

IDENTIFICATION OF SUSPECTED HAZARDOUS CHEMICAL CONTAMINANTS IN RECYCLED PASTRY PACKAGING*

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ABSTRACT

Background. The safe use of recycled paper and cardboard material for food packaging applications is an important area of investigation. Therefore, the aim of this study was to determine which hazardous chemical pollutants were found in paper and cardboard samples used for pastry packaging, and to measure the migration of pollutants over time into the pastries.

Material and methods. In this study, the presence of some organic pollutants in common confectionery packaging, and the effects of storage time and type of pastry on pollutant migration, were investigated.

Results. The results of the study indicate that harmful compounds such as benzophenone, pentachlorophenol, bis(2-ethylhexyl) phthalate and dibutyl phthalate are present at high concentrations in most recycled boxes used for pastry packaging.

Conclusion. Since the migration of some of the hazardous compounds from the packaging materials into the pastries under normal conditions was indicated, it is recommended that the procedure for preparing pastry packaging materials should be reconsidered and improved.

Keywords: recycled paper, packaging materials, pastry

INTRODUCTION

Food packaging is the most common way to store, transfer and transport foods in different environmental conditions, extend the shelf-life of products and protect foods from external pollution (Marsh and Bugusu, 2007). Paper and paper derivatives are one of the main sources of food packaging all over the world; the total mass of paper and cardboard used in packaging is approximately equal to the mass of all other packaging

materials combined (Song et al., 2000). Because of the current need to recycle materials in order to minimize the amount of worldwide waste, reclaimed fibers are utilized in many paper food-packaging materials. Paper and cardboard partly or fully produced from recycled fibers are already in use as packaging, which comes into direct contact with food products, in many countries. Recycled paper and cardboard is widely

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used for packaging dry foods (Ewender et al., 2013). However, paper and cardboard made from recovered fibers is often not as pure as virgin paper, because of residues that may not have been completely removed. Depending on the production process and the use of the paper before it was collected and recycled, it is exposed to numerous compounds that are harmful for human health. It is possible to find many of these compounds in paper and cardboard after the recycling process (Jamnicki et al., 2012). In these cases, packaging can become a new source of contaminants that can affect the organoleptic properties of foods and makes them hazardous to consumers (Triantafyllou et al., 2005). It is necessary to check the composition of the packaging and assess the potential migration of these compounds to the foods. Regarding food safety, recycled packaging materials need to comply with specific legislative requirements. A recently approved proposal by the Council of Europe requires the assessment and analysis of a series of contaminants in recycled paper and cardboard such as heavy metals, plasticizers, aromatic amines, poly aromatic hydrocarbons, benzophenone, etc. (Nerin and Asensio, 2007). The four most publicized contaminants, which are found in recycled papers and comprise major concerns for consumer health, are: benzophenone (a residue from UV-cured inks and lacquers used for printing on packaging), pentachlorophenol (a biocide used mainly for wood protection in pulp and paper mills), phthalates (from inks) and diisopropylnaphthalenes (DiPNs, from inkjet inks and carbonless copy papers) (Jamnicki et al., 2012). These kinds of contaminants have harmful effects on biological systems and can cause various diseases and disorders, even in relatively low concentrations (Grosse et al., 2011; McLean et al., 2007; Meeker et al., 2009). To our knowledge, there are few reports on the identification of organic pollutants in dry foodstuff packaging (Bononi and Tateo, 2009; Parigoridi et al., 2014). However, in these studies, the authors found some important organic pollutants in real recycled cardboard samples from various sources. The aim of this study was to prove that important organic pollutants are present in common confectionery packaging from the local market in Tehran, Iran. In addition, the effects of storage time and the type of pastry on the migration of organic pollutants under normal conditions of use were investigated.

