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Development of in-situ produced CO_2 switchable fatty acid microextraction based solidification of floating organic droplet for quantification of morin and quercetin in tea, vegetable and fruit juice samples by HPLC

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ABSTRACT

An in-situ produced CO_2 switchable fatty acid microextraction based on solidification of floating organic droplet (In-situ-CO_2-SFA-ME-SFO) was evaluated for microextraction of two antioxidant flavonoids (morin and quercetin) in tea, fruit juice and vegetable samples prior to HPLC-UV. Medium-chain fatty acids (e.g. nonanoic acid) were investigated as switchable hydrophilicity solvents via pH adjustment. Sodium carbonate (Na_2CO_3) was used to solubilize immiscible fatty acid in water as well as, to provide effervescence. The addition of H_2SO_4 into the solution led to the in-situ chemical reaction with excess Na_2CO_3 which resulted in effective dispersion of fatty acid through generated CO_2 and separation of phases. Subsequently, solidification of the fatty acid enabled facile separation without the need for sophisticated equipment. To optimize extraction process, the effects of some important parameters on the extraction recovery were investigated. At the optimum conditions, the limits of detection (LODs) and the limits of quantification (LOQs) were found 0.5 and 1.3 μg L^{-1} and 1.6 and 4.3 μg L^{-1} for morin and quercetin respectively. The preconcentration factors were 105 while, the relative standard deviations (RSDs %) of the method were < 3.5 % for both flavonoids. The recoveries of the analytes in tea, vegetable and fruit juice samples were in the range of 95.5 and 98.2%

1. Introduction

Morin,(2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one), and quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) are two major plant flavonoids which are widely distributed in human foods and herbs, particularly in tea, dark chocolates, onions, and beans [1]. The beneficial pharmacological properties of these polyphenols including anti-oxidant, anti-inflammatory, anti-cancer and cardioprotective effects are well established in a wide range of preclinical and clinical studies [2-4]. Given that morin and quercetin are structural isomers with close resemblance and comparable biological activities, it is indispensable to develop new sensitive and selective methodologies for their extraction and determination [4]. To this end, sample preparations before quantification of morin and quercetin in real samples attracted increasing attention in recent years. Such preparation techniques have at least three main advantages a) they decrease the complexity of the matrix of the samples, b) minimize the interferences with similar absorption peaks of other flavonoids and c) elevate the concentration to a proper level which can be reproducibly detected [5]. Traditional sample preparation methods such as liquid–liquid extraction (LLE) and solid phase extraction (SPE) are associated with some limitations such as tedious processes and consuming a lot of expensive and toxic solvents [6,7]. In recent years, liquid-phase microextraction (LPME) methods have been applied as separation procedures due to low extraction solvent consumption, simplicity, adaptability to a wide ranges of samples, analytes and analytical instrumentation [8,9]. Among LPME methods, dispersive liquid–liquid microextraction (DLLME) is known as a method based on fast dispersion of extraction and dispersing solvents into a sample solution containing the target analytes. DLLME has gained growing interest because it is simple, fast to

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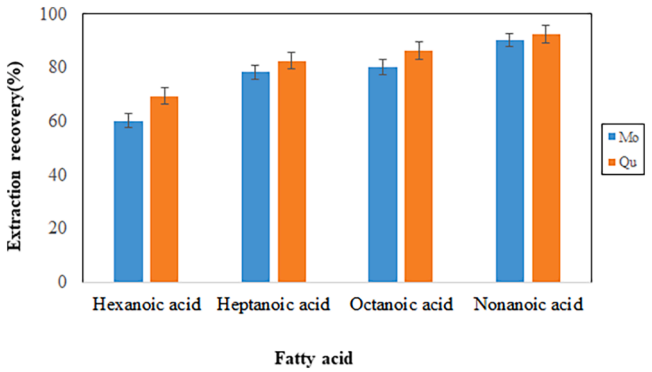

Fig. 1. Effect of medium-chain fatty acid types on the extraction recovery of quercetin and morin. Extraction conditions: 10 mL of sample solution, concentration quercetin and morin 50 μg L^{-1}, 400 μL of Na_2CO_3 (2 mol L^{-1}); 600 μL H_2SO_4 (3 mol L^{-1}); 150 μL of extraction solvent.

