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ORIGINAL ARTICLE

Analysis of Hydroxyl Methyl Furfuran by Graphene Oxide-reinforced Hollow Fiber Electromembrane Extraction in Food Samples

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KEYWORDS

Graphene oxide; Hollow fiber; Hydroxymethylfurfural; High-performance liquid chromatography; Electromembrane techniques

ABSTRACT: Cooking, sterilization and roasting are used in the food industry for different purposes, such as taste improvement, flavor addition, and shelf-life extension. The roasting stage plays an important role in the improvement of color and sensory properties of coffee; although as a result of this process the level of *Hydroxymethylfurfural* (HMF) is increased. The purpose of the this investigation was to analysis the level of HMF in instant coffee and infant formula samples through Electromembrane extraction (EME) techniques coupled with HPLC. To achieve the highest extraction efficiency, some critical extraction parameters were optimized, including the concentration of geraphen oxide (GO), the pH of the sample solution, the solution existing inside the lumen of hollow fiber, the extraction technique, and desorption time. A full validation was conducted for the method in terms of linearity, precision, and trueness. The method showed good linear ranges (0.1-100µg kg^{-1}) with correlation coefficients greater than 0.999-0.998 µg kg^{-1} for instant coffee and infant formula. The limit of quantification (LOD) value of the present method for instant coffee and infant formula was 0.1µg kg^{-1}. The method provided high recoveries ranging from 85.0% to 108.0% for instant coffee and infant formula. The results indicated that the method was rapid, efficient and environment-friendly in the determination of HMF in instant coffee and infant formula samples.

INTRODUCTION

Technological processes (e.g. thermal treatments) are used in the food industry to obtain desirable sensory properties, ensure microbiological safety, and reduce enzymatic activities [1]. When thermal processes are applied at high temperatures for a long time, food quality undergoes some changes. Such conditions also lead to a reduction in nutritional value, especially due to the depletion of protein/vitamins or the generation of harmful substances that may jeopardize health [2, 3]. As a result, optimizing the thermal process can help to maintain the balance between the safety and quality raw materials.

One of the thermal reactions is called the *Millard reaction*, which is a chemical reaction between amino acids and reducing sugars as a result of exposure to heat. This reaction plays a major role in different food preparation methods such as caramelisation, a kind of non-enzymatic

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browning. Furthermore, an important index for the reaction of Millard is the production of hydroxymethylfurfural (HMF) (formula $C_6H_6O_3$). Therefore, in a wide variety of foods, HMF is recognized as an indicator of the quality factor, resulting from overheating or long-term storage [4, 5]. Although HMF is a thermal processing product, its impact on human health remains an important issue. There are many discussions about the toxicity, genetic toxicity, mutagenicity, and carcinogenesis of HMF [6]. Some scientists believe that HMF is a natural component of traditional foods and does not pose any risk to human health [7], whereas others argue that HMF can function like a neurotoxin as it accumulates in the muscles and damages the nervous system.

From the perspective of food safety, HMF is produced in large quantities and its levels can exceed 1 g/kg in foodstuffs; however, honey is the only food with a legal limit for HMF concentrations [8]. Although Codex Alimentarius (Alinorm 01/25 2000) has proven that after processing and/or composition, the HMF content of honey should not exceed 80 mg/kg., the European Union recommends a low dosage of 40 mg/kg, with the exception of a dosage of 80 mg/kg for honey types from countries/areas with tropical temperatures [9]. Similarly, many researchers have investigated the amount of HMF in bread, instant coffee, honey, juice, raisins, milk, biscuits, jam, and breakfast cereals, because HMF can serve as a parameter reflecting the freshness and quality of foods [10, 11].

There are many different methods for extracting HMF from and quantifying it in foodstuffs, infant products, and instant coffee. Conventional methods include liquid-liquid extraction (LLE) [12, 13] and solid phase extraction (SPE) [12-14]. However, these methods involve some disadvantages as they are time-consuming and complex, need a large amount of organic solvents, generate secondary waste harmful for the environment, and require a derivatization procedure. Recently, the microextraction technique has been demonstrated as a relatively new extraction technique used for pre-concentration before conducting an appropriate analysis for complex matrixes. Low-level solvent exposure, short-time consumption, a low amount of sample needed, the clean-up free process, and high sensitivity are some of the advantages of microextraction techniques in comparison to the conventional methods.