MATERIALS AND METHODS

Sample collection

Thirty recycled pastry boxes (300–350 g/m²), the inner layers of which were covered with polyethylene film and used for pastry packaging, in direct contact with foodstuffs, were purchased from different confectionary stores situated in different parts of the city in winter and spring 2015. Additionally, four samples of each type of pastry (Danish pastry, marmalade pastry, puff pastry and sugar coated biscuit), 16 samples in total, were purchased from retail outlets in Tehran. The samples were purchased in March 2015. The selected pastry boxes were similar in shape, with the dimensions 23×23×7 cm³ and a capacity of 1 kg. The pastry pieces are usually packaged in such a way that all of them are in contact with the sides of the boxes. One third of each sample was pooled, homogenized and extracted on the day of purchase and used directly for analyses at time zero. The remaining contents of each sample were stored in the same recycled boxes at room temperature and analyzed after a period of storage (after 5 and then after 10 days). To make a reproducible sample, in each sample run, the pastry pieces were selected from different parts of the boxes.

Determination of fat content

The fat content of the samples was determined according to the method described previously (Nielsen, 2003). In brief, six samples of each pastry type were collected and 10 g of each dried sample was ground and placed in an extraction thimble and accurately weighed. 350 mL petroleum ether was added as an extraction solvent and extraction was carried out for 6 h on 6 samples separately. After air and vacuum drying, the cool, dried samples were re-weighed. Fat contents were measured by the loss in weight which the

Table 1. Fat content of pastry samples

Sample	Total fat content, %
Danish pastry	5.6
Marmalade pastry	4.2
Puff pastry	6.3
Sugar coated biscuit	3.8

samples demonstrated, after correction for moisture content as described (Nielsen, 2003). The results are indicated in Table 1.

Analytical procedure

All reagents used in the present work were of analytical grade. The calibration solution of target compounds (benzophenone (PhCO), pentachlorophenol (PCP), 2,7-diisopropylnaphthalene (2,7-DiPN), 2,6-diisopropylnaphthalene (2,6-DiPN), dibutyl phthalate (DBP) and bis(2-ethylhexyl) phthalate (DEHP) was prepared by diluting a stock solution of 1 mg/mL supplied by Merck. Stock solution of benzyl benzoate (1 µg/mL) was used as an internal standard (I.S.). Based on the physical and chemical properties of the analytes (Table 1), a multistep sample preparation method, including liquid-phase extraction, ultra-sonication, solvent evaporation and reconstitution was employed for cardboard and pastry samples. Approximately 3 g of each of the cardboard samples was cut into small pieces (5×5 mm), mixed with 3 g of anhydrous sodium sulphate and extracted twice with 30 mL dichloromethane in an ultrasonic bath for 30 min at 25°C. Then the mixture was filtered and washed with 5 mL dichloromethane twice. The organic phase was evaporated under nitrogen until dry, re-diluted with 0.5 mL methanol-dichloromethane (50:50, v/v) and stored in screw cap vials for further analysis (Song et al., 2000). The determination of the concentration of the organic contaminants in different kinds of pastries

was performed as follows: Prior to extraction, pastry samples were homogenized and a representative amount of the sample (10 g) was weighed and put in a 60 mL-vial. The extraction was performed by adding 40 mL of acetone/n-hexane (1:1), followed by centrifugation for 15 min at 5000 rpm. The supernatant was evaporated under nitrogen until dry, and the solvent was exchanged to 20 mL of dichloromethane. The final extract was brought to a volume of approximately 500 µL (Fierens et al., 2012).

An agilent gas chromatograph 6890 plus (Agilent Technologies, Palo Alto, CA, USA) equipped with a 5973 quadrupole mass spectrometer, was used for GC-MS analysis. The gas chromatograph was fitted with an HP-5 (MS) capillary column (30 m, 0.25 mm i.d., 0.25-µm film thickness). The instrumental temperatures were as follows: injector temperature; 250°C, and initial oven temperature; 60°C, which was maintained for 3 min and then increased to 150°C at a rate of 15°C/min, and finally increased to 290°C at a rate of 10°C/min, and maintained for 3 min. The inlet was operated in split-less mode. The temperature of the transfer line was maintained at 310°C. As carrier gas, helium (99.9999%) was used at 1 mL/min (constant flow). The source and quadrupole temperatures were kept at 230 and 150°C, respectively. The electronic beam energy of the mass spectrometer was set at 70 eV. The mass-selective detector was operated in electron impact mode, using selected ion monitoring (SIM). One quantitation and two qualifier ions were monitored

Table 2. Determinant physicochemical properties of the analyte compounds and their target ions for electron ionization mass spectrometry

