Rapid determination of benzophenone derivatives in cereals using FaPEx coupled with ultra–high-performance liquid chromatography–tandem mass spectrometry

Yu-Fang Huang [a,b,c,*], Jui-Ting Chien [a], Hsin-Chang Chen [d], Xuan-Rui Liu [a], Jui-Pin Chang [a], Jun-Jie Huang [a]

[a] Department of Safety, Health and Environmental Engineering, National United University, Miaoli, Taiwan
[b] Center for Chemical Hazards and Environmental Health Risk Research, National United University, Miaoli, Taiwan
[c] Institute of Food Safety and Health Risk Assessment, National Yang Ming Chiao Tung University, Taipei, Taiwan
[d] Institute of Food Safety and Health, National Taiwan University, Taipei, Taiwan

Abstract

A robust and sensitive analytical method was developed and validated for the simultaneous analysis of the levels of 10 benzophenone derivatives (BPs; BP, BP-1, BP-2, BP-3, BP-8, 2-hydroxybenzophenone [2-OHBP], 4-hydroxybenzophenone [4-OHBP], 4-methylbenzophenone [4-MBP], methyl-2-benzoylbenzoate [M2BB], and 4-benzoylbiphenyl [PBZ]) in 54 breakfast cereal samples. A fast pesticide extraction (FaPEx) technique coupled with isotope-labeled internal standards ultra–high-performance liquid chromatography–tandem mass spectrometry was employed. The developed method exhibited satisfactory linearity ($R^2 > 0.998$), high precision (intraday and interday relative standard deviations in the ranges of 1.4%–20.8% and 3.2%–23.9%, respectively), and a limit of detection ranging from 0.001 to 0.122 ng/g. BP and 4-MBP were detected in all samples, BP-3 was detected in >59% of the samples, M2BB was detected in 14% of the samples, and 4-OHBP was detected in 7% of the samples. The mean level (range) of BP was significantly higher in corn flakes [146.9 (25.3–1083.8) ng/g] than in oatmeal [22.8 (14.2–67.5) ng/g], and it contributed the most to the overall levels of the BPs, followed by 4-MBP. When the samples were stratified according to their packaging material, the mean level of BP was significantly higher in corn flake samples with plastic packaging (251.9 ng/g) than in corn flake and oatmeal samples with laminated aluminum foil packaging. Two samples of six-grain muesli contained remarkably high levels of BP (1084 and 1055 ng/g); both were nonorganic samples packaged in a polylactide bag. Future studies must examine the possible risks that these contaminants pose to human health.

Keywords: Benzophenone derivatives, Cereals, FaPEx, Ultra-high-performance liquid chromatography–tandem mass spectrometry

1. Introduction

Benzophenone (BP) and its derivatives (BPs) have been widely used as ultraviolet (UV) stabilizers in plastic and cardboard food packaging materials, as UV-curable inks in the printing of food packaging materials, and as sunscreen agents in cosmetic products [1]. The parent BP structure has two phenyl rings and a carbonyl group, on the basis of which 12 derivatives have been reported (BP-1 to BP-12), along with lesser-known derivatives such as 4-methylbenzophenone (4-MBP), methyl-2-benzoylbenzoate (M2BB), and 4-benzoylbiphenyl (PBZ) [2]. Of them, BP and 4-MBP are the most widely used initiators for printing ink [3]. Because of their

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* Corresponding author at: Department of Safety, Health and Environmental Engineering, National United University, Miaoli, Taiwan. Fax: +886 37 382281.
E-mail address: yfh@nuu.edu.tw (Y.-F. Huang).

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volatility, if no functional barrier is in place, then they can migrate from the packaging material into the food to contaminate the food product. In 2009, German and Belgian authorities reported that breakfast cereals contain up to 4210 ng/g BP and 3729 ng/g 4-MBP [4]. So far, no legislation has been passed regarding the use of printing inks on food contact materials. However, in the European Union (EU), the use of printing inks must comply with the general rules of Regulation (EC) No. 1935/2004 and adhere to good manufacturing practice to ensure that they do not transfer their constituents to food in quantities that can endanger human health [5]. According to Regulation (EU) No. 10/2011 on plastics, BP has a specific migration limit of 0.6 mg/kg of food [6]. In 2013, the International Agency for Research on Cancer classified BP as possibly carcinogenic to humans (Group 2B) on the basis of sufficient evidence in experimental animals of liver and kidney carcinogenicity [7].

Humans may be exposed to BPs through inhalation, dermal contact, and dietary consumption; in addition to their natural occurrence in food, BPs are used as a flavoring agent, can contaminate drinking water, and can migrate from food packaging to penetrate food products [7]. Although the levels of BPs in food may be low enough that they do not to pose an acute risk to humans, long-term exposure to BPs can have adverse effects. Given that BPs are being increasingly used and may act as endocrine disruptors, a straightforward and convenient analytical method for determining the BP levels in food products is required. Most methods that have been developed for determining BP content focus on using food simulants to quantify the levels of BPs that have infiltrated food products from their packaging materials [8,9]. Few methods exist for the simultaneous analysis of BPs in foods. Cereals and cereal products are essential for human nutrition because they are major sources of carbohydrates (approximately 75%) and contain 6%–15% of protein, fiber, and traces of minerals such as iron, vitamins B and E, niacin, riboflavin, and thiamine [10]. The demand for cereals and cereal products has been increasing, with the global cereal production reaching its highest level at 2742 million t per year [11].

The main methods for quantifying the BPs content in breakfast cereals and packaged foods (e.g., milk and other beverages) are high-performance liquid chromatography (HPLC) combined with UV detection [12], HPLC combined with diode-array detection [8,13], HPLC combined with mass spectrometry (MS) [4] or tandem MS (MS/MS) [13–16], and gas chromatography combined with MS [17,18]. The sample preparation methods include solvent extraction with acetonitrile [4,13] and pressurized liquid extraction (PLE) [18]; however, these techniques are time consuming and require a large volume of organic or toxic solvents. Solid-phase extraction (SPE) [17,19] and the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method [15] have been proposed for determining the quantities of photoinitiator BPs in breakfast cereal and packaged food products. However, some methods are limited in terms of instrument sensitivity or the number of BPs that can be simultaneously analyzed. Fast pesticide extraction (FaPEx) is a method used to extract pesticide residues in agricultural samples by using single-use prefilled sealed cartridges; this method is innovative, simple, and fast and is a simplified version of the QuEChERS method that is based on the same principles [20].

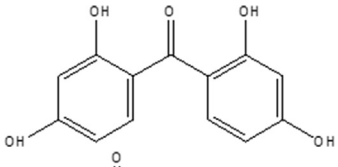
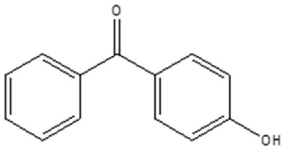
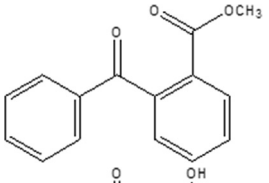
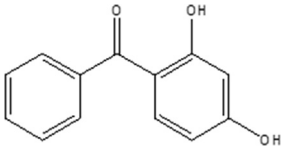
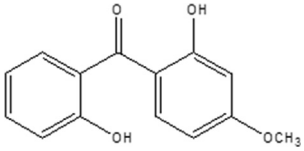
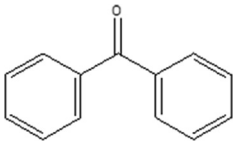
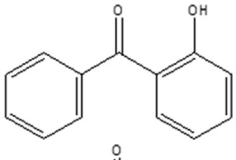
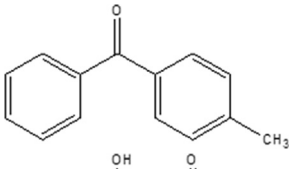
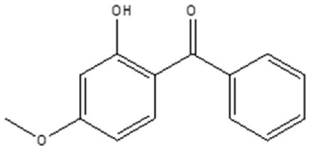
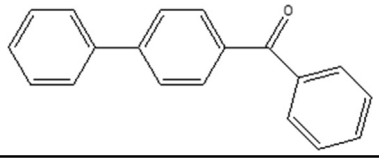
We developed a FaPEx technique coupled with ultra-HPLC (UHPLC)–MS/MS to simultaneously analyze the levels of 10 BPs (BP, BP-1, BP-2, BP-3, BP-8, 2-hydroxybenzophenone [2-OHBP], 4-hydroxybenzophenone [4-OHBP], 4-MBP, M2BB, and PBZ) in breakfast cereals. To achieve highly accurate quantification, overcome matrix effects, compensate for method recovery, and mitigate measurement uncertainty, the use of isotope-labeled internal standards (ILISs) for UHPLC–MS/MS is essential. The developed method was validated and applied to analyze the levels of different BPs in oatmeal and corn flake samples.

