separations

MDPI

Article

Green Chemistry Method for Analyzing Bisphenol A in Milk

Angela M. Encerrado Manriquez [1] and Wen-Yee Lee [2,*]

[1] Department of Environmental Toxicology, University of California, Davis, CA 95616, USA; amencerrado@ucdavis.edu
[2] Department of Chemistry and Biochemistry, The University of Texas at El Paso, El Paso, TX 79968, USA
* Correspondence: wylee@utep.edu; Tel.: +1-915-747-8413

Abstract: A simple, fast, green, and sensitive method for determining Bisphenol A (BPA) levels in commercial milk was developed using a solventless sample preparation technique known as stir bar sorptive extraction, coupled with thermal desorption–gas chromatography/mass spectrometry. BPA was selected due to its ubiquitous presence in the environment and its classification as an endocrine-disrupting chemical of concern (i.e., its ability to mimic hormone functions). Studies have reported that BPA can leach into various food sources, including milk, a dietary staple for infants. It is critical to have an effective and efficient process for monitoring the presence of BPA in milk to protect children's health. Current detection methods for BPA in milk are lengthy and tedious and tend to require the use of organic solvents for the extraction of BPA. This optimized "green" method provides an effective alternative for BPA detection in a challenging matrix, e.g., milk. Factors such as pH (1.5, 6, and 13), temperature (70–80 °C), and sonication (1 h, 2 h, and 3 h) were studied with a BPA-spiked whole milk sample (final concentration of 8 ppb) to optimize the extraction efficiency without the use of solvents. The developed methodology improves BPA recovery from whole milk by over 50%, with a detection limit in the parts per trillion range (45 ng/L). The sample preparation developed in this report rendered a more sensitive option for analyzing BPA in milk, with a limit of detection in the parts per trillion range (compared to low ppb) even though the recovery performance is not as good as in reported studies (54% vs. >85%); nonetheless, it provides a green alternative for future studies assessing BPA exposure through dairy products.

Keywords: Bisphenol A; milk; green chemistry; SBSE; GC/MS; method development

Academic Editor: Victoria Samanidou

Received: 13 December 2024
Revised: 15 January 2025
Accepted: 22 January 2025
Published: 25 January 2025

Citation: Encerrado Manriquez, A.M.; Lee, W.-Y. Green Chemistry Method for Analyzing Bisphenol A in Milk. *Separations* **2025**, *12*, 25. https://doi.org/10.3390/separations12020025

1. Introduction

Bisphenol A (4,4′-isopropylidenediphenol, 2,2′-bis(4-hydroxyphenyl)-propane, BPA) is a compound with extensive applications in the plastics industry. It can be found in polycarbonate plastics used for reusable bottles and food storage containers, polymers for medical devices, epoxy-based can coatings, and water supply pipes [1–3]. The wide applications have made BPA a mass-produced chemical worldwide, with over six million tons produced each year [4]. As a result, BPA is constantly released into the environment by humans, animals, and industrial activities [1]. Even though BPA is normally found in low concentrations, its ubiquity boosts the likelihood of contact through enhanced exposure routes, such as ingestion (drinking water and food), inhalation (atmosphere and dust), and transdermal (recreational water and potable water) [1,5–7]. Furthermore, BPA is an endocrine-disrupting chemical (EDC) as it can mimic hormone function in the body [8]. Studies have indicated that diet is the main source of exposure to EDCs such as BPA among children, who are especially vulnerable due to their undeveloped endocrine systems [9].

After ingestion, BPA has an estimated half-life of 6 h in the blood, then is excreted from the organism through pathways such as urine, human colostrum, breast milk, and saliva, while a part of the BPA is distributed to body storage areas, such as adipose tissue, where is then slowly released [10]. Nevertheless, the countless sources of exposure create continuous exposure to low doses of BPA in humans [9]. Consequently, it is estimated that more than 95% of the population have BPA in their bodies [11].

Currently, the use of BPA in the manufacture of polycarbonate drinking cups or feeding bottles intended for infants and young children is banned under EU Regulations, and the European Food Safety Authority (EFSA) has set a tolerable daily intake (TDI) level of 0.2 ng/kg bw per day, which is a reduction of a factor of 20,000 from the previous recommendation of 4 µg/kg body weight (bw) per day [12]. It should be noted that the US Food and Drug Administration (FDA) and The German Federal Institute for Risk Assessment (BfR) opposed EFSA's revision of the TDI for BPA and are maintaining the TDI (4 µg/kg body weight (bw)) for BPA [13].

BPA has been shown to be a significant contributor to several health issues, including breast cancer, prostate cancer, immunodeficiency, developmental defects, neonatal mortality, type 2 diabetes, obesity, and an increase in the risk of developing neurological diseases [5,14–18]. Furthermore, due to the lipophilicity of the molecule, BPA can accumulate in human breast milk, exposing even newborns who have never been directly exposed to EDCs via second-hand exposure by means of biological transfer through breast milk consumption [14,19–21]. With milk as their primary food source, the infant population's health is at risk of increased exposure to BPA and other EDCs, either directly or indirectly, in their food supplies [21]. To assess the impact of BPA on infants and assure consumers' safety, it has become important to develop efficient methodologies for the analysis of BPA in milk.

To extract BPA from milk, traditional sample preparation methods commonly make extensive use of organic solvents and are often labor intensive. For example, Kang et al. implemented an extraction technique for the determination of BPA in milk and various dairy products with large volumes of methanol, acetonitrile, and hexanes during an extensive extraction procedure [22]. Also, in Cao et al., the methodology for the extraction of BPA from infant formula involved multiple steps and the use of acetonitrile and methanol [23]. Rodriguez-Gomez et al. used a methodology for BPA detection in human breast milk that required separation of fat/proteins, extraction, concentration, and reconstitution. The method was extensive and very meticulous [24].

In an effort to streamline the analysis, this paper presents a green and solventless technique known as stir bar sorptive extraction (SBSE) to extract BPA from milk samples, followed by thermal desorption–gas chromatography/mass spectrometry (TD-GC/MS) for quantitative analysis. Factors such as pH, temperature, and sonication were investigated to optimize the extraction of BPA from milk. It was hypothesized that even though BPA can remain in the fat portion of milk, decomposition of the fat content will reduce the retention of BPA and hence improve the efficiency of its extraction from the matrix.

2. Materials and Methods

2.1. Chemicals and Reagents

Commercially available cow milk was used for all experiments. Homogenized non-fat, 2% fat, and whole-fat milk was purchased at local grocery stores and stored at 4 °C until sample preparation. Bisphenol A, a BPA (99+%) standard, was purchased from Sigma Aldrich, Inc. (St. Louis, MO, USA). A 10 ppm (mg/L) BPA stock solution was prepared in methanol (MeOH, LC-MS Grade, Omni Solve Millipore Sigma, Billerica, MA, USA). Mirex, as the internal standard, was purchased from Crescent Chemical (Islandia, NY, USA).

Hydrochloric acid (HCl, 37% ACS grade), sodium hydroxide (NaOH, ACS grade), and acetic acid anhydride (AAA, Reagent Plus ≥ 99%) were purchased from Sigma Aldrich, Inc. (St. Louis, MO, USA). Both HCl and NaOH were prepared in water at a 2 M concentration. Acetonitrile (LC/MS grade) was purchased from Fisher Chemical (Fair Lawn, NJ, USA). Sodium Carbonate (Na_2CO_3) was purchased from BDH Chemicals (Radnor, PA, USA). Sodium Chloride (NaCl), ACS reagent (≥99.0%), was purchased from Sigma Aldrich, Inc. (St. Louis, MO, USA). Sodium Ethylenediaminetetraacetic Acid (EDTA) Dihydrate was purchased from J.T. Baker Chemical CO (Phillipsburg, NJ, USA) and prepared in water at a 0.2 M concentration. Deionized (DI) water was produced using a Milli-Q system from Millipore (Bedford, MA, USA).

2.2. BPA Extraction from Milk

For the study, milk samples were pretreated (labeled as pre-extraction) prior to stir bar sorptive extraction (SBSE). The experimental conditions and procedures for both parts, (A) pre-extraction and (B) SBSE extraction, are illustrated in Figure 1.

