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Analytical Methods

Electromembrane extraction of diamine plastic restricted substances in soft drinks followed by capillary electrophoresis with contactless conductivity detection

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ABSTRACT

Ethane-1,2-diamine (EA) and hexane-1,6-diamine (HA) are two important plastic restricted substances commonly existing in food contact materials. A capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D) method has been developed for direct determination of above analytes, and the detection sensitivity has been significantly improved based on electromembrane extraction (EME). Under the optimum conditions, EA and HA could be well separated from their aliphatic diamine homologs as well as the common inorganic cations within 25 min. The limits of detection could reach sub-ng/mL level, and good linearity ($r > 0.998$) between peak area and analyte concentration could be obtained at three orders of magnitude. This EME/CE-C^4D method provided a novel application for determining these plastic restricted substances in different bottled soft drinks, providing an alternative for the sensitive analyses of diamine substances.

1. Introduction

With the enhancement of human health awareness, food safety has become one of the most important focal points around the world, among which the safety of food contact materials has being attracted much attention. Plastic is one kind of the most common materials used for food packaging due to its economy and convenience. However, some ingredients in plastic can contaminate food, and thereby affect human health. For example, ethane-1,2-diamine (EA) and hexane-1,6-diamine (HA) are two important raw materials for the production of plastics. Some researches have shown that excessive amounts of many compounds containing amino groups are harmful to health, for instance, strongly stimulating the eyes, respiratory tract mucosa and skin, and some might be the precursors of nitrosamines, many of which are known carcinogens (Beard & Noe, 1981). European Union (European Union Commission., 2011) and Korea (Korea Food, 2011) have limited the specific migration contents to 12.00 mg/L and 2.40 mg/L for EA and HA, respectively. Therefore, it is meaningful to develop a quick and sensitive method for the determination of diamine plastic restricted substances.

Since most compounds with amino group, particular for aliphatic amines, lack chromophores, the common methods widely used for amino-compound analyses are based on derivatization and chromatographic separation procedures followed by ultraviolet, laser-induced fluorescence (LIF), electrochemical or mass spectrometry detection (Almeida, Fernandes, & Cunha, 2012; Deng, Wang, & Zhang, 2010; Huang et al., 2009; Zhang, Liu, Wang, & Cheng, 2004). In China, GC-hydrogen flame ionization detector integrated with ethyl chloroformate derivatization has been recommended as the national standard method (GB/T 23296.17-2009) to determine EA and HA in food simulants. Capillary electrophoresis (CE) is one of the most powerful separation techniques due to its distinct advantages such as low running cost and environmental friendliness (Kvasnicka, 2007). To prevent long reaction time, tedious processes, and side products associated with derivatization, several direct methods have been carried out for this purpose by CE combined with amperometric detection (AD) (Ge et al., 2015; Li, Ge, Pan, Ye, & Chu, 2012; Sun, Yang, & Wang, 2003; Wang et al., 2003), contact conductivity detection (CCD) (Kvasnicka & Voldrich, 2006), and capacitively coupled contactless conductivity detection (C^4D) (Gong & Hauser, 2006; Li et al., 2014; Liu et al., 2014). Among these detectors, C^4D has been considered as an universal detection technique for CE since it effectively avoids the electrode surface fouling, isolates itself from high separation volt-

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age, and simplifies the detector design and electrode alignment (Fracassi da Silva & do Lago, 1998; Zemann, Schnell, Volgger, & Bonn, 1998). These direct methods evade derivatization procedure and greatly shorten the analytical time, however, the limits of detection (LODs) of most above methods are relatively high, which could not meet the requirements of trace analysis in complex system.

Electromembrane extraction (EME) (Pedersen-Bjergaard & Rasmussen, 2006) is proposed as a new concept for analytical sample preparation. Compared with other extraction methods including liquid-liquid extraction, solid-phase extraction and hollow-fiber liquid-phase microextraction, EME could provide much easier operation, lower consumption of organic solvents and analytical cost, or shorter extraction time. So, EME has been gradually applied to purification and concentration the target analytes in pharmaceutical, environmental and biological samples (Costa, 2014; Yamini, Seidi, & Rezazadeh, 2014). In our previous work, CE-C^4D coupled with EME has been used for monitoring several typical polyamines in saliva (Liu et al., 2014) and haloacetic acids in drinking water samples (Zhang et al., 2015).