2. Materials and methods

2.1. Chemicals and reagents

Table 1 lists the names, structures, chemical formulas, and log Kow of the BPs analysed in this study. The ILISs, d_5-BP-1, d_3-4-MBP, d_3-BP-8, $^{13}C_6$-diOHBP, and d_4-4-OHBP (purities of all compounds were >98%) were purchased from Toronto Research (North York, Toronto, Canada). BP-d_5, d_5-BP-3 (purities of both compounds were >98%), and Supelclean PSA SPE bulk packaging were obtained from Sigma–Aldrich. Anhydrous magnesium sulfate ($MgSO_4$, 99% purity) and high-performance LC-grade acetonitrile (ACN), formic acid (88% purity), acetic acid (99.7% purity), and LC–MS-grade methanol were obtained from JT Baker (Phillipsburg, NJ, USA). FaPEx-cer was obtained from Silicycle (Quebec, Canada; Taiwan Patent No. 1500915,

Table 1. Reference standard and internal standards.

Name	Chemical Structure	Chemical Formulas/Average Mass/Purity/Producer	log Kow
2,2′,4,4′-Tetrahydroxybenzophenone (BP-2)		$C_{13}H_{10}O_5$ 246.21 >98% Tokyo Chemical Industry	2.78
4-Hydroxybenzophenone (4-OHBP)		$C_{14}H_{12}O$ 198.22 98% Sigma–Aldrich	2.67
Methyl-2-Benzoylbenzoate (M2BB)		$C_{19}H_{14}O$ 240.25 97% Sigma–Aldrich	2.70
2,4-Dihydroxybenzophenone (BP-1)		$C_{13}H_{10}O_3$ 214.22 AccuStandard	2.96
2,2′-Dihydroxy-4-methoxybenzophenone (BP-8)		$C_{14}H_{12}O_4$ 244.24 >98% Tokyo Chemical Industry	3.82
Benzophenone (BP)		$C_{13}H_{10}O$ 182.22 99% Sigma–Aldrich	3.18
2-Hydroxybenzophenone (2-OHBP)		$C_{13}H_{10}O_2$ 198.22 99% Sigma–Aldrich	3.44
4-Methylbenzophenone (4-MBP)		$C_{13}H_{10}O_2$ 196.24 98.5% Sigma–Aldrich	3.5
2-Hydroxy-4-methoxybenzophenone (BP-3)		a$C_{14}H_{12}O_3$ 228.24 AccuStandard	3.79
4-Benzoylbiphenyl (PBZ)		$C_{15}H_{12}O_3$ 258.31 99% Alfa Aesar	4.91

USA Patent No. 9581579). Sodium chloride (NaCl; >99% purity) was purchased from PanReac (Castellar del Vallès, Barcelona, Spain). Sepra C18-E (50 μm, 65 Å) was obtained from Phenomenex (Torrance, CA, USA). Standard stock solutions of 1000 mg/L of each analyte were prepared in ACN and stored at −20 °C. Standard working solutions of 10 mg/L were prepared through the dilution of each

standard stock solution with methanol, and the solutions were then used to spike samples and prepare the calibration curves of solvents and cereal matrices. BP-d_5, d_5-BP-1, d_3-4-MBP, d_3-BP-8, $^{13}C_6$-diOHBP, d_4-4-OHBP, and d_5-BP-3 were used as ILIS for BP, BP-1, 4-MBP, BP-8, 2-OHBP, 4-OHBP, and BP-3, respectively. The analytes BP-2, M2BB, and PBZ lack specific internal standards (ISs); thus, d_4-4-OHBP was used as an IS for BP-2 and M2BB, and d_5-BP-3 was used as an IS for PBZ. The ILIS mixture was prepared at 500 mg/L and diluted to 20 μg/L.

2.2. Instrumentation

A Nexera UHPLC system (Shimadzu, Kyoto, Japan) was used for BPs analysis. A Waters Acquity UPLC BEH C_{18} column (1.7 μm, 2.1 mm × 100 mm) was used to separate the analyte at a flow rate of 0.3 mL/min. Mobile phase A was H_2O, and mobile phase B was LC–MS-grade methanol with 0.1% formic acid. The following elution gradient was applied: 20%–80% B for 3.5 min, 80% B held for 1 min, 80%–90% B for 1 min, 90% B held for 4 min, 90%–20% B for 0.1 min, and re-equilibration at 20% B for 3.9 min. The total analysis time was 13.5 min, and the injection volume was 10 μL. MS/MS analysis was employed using triple quadrupole MS (Shimadzu, LCMS-8045) with an electrospray ionization (ESI) source. Ions were monitored in the positive and negative multiple reaction monitoring (MRM) modes. LabSolution version 5.93 (Shimadzu, Kyoto, Japan) was used for data analysis.

2.3. Sample preparation and extraction

Two methods—FaPEx and QuEChERS—were compared to identify the most suitable sample preparation procedure. The FaPEx technique was developed by the Taiwan Agricultural Chemicals and Toxic Substances Research Institute and authorized by Uni-Onward. The major components involved in FaPEx are $MgSO_4$, PSA, C18, and graphitized carbon black [20]. FaPEx is an easy technique, and it has a short extraction time (<5 min) and produces remarkably low amounts of waste solvents and chemicals. The QuEChERS method typically includes two steps: extraction using ACN and salts for enhanced efficiency and clean-up using dispersive SPE. In these steps, sorbents such as $MgSO_4$, PSA, and C18 are used to remove excess water, pigments, and lipids/fatty acids, respectively.

(i) For the FaPEx procedure, 0.5 g of a homogenized cereal sample was placed in a 15-mL polypropylene (PP) tube. Next, 1 mL of pure water was added, and the sample was spiked with 8 ng/g ILIS and vortexed for 1 min. After the sample was allowed to stand for 30 min, 5 mL of ACN with 1% acetic acid was added to the sample; the mixture was vortexed for 30 s and centrifuged at 6000×*g* for 5 min. Next, the supernatant solution was transferred to a FaPEx-cer kit, with the liquid flow rate controlled at 1 drop/s and the dryness level controlled using a gentle nitrogen stream. Finally, the residue was reconstituted in 200 μL of methanol and filtered using 0.22-μm polytetrafluoroethylene filters.

(ii) The QuEChERS method, described previously [14,15], was performed in the present study with some modifications. In brief, cereal samples (5 g) were loaded into a 50-mL PP tube, and the ILISs (100 μL) was used as a surrogate (40 μg/L); the samples were then supplemented with deionized water (10 mL) and ACN (10 mL) with 1% acetic acid. The mixtures were shaken for 1 min, and anhydrous $MgSO_4$ (4 g) and NaCl (1 g) were added, followed by another 1 min of vigorous shaking. For samples without a clean-up procedure, the extract was then centrifuged at 6000×*g* for 5 min, and the supernatant was dried under a gentle nitrogen stream. Otherwise, the ACN extract was cleaned using $MgSO_4$ (1.2 g), C18-E (1.2 g), and PSA (0.4 g) sorbents. Finally, the residues were treated in the same manner as described in the FaPEx method.