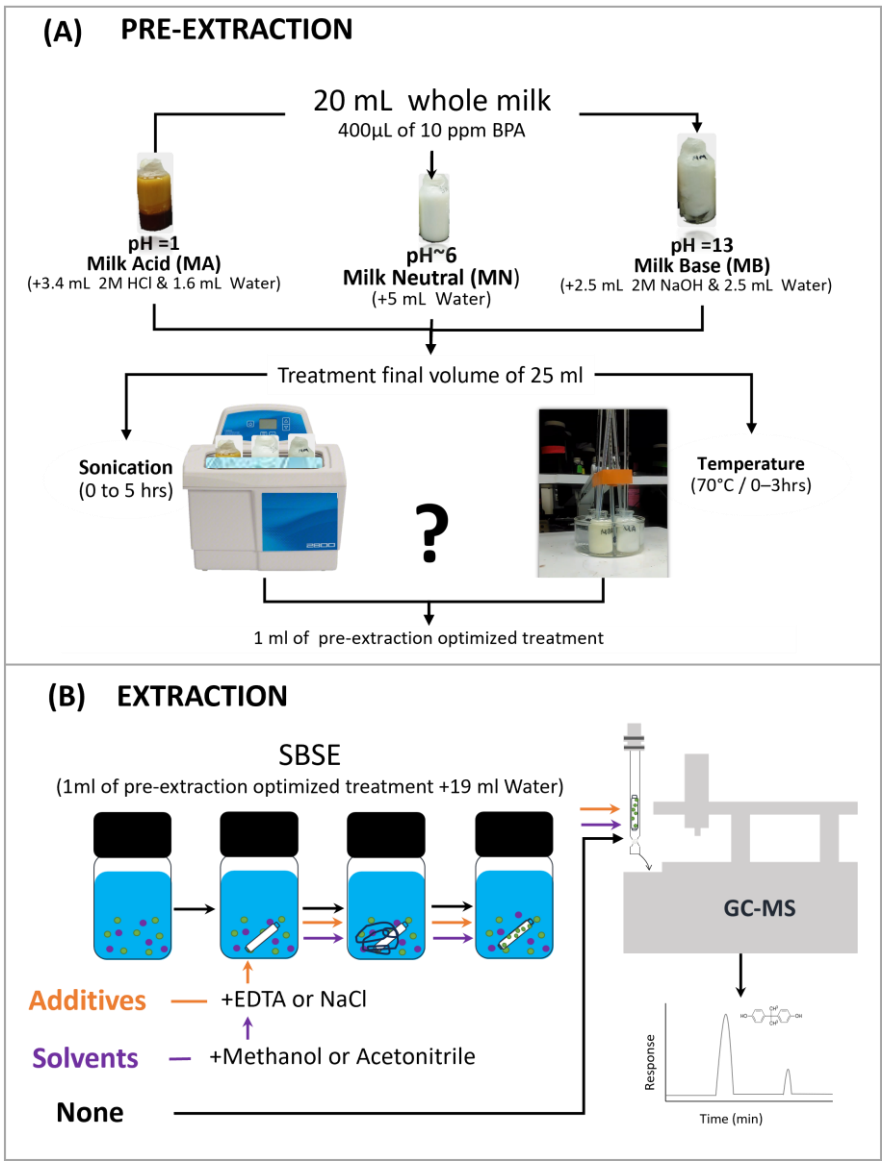

Figure 1. Diagrams showing the experimental design. (**A**) The sample preparation specifics and pre-extraction conditions that were studied. (**B**) The different extraction conditions that were studied to improve extraction efficiency during SBSE.

2.2.1. Pre-Extraction

Four pre-extraction conditions were investigated to improve the methodology—none (no treatment), pH, sonication, and temperature.

Effect of pH. Milk was treated under neutral (i.e., no treatment), basic, and acidic conditions. A 20 mL volume of whole milk was spiked with 400 µL of 10 ppm BPA solution. Each sample was labeled as milk in neutral (MN), milk in base (MB), and milk in acid (MA). For MN, 5 mL of deionized (DI) water was added; for MA, 3.4 mL of 2 M HCl and 1.6 mL of DI water were added; for MB, 2.5 mL of 2 M NaOH and 2.5 mL of DI water were added to reach a final volume of 25 mL. The target pH values for samples were measured to be 6, 13, and 1.5 for MN, MB, and MA, respectively. All pH measurements were taken using a pH meter (Agilent 3200P, Agilent Technologies, Santa Clara, CA, USA). A 1 mL volume of treated milk sample was used in the subsequent SBSE for the BPA analysis described in Section 2.2.2. In addition, DI water samples were prepared using the same process as blanks for comparison with the milk. All samples were prepared in duplicates.

Effect of Sonication. Sonication experiments were performed using a Bransonic® ultrasonic cleaner. During sonication, vials were sealed using Parafilm M All-Purpose Laboratory Film. The effect of sonication on BPA extraction from milk was studied for periods of 1 h to 3 h while the pH and temperature of the samples were monitored. Water temperature was monitored using the sonicator's temperature sensor and a thermometer inserted in the water bath. After sonication, vials were removed from the water bath, capped, and stored at 4 °C until SBSE analysis. One milliliter aliquots of milk samples subjected to sonication for different durations were used in the subsequent SBSE for BPA analysis described in Section 2.2.2.

Effect of Temperature. Experiments were completed using a Thermolyne Cimarec 3 Hot Plate Magnetic Stirrer. All samples were placed in a water bath. Temperatures in each sample and water bath were monitored and recorded. The target temperature range (70–80 °C) was chosen according to our preliminary experiments. Samples were heated for 3 h. A 5 mL aliquot was taken from each treatment at 1 and 2 h of heating, placed in a separate vial, and cooled to room temperature before SBSE analysis. One milliliter aliquots of treated milk samples heated for different durations were used in the subsequent SBSE for BPA analysis described in Section 2.2.2.

2.2.2. Stir Bar Sorptive Extraction (SBSE)

Prior to SBSE, all samples were brought to room temperature. A pre-conditioned stir bar (Twisters™ (polydimethylsiloxane (PDMS, 1 mm thickness), 10 mm length, GERSTEL, Linthicum, MD, USA) was used to extract BPA from milk using a previously developed method [25,26]. Briefly, 1 mL of pre-treated milk sample (i.e., MN, MB, or MA) was transferred into a 20 mL amber vial with 19 mL of DI water. Samples were then spiked with 200 µL of 1 ppm of mirex as the internal standard. In situ derivatization was incorporated by adding 200 mg of Na_2CO_3 followed by 200 µL of acetic acid anhydride. Finally, a preconditioned GERSTEL Twister™ was added to each sample, and all samples were stirred for 2 h at 1000 rpm on a GERSTEL Twister stir plate. After 2 h of stirring, the Twister was removed from the solution with sterilized forceps and thoroughly rinsed with DI water. The Twister™ was dried with lint-free wipes and individually placed into Thermal Desorption Tubes (TDTs) for BPA analysis by thermal desorption–gas chromatography/mass spectrometry (TD-GC/MS).

The factors tested in this study for BPA recovery by SBSE were solvents and additives.

Effect of Solvents. Two solvent systems, methanol (MeOH) and acetonitrile (ACN), were investigated for their potential to enhance the recovery of BPA from milk by SBSE. Twenty milliliters of solutions containing 1 mL of milk spiked with BPA to give final

concentrations of 1 and 5 ppb, and 19 mL of 0%, 10%, and 30% MeOH or can were prepared. The derivatizing agents and internal standard were then added for the extraction of BPA following the SBSE procedure as previously described.

Water samples (i.e., blank) were also prepared for comparison purposes. All samples were tested in triplicate.

Effect of Additives. Two additives, (1) EDTA and (2) NaCl, were tested for their effects on BPA recovery by SBSE. (1) EDTA was incorporated as an additive to study the effects of a chelating agent on the extraction of BPA from milk. Solutions containing 19 mL of DI water, 1 mL of milk, and 0.5 M EDTA were spiked with BPA to give a final concentration of 1 ppb. Controls without additives were also prepared. All samples were tested in triplicate. (2) NaCl. Samples were prepared using the optimized methodology for MN with 1 h sonication at 70 °C. Into a 20 mL amber vial was added 1 mL milk sample, 19 mL deionized water, and 500 mg NaCl. The derivatizing agents and internal standard were then added for the extraction of BPA following the SBSE procedure as previously described.