In this work, a newly developed EME/CE-C^4D method has been applied for sensitive determination of two diamine plastic restricted substances, EA and HA, in soft drinks. The target analytes were firstly extracted from the sample solution (7 mL, donor phase), through the supported liquid membrane (SLM), and then into an acceptor phase (~8 μL). The extracted solution could be directly analyzed by CE-C^4D, avoiding derivatization process. Various parameters affecting extraction efficiency, electrophoretic separation and detection were investigated, and the proposed method has been applied to determining the target analytes in bottled soft drinks including purified water, mineral water, carbonated and tea beverages.

2. Materials and methods

2.1. Reagents and samples

The standard compounds and organic solvents including bis (2-ethylhexyl) phosphate (DEHP), 1-ethyl-2-nitrobenzene (ENB) and 2-Nitrophenyl octylether (NPOE) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid (HAc), chloride salts and 18-crown-6 were purchased from J&K Chemical (Shanghai, China). All chemicals were of analytical grade, and deionized water with resistivity higher than 18 MΩ·cm was used in this work. The stock solution of each analyte (1.0 mg/mL) was prepared with deionized water. A fresh mixture standard solution was prepared daily by diluting the stock solution with running buffer (0.10 mol/L 18-crown-6/0.60 mol/L HAc buffer) to the desired concentration. Before use, all solutions were stored in a 4 °C refrigerator.

Bottled soft drinks were purchased from supermarkets (Shanghai, China). Four different kinds of bottled soft drinks, including purified water, mineral water, carbonated and tea beverages, totally 12 samples, were selected for model samples in this experiment. Purified water and mineral water need no any additional pre-treatment. Since carbonated and tea beverages are weak acid solutions, the samples should be adjusted with 0.10 mol/L NaOH to achieve neutralization before EME procedure. Each sample was conducted triple tests in parallel.

2.2. Electrophoretic conditions

The laboratory-built CZE-C^4D system was employed as described previously (Liu et al., 2014). The excitation frequency and amplitude of C^4D were set at 550 kHz and 80 Vpp (peak-to-peak voltage), respectively. The effective length of the capillary tube (23.5 μm id × 360 μm od, Polymicro Technologies, Phoenix, AZ, USA) was 88.0 cm to C^4D. The running buffer was 0.10 mol/L 18-crown-6/0.60 mol/L HAc buffer. The separation voltage was 16 kV and the injection time was 6 s (at 16 kV). All experiments were performed at room temperature. (The typical electropherogram of a mixture standard solution of two analytes and the main co-existing interferences was shown in Fig. S1 of 'Supplementary Material'.)

2.3. EME equipment and procedure

The equipment and extraction principle for EME were the same as described previously (Liu et al., 2014). (The illustration of EME device was shown in Fig. S2 of 'Supplementary Material'.) The primary optimization of EME procedure was conducted using a unified mixture standard solution. 7 mL of deionized water containing two diamines (5.0 ng/mL each) was added into the glass vial as the donor solution, the SLM consisted of the mixture organic phase (v_{DEHP}: v_{ENB} = 9:91), and about 8 μL of 4.0 mmol/L HCl solution was filled into hollow fiber as the acceptor phase. Two platinum electrodes were carefully inserted into the hollow fiber and the donor phase, respectively, acting as the cathode and anode. The magnetic stirrer was switched on to start the extraction at 500 rpm. The EME system was operated at 80 V by a power supply for 15 min. Subsequently, the magnetic stirrer and power supply were switched off, and the hollow fiber was taken out from the glass vial. The acceptor solution in the hollow fiber was withdrawn into a syringe, and ready for electrophoretic analysis.

3. Results and discussion

3.1. Optimization of EME procedure

To investigate the enrichment factors (EFs) of the target analytes, various parameters were optimized based on a univariate approach. The EF value was calculated according to the following equation: $EF = c_{a,final}/c_{d,initial}$, where $c_{d,initial}$ and $c_{a,final}$ were the initial concentration of the target analyte in donor solution and the final concentration in acceptor phase, respectively.