2.4. Validation procedure

The proposed method was validated according to guidelines established in the United States [21] and Taiwan [22] on the basis of evaluations of linearity, the matrix effect, the limit of detection (LOD), the limit of quantification (LOQ), precision, and accuracy. Blank cereal matrix samples with tin can or laminated aluminum foil packaging were analyzed using UHPLC–MS/MS, and the samples were discovered to have low BPs levels. Linearity was evaluated using solvent- and matrix-matched calibration standards covering six levels (0.4, 2, 4, 8, 12, and 20 μg/L, with an ILISs of 8 ng/g). Calibration curves of solvents and the matrix were obtained by plotting the quotients of the peak areas of 10 BPs and their corresponding ILISs versus the levels of the standards. UHPLC–MS/MS was employed to analyze 10 oatmeal samples and 10 corn flake

samples; of these, three samples of each type were selected as blank samples because they contained low BP levels. A mixture of all six samples was spiked to obtain two matrix-matched calibration curves, which revealed BPs levels of 0.4–20 ng/g. The matrix effect was evaluated through comparison of the slopes of standards in a solvent with matrix-matched standards. LOD and LOQ were defined as the levels with signal-to-noise ratios of 3 and 10, respectively. Blank cereal samples with a 20 ng/g spiking level were used to evaluate the precision and accuracy of the method. The relative standard deviation (RSD) was used to determine the intraday and interday precision. Intraday precision (n = 5) was assessed according to the SD in the recovery percentage of the spiked samples on a given day. Interday precision (n = 15) was determined by comparing the spiked samples across 3 days. Accuracy was evaluated according to the mean recovery for these spiked samples (n = 15).

2.5. Quantification of cereal samples

Fifty-four breakfast cereal samples (25 oatmeal samples and 29 corn flake samples) were purchased from supermarkets in Taiwan. Packaging materials were selected on the basis of the principle of proportionality, but corn flakes in tin can packaging were unavailable. A total of 7, 10, and 8 oatmeal samples and 0, 14, and 15 corn flake samples had a tin can, laminated aluminum foil bag, and plastic bag as their packaging material, respectively. We recorded sample data, including the sampling date, the type of packaging material (tin can, laminated aluminum foil bag, or plastic bag), whether the food product was organic or nonorganic, and whether the raw material was obtained from domestic or foreign sources. To reduce the risk of contamination from packaging materials and the amount of time the products spent in their packaging, blank matrix samples with a tin can or laminated aluminum foil bag as their packaging material were selected. In addition, cereal samples were collected soon after the manufacturing date and were analyzed within 1 week of the sampling date. Table S1 presents an overview of the samples and their grouping according to their type of by packaging material, their status as an organic or nonorganic food product, and the source of their raw materials (domestic or foreign).

2.6. Statistical analysis

The mean (SD, range) values of the experimental results were calculated. The nonparametric Mann–Whitney U test was used to compare BPs levels between the oatmeal and corn flakes samples. The Kruskal–Wallis test was used to evaluate the differences in BPs levels among packaging materials, between organic and nonorganic foods, and between foods with domestic and foreign raw materials. The Tukey honestly significant difference (HSD) for unequal N-test was used for *post hoc* comparisons. Statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA), and significance level was set as $p < 0.05$.

3. Results

3.1. FaPEx sample pretreatment

In the present study, we applied the FaPEx and QuEChERS methods with and without a clean-up procedure by spiking mixtures of oatmeal and corn flakes at a spiking level of 20 ng/g. For the oatmeal sample, FaPEx and extraction were performed using 4 g of anhydrous $MgSO_4$ and 1 g of NaCl, and the sample was tested: with or without a clean-up procedure (1.2 g of $MgSO_4$ + 1.2 g of C18 + 0.4 g of PSA). A comparison of the two techniques for the clean-up of BPs revealed that the FaPEx method had higher recovery, followed by the QuEChERS method with a clean-up procedure (Fig. 1A). Therefore, for the corn flake samples, we only compared the FaPEx and QuEChERS methods involving a clean-up procedure. The FaPEx method yielded higher recovery (Fig. 1B). Thus, in addition to all of its advantages, the QuEChERS approach reduces the labor time, processing time, and requirements for organic solvents, glassware, and specialized equipment.

3.2. Optimization of UHPLC–MS/MS

The MS settings were optimized through direct injection of the working solution at a concentration of 1 μg/mL. An ESI-positive mode was used for developing multiple analytes, except for BP-2. The retention time, MS parameters (e.g., ion transitions for quantification and confirmation), and collision energy of BPs obtained in the MRM mode are listed in Table 2. The chromatographic conditions were optimized (Fig. 2). Separation was performed on a Waters Acquity UPLC BEH C_{18} column, which resulted in a smooth peak and more effective separation of the oatmeal sample with spiking levels of 20 and 8 ng/g for BP standards and ILISs, respectively. Water combined with 0.1% formic acid in methanol was selected as the mobile phase because of its superiority to ACN in terms of sensitivity, peak

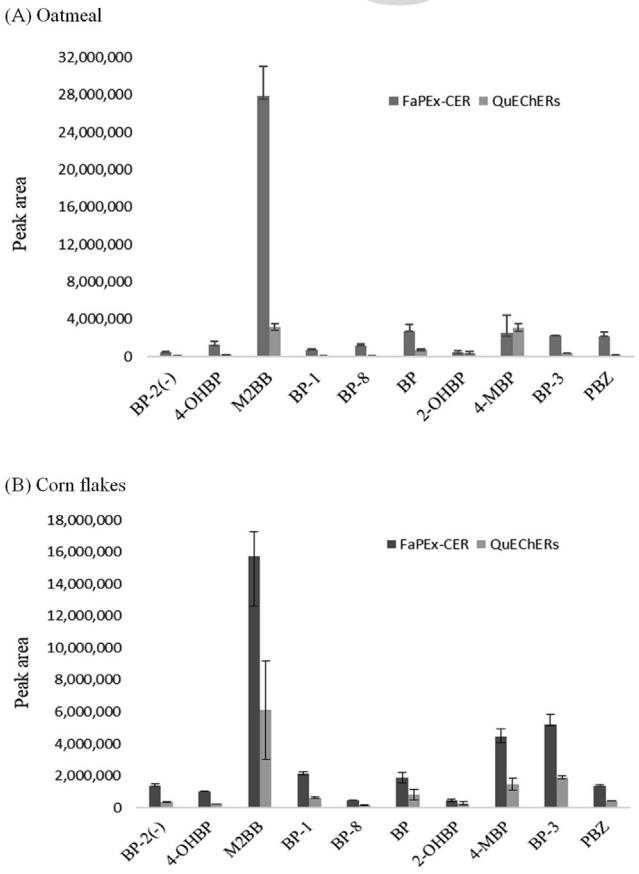


Fig. 1. Comparison of FaPEx-CER and QuEChERS methods using an extraction kit (4 g of anhydrous $MgSO_4$ and 1 g of NaCl) and a clean-up procedure (1.2 g $MgSO_4$ + 1.2 g C18 + 0.4 g PSA) in samples (n = 3 replicates) of (A) oatmeal and (B) corn flakes spiked with standards and IS (20 and 8 ng/g).

shapes, and greenness (Fig. 3). We ultimately used LC–MS-grade methanol with 0.1% formic acid and water as the mobile phase for BP analysis because of its high sensitivity (Fig. S1). Gradient elution was applied at a flow rate of 0.3 mL/min and with a total run time of 13.5 min.

3.3. Method validation

According to matrix-matched and solvent calibration curves, all analytes exhibited high linearity (R^2 > 0.997) in the range of 0.4–20 ng/g (Table 3). The matrix effect of 10 BPs was 87%–285% and 87%–135% in the oatmeal and corn flake samples, respectively, indicating that the matrix effect was present for BP-2, M2BB, and PBZ. Consequently, the matrix-matched standard solutions were selected for calibration. The LOD and LOQ were 0.001–0.289 and 0.003–0.867 ng/g, respectively. The average recovery range was 79%–121%, with intraday and interday RSDs of 1.4%–20.8% and 3.2%–23.9%, respectively. In the FaPEx method, the sample was directly treated with a FaPEx cartridge after homogenization, and the resulting cleaned filtrate was ready for injection and chromatographic analysis. Because of its ease of use and simplicity, FaPEx minimizes handling errors while providing high recovery yields.