2.3. Instrumental Analysis (TD-GC/MS)

After SBSE, the stir bars were removed and placed in a TDT, followed by thermal desorption in a Thermal Desorption Unit (TDU, GERSTEL) coupled with GC/MS. Instrumental settings were as follows: For thermal desorption, the TDU was programmed to have an initial temperature of 45 °C (held for 0.5 min) and ramped to a final temperature of 280 °C (held for 7 min) at a rate of 60 °C/min. The transfer line temperature was set at 300 °C. During the desorption, compounds were cryo-focused on a baffled glass liner in a cryo-injection system (CIS4) at −40 °C. Once desorption was completed, CIS was heated from −40 to 300 °C (held for 10 min) at a rate of 12 °C/s. Separation of BPA analytes was completed using an Agilent 6890/5973 GC/MS (Agilent, CA, USA) fitted with a HP-5MS capillary column (0.25 mm × 30 m × 0.25 um, Agilent, CA, USA). The GC oven was programmed to have an initial temperature of 60 °C and increased to 300 °C (held for 5 min) at a rate of 15 °C/min. Ultra-high purity helium was used as the carrier gas at a constant flow of 0.9 mL/min. For data analysis, the m/z values of the targeted analytes were 213 and 272, representing the acyl derivative of BPA and mirex (internal standard).

2.4. Quality Control

A calibration curve was prepared. The calibration standards were prepared in DI water with concentrations of 0.1 ppb, 0.25 ppb, 0.5 ppb, 0.75 ppb, 1 ppb, 3 ppb, and 5 ppb. All samples were analyzed in duplicate. Where the RSD was greater than 25%, a third sample was analyzed.

The method detection limit (MDL) [27] was calculated by analyzing 7 whole milk samples spiked with 1 ppb of BPA using the optimized procedure. The milk samples were of the same source as those used in method development. The MDL was determined to be 0.045 ppb.

To determine percent recovery, triplicates of whole milk samples spiked with 3 ppb and 5 ppb of BPA were analyzed using the optimized method. Percent recovery was calculated by comparing the measured concentrations of the milk samples with the BPA concentrations measured in water. The average percent recovery was 54%.

2.5. Statistical Analysis

All the data were statistically analyzed using R.Studio v.4.2.2. The data were checked for normality using a histogram, QQ-plot, and a Shapiro–Wilk normality test). Where the data were normally distributed, a one-way ANOVA test ("analysis of variance") was performed, followed by a Tukey test to identify significant differences between combinations of treatments. Where the data were not normally distributed, a non-parametric statistical

analysis was performed using a Kruskal–Wallis test to identify possible differences, followed by a Dunn test with a "Bonferroni" adjusted *p*-value. Comparisons were conducted across treatments to compare the efficiency of BPA extraction from milk. Values below 0.05 were considered statistically significant.

3. Results

3.1. Study of Matrix Effect

The optimized methodology for BPA extraction was developed using whole-fat milk since its fat content is comparable to that of breast milk (2–5 g/100 mL) [16]; therefore, the method was easily translatable to the matrix. First, to prove the degree of the matrix effect present when analyzing milk, four spiked samples were analyzed for BPA recovery (Table 1). The results were normalized to BPA extraction from water. A significant matrix effect was observed with increased fat content in the milk, with whole milk (8 g fat) showing a decrease in recovery of 65% compared to water.

Table 1. Comparisons of the extraction efficiency of BPA in neutral water, non-fat milk, 2% fat milk, and whole fat milk. Data shown are means of four replicates.

	Normalized Extraction Efficiency (% ± RSD [1])	Total Fat Content (g) [2]	Cholesterol (mg) [2]	Total Carbohydrates (g) [2]	Vitamin A (%) [2]	Calories [2]
D.I. Water	100	-	-	-	-	-
Non-fat milk	87 ± 5	-	5	13	6	90
2% fat milk	40 ± 4	5	20	12	10	130
Whole fat milk	35 ± 2	8	35	12	10	150

[1] RSD: relative standard deviation. [2] All nutrient information is based on a 240 mL volume and was obtained from the product label.

3.2. Optimization of Pre-Extraction Procedures

As previously shown in Figure 1, the method development was divided into two sections: pre-extraction and extraction. During the pre-extraction portion, the goal was to reduce the matrix effect by using sonication, pH, or temperature to break up the lipids in milk.

3.2.1. pH Adjustment

Three pH conditions were investigated during this experimental set: no pH adjusted (MN), pH adjusted to basic conditions (MB), or pH adjusted to acidic conditions (MA). All MA solutions were targeted to have a pH of around 1, while MB solutions had a pH of about 13. These pH values were set up at extreme levels so that the recovery of BPA could be evaluated across the different ionization forms of BPA [28].

Differences in BPA recovery were noticed under the three pH treatments (Figure 2). The efficiency of BPA extraction from milk was highest in neutral conditions, i.e., without pH adjustment. In the acidic and basic conditions, extraction of BPA was 89% and 63%, respectively, compared to the neutral condition. A *t*-test was performed to compare BPA recovery under acidic and basic conditions (n = 8) with respect to neutral milk. BPA recovery from milk under basic conditions (MB) was significantly lower than from the milk without pH adjustment (p-value = 0.06), while the recovery of BPA was not significantly different between MA and MN (p-value = 0.48). It is concluded that the efficiency of BPA extraction from milk was significantly better without adjusting the pH of milk when no other factors (such as sonication and heating) were considered.

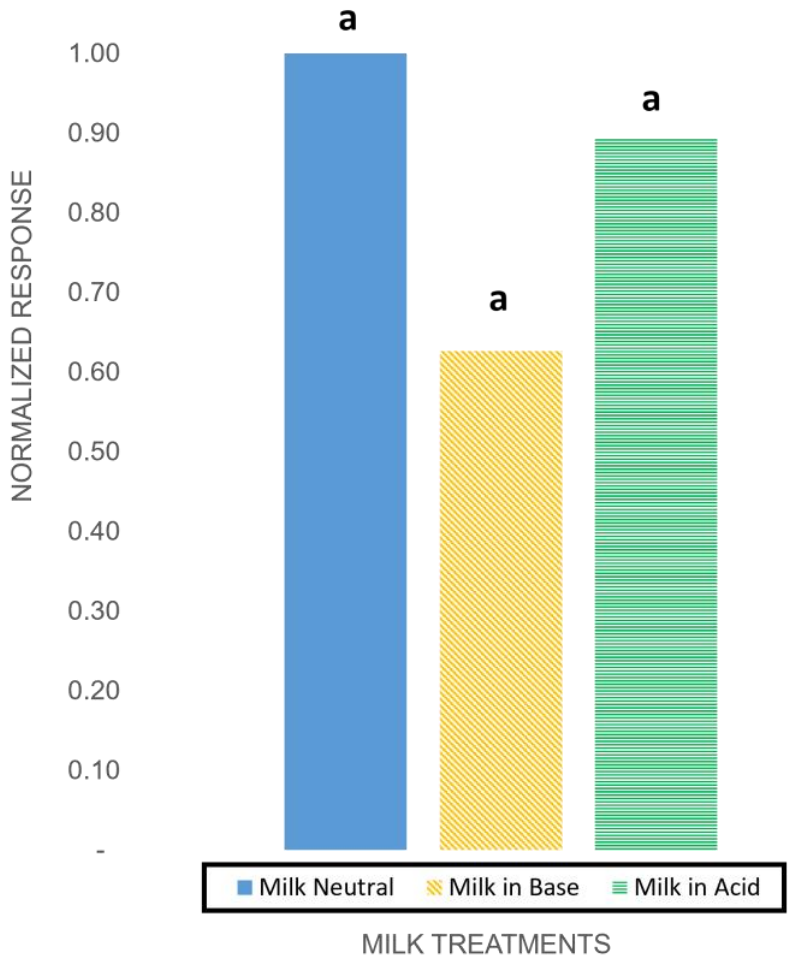

Figure 2. BPA recovery (based on instrument response) under neutral (MN), basic (MB), and acidic (MA) conditions. MA and MB results were normalized and compared to MN; therefore, the standard deviation was excluded from the dataset (n = 8). Statistical analysis revealed that the data did not follow a normal distribution, and a non-parametric test revealed no statistically significant difference across treatments (p.adj-value > 0.05). The same letter above each treatment indicates no significant differences at Tukey's test ($p \leq 0.05$).

