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Determination of free and total phthalates in commercial whole milk products in different packaging materials by gas chromatography-mass spectrometry

Jialu Lin, Wanxin Chen, Hangcui Zhu, and Chengjun Wang[1]
College of Chemistry and Materials Engineering, Wenzhou University, Wenzhou 325035, China

ABSTRACT

We developed a method for extraction and determination of free and total phthalate esters in commercial whole milk products. The free phthalates in milk samples were extracted with ethyl acetate after general pretreatment procedures including protein precipitation, centrifugation, and filtration. The bound phthalates in samples were first desorbed with the aid of ultrasound irradiation before extraction of total phthalates. The separation and determination of phthalates in extractsw was performed by gas chromatography coupled with mass spectrometric detection. The detection limits were in the range of 0.09 to 0.36 ng/g and the average recovery between 79.1 and 110.3%. The developed methods were applied to extract and determine phthalates in commercial whole milk products with different packaging materials, including plastic, glass, and metal. All samples contained several phthalates, including diethyl, diisobutyl, and bis(2-ethylhexyl) phthalates at concentrations between 2.60 and 156.4 ng/g. The identified phthalates occurred in both free and bound forms. The amounts of phthalates in milk samples packaged in glass and metal containers were much lower than those in plastic containers. Plastic packaging materials are a possible source of phthalate contamination in commercial whole milk products, and a considerable portion of bleached phthalates from packaging can be adsorbed on proteins and other solid components of milk.
Key words: phthalate, milk product, bound form, gas chromatography-mass spectrometry

INTRODUCTION

Phthalate esters are used primarily as plasticizers in polymeric materials to increase their flexibility through weak secondary molecular interactions with polymer chains. Because they are physically bound to the polymer chains, phthalate esters may migrate into foods during food processing and storage in plastic packing materials (Fasano et al., 2012). These compounds are listed as suspected endocrine disrupters or mutagens, which can have adverse effects on human health even at low levels (Hauser et al., 2007; Kamrin, 2009; Kimber and Dearman, 2010; López-Carrillo et al., 2010; Ferguson et al., 2011; Hu et al., 2013; Ventrice et al., 2013). Because it contains abundant vitamins, minerals, carbohydrates, lipids, and proteins, which are essential for human health, milk is considered one of the most nutritionally complete natural foods (Jenkins and McGuire, 2006; Soyeurt et al., 2010). Many specialized milk products such as cheese, yogurt, butter, and ice cream are popular in diets worldwide. However, most commercial milk products are packaged in plastic or other polymer materials. Therefore, it is extremely important for human health protection to evaluate and monitor phthalates in commercial milk products.

Recently, many studies have reported analysis of phthalates in milk or other food samples (Casajuana and Lacorte, 2004; Carrillo et al., 2007; Cao, 2008; Guo et al., 2012; Hsieh et al., 2013; Jia et al., 2014; Van Holderbeke et al., 2014). In general, techniques such as extraction with solvents and solid phase extraction are used to clean up and concentrate the milk samples before analysis. Following sample extraction or enrichment, GC-MS and HPLC-MS have been exclusively used for the separation and detection of phthalates (Feng et al., 2005; Feás et al., 2008; Guo, 2008; Xu et al., 2014). The HPLC-MS method is used less often than GC-MS, possibly because it uses more potential contamination sources such as plastic filters, tubing, and solvents for the mobile phase (Khedr, 2013). Although these methods have been successfully applied for the analysis of phthalates in whole milk and derivative products, most studies determined only free phthalates that occurred in solution of milk samples. However, a few studies have indicated that phthalates are highly hydrophobic and easily adsorbed onto the surface of proteins and lipids (Abraham and Acree, 2015). Theoretically, part of the migrated phthalates could exist as bound forms in milk products, and those phthalates should be measured to avoid underestimation of the total concentration of phthalates in milk samples. The adsorbed phthalates should be desorbed from the surface of proteins and

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[1]Corresponding author: wang.chengjun@yahoo.com

lipids in milk samples before the general pretreatment and preconcentration procedures.

The main objective of this work was to develop a method for extraction and determination of free and bound phthalate esters in milk samples, and to apply the developed method to evaluate free and bound phthalates in selected commercial whole milk products packaged in plastic, glass, and metal materials.

