Chapter 4

Conclusions

Packaged food can be very aggressive milieu and may interact strongly with materials that they contact, so it is possible for their chemical constituents to migrate into the food. Phthalate and adipate ester are the additive in the manufacturing of plastics that have the endocrine-disrupting properties (Harris *et al.*, 1997 and Hashimoto *et al.*, 2003). Because of the principal route of exposure of the general population to phthalate and adipate esters is through food consumption. Thus, for food safety, these compounds must not migrate into the food in unacceptable quantities.

The analysis of phthalate and adipate esters contaminated in packaged food by gas chromatography couple with flame ionization detector (GC-FID) was investigated, two migrants have been studied simultaneously: di (2-ethylhexyl) phthalate (DEHP) and di (2-ethylhexyl) adiapte (DEHA). Chromatographic separation was carried out by a fused silica HP-5MS capillary column, 30 m × 0.25 mm I. D., 0.25 μm film thickness of 5% phenyl and 95% dimethylpolysiloxane. Conditions for GC-FID technique were optimized and gave the values of,

Conditions		Optimum values
Flow rate:	He, carrier gas	1.2 mL min^{-1}
	H_2, fluel gas	30 mL min^{-1}
	Air, oxidant gas	300 mL min^{-1}
Column temperature program:		
	Initial temperature	110 °C
	Initial hold time	1 min
	Ramp rate	20 ° C min^{-1}
	Final temperature	300 ° C
	Final hold time	2 min

Conditions	Optimum values
Injector temperature	255 °C
Detector temperature	300 °C

These optimum conditions are appropriate for the simultaneous analysis of DEHP and DEHA. They provided very good resolution (R ≥1) and selectivity within a short analysis time (≤14.50 minutes). Wide linear dynamics range were obtained for both of DEHA and DEHP, *i.e.*, 25 ng mL^{-1} to 60 μg mL^{-1}, with high coefficient of determination ($R^2 > 0.99$). The limits of detection for DEHA and DEHP were 12 and 25 ng mL^{-1}, respectively. When comparing the LOD of this method with those reported (Table 57), this method gives lower LOD.

Table 57 LOD reported for the determination of DEHP

Analytical method	LOD (ng g^{-1})	Reference
GC-FID	5000	Rastogi, 1998
GC-MS	23	Tsumaru *et al.*, 2001
GC-MS	70	Petersen *et al.*, 2000
GC-MS	37	Tsumaru *et al.*, 2002
GC-FID	25	This work

Sour yellow, Red, Green, Masman and Panang curry pastes were sampling from the supermarkets. Because of their complex matrix, many compounds can be co-extracted with DEHP and DEHA. Therefore, sample preparation and clean up step is required. In this work, this step is based on ultrasonic extraction followed by solid phase extraction (SPE) using Florisil® cartridges. This SPE, Florisil® clean up procedure allows the matrix components to be retained on the sorbent material while the analytes pass through. The optimum conditions for extraction of DEHP and DEHA were obtained.

Conditions	Optimum values
Ultrasonic extraction:	
Extraction time	90 minutes
Extraction solvent	dichloromethane-cyclohexane 1:1 (v/v)
Volume of extraction solvent	20 mL
Solid phase extraction:	
Sample flow rate	3 mL min^{-1}
Drying time	6 minutes
Type of eluting solvent	acetone-hexane 10/90 (v/v)
Volume of eluting solvent	5 mL
Flow rate of eluting solvent	5 mL min^{-1}

The matrix interference was also studied and statistically confirmed for all types of curry paste sample. The results indicated that the matrix of curry paste samples were presented but chromatographically separated from DEHP and DEHA. Thus, the matrix match calibration curve was used for determination of DEHP and DEHA in the samples.

The method was validated to ensure the reliability of the results by using standards, spiked samples, reagent and method blanks. Validation parameters *i.e.,* recovery, method detection limit (MDL), limit of quantitation (LOQ) and precision were studied. High percentage recoveries were obtained in acceptable level (EPA method 8061, 1996), ranged from 93 to 100% and 88 to 99% for DEHP and DEHA at spiked concentration 0.5 and 5.0 μg mL^{-1} with relative standard deviations (RSD) lower than 8 and 10%, respectively. The method detection limit were from 27 and 30 ng mL^{-1} and the limit of quantitation for various curry paste samples are ranged from 90 to 100 ng mL^{-1}, respectively, for DEHP and DEHA.