3.2.2. Sonication (S)

Sonication was hypothesized to assist in the breakup of lipids and, in turn, improve the release of BPA from milk. Optimal sonication conditions were studied for all three pH treatments (MN, MA, and MB). Sonication was studied in one-hour intervals for a total of five hours. All the BPA recovery results were normalized to the neutral condition, i.e., milk without pH adjustment and no sonication. After 3 h, the recovery of the samples was reduced. Furthermore, the length of time sonicating (>3 h) was not conducive to the objective of developing a time-efficient protocol. As shown in Figure 3A, the highest BPA recovery was observed at 3 h of sonication in MB. While longer sonication seemed to improve the dissociation of lipids, it also caused the temperature to increase from 20 °C (Time 0) to 50–70 °C (at 3 h); therefore, the high recovery could also be related to the increase in temperature caused by sonication.

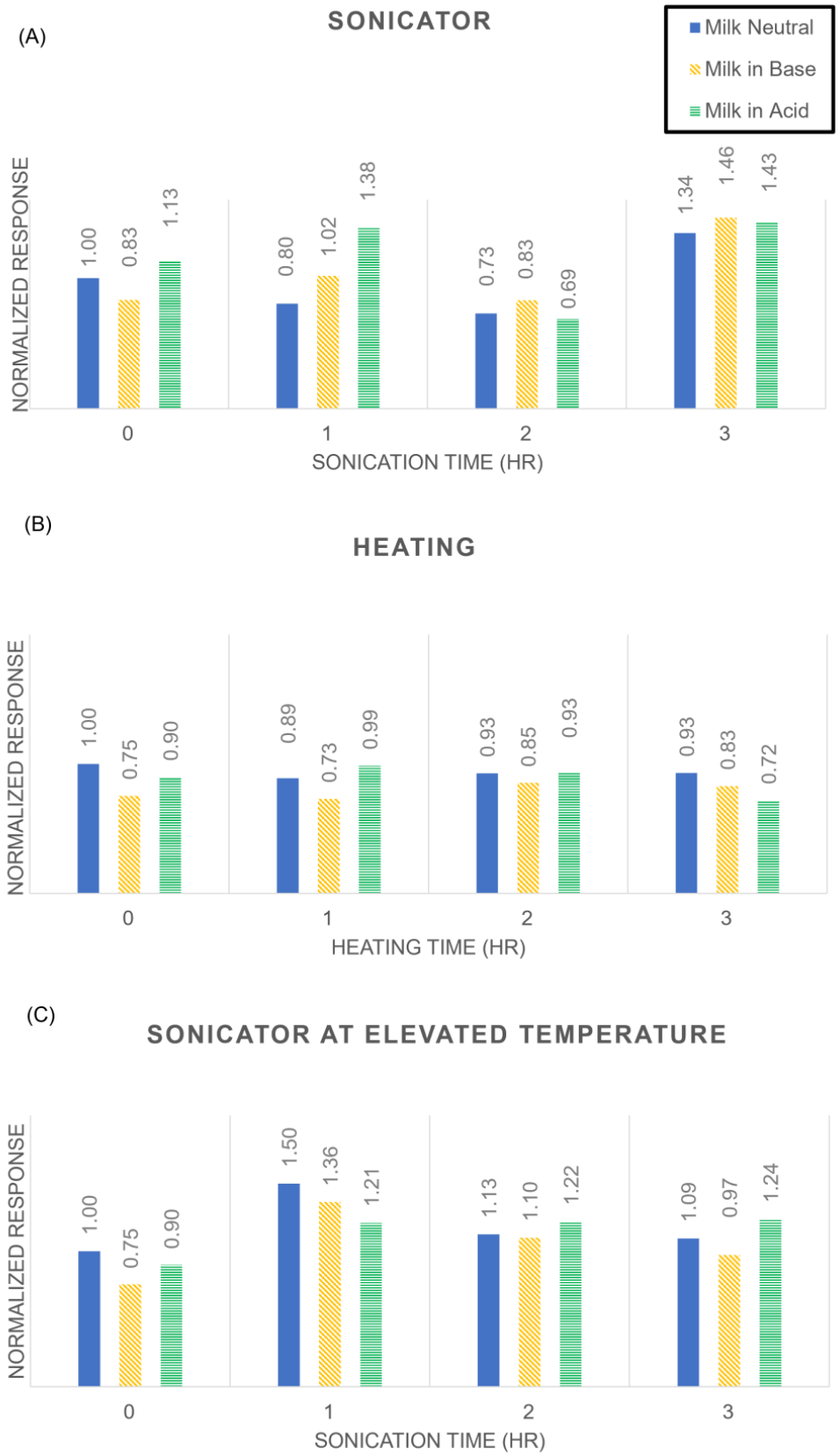

Figure 3. The effects of sonication time and temperature on BPA recovery from MN, MB, and MA. (**A**) Effects of 0–3 h of continuous sonication starting at 25 °C; (**B**) effects of temperature maintained at 60–80 °C for 3 h; and (**C**) combined effects of sonication (0–3 h) and temperature (69 °C). Responses were normalized against the control condition, as there was no pH adjustment or sonication. The standard error is not shown since all samples were normalized. A complete statistical analysis can be found in the Supplementary Section (Table S1).

3.2.3. Temperature (H)

To study the effect of temperature alone on the recovery of BPA, milk samples were heated on a hot plate at 70 °C, which was the maximum temperature reached during 3 h of sonication. Samples were taken every hour for a total of three hours, and the results were normalized to the control. The best BPA recovery was found to be in MA after one hour of heating, yielding a 99% recovery compared to the no-treatment milk (Figure 3B). The results indicate that temperature is a contributor to BPA extraction, but it is not the dominating factor since it did not increase recovery above the control.

Based on the two previous observations, sonication and heating were studied together. The results showed that a combination of 1 h of sonication and high temperature (70–80 °C) yielded the highest BPA from MN (Figure 3C), increasing the recovery by 50% above the control. Statistical analysis showed that sonication and heating had a significant effect on BPA recovery compared to sonication or heating alone. Furthermore, significant differences were seen between sonication or heating treatments alone and combined sonication and heating treatments (Table S1). The change in pH again did not seem to be a factor that improved extraction as much as sonication and heating did.

Overall, BPA recovery was improved in treatments with sonication alone or sonication and heating, but not with heating alone. It is interesting to point out that the appearance of milk under acidic or basic conditions changed as sonication time progressed (Figure S1). The solution was separated into clear liquid and chunks of white sticky layers in MA samples. The sample matrix was separated into a liquid and a solid layer in MB samples, and the color changed from white to orange (Figure S1). The color change could be attributed to the Maillard reaction, which is responsible for food's browning, taste, and aroma changes when cooked. Under basic conditions, different reactions and degradations took place, resulting in the formation of melanoidin, which gives the brown color [29].

3.3. Improving SBSE Extraction of BPA

From the pre-extraction parameters investigated (Figure 1), MN and 1 h of sonication at 70–80 °C were selected as the most optimal conditions for BPA extraction from whole milk. Following the diagram in Figure 1, 1 mL of the milk solution from the best condition (MN, 1 h sonication at 70–80 °C) was subjected to stir bar sorptive extraction (SBSE) to extract BPA. Three parameters were investigated to improve BPA extraction through SBSE: solvent amount, additive addition, and stirring time.

3.3.1. Solvents

Multiple acetonitrile (ACN) and methanol (MeOH) concentrations were studied for BPA extraction efficiency during SBSE. However, it was observed that ACN and MeOH did not improve the recovery of BPA from the milk sample compared to samples without the added solvent (Figure 4). In fact, extraction of BPA from the ACN and MeOH solvents indicated a negative trend: the greater the concentration of MeOH or ACN, the lower the BPA recovery by SBSE. It was hypothesized that this could be due to hydroxyl groups forming hydrogen bonds with BPA, thereby making the sorption of BPA to the stir bar less favorable during SBSE. No further statistical analysis of these data was conducted.