3.1.1. SLM composition

Since ENB could provide more efficient purification in preventing co-extracting cations across the SLM (Liu et al., 2014), the effects of different compositions of DEHP and ENB on the extraction efficiency were further investigated in this work (as shown in Fig. S3A of 'Supplementary Material'). The EFs of two diamines were firstly increased with increasing DEHP percentage in ENB; when the percentage reached to 9%, EFs of EA and HA could achieve the highest values; as the percentage continuously increased, EFs became decreased, which might be caused by the strong interaction of the ion-pair complex of target analytes with the organic phase (Gjelstad, Rasmussen, & Pedersen-Bjergaard, 2006). Consequently, ENB containing 9% DEHP was used as the SLM for next studies.

3.1.2. pH values of donor and acceptor phases

Since the pK_a values of two diamines are approximately 10.71, 7.56 (EA), and 11.86, 10.76 (HA), respectively, the pH value in donor phase should be lower than their pK_a values to attain the complete ionization of the basic analytes in EME progress (Liu et al., 2014). The effects of various pH values of acid solutions as well as neutral deionized water on EFs of the target analytes were examined as shown in Fig. 1A, and the results showed that neutral deionized water (pH = 7.00) provided highest EFs for all analytes. Possible explanation was the addition of HCl caused the increase

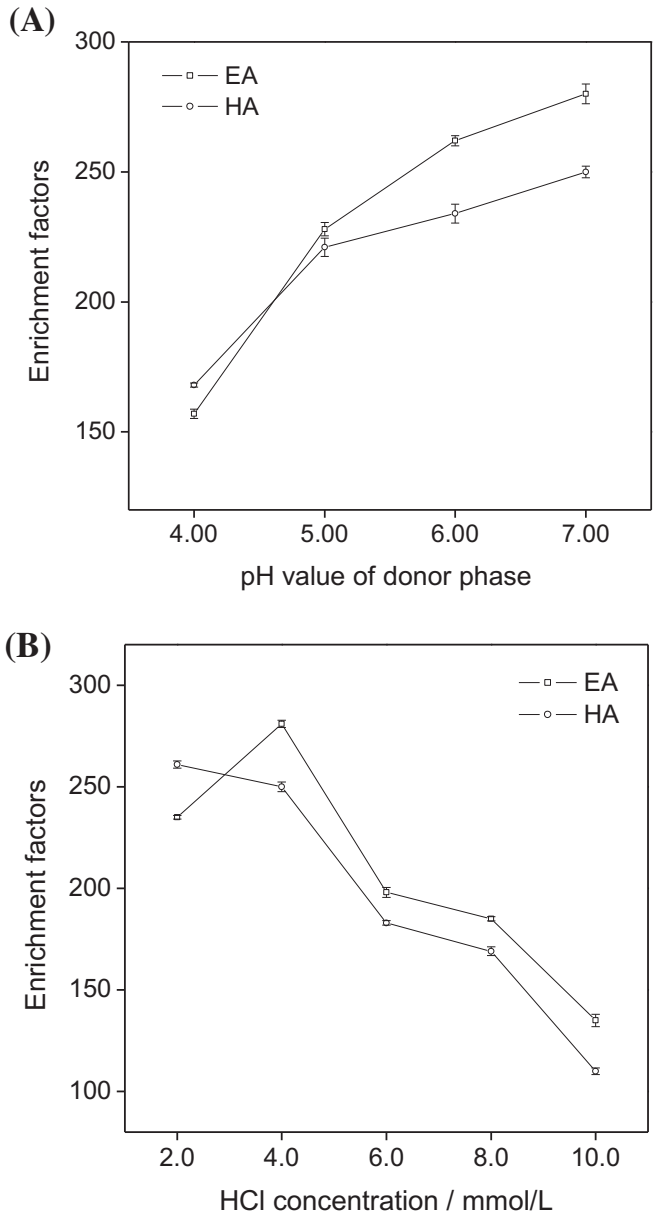

Fig. 1. Effects of (A) the pH value of donor phase, and (B) HCl concentration in acceptor phase on EFs of the target analytes. Other EME conditions: donor phase: neutral deionized water; SLM: v_{DEHP}: v_{ENB} = 9:91; acceptor phase: 4.0 mmol/L HCl; stirring speed: 500 rpm; extraction voltage: 10 V; extraction time: 10 min; concentration of two diamines: 5 ng/mL each.

of ionic strength of the donor phase, and then blocked the phase transfer of EA and HA, resulting in negative effect on extraction, while neutral deionized water could provide enough protonation and better phase migration for EA and HA. Therefore, neutral deionized water was selected as diluent for standard analytes for subsequent investigation.