Microextraction methods, such as solid-phase microextraction (SPME) and liquid-phase microextraction (LPME), are used to analyze HMF in honey samples [15-17]. Some other methods (e.g. dispersive liquid-liquid microextraction, DLLME) have been utilized to analyze HMF in milk-based (powdered) infant formulas [18]. Various instrumental HMF determination techniques have been reported including spectroscopy [19], liquid chromatography multi-stage mass spectrometer, high performance liquid chromatography with UV/vis detection, and headspace gas chromatography with mass spectrometry [20 - 22].

Electromembrane techniques (EME), proposed by Pedersen et. al., represents a newly modified liquid phase micro-extraction [23, 24]. EME has been successfully applied to the determination of a variety of analytes in environmental water, foods, drugs, and biological fluids [25]. The procedure is based on the migration of the analytes through the supported liquid membrane (SLM) by applying an electric potential difference between both sides of the membrane [26].

Coffee mix and infant formulas are complex samples because of their high protein and fat contents; therefore, effective cleaning techniques are essential before the HMF analysis [27]. Furthermore, Graphene Oxide (GO) is a carbon material with unique mechanical, thermal, and electrical properties. GO has a large surface area, which is a quality that has increased its application in modified electrochemical sensors and has introduced it as a novel adsorbent in microextraction techniques [28].

The purpose of the present investigation is to analyze the level of HMF in instant coffee and infant formula samples through Electromembrane techniques (EME) coupled with HPLC. To accomplish this, EME extraction is conducted through a hollow fiber reinforced with GO and is identified by an HPLC-UV detector.

MATERIALS AND METHODS

Chemicals and reagents

The HMF reference standard was obtained from Sigma-Aldrich (Germany). Graphite, sodium hydroxide, 1-Octanol, hydrochloric acid, acetonitrile and acetone were obtained from Merck, Darmstadt, Germany. Ultra-pure water was used from a Millipore system (Le Montsur-Lausanne), Switzerland. All the reagents and solvents were of analytical grade. PPQ3/2 polypropylene hollow fibers were purchased from Membrane Co. (Wuppertal, Germany) with an inner diameter of 0.6 mm, a thickness of 200 μm, and a pore size of 0.2 μm.

Standards preparation

HMF is freely soluble in water, methanol, ethanol, acetone, and ethyl acetate. The standard stock solution containing 1000 mg L^{-1} of HMF was provided in methanol; 10 mM of hydrochloric acid (HCL) solution was used to prepare the working standards for the calibration curve. The spiked samples were prepared at the concentration of 1 μg kg^{-1} by adding the HMF standard solution to the infant formula and instant coffee for accuracy assessment.

EME set-up and procedure

The hollow fibers were cut into 5.0 cm pieces, dipped in acetone or acetonitrile, and placed in an ultrasound bath for 10 seconds to remove the impurities and contaminants from the surface and inner lumen of the HF. The hollow fibers were dried in air. The GO synthesis was based on the Hummer method. The quality of the synthesis was confirmed through a scanning electron microscope (SEM; Tescan; Czech Republic) and through FT-IR. Briefly, 2 mg of GO in 1 ml 1-octanol was sonicated in an ultrasonic bath for 30 min to disperse GO in the solvent (Figure 1). The GO was then adsorbed onto the wall pores of the HF with 20 μl SLM using a Hamilton syringe. Afterwards, the HF was cleaned with ultrapure water to remove the excess GO from the lumen. The holes were filled with GO after SLM injection. These micrographs indicated that the GO was immobilized onto wall pores of the HF. The end of the hollow fibers was sealed using heat. Finally, 15 μL of acceptor solution (10 mM hydrochloric acid, pH was adjusted to 2.0) was placed into the liner lumen.