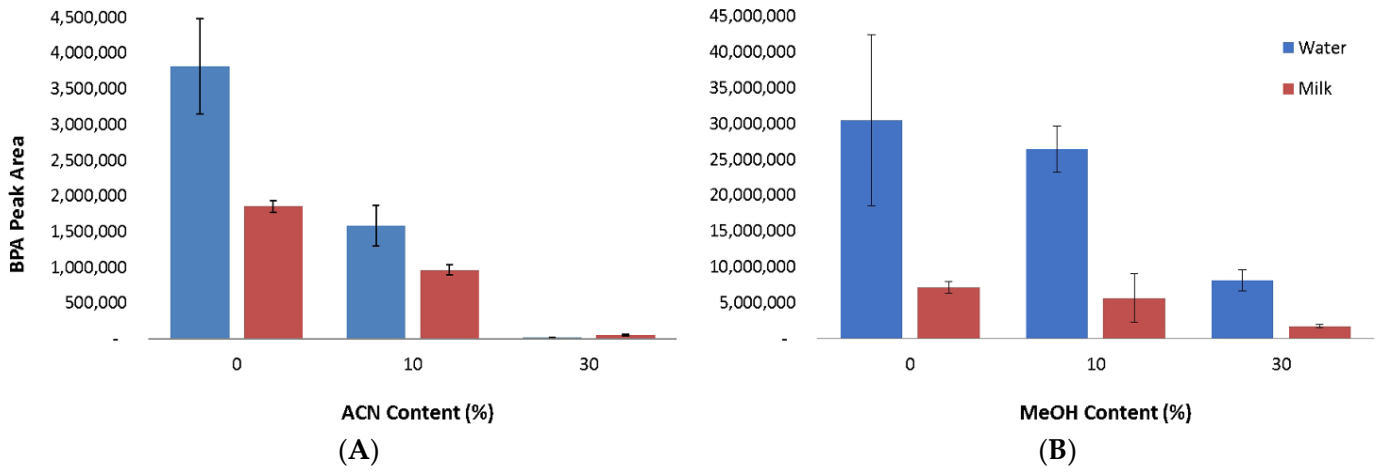

Figure 4. Recovery of BPA from different solvents using SBSE: (**A**) BPA recovered from water and milk samples with 0%, 10%, and 30% of ACN, and (**B**) BPA recovered from water and milk samples with 0%, 10%, and 30% of MeOH. BPA recovery was based on the instrument's response, i.e., BPA peak area in the chromatograms. Data are means of three replicates ± standard error (error bar).

3.3.2. Additives

Two types of additives, NaCl and EDTA, were tested for the recovery of BPA during SBSE. EDTA was added to 1 mL of milk spiked with BPA and 19 mL of water to give a final concentration of 1 ppb. As shown in Figure 5, BPA recovery from milk was greatly impaired by the addition of EDTA. In the case of NaCl, 500 mg of salt was added to the BPA milk solution. Adding NaCl during sample extraction did not improve BPA recovery from milk samples. As most BPA remains as neutral molecules, increasing the ionic strength of the SBSE solvent system did not affect the extraction of BPA by SBSE. No further statistical analysis of these data was conducted.

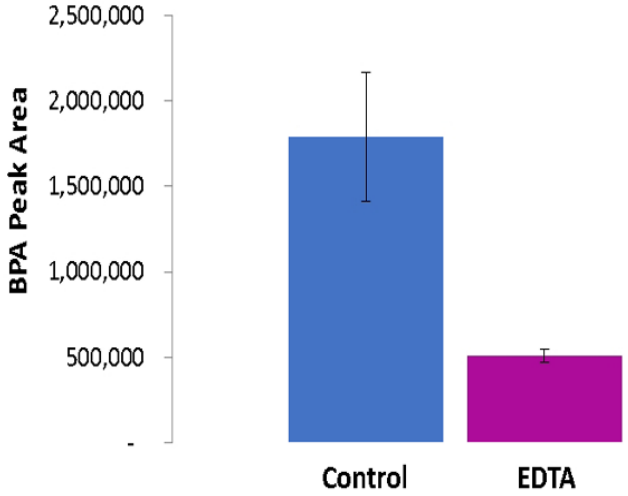

Figure 5. Recovery of BPA during SBSE through the addition of EDTA. Data are means of three replicates ± standard error (shown as error bars).

4. Discussion

Commercial milk in the U.S. is a homogeneous mixture generally composed of 87.7% water, 4.9% lactose (carbohydrates), 3.4% fat, 3.3% protein, and 0.7% minerals (ash) (Table 1) [15]. Because of BPA's high affinity for lipids (log Kow = 3.32), the fat content in milk can hinder its extraction. This study compared milk with three different fat contents (non-fat milk, 2% fat milk, whole-fat milk), and as expected, the recovery of BPA from

milk substantially decreased as fat content increased (Table 1). However, the presence of a matrix effect on the extraction of BPA from other food sources is not uncommon and has been previously reported; for example, Maragou et al. studied BPA in foods (mushrooms, pineapples, and tuna), with the recovery of BPA ranging from 51 to 57% [30]. The hydrophobic nature of BPA often compromises its transfer to the extractant during sample preparation [31].

To help in the extraction of BPA, changes in its chemical structure can be induced via pH changes. BPA becomes ionized across different pH levels [28], which can influence its extraction. BPA remains a neutral molecule, but under basic conditions, it can be ionized to form mono- (pH > 7.5) or divalent anions (pH > 9). In this study, pH values were set up at extreme levels (i.e., pH = 1 for MA and pH = 13 for MB) so that the recovery of BPA could be evaluated across the different ionization forms of BPA. Under MN and MA conditions, BPA was present in its molecular form, while it was present as divalent anions under MB conditions. Furthermore, the coating material, PDMS, on the stir bar is hydrophobic in nature; therefore, neutral BPA has a higher affinity for PDMS compared to its ionic form. As shown in Figure 2, BPA recovery under MN and MA conditions was similar and better than observed under the MB condition; however, no significant difference was found following statistical analysis.

In addition, factors such as heating, sonication, and additives to facilitate the recovery of BPA from milk have been previously reported in the literature. For example, Rodrigues et al. [32] reported a temperature rise to 90 °C for 45 min with stirring and no NaCl addition, resulting in greater BPA recovery from milk samples. We also observed the positive effects of heating and sonication on BPA recovery (Figure 3C), with up to 150% improvement compared to recovery performed in an experiment without heating and sonication treatment. However, longer heating time did not improve the recovery of BPA (Figure 3B). We suspect that prolonged heating and sonication time might have deleterious effects on milk or BPA, possibly leading to its decomposition during the process [33].

During SBSE, the addition of organic solvents such as methanol destabilized the milk's emulsion [34], improving the release of BPA to the stir bar. Although adding methanol during the extraction of organic compounds during SBSE has been reported to improve the extraction efficiency of the organic compounds from various matrices [35–37], we did not observe the same effect of methanol on the recovery of BPA from milk (Figure 4). Extraction of BPA in the ACN and MeOH solvents had a negative trend: the greater the concentration of MeOH, the lower the BPA recovery by SBSE. This could be due to hydroxyl groups forming hydrogen bonds with BPA, thereby making the sorption of BPA to the stir bar less favorable during SBSE.

Particles known as casein micelles constitute the largest protein component found in most types of milk [38,39]. These casein micelles form complexes with important minerals, including calcium. It has been reported that EDTA can disrupt casein micelles by destroying their calcium core, subsequently freeing any entrapped protein from casein aggregates [40]. The key interactions that are considered to be involved in maintaining the caseins are hydrophobic interactions, electrostatic attractions, and Ca bridges [41]. Alteration of protein–mineral equilibria by chelation was also demonstrated in a study by McCarthy et al. [42], where various calcium-chelating agents were found to impact the physical properties of milk powder concentrate dispersion, thereby increasing its solubility. Even though other studies have shown that EDTA can induce the dissociation of casein [41,43], our results indicate that this phenomenon is ineffective at improving BPA recovery from milk. As seen in Figure 5, BPA recovery from milk was greatly impaired by the addition of EDTA. We suspect that under high-temperature conditions, milk can increase the release

of denatured whey proteins [41] and the whey proteins can absorb BPA, resulting in a decrease in BPA extraction [44].

Overall, several methodologies have been developed for the analysis of BPA in milk products (Table 2). However, most of these approaches are resource-intensive, requiring various solvents to separate BPA from lipids for extraction. For example, Khedr et al. [39] reported an optimized extraction technique for BPA detection in milk power that required the use of NH_3, CH_2Cl_2, and methanol (MeOH). Samanidou et al. used fabric phase sorptive extraction for BPA extraction from milk using methods requiring formic acid, MeOH, and acetonitrile (ACN) [45]. Rodríguez-Gómez et al. [24] used SBSE, GC-MS/MS, ACN, NaCl, ethyl acetate, and BSTFA 1%TMCS. Souza et al. [46] used QuEChERS as the extraction technique, which, although it promises to be quick and easy, also requires a lot of solvents (ACN, NaCl, MeOH, $MgSO_4$, NH_4OH, hexane). In contrast, our method did not require the use of any solvent; in fact, solvents hindered the extraction of BPA (Figure 4).

