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Analytical Methods

A novel extraction method for β-carotene and other carotenoids in fruit juices using air-assisted, low-density solvent-based liquid–liquid microextraction and solidified floating organic droplets

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Sodium chloride (PubChem CID: 5234)
Calcium chloride (PubChem CID: 5284359)
Aluminium chloride (PubChem CID: 24012)
Sodium nitrate (PubChem CID: 24268)
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ABSTRACT

Green extraction using air-assisted, low-density solvent-based liquid–liquid microextraction and solidified floating organic droplets (AA-LDS-LLME-SFOD) prior to spectrophotometry was successfully applied for quantitation of carotenoids in fruit juices. Under optimal conditions, β-carotene could be quantified with a linear response up to a concentration of 60 μg mL^{-1}. The procedure was performed in a microcentrifuge tube with 40 μL of 1-dodecanol as the extraction solvent and a 1.0 mL juice sample containing 8% NaCl under seven extraction cycles of air pumping by syringe. This method was validated based on linearity (0.2–30 μg mL^{-1}, R^2 0.998), limit of detection (0.04 μg mL^{-1}) and limit of quantification (0.13 μg mL^{-1}). The precision, expressed as the relative standard deviation (RSD) of the calibration curve slope ($n = 12$), for inter-day and intra-day analysis was 4.85% and 7.92%, respectively. Recovery of β-carotene was in the range of 93.6–101.5%. The newly proposed method is simple, rapid and environmentally friendly, particularly as a useful screening test for food analysis.

1. Introduction

Fruits are a major dietary source of carotenoids and have been studied extensively. Although some fruits contain either non-significant amounts or small amounts of the carotenoids that are usually found in chloroplasts, others may have larger amounts of different carotenoids. An interest in carotenoids and their human health effects has existed for many decades since the linkage between β-carotene (a member of the carotenoid family) and vitamin A was first demonstrated. The dietary importance of β-carotene as provitamin A (Vitamin A precursors like alpha carotene, beta carotene and beta cryptoxanthin) has also been established (Britton & Khachik, 2009; Qiu, Chen, & Li, 2009; Sathya, Sumathi, & John Joel, 2014). β-carotene is a fat-soluble compound that is naturally present in many fruits, flowers, grains, oils,

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vegetables, roots, leaves, and seeds, and in most cases is responsible for red, orange and yellow colorations (Mellado-Ortega & Hornero-Méndez, 2016). The conjugated double-bond system of β-carotene determines the photochemical properties and chemical reactivity that form the basis of most of its functions. Light absorption is the basis of detection and analysis. The excitation, energy transfer and quenching are relevant to protective roles in the eye and skin which are essential for vision and growth (Haftel, Berkovich, & Reifen, 2015; Petrogianni, Kanellakis, Moschonis, & Manios, 2014; Zaccari, Cabrera, Ramos, & Saadoun, 2015). The susceptibility of the electron-rich polyene chain to degradation by electrophilic reagents and oxidizing free radicals is a fundamental characteristic of β-carotene as antioxidant or prooxidant (Phan-Thi, Durand, Prost, Prost, & Waché, 2016). It acts as an antioxidant by scavenging peroxyl radicals, thus inhibiting lipid peroxidation (Majumdar, Roy, Bejjanki, & Bhaskar, 2015), which affords protection against illnesses such as cancer and heart disease and slows the ageing process (Akinosho & Wicker, 2015; Brabcová, Hlaváčková, Šatínský, & Solich, 2013; Rodriguez-Amaya & Kimura, 2004; Sricharoen, Techawongstein, & Chanthai, 2015).

In recent years, the use of natural colorants has been steadily increasing primarily because of changes in consumer preference toward more natural products known to exhibit specific functional properties (Singh, Shakil, Kumar, Walia, & Kar, 2015). β-Carotene is also used as a colorant in manufactured food products and genetically modified (GM) crops, including 'Golden Rice' engineered to accumulate β-carotene (Buu, 2003). However, most analytical systems are not capable of performing direct analysis of a real sample without sample pretreatment. Depending on sample type, the selectivity and sensitivity of a proposed method may require the removal of potential interfering substances, isolation, and/or preconcentration of the analyte. Therefore, several extraction methods such as liquid–liquid extraction (Sarungallo, Hariyadi, Andarwulan, Purnomo, & Wada, 2015), solid-phase extraction (Kozukue & Friedman, 2003), supercritical fluid extraction (Gómez-Prieto, Caja, Herraiz, & Santa-María, 2003; Rozzi, Singh, Vierling, & Watkins, 2002) and dispersive liquid–liquid microextraction (Viñas, Bravo-Bravo, López-García, & Hernández-Córdoba, 2013) have been applied for the extraction of β-carotene from different matrices. Currently, solidified floating organic droplet microextraction is increasingly applied in sample preparation procedures (Viñas, Campillo, & Andruch, 2015).