In order to make the ion balance benefit for the phase transfer of the target analytes, the pH value of the acceptor phase should be lower than that of the donor phase (Kuban, Slampova, & Bocek, 2010). So, the effects of HCl and HAc solution as acceptor phase on EFs were studied, respectively. The results showed that under the same pH value, HCl solution could provide much sharper peak shape than HAc solution; when 4.0 mmol/L HCl was used as the acceptor phase, two analytes could gain relative high enrichment efficiency as shown in Fig. 1B. Besides, the experimental results showed that addition of 18-crown-6 benefitted the enrichment of EA, while HA got the opposite effect. So, 4.0 mmol/L HCl was selected as the optimum acceptor phase considering the EFs of both analytes.

Besides, extraction voltage, stirring speed and extraction time were also investigated, respectively. Through the above optimization experiments, the optimum EME conditions for the samples with weak ionic strength were as follows: donor phase: neutral deionized water, SLM: $v_{DEHP:}$ v_{ENB} = 9:91, acceptor phase: 4.0 mmol/L HCl, extraction voltage: 80 V, stirring speed: 500 rpm, and extraction time: 15 min. Under the optimum EME conditions, the EFs could achieve 785-fold (EA) and 675-fold (HA) in deionized water, respectively.

3.1.3. Sample matrix

Since mineral water and beverage samples contain various ions and possess much higher ionic strength, the effects of sample matrix on the extraction efficiency were also investigated in different real samples. The experimental results showed that the sample matrix has a large influence on extraction voltage. When the applied voltage exceeded 10 V, the donor phase was prone to produce bubbles, resulting in the instability of SLM. Therefore, considering the extraction efficiency and the stability of SLM, 10 V was selected for extraction of the samples with higher ionic strength, i.e. the mineral water and beverages tested in this work, and other extraction conditions were the same as above optimum EME parameters.

3.2. Method validation

3.2.1. Linearity, LODs and limits of quantification (LOQs) of the analytes

In considering the influence of sample matrix, the regression analyses of the target analytes were carried out in different sample matrices. So, a series of different concentrations of the target analytes (0.20 ng/mL ~ 0.10 μg/mL) were tested in four selected sample matrices (namely purified water, mineral water, carbonated beverage and tea beverage), respectively, in order to detect the linearity of two diamines. And each experimental concentration was conducted triple tests in parallel. The correlation between peak area and concentration of each analyte was subjected to regression analysis, and the calibration equations and correlation coefficients were listed in Table 1. The results showed that two diamines could be pre-concentrated up to 718-fold (EA) and 660-fold (HA) in purified water sample based on EME procedure, and the LODs and LOQs could achieve 0.038 ng/mL (S/N = 3) and 0.13 ng/mL (S/N = 10), respectively. Although the EFs could only obtain 62 ~ 234-fold in the mineral water and beverage samples due to the matrix effects of co-extraction inorganic cations, the LODs could still achieve the level of 0.1 ng/mL, which are far lower than the maximum values of the provision. (The comparison of this method with the reported methods for the determination of amine-group compounds were listed in Table S1 of 'Supplementary Material'.).

3.2.2. Precision

The reproducibility of this CE-C^4D method was evaluated by intraday and interday precision at three different concentrations (0.5, 5.0 and 20 μg/mL), respectively. The relative standard deviation (RSD) was used as a measure of precision (as shown in Table S2 of 'Supplementary Material'). The assay results showed that the RSDs of peak area and migration time for intraday precision (n = 7) were within 2.0% and 1.0%, respectively, and the interday precision were within 3.8%. Furthermore, EME reproducibility was also evaluated using the tested samples, and the spiked concentration of each target analyte was 5.0 ng/mL in the drinking

Table 1
The regression equations, linearity, LODs, LOQs and the repeatability data of two diamines.