3.4. Method applications

Table 4 summarizes the detection frequencies and mean (SD, range) levels of BPs in samples of oatmeal (n = 25) and corn flakes (n = 29). Of the 10 BPs, three were detected in the range of 59%–100% in the cereal samples; exceptions were BP-1, BP-2, BP-8, 2-OHBP, and 4-MBP, which were detected at percentages lower than the LOD. BP and 4-MBP were detected in all samples, and BP-3 was detected in >59% of the samples. The BP levels had a significantly higher mean ± SD (range) in corn flakes (146.9 ± 276.4 [25.3–1083.8] ng/g) than in oatmeal (22.8 ± 11.3 [14.2–67.5] ng/g; $p < 0.0001$). Furthermore, BP contributed the most to the total BP level; the second greatest contributor was 4-MBP, which had levels of 5.8 ± 12.6 (1.2–65.8) and 3.1 ± 2.4 (1.3–12.0) ng/g in the oatmeal and corn flake samples, respectively. BP-3 had low levels of 0.2 ± 0.2 (0.1–1.0) and 1.4 ± 1.8 (0.8–8.5) in the oatmeal and corn flake samples, respectively. In addition, M2BB and 4-OHBP were detected in 14% and 7% of the corn flake samples, with levels of 6.1 ± 7.7 (0.5–17.3) and 6.4 ± 4.6 (3.1–9.7) ng/g, respectively.

4. Discussion

4.1. Comparison of analytical methods

To the best of our knowledge, this is the first study to simultaneously examine the levels of 10 BPs in samples of oatmeal and corn flakes from Taiwan. Chang et al. (2019) developed the QuEChERS method without a clean-up procedure and then used UPLC–MS/MS to analyze 30 photoinitiators in breakfast cereal and packaged juice [15]. However, the LOQ was 2–40 ng/g for cereal samples. Gallart-Ayala et al. (2011) developed a QuEChERS method with a clean-up procedure and SPE by using an HLB cartridge and then used HPLC–MS/MS to analyze 11 photoinitiators [14]. However, only BP and PBZ could be analyzed. The techniques used for extracting BPs from cereal samples are PLE [18]; solvent extraction using ACN or dichloromethane [4,13], followed by SPE [17]; SPE with HLB cartridge [14]; and QuEChERS [14,15] (Table 5). Solvent

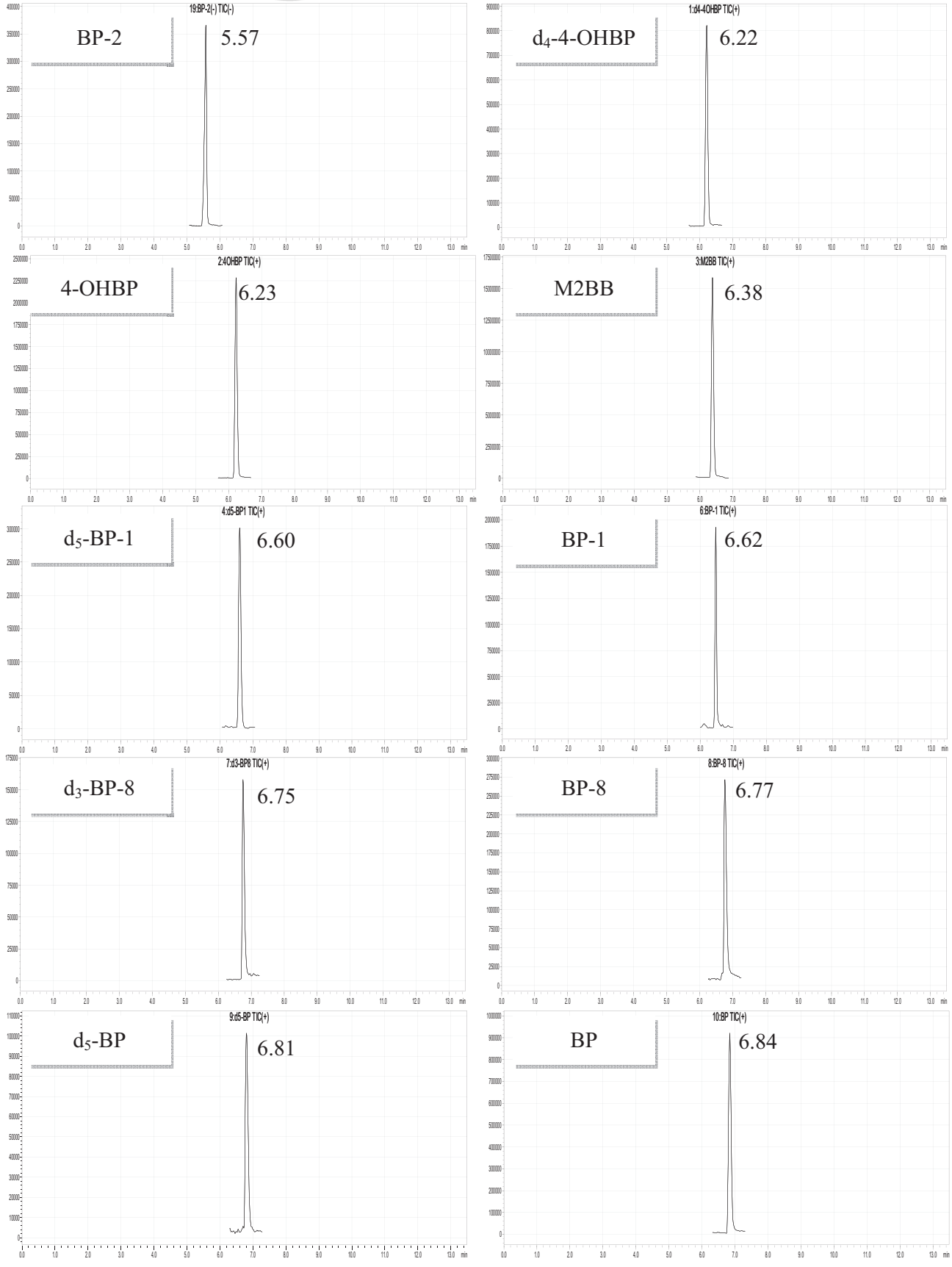


Fig. 2. Representative chromatograms of an oatmeal sample spiked with 20 ng/g of the BPs standards and 8 ng/g of the IS.