Other extraction methodologies have been reported using molecular imprinted polymers. However, the polymerization procedures presented in these methodologies tend to be lengthy and sometimes tedious. For example, Alexiadou et al. [47] used molecularly imprinted polymers for solid phase extraction cartridges (MISPE), and Zhan et al. [48] used dummy molecularly imprinted polymer-coated stir bars for SBSE (SBSE DMIPs-SB). In these techniques, several steps must be followed to prepare the coated extraction tools prior to the extraction of the analyte. Our methodology, in contrast, uses a commercially available stir bar that can be easily reused.

Furthermore, in this study, the optimized method had an LOD of 0.045 ppb when taking into account the matrix effect, which is higher than that reported by Maragou et al. [49] and Khedr et al. [39]—these studies used liquid chromatography-based analytical techniques and SPE as extraction techniques and reported LODs of 1.7 and 3.2, respectively. Filippou et al. [50] used the magnetic solid phase extraction–HPLC method for bisphenol A extraction from milk and reported an LOD of 0.75 ppb. Yang et al. [51] used Au nanoparticles and Surface-enhanced Raman spectroscopy (SERS) as the analytical technique for the detection of BPA in milk and reported an LOD of 0.98 ppb.

Although our methodology's recovery was 54%, this was taking into consideration the matrix effect. It should be noted that there are discrepancies in how the recovery was determined across methods, which could also fuel these differences. For example, Samanidou et al. [45] evaluated spiked blank milk samples that contained 0% fat; Alexiadou et al. [47] used aqueous and spiked blank milk with 3.5% fat for method validation, while in this article, whole-fat milk (8% fat) was used. Mercogliano et al. [52] used HPLC-FLD and SPE, which is the most competitive method compared to ours, with the use of a reduced number of solvents (ACN, MeOH), a low LOD (0.03 ppb), and a high recovery percentage (78.4–107.2%). However, for GC/MS, our method is one of the most complete green chemistry methodologies developed in the current literature.

Table 2. Comparison of BPA recovery (%) from our developed method with other reported methods. Abbreviations used in the table: AR (absolute recovery), RR (relative recovery), cc (calibration curve), I.S. (internal standard), Co (initial BPA concentration), Ce (equilibrium BPA concentration), and SERS (surface-enhanced Raman spectroscopy).

Analytical Technique	Extraction Technique	Chemicals Used for Extraction	Sample	LOD	Recovery			Ref.
				(ppb)	(%)	Calculation	Calibration Curve (cc)	
LC-ESI-MS	SPE	MeOH	Canned Condensed Milk	1.70	AR = 52 RR = 101	AR: (slope of cc from spiked milk samples/slope of aqueous external cc) × 100 RR: (slope of cc from spiked milk samples/slope of aqueous I.S. cc) × 100	AR: y = (analyte peak area) × 10^{-3} RR: y = (analyte peak area/I.S. peak area) × 10^{3} The cc for standard solutions was generated in an aqueous matrix.	[49]
HPLC-UV	Ultrasonic Magnetic Solid Phase Dispersive Extraction	ACN, MeOH, Hexanes	Milk	0.75	RR = 89.1–99.4	RR: ((Co-Ce)/Co) × 100	(Peak areas) vs. (Concentrations of analyte) An adjusted cc was generated using skimmed milk as the matrix.	[50]
GC-MS/MS	SBSE	ACN, NaCl, Ethyl Acetate, and BSTFA 1%TMCS	Breast milk	0.20	100–110	(Measured [BPA]/Spiked [BPA]) × 100	(Analyte peak area/surrogate peak area) vs. concentration analyte matrix-matched calibration was conducted for all compounds.	[24]
UHPLC-MS/MS				0.10	99–109			
HPLC-ESI-MS	SPE	NH_3, CH_4Cl_2, MeOH	Powder Milk	3.20	83–102.5	(Measured [BPA]/Spiked [BPA]) × 100	(Relative peak area of the analyte/I.S.) vs. concentration of the analyte.	[39]
HPLC FDL	SBSE (DMIPs-SB)	MeOH	Milk	0.00684	85	((Measured [BPA] –Blank [BPA])/Spiked [BPA]) × 100 Matrix effect was reduced by a five-fold dilution of milk samples.	(Peak areas) vs. (Concentrations of analyte) The cc was generated in an aqueous matrix.	[48]
HPLC-FDL, LC-MS	MISPE	MeOH	Milk	0.20	109	((C spiked sample—C non-spiked sample)/C added) × 100	y = (Analyte peak area/I.S. peak area) vs. Analyte concentration. Aqueous standard solutions and spiked blank milk (3.5% fat).	[47]
SERS	Halide-modified Au NPs	MeOH	Milk	0.98	89.5–100.2	(Measured [BPA]/Spiked [BPA]) × 100	LogC of [BPA] (mol/L) vs. Intensity of SERS peak at 641 cm^{-1} (a.u.) $I_{SERS} = 42.79 \log C_{BPA} + 404.03$ The cc was generated using supernatants of spiked milk.	[51]
HPLC-UV	FPSE	Formic Acid, MeOH, ACN	Milk	16.7	90–107	(Measured [BPA]/Spiked [BPA]) × 100	(Peak areas) vs. (Concentrations of the analyte). The cc was conducted using spiked blank milk samples (0% fat).	[45]
UHPLC-MS/MS	QuEChERS	ACN, NaCl, MeOH, $MgSO_4$, NH_4OH, Hexane	Whole Milk	0.12	78–94	(Measured [BPA]/Spiked [BPA]) × 100	(Peak areas) vs. (Concentrations of analyte) The cc was generated in the matrix and in MeOH:H_2O	[46]
HPLC-FLD	SPE	ACN, MeOH	Milk	0.03	78.4–107.2	(Measured [BPA]/Spiked [BPA]) × 100	Only an external cc was generated	[52]
GC-MS	SBSE	Acetic anhydride, Na_2CO_3	Milk	0.045	54	(Measured [BPA]/Spiked [BPA]) × 100	(Peak areas) vs. (Concentrations of analyte) The cc was generated in an aqueous matrix.	This Study

5. Conclusions

Following green chemistry principles, this study developed a solventless and simple extraction methodology for monitoring BPA levels in milk. The LOD of this methodology is comparable to previously reported methodologies and opens the door for further growth of green chemistry methodologies.

For this study, whole milk was used as the sample matrix, and three independent variables were studied during treatment preparation: pH, temperature, and sonication time. Our results showed that adjustment of the pH of milk as a pre-treatment did not increase BPA recovery and optimized BPA recovery from milk was obtained at neutral pH (i.e., no pH treatment) and one hour of sonication at 70–80 °C, with recovery under these conditions being increased by 150% compared to no sonication of neutral whole milk (Figure 2). Compared to extracting BPA from DI water, the recovery of BPA from whole milk was improved from 31% to 54% using the optimized method. The addition of solvents and additives was studied to improve the efficiency of SBSE for BPA extraction, but these factors did not improve the extraction efficiency.

This research developed and optimized a methodology for analyzing and monitoring BPA in whole milk, a matrix with a high lipid content. Our method provides a solventless and straightforward sample preparation procedure, making its application in sample analysis more attractive. This method can be applied to further research on analyzing BPA in breast milk to understand the dietary intake of BPA in infants and to investigate its potential impacts on their health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations12020025/s1, Figure S1: The change in the appearance of milk solution treated with acid after 1 and 2 h of sonication; Table S1: One-way ANOVA showing differences between groups. Confidence level = 0.95, p-value < 0.05. Significantly different means are represented by different letters (group), while non-significant differences share the same symbol.