Compound name	Molecular formula	Molecular weight g/mol	Boiling point at 760 mm Hg °C	Mass spectrometry quantification ion (m/z)	Mass spectrometry confirmation ions (m/z)
Benzophenone	C ₁₃ H ₁₀ O	182.22	305	182	153, 105
Pentachlorophenol	C ₆ HCl ₅ O	266.32	310	266	268, 264
Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390.55	386	279	167, 149
Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278.34	340	149	223, 205
2,6-diisopropylnaphthalene	C ₁₆ H ₂₀	212.33	279	197	155, 212
2,7-diisopropylnaphthalene	C ₁₆ H ₂₀	212.33	305	197	155, 212

Table 3. Recovery values estimated for cardboard and pastry samples

Compound name	Cardboard		Pastry
	recovery, %	SD of estimated recovery values	recovery, %
Benzophenone	85.6	2.39	79.4
Pentachlorophenol	87.4	3.25	80.2
Bis(2-ethylhexyl) phthalate	88.6	2.51	81.4
Dibutyl phthalate	86.7	2.84	82.7
2,6-diisopropylnaphthalene	82.3	3.12	83.4
2,7-diisopropylnaphthalene	83.6	3.22	82.7

for each compound. The dwell time of each ion was set at 100 ms (Table 2). In order to perform quantification, calibration samples in the range of 0.1–200 µg/mL were prepared by spiking calculated amounts of the analytes into 0.5 mL of final extraction solvents for each of the cardboard and pastry matrices. Calibration samples were prepared in five replicates and the results (ratio of peak areas versus nominal concentrations) were used to obtain the calibration equation. The quality of linear regression analysis was evaluated by comparing the results of determined concentrations to the nominal ones. The original concentrations of the analytes in each sample were determined based on estimated recovery values indicated in Table 3. To calculate the recovery, mean peak areas of the analytes were determined for spiked samples at different concentrations ($n = 6$) using the above-mentioned extraction and quantification methods, and the determined values were compared with those of quality control samples containing the analytes in the extraction solvent at the same nominal concentration.

Statistics

All statistical analyses were carried out by SPSS for Windows (version 18). The data determined was expressed as the mean of three replicate determinations and presented as means \pm SD. Data from each treatment or control group was analyzed for differences by using repeated measures analysis. Tukey's all-pairwise-comparison test was used to identify differences between different groups. The $P < 0.05$ criteria was considered statistically significant.

RESULTS

Table 4 shows the concentrations of compounds found in the analysis of the 30 box samples. Results were compared with the limits proposed by the Iranian National Standardization Organization (INSO) for cardboard materials used in food packaging (INSO, 2015). Since the maximum limit is expressed as a weight/area unit in the INSO guideline, a conversion of the analytical results (which are based on weight/weight) to weight/area units had to be done. The conversion was done by taking into account the actual weight [g] of analyzed paper (equation 1; Jannicki et al., 2012):

$$Q_a = (Q_m \cdot G) / 10^5 \quad (1)$$

where:

Q_a – the concentration of the substance in paper, mg/dm²,

Q_m – the concentration of the substance in paper, mg/kg,

G – the weight of the paper, g/m².

Q_a is the maximum quantity of the contaminant allowed in the packaging, if it is assumed that 100% of it will migrate into the foodstuff.

The results of box sample analysis are indicated in Table 4. DEHP was the most detected compound (identified in 100% of the 30 samples), followed by DBP (75%), benzophenone (PhCO; 69%) and PCP (58%). On the other hand, 2,6-DiPN and 2,7-DiPN were not detected in the analyzed samples. The concentration

Table 4. Mean concentrations of selected organic compounds in pastry boxes packaging materials, mg/dm²

Organic compounds	Mean ±SD	Range	Detection limit	Maximum permitted quantity
Benzophenone	0.018 ±0.009	0.004–0.035	0.002	— ^a
PCP	0.027 ±0.013	0.007–0.044	0.001	0.15
DEHP	0.123 ±0.091	0.025–0.263	0.002	— ^a
DBP	0.045 ±0.031	0.009–0.096	0.005	— ^a

^aNot established.

of PCP in all samples was below the proposed limit (INSO, 2015).