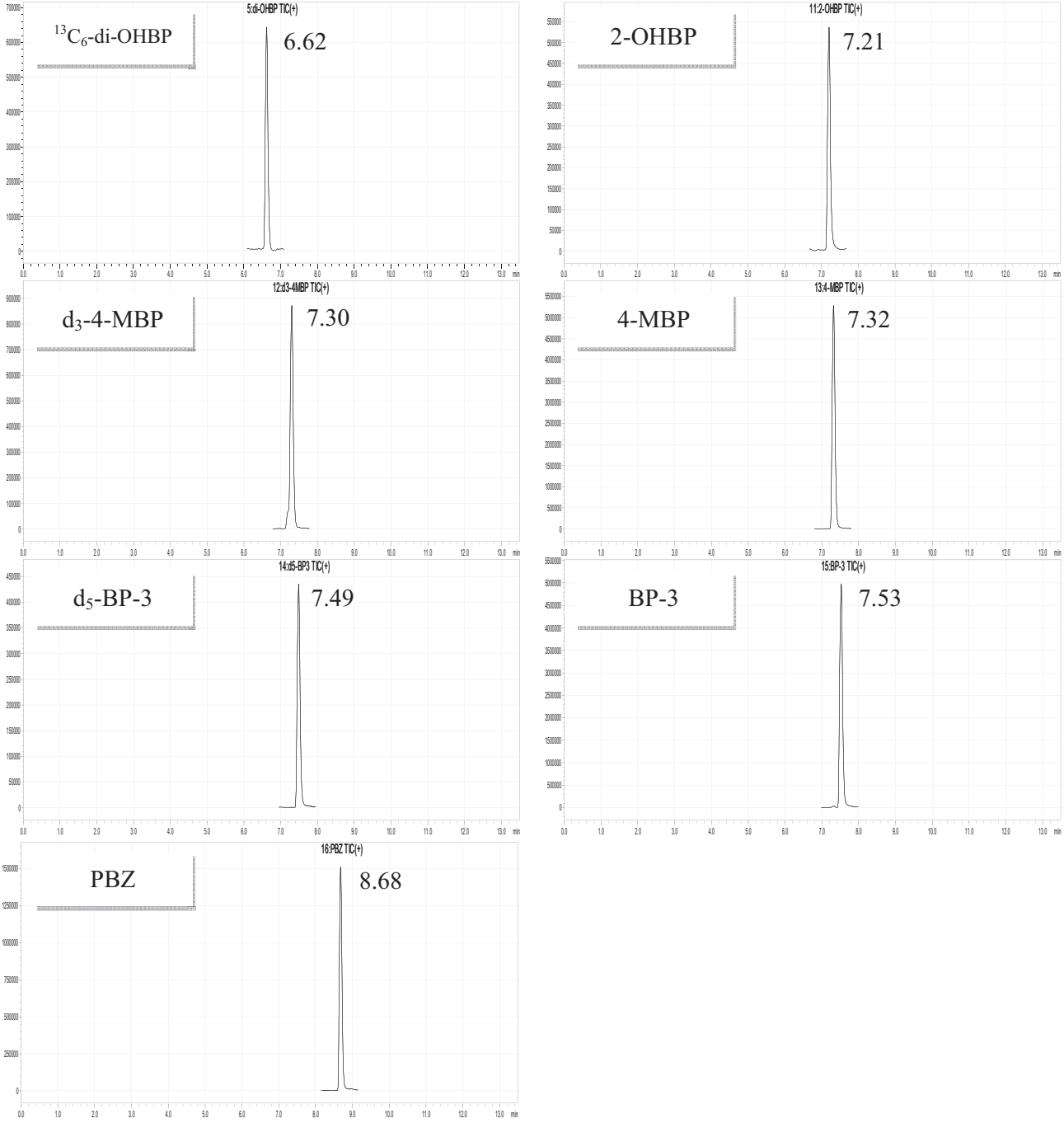


Fig. 2. (Continued).

Table 2. Retention time, selected ion/transitions, and multiple reaction monitoring (MRM) mode.

Analytes	RT (min)	Cone voltage Q1(V)	Precursor ion (m/z)> Product ion (m/z)	Collision energy (eV)	Analytes	RT (min)	Cone voltage Q1(V)	Precursor ion (m/z)> Product ion (m/z)	Collision energy (eV)
BP-2[a]	5.64	-12	245.00 > 135.00	-13	PBZ[g]	8.78	10	259.00 > 105.00	18
		-16	245.00 > 109.00	-21			29	259.00 > 77.05	40
							29	259.00 > 181.05	18
4-OHBP[a]	6.20	10	199.00 > 121.05	18	d_4-4-OHBP[a1]	6.19	23	203.10 > 125.15	18
		10	199.00 > 77.10	38			23	203.10 > 105.10	18
							24	203.10 > 77.10	37
M2BB[a]	6.37	17	240.25 > 209.10	10	d_5-BP-1[b1]	6.59	25	220.10 > 137.00	19
		17	240.25 > 152.00	38			25	220.10 > 138.00	18
							24	220.10 > 23.00	49
BP-1[b]	6.61	23	214.90 > 137.00	19	d_3-BP-8[c1]	6.77	28	248.10 > 121.10	17
		15	214.90 > 105.00	20			30	248.10 > 154.05	21
		23	214.90 > 81.05	38					
BP-8[c]	6.79	13	245.00 > 121.10	16	d_5-BP[d1]	6.80	21	188.10 > 105.10	16
		13	245.00 > 151.00	18			22	188.10 > 110.10	16
							22	188.10 > 82.15	31
BP [d]	6.83	19	183.00 > 105.05	16	$^{13}C_6$-diOHBP [e1]	6.61	11	221.00 > 137.00	21
		19	183.00 > 77.15	33			24	221.00 > 81.00	37
							11	221.00 > 111.05	20
2-OHBP [e]	7.22	11	199.20 > 121.00	16	d_3-4-MBP[f1]	7.31	12	200.20 > 105.10	16
		11	199.20 > 93.00	27			12	200.20 > 77.10	34
							12	200.20 > 122.15	16
4-MBP[f]	7.33	21	197.00 > 105.10	15	d_5-BP-3[g1]	7.51	27	234.10 > 151.00	20
		10	197.00 > 77.10	33			26	234.10 > 81.95	41
		10	197.00 > 119.10	15			12	234.10 > 110.05	19
BP-3[g]	7.54	25	229.00 > 151.05	19					
		11	229.00 > 105.05	19					
		24	229.00 > 77.05	39					

RT: retention time; a[1]: The use of a IS for a; b[1]: The use of a IS for b; c[1]: The use of a IS for c; d[1]: The use of a IS for d; e[1]: The use of a IS for e; f[1]: The use of a IS for f; g[1]: The use of a IS for g.

extraction requires a large amount of organic solvents and is time consuming, with an LOD and LOQ of 2.5–38 and 7.5–113 ng/g, respectively [4,13]. Compared with the aforementioned techniques, the PLE, SPE, and the QuEChERS methods require a lower volume of solvents; these methods have also been used to extract BPs from cereal matrices, with an LOQ of 2.3–60, 10, and 0.7–60 ng/g for BP, 4-MBP, and PBZ extraction, respectively, and recoveries ranging from 74% to 98%. Therefore, in this study, we established a method that provides satisfactory results, with a low LOD and high precision for the simultaneous determination of 10 BPs in cereals. In addition, we used ILISs to achieve highly accurate quantification by compensating for matrix effects and method recovery and by reducing measurement uncertainty. However, because of the high costs of ILISs, the corresponding ILISs could not be used for all analytes.

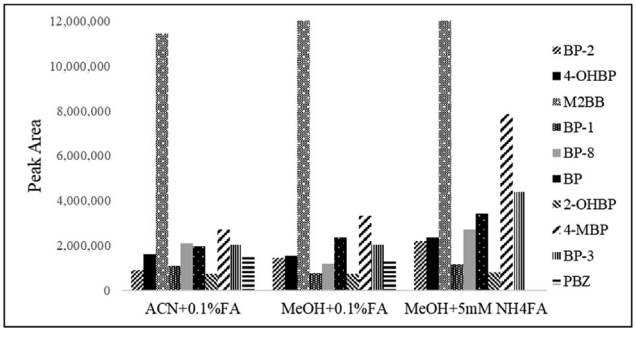


Fig. 3. Comparison of the peak areas in BPs standards of 50 pg/μL across the different mobile phases: ACN containing 0.1% formic acid and water; MeOH containing 0.1% formic acid and water; and MeOH containing 5 mM ammonium formate and water.

4.2. Comparison of the distributions of BPs

The developed method was applied for analysis of the BPs in 54 cereal samples. The results revealed that BP, 4-MBP, and BP-3 were the most prevalent. Few studies have reported the levels of BPs in cereal samples [4,13–18]. The results of the present study revealed that BP and 4-MBP were the most frequently detected photoinitiators in the cereal samples, which is in agreement with the results of studies conducted in Belgium [4,16], Germany [13], Spain [14], and Switzerland [18] (Table 5). The BPs levels detected in this study were higher than those measured in Belgium and Spain, which were in the ranges of ND–20 ng/g [16] and 29–40 ng/g [14], respectively, but were lower than those reported in

Table 3. Matrix-matched calibration curve in cereals.