For qualitative and quantitative analysis of DEHP and DEHA in packaged food samples. Ten curry paste samples in contact with plastic films (curry paste in packaged) were analyted and evaluated by using the matrix match calibration curve. The concentrations of DEHP in all curry paste samples were in the range from 0.12 to 0.61 μg g^{-1}. For DEHA, it could not be detected by matrix match calibration curve. It is possible that the amount of DEHA was lower than the method detection

limit. Therefore, the confirmation was carried out by standard addition method where the results showed the concentration levels of DEHA in all 10 curry paste samples were in the range from 4.0 to 26.4 ng g^{-1}. The concentrations of DEHP in all types of curry paste samples were extremely high when compared with DEHA.

The safety limits for phthalate and adipate esters that may migrate from food packaging into food were set by the European Economic Community Scientific Committee for Food (EEC SCF). They established a tolerable daily intake (TDI) of 0.3 and 0.05 mg kg^{-1} body weight (bw) day^{-1} for DEHA and DEHP, respectively. Assuming that one packaged (50 g weight) of each curry paste can be used to prepare curry for 10 adults and 50 childs, base on normal Thai who consume moderate spices curry. An adult (60 kg body weight) and a child (20 kg body weight) would have curry paste intake of 5 and 1 g per day, respectively. The calculated tolerable daily intake (TDI) of DEHP from curry paste samples were found in the range of 1.00×10^{-5} to 5.08×10^{-5} and 1.80×10^{-5} to 8.50×10^{-6} mg kg^{-1} bw day^{-1}. For DEHA, the TDI were in the range from 1.18×10^{-6} to 3.33×10^{-7} and 1.11×10^{-6} to 9.80×10^{-7} mg kg^{-1} bw day^{-1} for an adult and a child respectively. The amount of DEHP and DEHA found in all curry paste samples in term of TDI do not exceed the levels set by the EEC SCF.

The combination of ultrasonic extraction followed by solid phase extraction for sample preparation has several advantages. Ultrasonic extraction is an inexpensive, easy to operate and efficient alternative to conventional extraction techniques. The main benefits of the use of ultrasound in solid liquid extraction include the increase of extraction yield, faster kinetics and more complete extraction. The mechanical effects of ultrasound induce a greater penetration of solvent into samples and improve mass transfer. These lead to the enhancement of extraction with ultrasonic power. When comparing the sample preparation method of this work with those reported in Table 57, it can be seen that this method used less amount of sample (3 g) and volume of extraction solvent (20 mL) than other methods. The recovery in this study (88-100%) was better than obtained by Petersen and Breindahl (2000) and Tsumura *et al.* (2000) and these values were closed to those obtained by Tsumura and coworkers (2002). For solid phase extraction, it can be accomplished more rapidly, easy to perform, requires less organic solvent, reduces the need for large

concentration steps and greatly improve extraction selectivity. Multiple samples can be treated in parallel. SPE can also be automated couple on-line to GC or many instruments. It can also be safer to perform for the analyst because potential exposure to organic solvents is decreased dramatically with this technique.

Table 58 Comparison between proposed method and another sample preparation method for analysis of phthalate and adipate esters in food

Sample	**Amount of sample**	**Volume of extraction solvent (mL)**	**Recovery (%)**	**Reference**
Fresh meat	10 g	125 (acetone:pentane)	106	Petersen and Naamansen, 1998
Baby food	5-30 g	100 (pentane)	76-87	Petersen and Breindahl, 2000
Model breakfast	5 g	300 (acetonitrile)	79-81	Tsumura *et al.*, 2000
Retort-pounched baby food	50 g	200 (acetonitrile)	82-102	Tsumura *et al.*, 2002
Infant formula	3 mL	100 (ethyl acetate:cyclohexane)	93-100	Mortensen *et al.*, 2005
Curry paste	3 g	20 (cyclohexane:dichloromethane)	88-100	This work

In conclusion, the proposed method can be used for qualitative and quantitative analysis of di (2-ethylhexyl) phthalate (DEHP) and di (2-ethylhexyl) adiapte (DEHA) contaminated in packaged food with good recovery and precision. This method is simple, cost effective and can be used for routine analysis. Moreover, it could be applied for analysis of DEHP and DEHA in other packaged food.