15 mL of the sample solutions were placed into the 20-mL extraction glass tube with a screw cap. A POWER-PAC-3000 power supply (BIO-RAD) and two platinum wire electrodes (diameter 0.2 mm) were used. For the extraction of HMF, the cathode was located in the acceptor solution and the anode was located in the sample solution. Then, both of the electrodes were connected to the power supply and the extraction process was conducted by applying an optimum voltage of 50 V between the electrodes. The sample solution was stirred using a stirrer at 500 rpm. When the extraction was over, the power supply and magnetic stirrer were turned off. The acceptor solution was immediately collected by the Hamilton syringe and analyzed through HPLC-UV.

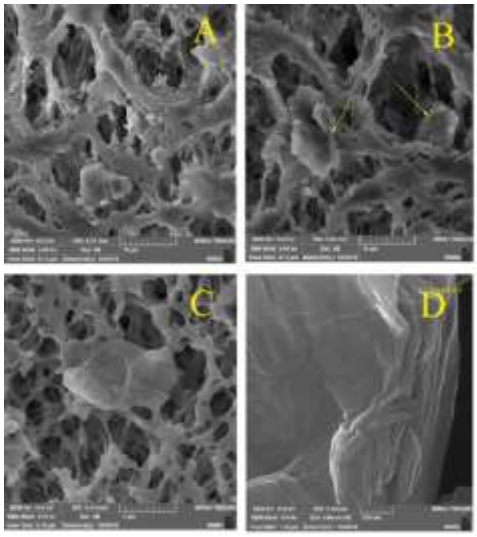

Figure 1. Scanned images produced by the electron microscope of GO-HF (A, B and C) and GO (D).

Analytical conditions

The analysis was conducted using a Dionex UltiMate 3000 HPLC system from the American company Dionex, including the UltiMate 3000 pump. The Chromeleon software from Dionex was used to control the tools and the data processing. The analyte was detected through the PDA detector (VWD-3400) with a wavelength of 285 nm. C18 column (250mm × 4.6mm, 3µm) was selected from the Waters Companies to conduct the separation. The volume of the injection was 10 µl with a flow rate of 0.5 ml/min. The mobile phases containing water and methanol with a ratio of 80 and 20(V/V) were used to enhance the separation of HMF.

Validation of results

The analytical method validation parameters including accuracy, precision, LOQ were determined (Table 1).

Table 1. The figures showing the advantages of the proposed method and its comparison with other methods

Method	Matrix	Analyte	Calibration range ($\mu g kg^{-1}$)	R2	LOQ ($\mu g kg^{-1}$)	Reference
RP-HPLC-UV[a]	Pestil, Jam, Marmalade	HMF	1000-1200	0.999	200	37
SPE-LC-MS[b]	Cereal and milk-based	HMF	50-2000	> 0.99	-	38
DLLME-HPLC-UV[c]	Powdered, ready-to-eat soups, fruit puree and juices	F	0.2-200	0.9902	4.4	20
		HMF		0.9915	6.7	
Nano-EME[d]	infant formula	HMF	0.1-100	0.999	0.1	This study
	instant coffee			0.998		

[a] Revers phase-high performance liquid chromatography, [b] Solid phase extraction-liquid chromatography-mass spectrometry, [c] Dispersive liquid-liquid microextraction coupled with high performance liquid chromatography, [d] The proposed method

Selectivity

The chromatograms were obtained by EME-HPLC-UV for the HMF standard solution and HMF levels in the instant coffee sample were compared for selectivity analysis.

RESULTS AND DISCUSSION

Optimization of the EME method

In order to obtain high enrichment and extraction efficiency of the analyte, the main parameters were optimized. The chemical properties of the organic solvents which used as

SLM were important in extraction efficacy, and some of them were critical for a successful and reliable electro-kinetic cross-membrane extraction. These parameters were low solubility of the aqueous phase, and an appropriate partition coefficient for the analyte and non–volatile properties of SLM [29]. GO must be well dispersed in the organic solvent [30]. Three different solvents were investigated including 1-octanol, n-hexane, and ethyl acetate. Among the tested solvents, GO was being well dispersed in 1-octanol and was more effective for HMF extraction.