Classification	Compound	Matrix-matched Calibration curve	R^2	Calibration curve in solvent	R^2	ME (%)	LOD (ng/g)	LOQ (ng/g)	RSD		Recovery (%, n=15)
									Intra-day (%, n=5)	Inter-day (%, n=15)	
Oatmeal	BP-2	y = 0.065x-0.0707	0.998	y = 0.0443x-0.0839	0.997	147	0.015	0.045	5.0	7.2	88
	4-OHBP	y = 0.0749-0.0172	0.999	y = 0.0774x-0.0456	0.999	97	0.043	0.129	4.2	6.8	95
	M2BB	y = 0.6794-0.7054	0.998	y = 0.523x-0.9177	0.997	130	0.277	0.831	4.3	8.0	120
	BP-1	y =0.1573x+0.0443	0.999	y = 0.137x-0.1265	0.999	115	0.044	0.132	5.9	8.9	94
	BP-8	y =0.0802x+0.0592	0.999	y =0.0821x+0.0079	0.999	98	0.083	0.249	2.2	3.2	98
	BP	y = 0.116x+0.6518	0.998	y =0.1208x+0.7948	0.999	96	0.001	0.003	11.0	23.9	80
	2-OHBP	y =0.0147x-0.0024	0.999	y = 0.0169-0.0208	0.999	87	0.142	0.426	6.3	6.8	109
	4-MBP	y = 0.0983x+0.448	0.999	y = 0.1103x-0.0595	0.999	89	0.009	0.027	1.4	4.5	120
	BP-3	y = 0.095x-0.0335	0.999	y = 0.1055x-0.0572	0.999	90	0.051	0.153	3.4	6.2	103
	PBZ	y = 0.1251x-0.4051	0.989	y = 0.0439x-0.0727	0.999	285	0.046	0.138	5.0	11.0	121
Corn flakes	BP-2	y = 0.0319x-0.0085	0.999	y = 0.0237x-0.0156	0.999	135	0.015	0.045	5.0	11.6	92
	4-OHBP	y = 0.0312x+0.0227	0.999	y = 0.036x+0.0139	0.999	87	0.051	0.153	2.6	10.0	100
	M2BB	y = 0.1296x-0.0973	0.999	y = 0.1411x-0.0006	0.999	92	0.289	0.867	6.0	11.1	114
	BP-1	y = 0.0658x-0.0256	0.999	y = 0.0612x-0.016	0.999	108	0.043	0.129	1.5	8.1	93
	BP-8	y = 0.0708x+0.0836	0.998	y = 0.0712x+0.0087	0.998	99	0.074	0.222	2.8	11.8	95
	BP	y = 0.1022x-0.232	0.999	y = 0.1026x+0.1438	0.999	100	0.001	0.003	19.0	23.0	103
	2-OHBP	y = 0.0123x-0.0192	0.998	y = 0.0123x-0.0034	0.999	100	0.145	0.435	5.1	12.6	90
	4-MBP	y = 0.0577x-0.015	0.999	y = 0.0532x-0.0216	0.999	108	0.009	0.027	4.8	11.6	115
	BP-3	y = 0.0897x-0.0345	0.999	y = 0.0869x+0.008	0.999	103	0.070	0.210	3.6	8.0	101
	PBZ	y = 0.0391x-0.0302	0.999	y = 0.0525x-0.0238	0.999	74	0.049	0.147	20.8	21.4	79

ME: Matrix effect; Intra- or Inter-day RSD: Precision expressed as intra- or inter-day relative standard deviation of spiked 20 ng/g.
RE: Mean recovery (n = 15) at final spiked level of 20 ng/g for each analyte.

Table 4. BPs levels in cereal samples of oatmeal and corn flakes (ng/g).

Compounds	Oatmeal (n = 25)			Corn flakes (n = 29)			p-value[a]
	DF (%)	Mean (SD)	Range	DF (%)	Mean (SD)	Range	
BP	100	22.8 (11.3)	14.2–67.5	100	146.9 (276.4)	25.3–1083.8	<0.0001
4-MBP	100	5.8 (12.6)	1.2–65.8	100	3.1 (2.4)	1.3–12.0	0.43
BP-3	60	0.2 (0.2)	0.1–1.0	59	1.4 (1.8)	0.8–8.5	<0.0001
M2BB	0	–	–	14	6.1 (7.7)	0.5–17.3	–
4-OHBP	0	–	–	7	6.4 (4.6)	3.1–9.7	–

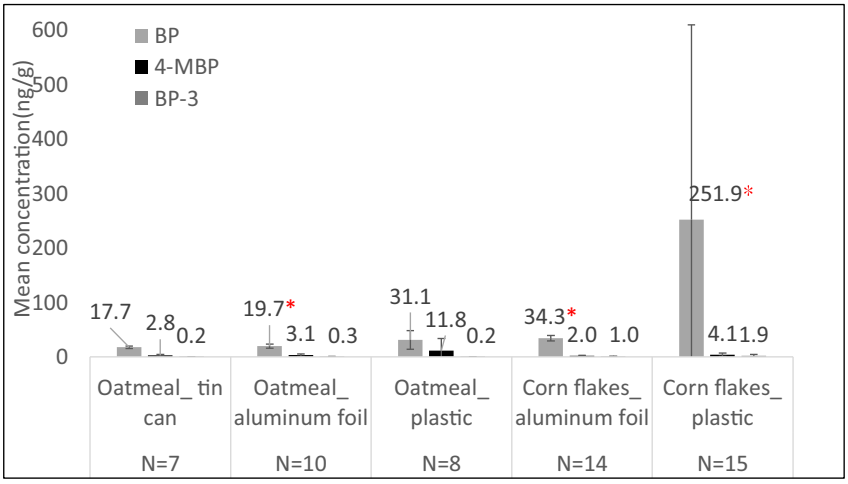
DF: Detection frequency; SD: Standard deviation.
[a] Mann–Whitney U test.

Table 5. Comparisons of BPs levels in breakfast cereals.

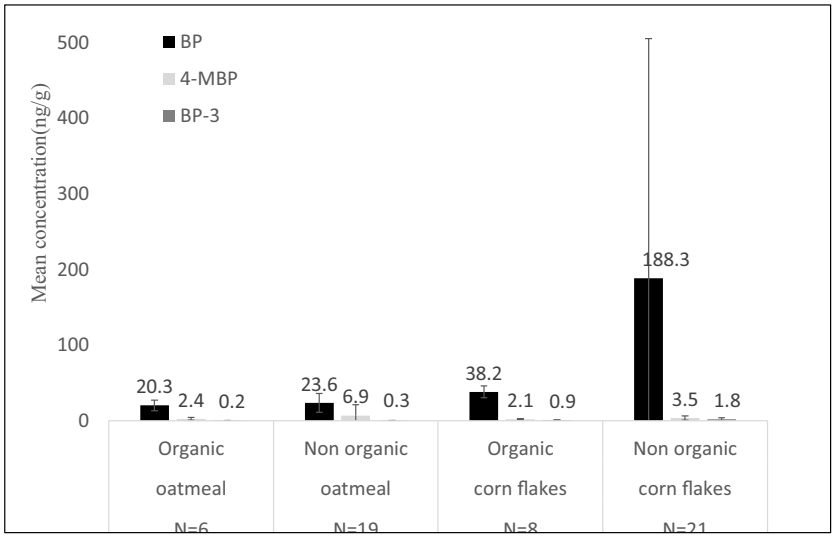
Reference	Country	N	Pretreatment	Instrument	Analyte	Recovery (%)	LOD/LOQ (ng/g)	Range (ng/g)
EFSA (2009)	Belgium	17	ACN extraction	HPLC-MS or GC–MS	4-MBP	–	-/50	795–5400
Hoeck Van et al. (2010)	Belgium	–	DCM & ACN extraction, followed by SPE with HLB cartridge	GC–MS	BP 4-MBP	74 98	2/–	Not available
Gallart-Ayala et al. (2011)	Spain	3	QuEChERS, SPE with HLB cartridge	HPLC-MS/MS	BP PBZ	92–97 88–91	–/2.3 –/0.7	29–40 ND
Jung et al. (2013)	Germany	5	ACN extraction	HPLC-MS/MS	BP 4-MBP	94 89	38/113 2.5/7.5	3367–3413 65–8073
Bugey et al. (2013)	Swiss	82	Pressurized liquid extraction	GC–MS	BP	–	–/60	$5 \times 10^6 - 7 \times 10^6$
Houwe Van Den et al. (2016)	Belgium	49	ACN extraction, followed by SPE with HLB cartridge	UPLC-MS/MS	BP 4-MBP	95 96	0.3/1.6 0.1/0.6	ND-20 Not available
Chang et al. (2019)	Taiwan	5	QuEChERS	UPLC-MS/MS	BP 4-MBP	– 62–120	–/20 –/10	ND ND
This study	Taiwan	54	FaPEx	UHPLC-MS/MS	10 BPs: BP 4-MBP BP-3	62–99	0.001–0.3/0.03–0.8	14–1084 1–66 0.1–8

