citrus essential oils. Analytical grade β-carotene, chlorophyll a and D-limonene (Sigma–Aldrich Corporation, St. Louis, MO, USA) were used as received.

2.2. Absorbance measurements

Absorbance measurements were performed with a UV–Vis spectrophotometer (Agilent Technologies, Inc., CA, USA, model 8453) in a semi-micro quartz cuvette with a 10-mm path length (Starna Cells, Inc., CA, USA, model 29B-Q-10). The measurements were repeated ten times for each sample and performed at room temperature (25 °C).

2.3. TWRC-PPE spectroscopy theory

The TWRC-PPE experimental setup for making optical absorption coefficient measurements using a sample thickness scan is shown in Fig. 1. The sample thickness scan consisted of recording the TWRC-PPE signal for different liquid sample thicknesses, or l, at a fixed modulation frequency. This method involves the use of a micro-linear stage to change the liquid thickness in the sample container.

According to Balderas-López (2012), the photopyroelectric signal V_p (assuming β_s as the optical absorption coefficient of the

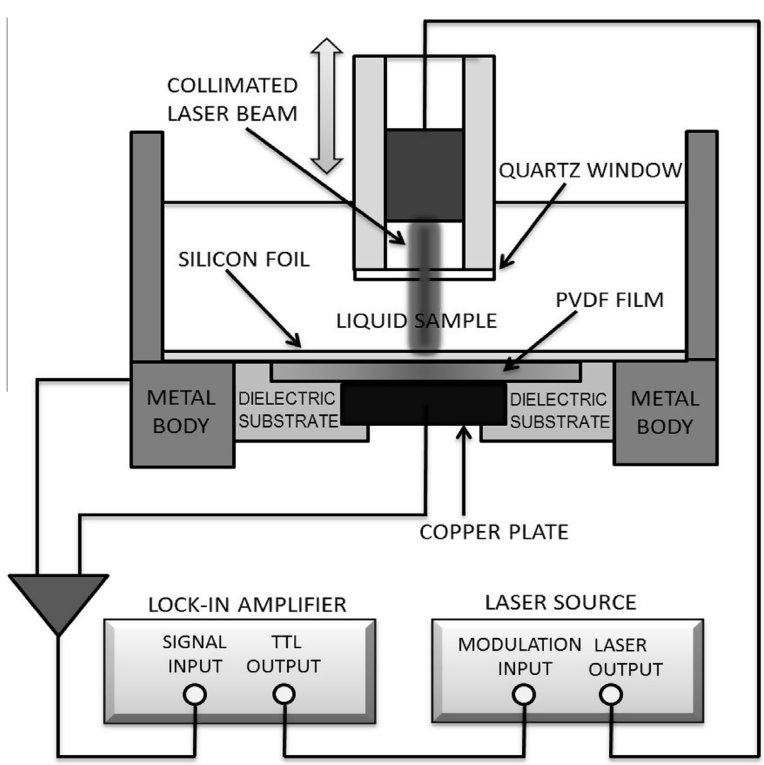

Fig. 1. A schematic view of the experimental set-up for TWRC-PPE measurements.