The adsorption capacity was affected by the concentration of GO in the acceptor phase. In this study, wide concentration ranges from 0 to 3 mg mL^{-1} were tested. The optimal concentration of the GO was 2 mg ml^{-1}. The pH of the sample and the acceptor solution were also optimized within the range of 1 to 7.5. The best pH was 5.5 for the sample solution and 2 for the acceptor solution (Figure 2.A and 2.B).

This study relied on GO as a SLM to increase electrical conductivity. It has been shown that using high voltages would lead to an increase in the air bubble and a decrease in the recovery of reaction [31]. As shown in Figure 2C, the most efficient extraction occurred at 60v. The extraction time and stirring rate were the factors affecting the increase of the kinetics and the yield of extraction. The stirring rate enhanced the mass transfer of the analyte and reduced the thickness of the double layer around the interface on both sides of the SLM [32]. Moreover, the extraction time was a very important parameter of the EME technique. As shown in Figure 2D, the extraction time was investigated within 2-20 min. The best extraction time was 15 min, in line with Balchen and colleagues' study [33].

The stirring rate which was applied for the HMF extraction was between 500 and 1500 rpm and the optimum rate was identified at 700 rpm. The results confirmed that the agitation of the sample enhanced the extraction procedure. However, higher stirring rates (>750 rpm) resulted in massive air bubbles and decreased the pre-concentration factors (Figure 2E). The data in this study confirmed the observation of Yamani and Rezazadeh 2014 [34].

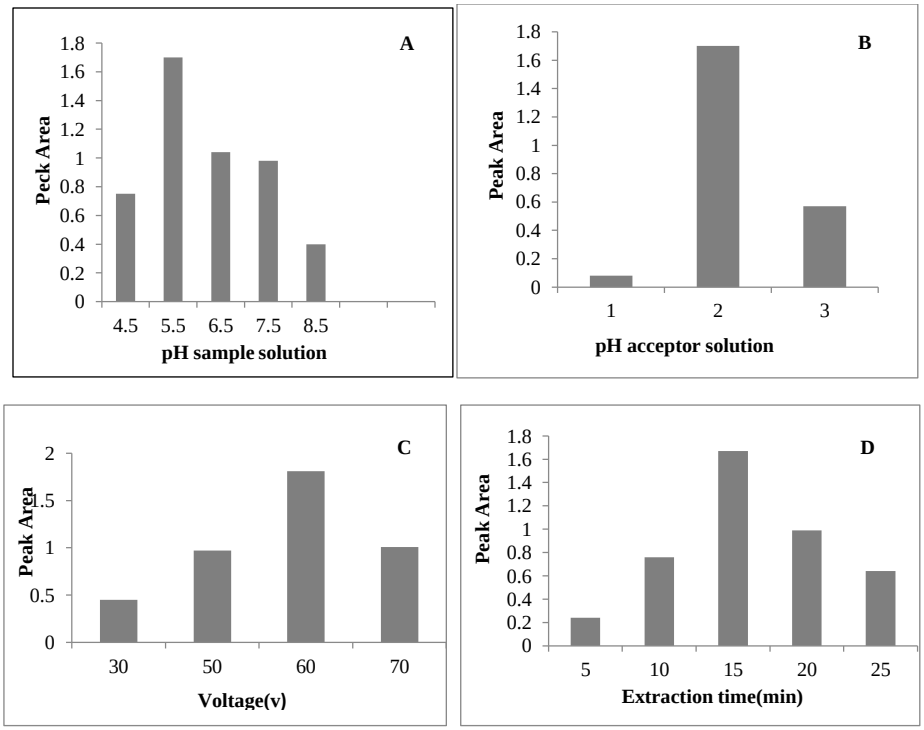

Figure 2. (A) Effect of the pH of the sample solution; (B) effect of the pH of the donor solution; (C) effect of the applied potential; (D) EFFECT of the extraction time; (E) effect of stirring rate on the GO-EME efficiency of HMF.