The use of this technique leads to simplification and acceleration of the sample preparation process, which is important in all analytical steps to obtain accurate results. To overcome this drawback, some dispersive solvent-free techniques have been developed in which the extraction solvents are dispersed into the aqueous sample with the assistance of vortexing (Zacharis, Christophoridis, & Fytianos, 2012), sonicating (Jia et al., 2010), magnetic stirring (Zhang, Shi, Yu, & Feng, 2011), or surfactant addition (Wu et al., 2010).

This paper presents for the first time the development of a novel air-assisted and low-density solvent-based liquid–liquid microextraction based on solidified floating organic droplets (AA-LDS-LLME-SFOD) prior to β-carotene determination by microscale UV–Visible spectrophotometry. This method combines the advantages of AA-LLME and dispersive liquid–liquid microextraction based on the solidification of floating organic droplets (DLLME-SFOD). Therefore, the main advantage of this proposed method is the reduction of time and sample manipulation required during sample preparation steps. A small volume of low-density extraction solvent with a melting point near room temperature is dispersed into the sample solution in the absence of the disperser solvent. The emulsion is rapidly formed by air pumping the solution mixture using a syringe. The technique was optimized for analytical performance and the procedure was applied for the quantitation of β-carotene in different commercially available fruit juices.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents used were of analytical reagent grade or better. Standard β-carotene and extraction solvents (1-undecanol, 1-dodecanol, and 2-dodecanol) were purchased from Sigma–Aldrich (USA). Sodium chloride, calcium chloride, aluminium chloride, and sodium nitrate were purchased from Carlo Erba (Italy). Sodium sulphate, disodium hydrogen phosphate, hydrochloric acid and sodium hydroxide were obtained from QRec™ (New Zealand). Tetrahydrofuran (THF) was purchased from UNILAB (Australia). Deionized water with a resistivity of 18.2 MΩ cm obtained from a Millipore water purification system (Molsheim, France) was used throughout. The stock standard solution of the analyte was prepared in THF at a concentration of 1000 μg mL^{-1} and stored at −20 °C in screw top amber vials that were protected from light. The standard working solutions were prepared daily by an appropriate dilution of the stock standard solution with THF to the required concentrations.

Twenty-five samples of commercial fruit juices (100%) from 17 types of fruits were purchased from a supermarket in Khon Kaen, Thailand. Some fruit juices bore brand names available at Tesco Lotus, 7-Eleven, and Tops retail locations. The fruit juice samples were transferred into polytetrafluoroethylene (PTFE) containers and centrifuged for 10 min at 4000 rpm to form condensed sediments. After centrifuging, the resulting supernatant was subsequently processed by the AA-LDS-LLME-SFOD method.

2.2. Apparatus

All absorption spectra and absorbance measurements were performed using a UV–Visible spectrophotometer (Agilent Technologies Cary 60, Germany) and a 10 mm optical path length using a 750 μL quartz cuvette (Fisher Scientific, USA).

2.3. Analytical procedures

The experimental procedure of the AA-LDS-LLME-SFOD technique is illustrated in Fig. 1. First, 1000 μL of the aqueous sample solution without pH adjustment was transferred to a 1500 μL microcentrifuge tube with a conical bottom and spiked at the given concentrations of the target analyte (5 μg mL^{-1}). The ionic strength of the solution was adjusted by addition of 8% (w/v) NaCl. Next, 40 μL of 1-dodecanol (an extraction solvent) was added and the mixture was rapidly drawn into a 3 mL plastic syringe from the tube and then ejected 7 times repeatedly via a syringe needle. This caused the solution to become increasingly turbid. After performing the predetermined number of aspiration–dispersion cycles, phase separation could be achieved by micro-centrifuging of the cloudy solution at 3000 rpm (50 Hz) for 3 min. After centrifugation, the tube was immersed in an ice-water bath for 2 min, the low density of the floated organic solvent was solidified (ring shape) on top of the surface of the aqueous solution after a short period of time, and any liquid phase was removed using the same syringe. Next, the solidified ring was quickly melted at room temperature and the extract was diluted to 1000 μL with THF and analysed using a 750 μL quartz cuvette with a UV–Visible spectrophotometer at 460 nm. Each replicate of the sample extraction procedure was performed using only one syringe and one microcentrifuge tube. Three replicates were run in all cases to enhance the precision of the test.