–: No data; ND: Not detected.

(A) Packaging material



(B) organic or nonorganic contents



(C) Domestic or foreign sources

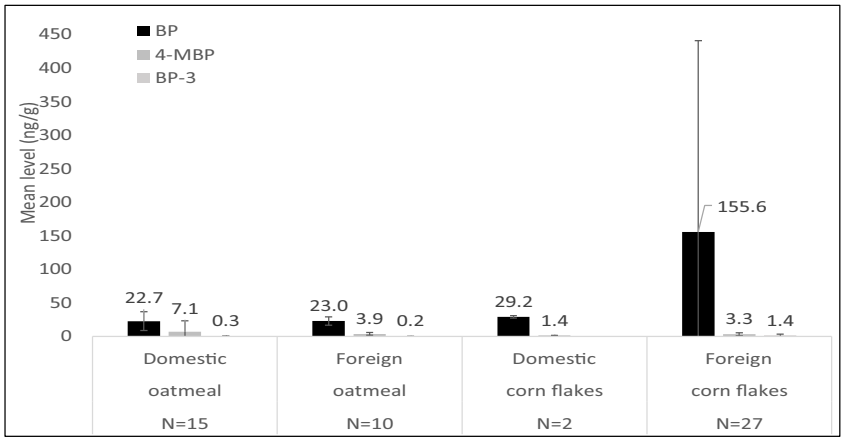
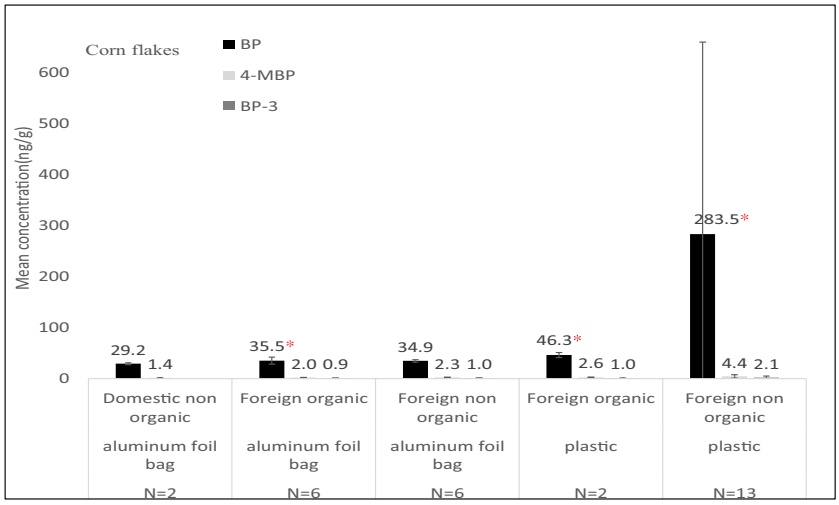


Fig. 4. (A)–(E) Levels of BPs in oatmeal (n = 25) and corn flake (n = 29) samples according to the packaging material, whether the food was organic or nonorganic, and whether the raw materials were from domestic or foreign sources. $p < 0.05$.*

(D) Source, packaging material, and (non-)organic contents in corn flakes



(E) Source, packaging material, and (non-)organic contents in oatmeal

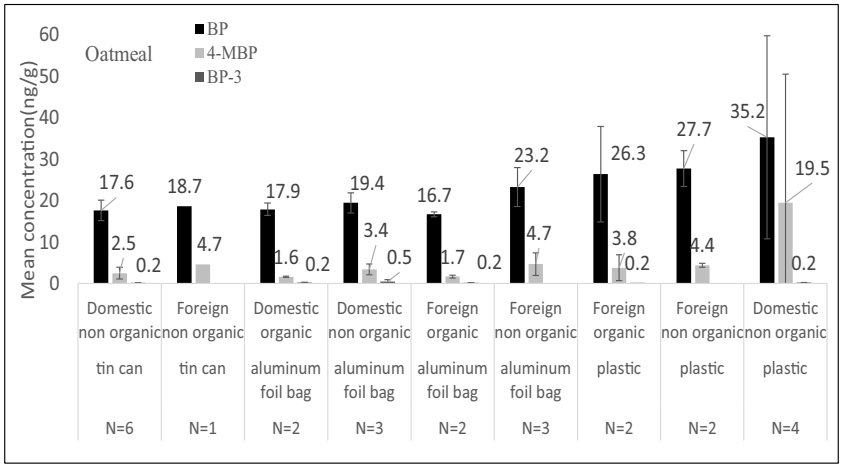


Fig. 4. (Continued).

Switzerland and Germany, which were in the ranges of 5–7 × 10^6 ng/g [18] and 3367–3413 ng/g [13] respectively. The 4-MBP levels detected in this study were lower than those reported in Belgium and Germany, which were in the ranges of 795–5400 ng/g [4] and 65–8073 ng/g [13], respectively. The use of printed cardboard with additional plastic wrapping as the packaging material and food contamination from packaging may contribute to the ubiquitously high levels of BP or 4-MBP in cereals [13,18].

4.3. Relationships between BPs levels and packaging materials

Fig. 4 illustrates the BP, 4-MBP, and BP-3 levels in the cereal samples according to the packaging material, whether the food was organic or nonorganic, and whether the raw materials were from domestic or foreign sources. When stratified according to the packaging material, corn flake samples with plastic packaging exhibited a significantly higher mean BP level (251.9 ng/g) than the mean BP levels of corn flake and oatmeal samples with aluminum foil packaging (34.3 and 19.7 ng/g, $p = 0.036$ and $p = 0.046$, respectively; Fig. 4A). This finding is in partial agreement with the previous findings [3,4] that BP levels are high in packaging materials. The mean BP levels in the samples did not differ significantly between organic and nonorganic samples or between samples with foreign and domestic ingredients (Fig. 4B, C). In addition, the mean BP level was significantly higher in nonorganic corn flake samples from foreign sources with plastic packaging (283.5 ng/g) than in organic samples with plastic or laminated aluminum foil packaging (46.3 and 35.5

ng/g, $p = 0.029$ and $p = 0.036$, respectively; Fig. 4D). No significant difference was observed in the mean BP levels of oatmeal samples with respect to the source of ingredients (foreign or domestic), type of packaging material, or status as organic or nonorganic food (Fig. 4E).

As indicated in Table S1, two samples of six-grain muesli contained significant levels of BP (1084 and 1055 ng/g); these samples were nonorganic and packaged in a polylactide bag, with raw material sourced from Germany. The highest 4-MBP level (66 ng/g) was observed in a nonorganic sample of fine oat flakes with plastic packaging sourced from Taiwan. The composition of the plastic bags was unknown. No sample had cardboard packaging, and we did not analyze the packaging material to evaluate whether the BPs were from the packaging material. Overall, however, our data indicate that BP and 4-MBP levels may be related to the food's packaging material and whether it is organic.