MATERIALS AND METHODS

Chemicals and Standards

Standards of the individual phthalate esters [dimethyl phthalate (**DMP**), diethyl phthalate (**DEP**), diallyl phthalate (**DAP**), diisobutyl phthalate (**DIBP**), dibutyl phthalate (**DBP**), benzyl butyl phthalate (**BBP**), dicyclohexyl phthalate (**DCHP**), bis(2-ethylhexyl) phthalate (**DEHP**), di-n-octyl phthalate (**DNOP**)] used for this study were purchased from Tokyo Kasei Kogyo Co. Ltd. (Shanghai, China). The HPLC-grade methanol and ethyl acetate were obtained from Tjshield Chemicals (Tianjin, China). Hydrochloric acid, sulfuric acid, glacial acetic acid, and magnesium sulfate were supplied by Zhejiang Zhongxing Chemical Reagent Co. Ltd. (Jinhua, China). All solvents and reagents used in this study were of analytical grade unless otherwise specified. All water and reagents were checked for contamination with phthalates before use. A mixed standard stock solution (50.0 mg/mL) containing the 9 phthalates was prepared in anhydrous methanol and stored at 4°C in the dark. The working standard solutions were prepared freshly in methanol at concentrations of 0.00, 5.00, 10.0, 25.0, and 50.0 mg/L by diluting the stock standard solution.

Sample Preparation

Three milk samples with different package materials—metal, glass, and plastic—were purchased from a local supermarket and stored at 4°C until used in this study. Before extraction of free phthalates, the proteins and lipids in milk samples need to be removed because those components could affect extraction efficiency and interfere with the instrumental analysis. In detail, a milk sample (5.0 mL) was pipetted into a 10-mL glass centrifuge tube; then, 1.0 mL of 10% (vol/vol) acetic acid was added, mixed well, and heated in a sand bath at 70°C for 10 min. The mixture was then cooled to room temperature and centrifuged for 10 min at 1,500 × *g*. An aliquot of the supernatant was decanted out and filtered through a 0.45-μm nylon filter membrane. Then, 5 mL of filtrate was pipetted into a 10-mL glass vial and extracted with 1.0 mL of ethyl acetate 3 times. The ethyl acetate extracts were combined, passed through anhydrous $MgSO_4$ packed in a Pasteur pipet to remove the residual water, and collected into a 5-mL glass vial. The extracts were then completely dried under a stream of nitrogen gas. Finally, 0.5 mL of methanol was added and vortexed for 1.0 min and the solution was transferred into a clean 2-mL mini-vial for instrumental analysis.

To analyze the total phthalates in milk samples, the bound phthalates adsorbed on the proteins and other solid components must be desorbed before pretreatment to remove general protein and lipids. Previously, ultrasonic radiation, a type of low frequency energy, was extensively studied for improving almost all sample preparation and pretreatment processes, including cleaning, degassing, digestion, leaching, crystallization, precipitation, and extraction (Shen, 2005; Zuo et al., 2008; Wang and Zuo, 2011). In this study, milk samples (5.0 mL) were first added to 1.0 mL of 10% (vol/vol) acetic acid and pretreated using ultrasonic irradiation generated by an ultrasound producer (model AS20500BDT with digital timer, heat and power control, Automatic Science Instrument, Tianjin, China) at 60°C for 30 min. Then, the samples were centrifuged, filtered, extracted, dried, redissolved, and transferred for instrumental analysis as described for extraction of free phthalates.

Analytical Methods

Instrumental analysis was carried out on a Shimadzu GCMS-QP2010 plus gas chromatography-mass spectrometer equipped with an auto-sampler (Shimadzu, Tokyo, Japan). The phthalate compounds in standards and extracts of milk samples were separated on a 30 m × 0.32 mm i.d., 0.25-μm film DB-5 fused-silica capillary column (J&W Scientific, Folsom, CA). The column temperature was initially held at 120°C for 4 min, and then programmed to 220°C at a rate of 5°C per min, from 220°C to 300°C at a rate of 20°C per min, and with a final hold time of 6 min. Helium was used as the carrier gas and the column head pressure maintained at 14.5 psi (~100 kPa). The injector and detector temperature were maintained at 280°C and 305°C, respectively, and the injection volume was 2 μL with splitless mode; electron impact ionization energy was 70 eV.

RESULTS AND DISCUSSION

Method Evaluation

The phthalates in extracts of milk samples were identified by matching GC retention times against