Author Contributions: Conceptualization and methodology, A.M.E.M. and W.-Y.L.; validation, A.M.E.M. and W.-Y.L.; formal analysis, A.M.E.M. and W.-Y.L.; investigation, A.M.E.M.; resources, A.M.E.M. and W.-Y.L.; data curation, A.M.E.M. and W.-Y.L.; writing—original draft preparation, A.M.E.M.; writing—review and editing, A.M.E.M. and W.-Y.L.; supervision, W.-Y.L.; project administration, W.-Y.L.; funding acquisition, A.M.E.M. and W.-Y.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by the National Cancer Institute of the National Institutes of Health under Award Number SC1CA245675.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to acknowledge the Campus Office of Undergraduate Research Initiative and the Vice President for Student Affairs at the University of Texas at El Paso, the Environmental Science Training in Environmental Research and Academic Success (TIERA) program, for providing stipend support. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Russo, G.; Barbato, F.; Mita, D.G.; Grumetto, L. Occurrence of Bisphenol A and Its Analogues in Some Foodstuff Marketed in Europe. *Food Chem. Toxicol.* **2019**, *131*, 110575. [CrossRef] [PubMed]
2. Liao, C.; Kannan, K. Widespread Occurrence of Bisphenol A in Paper and Paper Products: Implications for Human Exposure. *Environ. Sci. Technol.* **2011**, *45*, 9372–9379. [CrossRef] [PubMed]

3. Belfroid, A.; Van Velzen, M.; van der Horst, B.; Vethaak, D. Occurrence of Bisphenol A in Surface Water and Uptake in Fish: Evaluation of Field Measurements. *Chemosphere* **2002**, *49*, 97–103. [CrossRef] [PubMed]
4. Tsai, W.-T. Survey on the Environmental Risks of Bisphenol A and Its Relevant Regulations in Taiwan: An Environmental Endocrine-Disrupting Chemical of Increasing Concern. *Toxics* **2023**, *11*, 722. [CrossRef]
5. Huang, Y.Q.; Wong, C.K.C.; Zheng, J.S.; Bouwman, H.; Barra, R.; Wahlström, B.; Neretin, L.; Wong, M.H. Bisphenol A (BPA) in China: A Review of Sources, Environmental Levels, and Potential Human Health Impacts. *Environ. Int.* **2012**, *42*, 91–99. [CrossRef]
6. Khan, M.R.; Ouladsmane, M.; Alammari, A.M.; Azam, M. Bisphenol A Leaches from Packaging to Fruit Juice Commercially Available in Markets. *Food Packag. Shelf Life* **2021**, *28*, 100678. [CrossRef]
7. Wang, X.; Nag, R.; Brunton, N.P.; Siddique, M.A.B.; Harrison, S.M.; Monahan, F.J.; Cummins, E. Human Health Risk Assessment of Bisphenol A (BPA) through Meat Products. *Environ. Res.* **2022**, *213*, 113734. [CrossRef]
8. Rubin, B.S. Bisphenol A: An Endocrine Disruptor with Widespread Exposure and Multiple Effects. *J. Steroid Biochem. Mol. Biol.* **2011**, *127*, 27–34. [CrossRef]
9. Calafat, A.M.; Ye, X.; Wong, L.-Y.; Reidy, J.A.; Needham, L.L. Exposure of the U.S. Population to Bisphenol A and 4-*Tertiary*-Octylphenol: 2003–2004. *Environ. Health Perspect.* **2008**, *116*, 39–44. [CrossRef]
10. Fernandez, M.F.; Arrebola, J.P.; Taoufiki, J.; Navalón, A.; Ballesteros, O.; Pulgar, R.; Vilchez, J.L.; Olea, N. Bisphenol-A and Chlorinated Derivatives in Adipose Tissue of Women. *Reprod. Toxicol.* **2007**, *24*, 259–264. [CrossRef]
11. Chevalier, N.; Fénichel, P. Bisphenol A: Targeting Metabolic Tissues. *Rev. Endocr. Metab. Disord.* **2015**, *16*, 299–309. [CrossRef] [PubMed]
12. Ramírez, V.; Merkel, S.; Tietz, T.; Rivas, A. Risk Assessment of Food Contact Materials. *EFSA J.* **2023**, *21*, e211015. [CrossRef] [PubMed]
13. Vom Saal, F.S.; Antoniou, M.; Belcher, S.M.; Bergman, A.; Bhandari, R.K.; Birnbaum, L.S.; Cohen, A.; Collins, T.J.; Demeneix, B.; Fine, A.M.; et al. The Conflict Between Regulatory Agencies over the 20,000-Fold Lowering of the Tolerable Daily Intake (TDI) for Bisphenol A (BPA) by the European Food Safety Authority (EFSA). *Environ. Health Perspect.* **2024**, *132*, 045001. [CrossRef] [PubMed]
14. Wielogórska, E.; Elliott, C.T.; Danaher, M.; Chevallier, O.; Connolly, L. Validation of an Ultra High Performance Liquid Chromatography–Tandem Mass Spectrometry Method for Detection and Quantitation of 19 Endocrine Disruptors in Milk. *Food Control* **2015**, *48*, 48–55. [CrossRef]
15. Baluka, S.A.; Rumbeiha, W.K. Bisphenol A and Food Safety: Lessons from Developed to Developing Countries. *Food Chem. Toxicol.* **2016**, *92*, 58–63. [CrossRef]
16. Erler, C.; Novak, J. Bisphenol A Exposure: Human Risk and Health Policy. *J. Pediatr. Nurs.* **2010**, *25*, 400–407. [CrossRef]
17. Pérez-Bermejo, M.; Mas-Pérez, I.; Murillo-Llorente, M.T. The Role of the Bisphenol A in Diabetes and Obesity. *Biomedicines* **2021**, *9*, 666. [CrossRef]
18. Costa, H.E.; Cairrao, E. Effect of Bisphenol A on the Neurological System: A Review Update. *Arch. Toxicol.* **2024**, *98*, 1–73. [CrossRef]
19. Altamirano, G.A.; Muñoz-de-Toro, M.; Luque, E.H.; Gómez, A.L.; Delconte, M.B.; Kass, L. Milk Lipid Composition Is Modified by Perinatal Exposure to Bisphenol A. *Mol. Cell. Endocrinol.* **2015**, *411*, 258–267. [CrossRef]
20. Mercogliano, R.; Santonicola, S. Investigation on Bisphenol A Levels in Human Milk and Dairy Supply Chain: A Review. *Food Chem. Toxicol.* **2018**, *114*, 98–107. [CrossRef]
21. Jin, H.; Xie, J.; Mao, L.; Zhao, M.; Bai, X.; Wen, J.; Shen, T.; Wu, P. Bisphenol Analogue Concentrations in Human Breast Milk and Their Associations with Postnatal Infant Growth. *Environ. Pollut.* **2020**, *259*, 113779. [CrossRef] [PubMed]
22. Kang, J.-H.; Kondo, F. Determination of Bisphenol A in Milk and Dairy Products by High-Performance Liquid Chromatography with Fluorescence Detection. *J. Food Prot.* **2003**, *66*, 1439–1443. [CrossRef] [PubMed]
23. Cao, X.-L.; Dufresne, G.; Belisle, S.; Clement, G.; Falicki, M.; Beraldin, F.; Rulibikiye, A. Levels of Bisphenol A in Canned Liquid Infant Formula Products in Canada and Dietary Intake Estimates. *J. Agric. Food Chem.* **2008**, *56*, 7919–7924. [CrossRef]
24. Rodríguez-Gómez, R.; Zafra-Gómez, A.; Camino-Sánchez, F.J.; Ballesteros, O.; Navalón, A. Gas Chromatography and Ultra High Performance Liquid Chromatography Tandem Mass Spectrometry Methods for the Determination of Selected Endocrine Disrupting Chemicals in Human Breast Milk After Stir-Bar Sorptive Extraction. *J. Chromatogr. A* **2014**, *1349*, 69–79. [CrossRef]
25. Balsiger, H.A.; de la Torre, R.; Lee, W.-Y.; Cox, M.B. A Four-Hour Yeast Bioassay for the Direct Measure of Estrogenic Activity in Wastewater Without Sample Extraction, Concentration, or Sterilization. *Sci. Total Environ.* **2010**, *408*, 1422–1429. [CrossRef]
26. Kawaguchi, M.; Inoue, K.; Yoshimura, M.; Ito, R.; Sakui, N.; Okanouchi, N.; Nakazawa, H. Determination of Bisphenol A in River Water and Body Fluid Samples by Stir Bar Sorptive Extraction with in Situ Derivatization and Thermal Desorption-Gas Chromatography–Mass Spectrometry. *J. Chromatogr. B* **2004**, *805*, 41–48. [CrossRef]