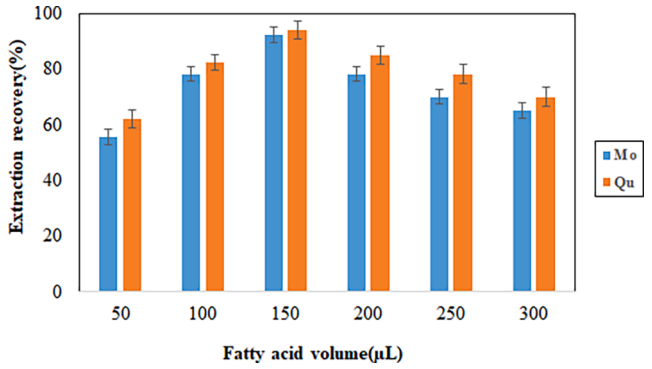

Fig. 2. Effect of volumes of fatty acid on the extraction recovery of quercetin and morin. Extraction conditions: 10 mL of sample solution, concentration of quercetin and morin 50 μg L^{-1}, 400 μL of Na_2CO_3 (2 mol L^{-1}); 600 μL H_2SO_4 (3 mol L^{-1}); extraction solvent, nonionic acid.

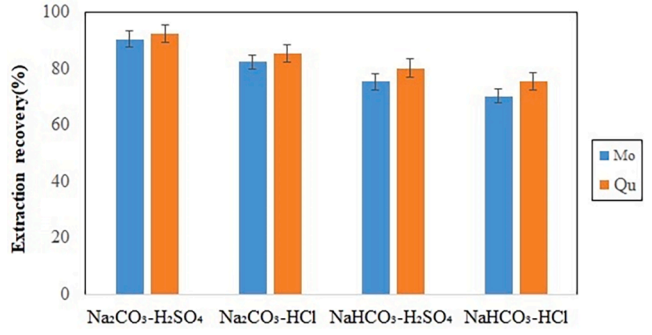

Fig. 3. Effect of effervescency agents and proton donor agents on the extraction recovery of quercetin and morin. Extraction conditions: 10 mL of sample solution, concentration of quercetin and morin 50 μg L^{-1}, extraction solvent, 150 μL of nonionic acid.

operate and requires low amount of organic solvent [10]. However, traditional DLLME accompanies by several disadvantages such as the utility of hazardous halogenated hydrocarbons as extractants with densities higher than water [11], and dispersive solvents that decrease extraction efficiency [12,13]. To overcome these drawbacks, trends in DLLME have been shifted towards replacing toxic solvents with more environmentally friendly alternatives and the elimination of dispersive solvents [13]. Solidification of floating organic droplets (SFO) has been developed as a version of DLLME in which the extraction solvents with lower density than water and low melting point are used [13-15].

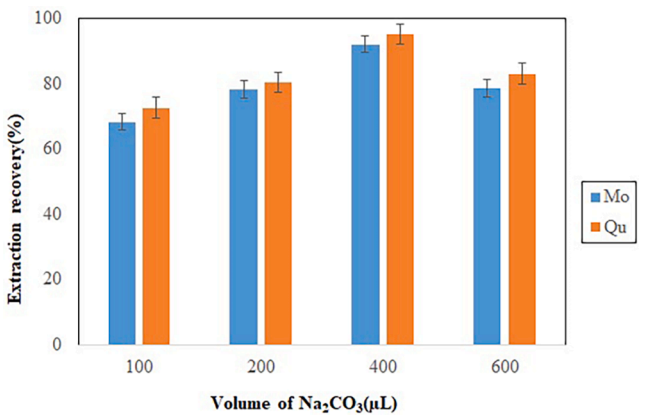

Fig. 4. Effect of volume of effervescent agent (Na_2CO_3) on the extraction recovery of quercetin and morin. Extraction conditions: 10 mL of sample solution, concentration of quercetin and morin 50 μg L^{-1}, extraction solvent, 150 μL of nonionic acid, 600 μL H_2SO_4 (3 mol L^{-1}).

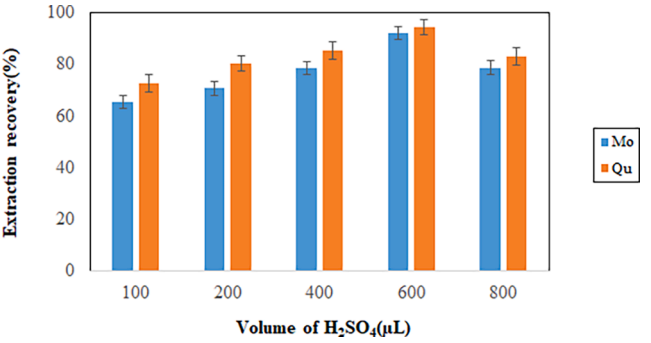

Fig. 5. Effect of volume of the proton donor agent (H_2SO_4) on the extraction recovery of quercetin and morin. Extraction conditions: 10 mL of sample solution, concentration of quercetin and morin 50 μg L^{-1}, extraction solvent, 150 μL of nonionic acid, 400 μL of Na_2CO_3 (2 mol L^{-1}).

Table 1
Analytical performance of In situ-CO_2-SFA-ME-SFO method for determination of two flavonoids.

