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Removal of three kinds of phthalates from sweet orange oil by molecular distillation

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ABSTRACT

The removal of phthalates from essential oils is necessary due to the wide use of plastic products in daily life and severe adverse effects. In this study, molecular distillation techniques were used to remove phthalates from sweet orange oil (*Citrussinensis* (L.) Osbeck). Three parameters were investigated to optimize this process. An evaporation temperature of 50 °C, evaporator pressure of 5 kPa and a feed flow rate of 0.75 ml/min were identified as optimal parameters for this method. After distillation, obvious decreases in bis (2-ethylhexyl) phthalate (DEHP), diisobutyl phthalate (DIBP) and dibutyl phthalate (DBP) levels were observed. Phthalates were efficiently removed from sweet orange oil using the developed molecular distillation technique indicating that this approach is suitable for the effective removal of harmful substances from essential oils.

1. Introduction

Phthalic acid diesters (dialkyl or alkyl aryl esters of 1, 2-benzenedicarboxylic acid, PAEs), have been intensively researched for several decades, and it has come to public view since the phthalate disaster revealed by Taiwanese researchers in 2010. For around 30 years, illegal manufacturers had been substituting the non-food additive, DEHP {di(2-ethylhexyl)phthalate}, for more expensive ingredients such as palm oil. This carcinogenic toxin was used as a filler to make items more opaque or richer in color and had contaminated at least 500 food/beverage products released into the Taiwanese market. This practice was adopted particularly for fat-soluble foods including food additives such as essential oils. DEHP is a type of PAE used to increase the flexibility and extensibility of polyvinyl chloride (PVC) and polyvinylidenchloride (PVCD). In the case of food grade plastics, PAEs are not bound covalently to the vinyl polymer matrix (Lau and Wong 2000). The lipophilic nature of these substances favors their migration into oil (Di Bella, Saittab, La Pera, Alfa, & Dugo, 2004) and fat-soluble food (Hamdani and Feigenbaum 1996). Ingestion from foodstuffs is thought to be the main source of some kinds of PAEs in the human body (Guo & Kannan, 2011).

DEHP causes liver cancer in rats and mice (Wams, 1987; U.S. Department of Health & Human Services 1992). Other phthalates such as BBP, DNBP (di-n-butyl phthalate), DEHP and DEP (dimethyl phthalate), have shown the ability to interfere with normal development and the reproductive system (Swan et al. 2005) in young mammals. In Europe, the total tolerable daily intake (TTDI) per person of total phthalates has been estimated to be 0.3 mg/kg bw/d. The estimated TTDIs of DEHP and BBP are 0.05 mg/kg bw/d and 0.1 mg/kg bw/d of DBP. The ministry of health of China has set the maximum contaminant at 1.5 mg/kg for DEHP, 9.0 mg/kg for DINP, 0.3 mg/kg for DBP in food or food additive, according to 2007/19/EC in Europe. As a result, intake of phthalates has become an urgent issue.

Sweet orange oil extracted from *Citrussinensis* (L.) Osbeck, is used as a natural alternative to synthetic flavors and fragrances in the food, cosmetic and tobacco industries. Dietary intake was the main source of exposure to all phthalates in China (Guo & Kannan, 2011). Sweet orange oil is an important addictive, the quality of it

Abbreviations: DMP, dimethyl phthalate; DEP, diethyl phthalate; DBP, dibutyl phthalate; DIBP, diisobutyl phthalate; DMEP, bis(2-methoxyethyl) phthalate; BMPP, bis(4-methyl-2-pentyl) phthalate; DEEP, bis(2-ethoxyethyl) phthalate; DPP, dipentyl phthalate; DHXP, dihexyl phthalate; BBP, benzyl butyl phthalate; DBEP, bis(2-n-butoxyethyl) phthalate; DCHP, dicyclohexyl phthalate; DEHP, bis (2-ethylhexyl) phthalate; DNOP, di-n-octyl phthalate; DNP, dinonyl phthalate; PAEs, Phthalic acid diesters; PVC, polyvinyl chloride; PVCD, polyvinylidenchloride; DNBP, di-n-butyl phthalate; TTDI, the total tolerable daily intake; MD, molecular distillation technique; DS, distillate stream; ET, Evaporator temperature; FFR, Feed flow rate; SOO, sweet orange oil.

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would play a crucial role in food safety. Plasticizer residues have been found in bergamot essential oils from Calabria Italy (Di Bella et al., 2004). The removal of phthalates from sewage using microbiological methods (Wang, Fan, & Gu, 2002) or by absorption using adsorbent, such as active carbon (Venkata Mohan, Shailaja, Rama Krishna, & Sarma, 2007), chitosan (Chen & Chung, 2006), biomass (Fang & Zheng, 2004) were the main methods to deal with phthalates. However, these methods are associated with limitations when applied to food additives or essential oils. The removal of phthalates from sweet orange oil has not been reported until now.