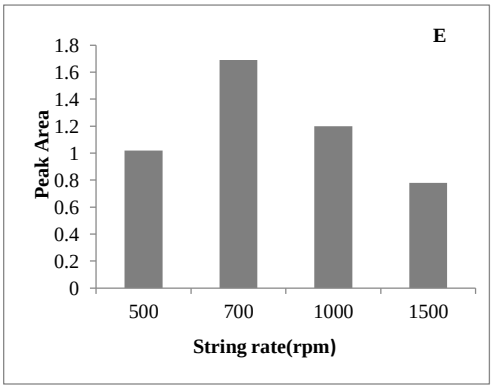

Figure 2. Continued

Evaluation of the GO-EME Method

To assess the practical applicability of the GO-EME, the advantages of this method were investigated under the best conditions (2 mg/mL GO with 1octanol solvent as SLM, stirring rate of 700 rpm, voltage of 60 V, 20 min extraction time, and a donor phase pH of 2). The analyte was detected through HPLC. The chromatograms of the standard solution sample are illustrated in Figure 3. The repeatability of the method was determined by performing three extractions of samples spiked with 1μg kg^{-1} for the HMH, and the RSD of the method for instant coffee and infant formula was 7.4% and 13.4%, respectively. The results are shown in Table 2.

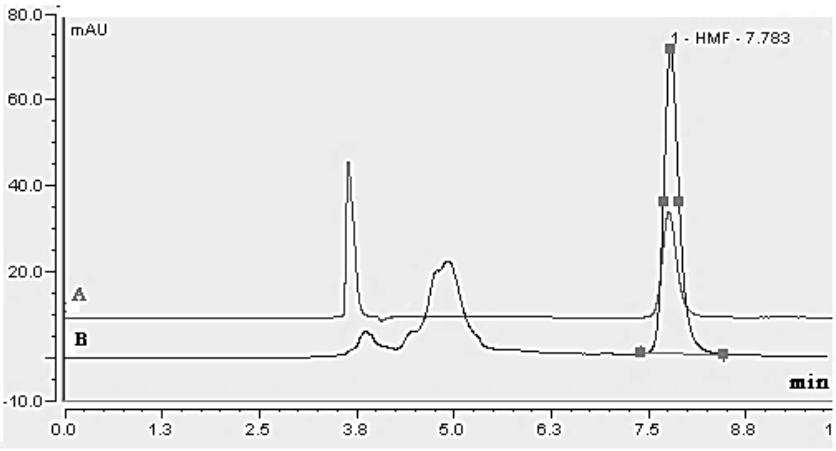

Figure 3. Chromatograms of HMF (A) Standard HMF (B) Instant coffee

The performance parameters of the GO-EME technique, such as the linearity, low limits of determination (LOD), and limits of quantification (LOQ) for the HMF were evaluated under the optimum extraction conditions. Acceptable linear ranges (0.1-100 μgkg^{-1}) were obtained with a correlation ratio of 0.999-0.998 μgkg^{-1} between the instant coffee and the infant formula. The results are shown in Table 1. Figure 3, shows that no interference occurred at the retention time of HMF in the solvent and the sample, indicating that the proposed method was selective for HMF analysis.

Table 2. HMF recovery tests performed on instant coffee, infant formula, and water samples.

Sample	N replicates	Average Recovery%	Average RSD%
Instant coffee	3	85.1	7.4
Infant formula	3	103	13.4

CONCLUSIONS

This study proposed an effective and sensitive method to determine HMF in foods using graphene oxide-reinforced hollow fiber EME-Go. After the extraction procedure, the analyte was desorbed and analyzed by high-performance liquid chromatography-photodiode array detection (HPLC-UV-vis). The GO was dispersed in 1-octanol through ultrasonication and then immobilized into the pores of a hollow fiber (HF).

The results indicated that the method was simple, sensitive, environment-friendly, and cost-effective, while showing an excellent clean-up ability for the extraction of HMF in different samples. The results also demonstrated that the EME-GO combination could be a promising method for the enrichment and clean-up of HMF in instant coffee and infant formula samples (Table 1).

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