In addition, the effects of storage time and type of pastry on the migration of selected organic pollutants into the pastry were investigated. The experiments were conducted over 15 days, with interim

measurements being taken on days 1, 10 and 15 at room temperature (25°C). All the migration measurements were made in duplicate. Table 5 illustrates the levels of contaminants in pastries after different storage periods. Benzophenone (PhCO), PCP, 2,6-DiPN and 2,7-DiPN were not detected in any of samples.

Table 5. The levels of selected organic compounds in various pastries after different storage periods, µg/kg

Group	Day	Benzophenone	PCP	DEHP	DBP
Danish pastry	1	ND ^a	ND ^a	1.74 ±0.28	0.84 ±0.08
	5	ND ^a	ND ^a	1.97 ±0.13	0.94 ±0.06
	10	ND ^a	ND ^a	2.11 ±0.17	1.13 ±0.10
	15	ND ^a	ND ^a	2.21 ±0.19	1.20 ±0.09
Marmalade pastry	1	ND ^a	ND ^a	3.12 ±0.14	1.58 ±0.05
	5	ND ^a	ND ^a	3.41 ±0.21	1.71 ±0.11
	10	ND ^a	ND ^a	3.76 ±0.15	1.82 ±0.08
	15	ND ^a	ND ^a	3.92 ±0.17	2.03 ±0.06
Puff pastry	1	ND ^a	ND ^a	2.47 ±0.10	0.75 ±0.05
	5	ND ^a	ND ^a	2.56 ±0.09	0.87 ±0.08
	10	ND ^a	ND ^a	2.64 ±0.13	1.01 ±0.11
	15	ND ^a	ND ^a	2.72 ±0.11	1.09 ±0.09
Sugar coated biscuit	1	ND ^a	ND ^a	2.03 ±0.07	0.59 ±0.07
	5	ND ^a	ND ^a	2.13 ±0.11	0.67 ±0.04
	10	ND ^a	ND ^a	2.21 ±0.09	0.74 ±0.08
	15	ND ^a	ND ^a	2.29 ±0.12	0.80 ±0.06

^aNot established.

DISCUSSION

During the present study, 30 commercially available recycled pastry boxes were checked for the presence of 6 substances (benzophenone (PhCO), PCP, DEHP, DBP, 2,6-DiPN and 2,7-DiPN) which are usually found as residues after recycling, so as to evaluate their suitability for use as packaging materials in direct contact with foodstuffs. Instead of applying the conventionally used methods, involving the analysis of each group of compounds separately, a modified analytical method was developed based on ultrasound-assisted solvent extraction followed by GC-MS analysis to identify and quantify all 6 substances in one single trial run. In this regard, diisopropylnaphthalenes posed a challenge to the experiment, since up to 10 diisopropylnaphthalenes can theoretically be obtained during synthesis; therefore separation is a crucial issue for the selective identification of each one. (Bouvier et al., 2009). However, separation of all DiPN isomers is neither within the scope of this study nor within the abilities of routine benchtop GC-MS instruments. Since the identification of any residues of DiNP in the form of an isolated isomer or a mixture of isomers seems sufficient for the purpose of this study, we decided to detect representative signals for this group of substances. Of the diisopropylnaphthalenes, if 2,6-diisopropylnaphthalene and its most frequent co-occurring isomer 2,7-diisopropylnaphthalene are discovered, this is usually considered sufficient to reassess the method of production in order to determine the ratio of isomers produced and the level of residues in the environment, in order to estimate of the level of pollution, therefore the method was developed in a way that allowed partial separation of the two important 2,6-DiPN and 2,7-DiPN isomers (Vápenka et al., 2016).