Samples		Analytes	r	Linear range (ng/mL)	LODs (ng/mL)	LOQs (ng/mL)	EFs	RSD[a] (n = 5%)	
								Peak area	Migration time
Drinking water	Purified water	EA	0.9993	0.20–10	0.038	0.13	718	3.8	1.5
		HA	0.9992	0.20–10	0.040	0.13	660	5.2	2.1
	Mineral water	EA	0.9997	0.50–50	0.12	0.40	234	4.5	2.0
		HA	0.9992	0.50–50	0.14	0.47	217	5.0	2.4
Beverages	Carbonated beverage	EA	0.9998	2.0–100	0.35	1.2	82	6.6	1.7
		HA	0.9995	2.0–100	0.25	0.83	145	5.8	2.3
	Tea beverage	EA	0.9997	2.0–100	0.39	1.3	62	5.4	1.9
		HA	0.9991	2.0–100	0.32	1.1	85	6.1	2.7

[a] The spiked concentration of the target analytes was 5.0 ng/mL in drinking water and 10 ng/mL in the tested beverages, respectively.

water samples and 10 ng/mL in the tested beverages, respectively. As shown in Table 1, the RSDs of peak area and migration time for two diamines were in the range of 3.8 ~ 6.6% and 1.5 ~ 2.7%, respectively, indicating the EME/CE-C^4D method could provide relatively good repeatability.

3.2.3. Accuracy

To further evaluate the reliability of this proposed method, recovery experiments were also performed by a standard addition method with the real samples (The recovery data were listed in Table S3 of 'Supplementary Material'). The average recovery data at three different concentrations were in the range of 83–113% with corresponding RSDs of 1.9–4.6%, indicating that this EME/CE-C^4D method was accurate enough for the determination of EA and HA.

3.3. Analyses of real samples

Under the optimum conditions, the proposed EME/CE-C^4D method was applied to determining the diamine plastic restricted substances in bottled soft drinks including purified water, mineral water, carbonated beverage and tea beverage. The typical sample electropherograms were shown in Fig. 2 A-B, respectively. In Fig. 2, the electropherograms labeled with 'a' represented blank samples, and those labeled with b and c were the spiked samples with different concentrations. By a standard addition method and comparing the migration times of target analytes with those of the mixture standard solution (as shown in Fig. S1), EA and HA were determined in different soft drinks. From the sample electropherograms, we can see that EME could provide good purification for complex samples, and the target analytes could be well separated from the main co-extraction substances in the real samples under the selected experimental conditions.

The test data for the bottled drink samples were summarized in Table 2. The results showed that the detection contents of the target analytes in the bottled drinking water samples were in the range of 0.14–0.98 ng/mL, which were consistent with the reported values (0.08–0.47 ng/mL in bottled drinking water) based on online preconcentration/CE-AD (Ge et al., 2015) which has no response to inorganic cations and anions, and this fact could further indicate that the potential coexisting inorganic mental ions or cations did not affect the determination of the target analytes; the analyte contents detected in the bottled soft beverage samples were in the range of 1.7–3.0 ng/mL, which were obviously higher than those in above bottled drinking water samples (0.14–0.98 ng/mL). A possible explanation was that the soft beverage is often weakly acidic, which makes the diamines more easily to release from the plastic packaging materials. Besides, the detected values of the target analytes in the tested samples were much lower than the maximum values of the provision.