27. U.S. Environmental Protection Agency. Procedures for Detection and Quantitation—Documents. Available online: https://www.epa.gov/cwa-methods/procedures-detection-and-quantitation-documents (accessed on 15 January 2025).
28. Dong, Y.; Wu, D.; Chen, X.; Lin, Y. Adsorption of Bisphenol A from Water by Surfactant-Modified Zeolite. *J. Colloid Interface Sci.* **2010**, *348*, 585–590. [CrossRef]
29. Martins, S.I.F.S.; Jongen, W.M.F.; Van Boekel, M.A.J.S. A Review of Maillard Reaction in Food and Implications to Kinetic Modelling. *Trends Food Sci. Technol.* **2000**, *11*, 364–373. [CrossRef]
30. Maragou, N.C.; Thomaidis, N.S.; Theodoridis, G.A.; Lampi, E.N.; Koupparis, M.A. Determination of Bisphenol A in Canned Food by Microwave Assisted Extraction, Molecularly Imprinted Polymer-Solid Phase Extraction and Liquid Chromatography-Mass Spectrometry. *J. Chromatogr. B* **2020**, *1137*, 121938. [CrossRef]
31. Lucena, R.; Gallego, M.; Cárdenas, S.; Valcárcel, M. Autoanalyzer for Milk Quality Control Based on the Lactose, Fat, and Total Protein Contents. *Anal. Chem.* **2003**, *75*, 1425–1429. [CrossRef]
32. Rodrigues, F.d.M.; Mesquita, P.R.R.; de Oliveira, L.S.; de Oliveira, F.S.; Filho, A.M.; Pereira, P.A.d.P.; de Andrade, J.B. Development of a Headspace Solid-Phase Microextraction/Gas Chromatography–Mass Spectrometry Method for Determination of Organophosphorus Pesticide Residues in Cow Milk. *Microchem. J.* **2011**, *98*, 56–61. [CrossRef]
33. Guo, Z.; Feng, R. Ultrasonic Irradiation-Induced Degradation of Low-Concentration Bisphenol A in Aqueous Solution. *J. Hazard. Mater.* **2009**, *163*, 855–860. [CrossRef]
34. Casajuana, N.; Lacorte, S. New Methodology for the Determination of Phthalate Esters, Bisphenol A, Bisphenol A Diglycidyl Ether, and Nonylphenol in Commercial Whole Milk Samples. *J. Agric. Food Chem.* **2004**, *52*, 3702–3707. [CrossRef] [PubMed]
35. Barriada-Pereira, M.; Serôdio, P.; González-Castro, M.J.; Nogueira, J.M.F. Determination of Organochlorine Pesticides in Vegetable Matrices by Stir Bar Sorptive Extraction with Liquid Desorption and Large Volume Injection-Gas Chromatography–Mass Spectrometry towards Compliance with European Union Directives. *J. Chromatogr. A* **2010**, *1217*, 119–126. [CrossRef] [PubMed]
36. Habib, A.; Landa, E.N.; Holbrook, K.L.; Walker, W.S.; Lee, W.-Y. Rapid, Efficient, and Green Analytical Technique for Determination of Fluorotelomer Alcohol in Water by Stir Bar Sorptive Extraction. *Chemosphere* **2023**, *338*, 139439. [CrossRef]
37. De La Torre-Roche, R.J.; Lee, W.-Y.; Campos-Díaz, S.I. Soil-Borne Polycyclic Aromatic Hydrocarbons in El Paso, Texas: Analysis of a Potential Problem in the United States/Mexico Border Region. *J. Hazard. Mater.* **2009**, *163*, 946–958. [CrossRef]
38. Müller-Buschbaum, P.; Gebhardt, R.; Roth, S.V.; Metwalli, E.; Doster, W. Effect of Calcium Concentration on the Structure of Casein Micelles in Thin Films. *Biophys. J.* **2007**, *93*, 960–968. [CrossRef]
39. Khedr, A. Optimized Extraction Method for LC–MS Determination of Bisphenol A, Melamine and Di(2-Ethylhexyl) Phthalate in Selected Soft Drinks, Syringes, and Milk Powder. *J. Chromatogr. B* **2013**, *930*, 98–103. [CrossRef]
40. Morcol, T.; He, Q.; Bell, S.J.D. Model Process for Removal of Caseins from Milk of Transgenic Animals. *Biotechnol. Prog.* **2001**, *17*, 577–582. [CrossRef]
41. Alessi, A.; Fontana, A.; Risso, P.; Gatti, C.; Pires, M. Identification of Interactions Involved in Rennet Gel Structures Using Dissociating Chemical Agents. *Colloid Polym. Sci.* **2007**, *285*, 1281–1286. [CrossRef]
42. McCarthy, N.A.; Power, O.; Wijayanti, H.B.; Kelly, P.M.; Mao, L.; Fenelon, M.A. Effects of Calcium Chelating Agents on the Solubility of Milk Protein Concentrate. *Int. J. Dairy Technol.* **2017**, *70*, 415–423. [CrossRef]
43. Ward, B.R.; Goddard, S.J.; Augustin, M.-A.; McKinnon, I.R. EDTA-Induced Dissociation of Casein Micelles and Its Effect on Foaming Properties of Milk. *J. Dairy Res.* **1997**, *64*, 495–504. [CrossRef]
44. Peydayesh, M.; Suter, M.K.; Bolisetty, S.; Boulos, S.; Handschin, S.; Nyström, L.; Mezzenga, R. Amyloid Fibrils Aerogel for Sustainable Removal of Organic Contaminants from Water. *Adv. Mater.* **2020**, *32*, 1907932. [CrossRef]
45. Samanidou, V.; Filippou, O.; Marinou, E.; Kabir, A.; Furton, K.G. Sol–Gel-Graphene-Based Fabric-Phase Sorptive Extraction for Cow and Human Breast Milk Sample Cleanup for Screening Bisphenol A and Residual Dental Restorative Material Before Analysis by HPLC with Diode Array Detection. *J. Sep. Sci.* **2017**, *40*, 2612–2619. [CrossRef]
46. Souza, P.S.; Krauss, T.M.; Sartori, A.V.; Abrantes, S.M.P. Simplified QuEChERS Technique Followed by UHPLC-MS/MS Analysis for the Determination Bisphenol A in Whole and Powdered Milk. *Int. Food Res. J.* **2023**, *30*, 524–535. [CrossRef]
47. Alexiadou, D.K.; Maragou, N.C.; Thomaidis, N.S.; Theodoridis, G.A.; Koupparis, M.A. Molecularly Imprinted Polymers for Bisphenol A for HPLC and SPE from Water and Milk. *J. Sep. Sci.* **2008**, *31*, 2272–2282. [CrossRef]
48. Zhan, W.; Wei, F.; Xu, G.; Cai, Z.; Du, S.; Zhou, X.; Li, F.; Hu, Q. Highly Selective Stir Bar Coated with Dummy Molecularly Imprinted Polymers for Trace Analysis of Bisphenol A in Milk. *J. Sep. Sci.* **2012**, *35*, 1036–1043. [CrossRef]
49. Maragou, N.C.; Lampi, E.N.; Thomaidis, N.S.; Koupparis, M.A. Determination of Bisphenol A in Milk by Solid Phase Extraction and Liquid Chromatography–Mass Spectrometry. *J. Chromatogr. A* **2006**, *1129*, 165–173. [CrossRef]
50. Filippou, O.; Deliyanni, E.A.; Samanidou, V.F. Fabrication and Evaluation of Magnetic Activated Carbon as Adsorbent for Ultrasonic Assisted Magnetic Solid Phase Dispersive Extraction of Bisphenol A from Milk Prior to High Performance Liquid Chromatographic Analysis with Ultraviolet Detection. *J. Chromatogr. A* **2017**, *1479*, 20–31. [CrossRef]

51. Yang, L.; Chen, Y.; Shen, Y.; Yang, M.; Li, X.; Han, X.; Jiang, X.; Zhao, B. SERS Strategy Based on the Modified Au Nanoparticles for Highly Sensitive Detection of Bisphenol A Residues in Milk. *Talanta* **2018**, *179*, 37–42. [CrossRef]
52. Mercogliano, R.; Santonicola, S.; Albrizio, S.; Ferrante, M.C. Occurrence of Bisphenol A in the Milk Chain: A Monitoring Model for Risk Assessment at a Dairy Company. *J. Dairy Sci.* **2021**, *104*, 5125–5132. [CrossRef]