Representative chromatograms of a spiked virgin cardboard sample and a real box and pastry sample are demonstrated in Figure 1. It is shown that evidence of the relevant compounds can be successfully demonstrated. The level of benzophenone determined in this study was more than that obtained for recycled cardboards in Greece (Parigoridi et al., 2014), whereas it is similar to levels which are reported for cardboard used in cartons (Anderson and Castle, 2003). Benzophenone may be sourced from UV-cured inks and

lacquers used to print on the packaging. It may also be present if the cardboard is made from recycled fibers recovered from printed materials. In comparison with a similar study, which reported the level of DEHP and DBP in packaging materials sold on the Belgian market (Fierens et al., 2012), lower levels of the phthalates were found in the analyzed recycled boxes. Phthalates, which are normally used as plasticizers, are not deliberately added to paper and cardboard as plasticizers, but can be used in lacquers, adhesives, glues and printing inks. The concentration of PCP in the samples was much lower than the maximum permitted level ($<0.15 \text{ mg/dm}^2$). Thus, according to the INSO established limit, the detected levels of phthalates and pentachlorophenol in the samples are below the level that could present a risk to human health. The levels of 2,6-DiPN and 2,7-DiPN were actually below the quantification limit of the instrument (0.001 and 0.002 mg/dm^2 , respectively), which leads to the conclusion that the concentration of these residues would be within the safe range.

In this article, we also studied the migration of the organic contaminants into pastries in order to generate a scientific understanding of the physicochemical behavior of chemical contaminants on paper and cardboard fibers, as a basis for a safety evaluation and the drawing up of criteria for the appropriate use of recycled fibers for food packaging.

According to these results, the migration of Benzophenone (PhCO), PCP, 2,6-DiPN and 2,7-DiPN from the recycled package into the pastries was not observed. However, a portion of the samples appeared to have been contaminated with DEHP and DBP through their packaging, as indicated in Table 5. This observation can be attributed to the very low initial concentration levels of Benzophenone (PhCO), PCP, 2,6-DiPN and 2,7-DiPN in the recycled packaging. It seems that the high concentration of phthalates in the cardboard could result in a higher level of migration into the pastry. Based on the results (Table 5), the highest concentration of DEHP and DBP was observed in marmalade pastry. These results indicate that some organic compounds could migrate more easily from the package materials into the pastries when the pastry surfaces are sticky, which causes more adhesion of the pastry surface to the inner cover of the package. On the other hand, the wet adhesion of pastry to the package