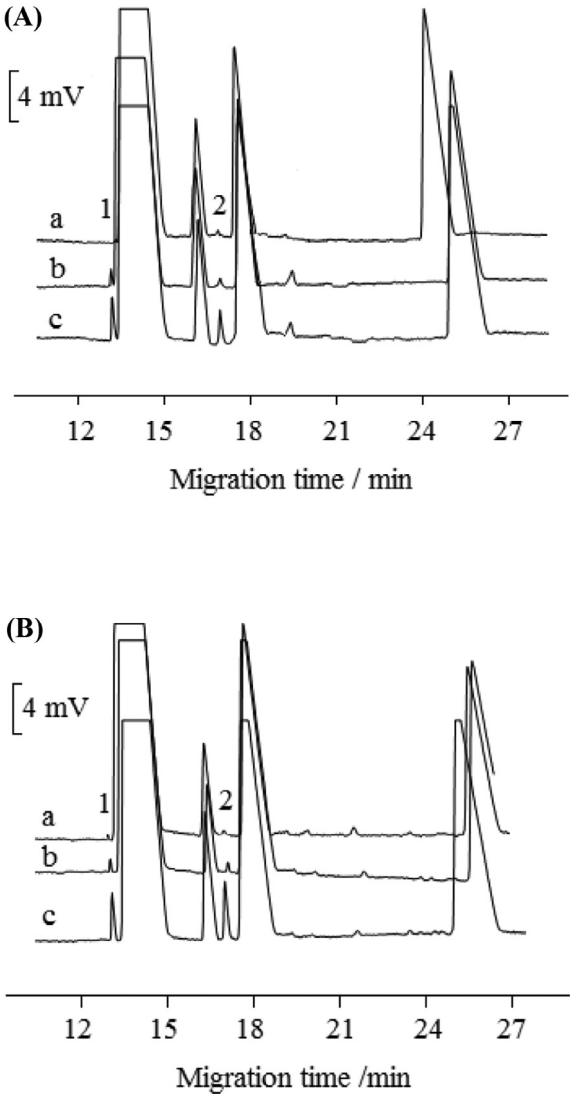

Fig. 2. Typical sample electropherograms of (A) bottled mineral water (a. blank sample, b. spiked sample with 1.0 ng/mL, and c. spiked sample with 5.0 ng/mL) and (B) bottled carbonated beverage (a., blank sample b. spiked sample with 5.0 ng/mL, and c. spiked sample with 20 ng/mL). CE-C^4D conditions: effective length of the capillary tube (23.5 μm id × 360 μm od): 88.0 cm for C^4D; excitation frequency of C^4D: 550 kHz; peak-to-peak voltage of C^4D: 80 Vpp; running buffer, 0.10 mol L^{-1} 18-crown-6/0.60 mol L^{-1} HAc buffer; separation voltage, 16 kV; injection time, 6 s (at 16 kV); and peak identifications, (1) EA, (2) HA. Extraction voltage for purified water (A) was 80 V, extraction voltage for other samples was 10 V, extraction time was 15 min, and other EME conditions were the same as those in Fig. 1.

Table 2
Assay results of diamine plastic restricted substances in real samples ($n = 3$).[a]

Samples		Numbers	EA (ng/mL)	HA (ng/mL)
Drinking water	Purified water	NO.1	0.26	0.57
		NO.2	0.34	0.14
		NO.3	0.14	0.30
	Mineral water	NO.1	0.30	0.98
		NO.2	0.40	/
		NO.3	/[b]	0.51
Beverages	Carbonated beverage	NO.1	1.7	2.1
		NO.2	/	/
		NO.3	2.4	/
	Tea beverage	NO.1	2.5	2.2
		NO.2	/	3.0
		NO.3	/	/

[a] EME/CE-C^4D conditions were the same as those in Fig. 2.
[b] '/' meant the content of the target analyte in the tested sample was lower than the LOD value of this method.

Above experimental results indicated that this proposed method was applicable to the analyses of bottled soft drinks, which was suitable not only for water samples with lower ionic strength (such as purified drinking water), but also for those with higher ionic strength (such as mineral water and beverages). In considering the fact that pH and ionic strength significantly influence on the enrichment efficiency of EME of diamines, appropriate pH regulation was required to optimize for specific samples in order to maintain the pH value of the tested samples at 7.00, and the regression analyses of the target analytes should be carried out in the corresponding sample matrix in order to ensure reproducibility and recovery.

4. Conclusion

Summing up, this work investigated the migration contents of EA and HA in bottled soft drinks from plastic containers by the developed EME/CE-C^4D method. EME procedure could provide better purification and relatively higher EFs than online field amplified sample stacking technology (Ge et al., 2015) for complex samples. This proposed method could attain not only equivalent or superior LODs for aliphatic diamines, but also relatively good recoveries ($83 \sim 113\%$) for the bottled soft drink samples. The EME/CE-C^4D method provides a fast and direct approach for sensitive detection of diamine plastic restricted substances, which could be conveniently used in food safety analysis.

Conflict of interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.11.084.

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