5. Conclusions

This is the first time that a FaPEx UHPLC–MS/MS method has been successfully employed for the simultaneous determination of the levels of 10 BPs in breakfast cereal samples. The method demonstrated satisfactory results, with a low LOD below the nanogram per gram level and high precision and recovery for BP analysis in oatmeal and corn flakes. BP and 4-MBP were the most abundant BPs in the analyzed samples, and the highest BP levels were observed in two samples of nonorganic six-grain muesli in plastic packaging. The observation of trace levels of BP-3 in the samples indicates the need for the development of analytical methods with high specificity and sensitivity, and the proposed method meets this requirement. Taken together, our findings indicate that the FaPEx UHPLC–MS/MS method is a fast, simple, and robust approach for the analysis of BPs in cereal samples.

Conflicts of interest

None.

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Appendix A. Supplementary material

Table S1. Overview of the samples and their grouping according to their type of by packaging material, their status as an organic or nonorganic food product, and the source of their raw materials (domestic or foreign)

No.	Sample type	Package type[a]	Sample come from	Organic/ Nonorganic	Sampling date	Analytes (ng/g)				
						BP	4-MBP	BP-3	M2BB	4-OHBP
1	Oatmeal	Tin can	Domestic	Nonorganic	10.01.2020	21.24	3.44	0.19	ND	ND
2	(n = 25)	(n = 7)	Domestic	Nonorganic	10.01.2020	18.39	4.76	ND	ND	ND
3			Domestic	Nonorganic	10.01.2020	15.48	1.21	0.12	ND	ND
4			Domestic	Nonorganic	10.01.2020	14.41	1.29	0.16	ND	ND
5			Domestic	Nonorganic	10.01.2020	18.80	2.72	0.18	ND	ND
6			Domestic	Nonorganic	10.01.2020	17.25	1.60	0.13	ND	ND
7			Foreign	Nonorganic	10.01.2020	18.65	4.65	ND	ND	ND
8		Aluminum foil bag	Domestic	Nonorganic	10.02.2020	18.22	1.91	0.26	ND	ND
9		(n = 10)	Domestic	Nonorganic	10.02.2020	17.88	4.22	0.25	ND	ND
10			Domestic	Nonorganic	10.02.2020	22.23	4.08	1.02	ND	ND
11			Domestic	Organic	10.02.2020	16.80	1.52	0.18	ND	ND
12			Domestic	Organic	10.02.2020	18.96	1.77	0.27	ND	ND
13			Foreign	Organic	10.02.2020	17.06	1.45	0.15	ND	ND
14			Foreign	Organic	10.02.2020	16.26	1.90	0.15	ND	ND
15			Foreign	Nonorganic	10.02.2020	28.10	7.85	ND	ND	ND
16			Foreign	Nonorganic	10.02.2020	18.76	2.94	ND	ND	ND
17			Foreign	Nonorganic	10.02.2020	22.83	3.33	ND	ND	ND
18		Plastic bag	Foreign	Organic	10.02.2020	34.47	6.02	ND	ND	ND
19		(n = 8)	Foreign	Organic	10.02.2020	18.22	1.59	0.16	ND	ND
20			Foreign	Nonorganic	10.02.2020	24.67	4.74	ND	ND	ND
21			Foreign	Nonorganic	10.02.2020	30.73	4.10	ND	ND	ND
22			Domestic	Nonorganic	10.02.2020	40.62	3.76	0.26	ND	ND
23			Domestic	Nonorganic	10.02.2020	67.51	65.80	0.22	ND	ND
24			Domestic	Nonorganic	10.02.2020	18.47	7.01	ND	ND	ND
25			Domestic	Nonorganic	10.02.2020	14.19	1.32	ND	ND	ND

(continued on next page)

Table S1. (continued)

No.	Sample type	Package type[a]	Sample come from	Organic/ Nonorganic	Sampling date	Analytes (ng/g)				
						BP	4-MBP	BP-3	M2BB	4-OHBP
26	Corn flakes	Aluminum foil bag	Foreign	Nonorganic	10.01.2020	35.03	2.65	0.96	ND	ND
27	(n = 29)	(n = 14)	Foreign	Nonorganic	10.01.2020	32.29	2.32	0.99	ND	ND
28			Foreign	Nonorganic	10.01.2020	33.93	2.29	ND	ND	ND
29			Foreign	Nonorganic	10.01.2020	36.29	2.16	ND	ND	ND
30			Foreign	Nonorganic	18.02.2020	38.58	2.89	1.00	ND	ND
31			Foreign	Nonorganic	18.02.2020	33.05	1.54	ND	ND	ND
32			Domestic	Nonorganic	10.01.2020	27.96	1.34	ND	ND	ND
33			Domestic	Nonorganic	10.01.2020	30.52	1.45	ND	ND	ND
34			Foreign	Organic	18.02.2020	41.72	2.04	1.00	ND	9.66
35			Foreign	Organic	18.02.2020	34.67	1.59	0.94	ND	ND
36			Foreign	Organic	18.02.2020	43.49	2.45	0.93	ND	ND
37			Foreign	Organic	18.02.2020	25.29	1.36	0.84	ND	ND
38			Foreign	Organic	18.02.2020	37.39	2.63	ND	ND	ND
39			Foreign	Organic	18.02.2020	30.38	1.90	0.98	ND	ND
40		Plastic bag	Foreign	Nonorganic	18.02.2020	550.40	9.59	8.53	17.33	ND
41		(n = 15)	Foreign	Nonorganic	18.02.2020	43.49	2.71	0.96	ND	ND
42			Foreign	Nonorganic	18.02.2020	182.18	12.04	1.03	ND	ND
43			Foreign	Nonorganic	18.02.2020	51.26	3.10	ND	1.60	3.11
44			Foreign	Nonorganic	18.02.2020	104.91	5.49	ND	ND	ND
45			Foreign	Nonorganic	18.02.2020	39.00	2.28	0.93	ND	ND
46			Foreign	Nonorganic	18.02.2020	63.96	4.46	0.91	ND	ND
47			Foreign	Nonorganic	18.02.2020	55.06	3.33	ND	ND	ND
48			Foreign	Nonorganic	18.02.2020	56.00	4.97	1.25	0.52	ND
49			Foreign	Nonorganic	18.02.2020	123.07	3.80	1.19	4.79	ND
50			Foreign	Nonorganic	18.02.2020	277.52	1.59	ND	ND	ND
51			Foreign	Nonorganic	18.02.2020	1055.44	1.40	ND	ND	ND
52			Foreign	Nonorganic	18.02.2020	1083.84	2.25	ND	ND	ND
53			Foreign	Organic	18.02.2020	49.89	2.81	1.06	ND	ND
54			Foreign	Organic	18.02.2020	42.79	2.34	0.86	ND	ND

[a]The numbers of samples for tin can, laminated aluminum foil bag, and plastic bag packaging materials were 7, 10, and 8 for oatmeal and 0, 14, and 15 for corn flakes, respectively.

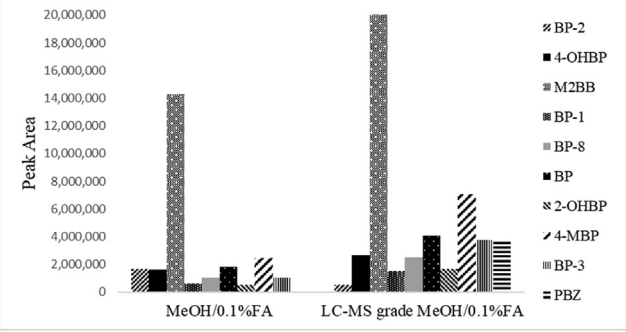

Fig. S1. Comparison of the peak areas in BPs standards of 50 pg/μL between HPLC or LC–MS grade MeOH containing 0.1% formic acid and water.

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