A molecular distillation technique (MD), also known as short path distillation, is a useful and mild method for separation and purification of heat-sensitive materials, as well as for those compounds with high molecular weight and low vapor pressure. This method is characterized by a small distance between the evaporator and the condenser (20–50 mm) (Lutisan, Cvengros, & Micov, 2002), high vacuum in the distillation space and short exposure of the distilled liquid to the operating temperatures. Moreover, this process does not use any solvent as separating agent thus avoiding solvent pollution of the material. MD has been used to concentrate tocopherols from grape seed oil (Miriam, Gonzalo, & María del Carmen, 2007) and separate free fatty acids from vegetable oil (Martins, Ito, Batistella, & Maciel, 2006). Our team has used MD to purify and refine volatile components, such as patchouli oil (Hu et al., 2004), ginger oil (Wang, Hu, Huang, Yang, & Xuan, 2003) and cinnamon oil (Yang et al., 2006) and furthermore, investigated the properties of low polar compounds and medium polar components (Yang et al., 2006). In this study, we investigated the efficiency of MD technology, which is considered to be a gentle and ecologically beneficial technology, for removal of phthalates from essential oil. Optimization of evaporator temperatures, pressure and feed flow rates were investigated.

2. Materials and methods

2.1. Chemicals and reagents

Raw sweet orange oil was provided by the Zhongshan Unicare Natural Medicine Co., Ltd. Zhongshan, Guangdong, China. The oil was stored at 4 °C and examined for the presence of phthalates. Before distillation, the raw oil was analyzed by GC–MS. Three types of phthalates: (DIBP, DBP, DEHP) were detected in the sample using the Chinese standard method (G/B 21911-2088). To verify the efficiency of this method, three standard plasticizers were added into the oil to ensure that the content of PAEs exceeded 50 mg/kg. The original and spiked samples were then analyzed by GC–MS. Final concentrations were detected as follows: DHEP, 63.1909 mg/kg; DBP, 79.9134 mg/kg; DIBP, 105.6132 mg/kg.

To prevent secondary contamination of solvents and glassware, all laboratory glassware were soaked in re-distilled acetone (checked for the presence of phthalates) after washing with a detergent. All glassware was then subjected to 105 °C for 2 h, covered with aluminum foil and stored. Clean glassware was rinsed again before use. All contact with plastic material was avoided.

Ethanol (analytical grade) and *n*-hexane (HPLC-grade) were obtained from J&K China Chemical Co. Ltd. Helium (99.99% purity), DBP (99.0% purity), DIBP (99.0% purity) and DEHP (98.5% purity) were purchased as neat compounds (Dr. Ehrens torfer GmbH, Germany) and stored at 4 °C. Standard mixtures were prepared in hexane.

2.2. Molecular distillation equipment

Distillation was performed with a laboratory wiped film molecule distillation equipment MD-S80-III (Handway, Foshan China). The feeding device, discharge of the distillate and residue were performed in glass flasks, while other important parts of the equipment were made of steel. An oil filled jacket circulated heater was used to regulate the temperature of the evaporator while water was used to cool the condenser. Three vacuum systems were used: a water-ring pump, a mechanical pump, and a diffusion pump.

Sweet orange oil and phthalates were collected separately in the distillate and residue streams, respectively. A condensation temperature of 4 °C and a wiper speed of 300 rpm were used throughout the study.

To avoid secondary contamination, the apparatus would be washed with re-distilled acetone and pumped with water-ring pump for 0.5 h before distillation.

2.3. Standard calibration curve

Individual stock solutions (approximately 1000 mg/kg) were prepared in hexane from the neat compounds in liquid form and stored in −4 °C in the dark. Standard solutions (0.05, 0.1, 0.2, 0.5, 1, 2 and 5 mg/kg) were prepared as low concentration samples in addition to six diluted other solutions (10, 20, 40, 60, 80 and 100 mg/kg).

2.4. Gas chromatography–mass spectrometry analysis

All samples were dissolved (0.25–0.5 g) with 4 ml hexane (HPLC grade) in glass tube for analysis. 4 ml Hexane is for blank. The phthalates in oil were analyzed by gas chromatography–mass spectrometry (the GC–MS) method which was carried out according to the Chinese standard method (GB/T 21911-2088 determination of phthalate esters in food).

Each sample was analyzed by GC–MS (Thermofisher DSQ-II, San Jose, CA, USA) equipped with the NIST library and data were processed with the Xcalibur software, version 2.0.7 (ThermoFinnigan, San Jose, CA, USA). A DB-5 capillary column (30 m × 0.2 mm), coated with 0.25 um film was used with the following temperature program: initial temperature of 60 °C for 1 min, increased to 220 °C (20 °C/min) and held for 1 min, increased to 280 °C (5 °C/min) and held for 4 min. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. Each sample (1 μl) was injected in splitless mode and helium (99.9% purity) was used as the carrier gas at a constant flow rate of 1 ml/min.

MS scan conditions: interface and source temperatures, 280 °C; ionization energy (E), 70 eV; solvent delay, 5 min. The detector was operated in a selected ion monitoring mode (SIM, monitored *m*/*z*, DBP 149, 223, 205, 121; DIBP 149, 223, 205, 167; DEHP 149, 167, 279, 113).

Components of sweet orange oil were analyzed using the same GC–MS equipment. Helium was used as the carrier gas at a flow rate of 1 ml/min and a pressure of 100 kPa. The oven temperature was programmed from 80 °C (1 min) to 250 °C (10 min) at rate of 5 °C/min. An electron ionization mode (ionization energy: 70 eV) was used for GC–MS detection. The injector temperature was set at 280 °C and the source and interface temperatures were set at 280 °C. The scanning mass range varied from 40 *m*/*z* to 650 *m*/*z*. A sample volume of 1 μl was injected into the column with the split ratio of 1:100. Data was analyzed using the Xcalibur software and matched with the NIST mass spectral library database.

3. Results and discussion

3.1. GC–MS of standard compounds and standard calibration curve

In order to detect the spiked oil which with a high concentration of phthalates and the refined oil, two different calibration curves