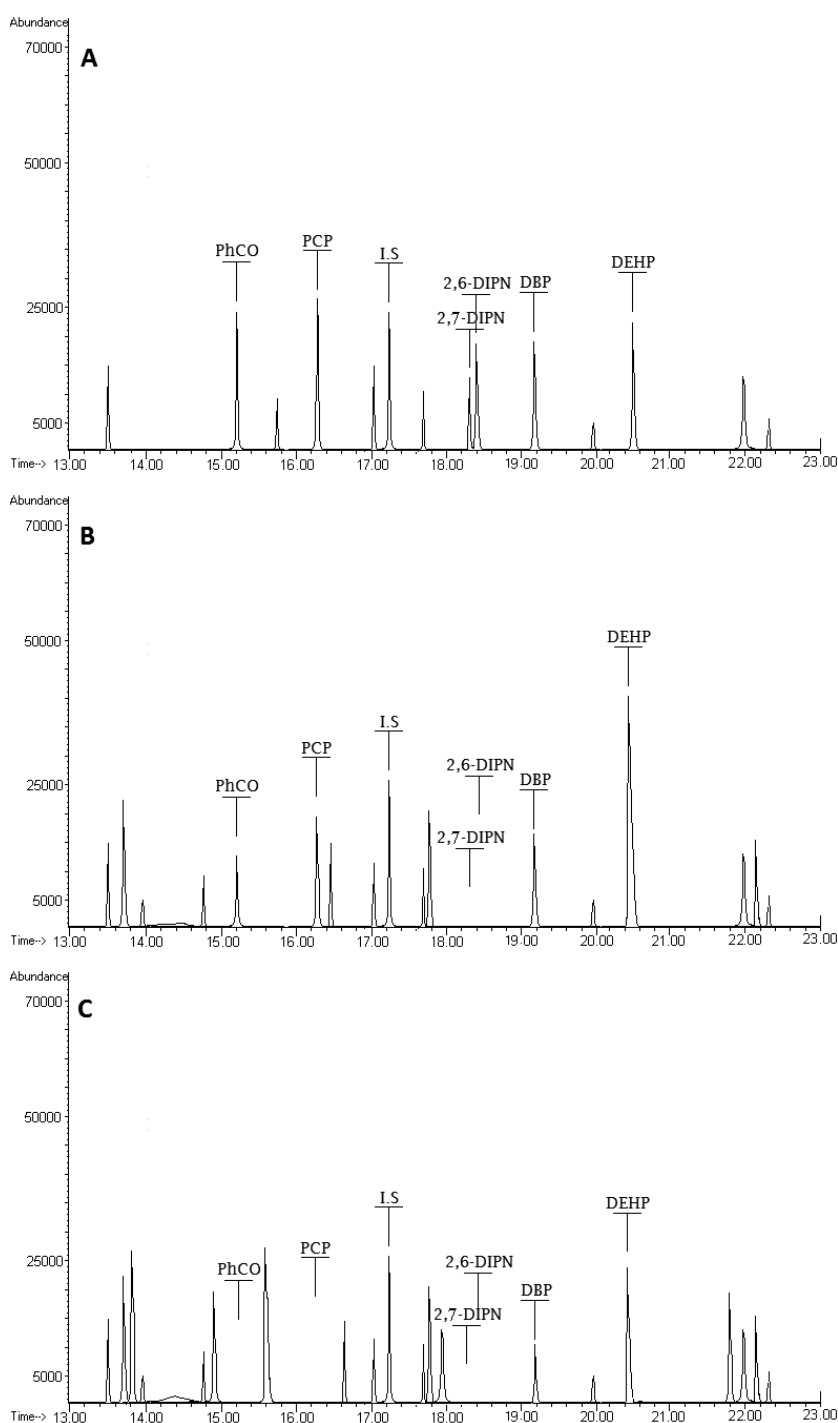


Fig. 1. Representative chromatogram of the organic pollutants identified in (A) spiked virgin cardboard sample: benzophenone (PhCO), pentachlorophenol (PCP), benzyl benzoate (I.S), 2,7-diisopropylnaphthalene (2,7 DiPN), 2,6-diisopropylnaphthalene (2,6 DiPN), dibutyl phthalate (DBP), bis(2-ethylhexyl) phthalate (DEHP); (B) a real pastry box sample and (C) a real pastry sample

surfaces would allow a different contaminant in the outer package layer to migrate into the pastry, because the packages are usually so poorly designed that their inner coatings cannot maintain their structural integrity. We have not been successful in finding a relationship between the levels of migrated compounds and fat content of the pastries. As indicated in Table 1, the fat content of the pastries decreased in the following order: puff pastry > Danish pastry > marmalade pastry > sugar coated biscuit. But the total phthalate residue decreased in the following order: marmalade pastry > puff pastry > sugar coated biscuit > Danish pastry. The reason could be the multifactorial nature of compound migration from the package layers to the pastries, which determines the quantity and quality of pollutant migration in different situations. For example, it seems that direct contact with the surface of the box, which affected a larger area of marmalade pastry and sugar coated biscuit samples than Danish pastry, was more influential than fat content. On the other hand, surface area of the puff pastry which had contact with the box was higher than that of marmalade pastry relative to the amount of total phthalate residue, as was its fat content, which can be considered evidence for the effect of the “wet surface adhesion” factor.

Storage time also affected the concentration of DEHP and DBP in the pastries from days 0 to 15, and the highest DEHP and DBP concentrations were observed on day 15. Our results concerning the effect of contact time are also in accordance with previous findings (Vápenka et al., 2016). Therefore, it is recommended that pastries, especially those with sticky surfaces, should not to be stored in their original cardboard-based packing for more than few days.

CONCLUSION

In this study, harmful organic compounds such as DEHP and DBP were detected at high concentrations in most recycled boxes which are used for pastry packaging. In addition, these results indicate that these kinds of contaminants could migrate from the recycled packages into pastries under normal conditions of use. Therefore, it seems essential to reconsider the procedure for preparing recycled cardboard for food packaging. Some practical options (individually or in combination) which can keep the level of

migrated compounds within acceptable limits are as follows: source control by sorting used fibers into different grades, using effective and improved cleaning methods for preparing recycled papers (such as flotation deinking), frequent testing of batches/lots, using multi-layer barriers to prevent or reduce the amount of migrated compounds and the use of recycled cardboard only for indirect contact applications and/or at low temperatures.

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