Analyte	LOD[a] (μg L^{-1})	LOQ[b] (μg L^{-1})	LR[c](μg L^{-1})	R^2	RSD% (n = 6)	PF[d]	% ER[e]
Quercetin	1.3	4.3	4.3–500	0.9967	3.1	105	95.9
Morin	0.5	1.6	1.6–500	0.9979	2.8	105	96.7

[a] Limit of Detection (S/N = 3)
[b] Limit of quantification (S/N = 10)
[c] Linear range
[d] Preconcentration factor
[e] Extraction recovery

Nevertheless, only a few extraction solvents satisfy the requirements of SFO, therefore, a huge endeavor has been made to find suitable solvents for this purpose [16,17]. Hence, green solvents such as switchable hydrophilic solvents (SHS) have attracted growing attention as an extractant substitute[17–23]. These solvents can be switched between two forms of poor and proper miscible states in water upon the addition or removal of atmospheric CO_2. A medium-chain fatty acid whose hydrophobicity can be reversibly altered by changing pH is a suitable SHS candidate for LPME [23]. Some medium-chain saturated fatty acids have low freezing points and therefore can fulfill the condition required for SFO [17]. Currently, to achieve the dispersion process, a novel approach of DLLME so-called effervescence assisted dispersive liquid–liquid

Table 2
Analysis of two flavonoid in distillated water, tea, vegetable and fruit juice samples spiked at different concentrations using In situ-CO_2-SFA-ME-SFO method. (n = 3).

Sample	C_{added}(μg L^{-1})	C_{found} (μg L^{-1})	RSD (%)	RR (%)	C_{added}(μg L^{-1})	C_{found} (μg L^{-1})	RSD (%)	RR (%)
	Quercetin				Morin			
Distillated water	–	–	–	–	–	–	–	–
	10	9.5	3.7	95.3	10	9.4	2.9	94.0
	50	49.9	2.6	99.8	50	48.2	3.1	96.4
	200	192.2	3.1	96.1	200	189.6	4.1	94.8
Black tea*	–	32.1	4.2	–	–	36.8	–	–
	10	41.7	3.8	96.0	10	46.5	6.1	97.1
	50	82.6	4.9	101.2	50	85.7	3.4	97.8
	200	233.5	2.8	100.7	200	243.6	4.8	103.4
Green tea*	–	54.3	3.9	–	–	54.2	–	–
	10	64.0	4.1	96.6	10	63.7	2.9	95.3
	50	102.9	5.0	97.3	50	102.3	4.1	96.3
	200	247.9	1.8	96.8	200	250.0	4.3	97.9
Apple	–	17.3	2.8	–	–	14.5	–	–
	10	26.8	3.5	95.3	10	24.2	4.4	96.6
	50	67.2	6.4	99.7	50	62.1	5.3	95.3
	200	207.1	5.9	94.9	200	212.1	1.9	98.8
Orange	–	9.8	5.1	–	–	14.1	–	–
	10	19.6	2.5	97.6	10	23.6	4.8	94.7
	50	57.7	4.2	95.8	50	61.4	4.1	94.5
	200	201.2	4.2	95.7	200	207.9	4.9	96.9
cherry	–	21.8	3.6	–	–	25.2	–	–
	10	31.3	3.1	94.9	10	34.7	2.4	95.3
	50	73.5	2.9	103.4	50	72.5	6.0	94.6
	200	224.2	4.7	101.2	200	219.0	5.2	102.3
Onion	–	19.3	3.3	–	–	21.7	–	–
	10	29.1	2.8	98.3	10	31.9	3.5	101.7
	50	69.9	4.6	101.3	50	69.6	3.5	95.8
	200	212.9	3.9	96.8	200	214.1	4.6	96.2

* μg kg^{-1}

microextraction (EA-DLLME) has been developed based on in-situ CO_2 generation [14]. In this method, the CO_2 bubbles produced through the reaction of low cost and low-toxic agents that homogeneously disperse the extraction solvent into the sample solution [14]. In other words, a relatively strong base (e.g. Na_2CO_3) and an acid (e.g. H_2SO_4) act as effervescent and proton donor agents respectively to assist the fast dispersion of the extraction solvent into the sample. In this view, we developed an in situ-produced CO_2 switchable fatty acid micro-extraction based on solidification of floating organic droplet (In situ-CO_2-SFA-ME-SFO) for microextraction of quercetin and morin followed by HPLC-UV analysis. In this method, we used medium-chain fatty acids as the SHS with SFO property. Na_2CO_3 was utilized to solubilize the water-immiscible fatty acid form and to provide effervescence, thereby increased the extraction efficiency. In addition, the efficacy of the developed procedure was evaluated in the determination of quercetin and morin for tea, vegetable and fruit juice samples.

2. Experimental

2.1. Chemicals

Chemicals including hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, quercetin and morin standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). 100 mg L^{-1} stock solutions of quercetin (Qu) and morin (Mo) in ethanol (GC-grade) were prepared and stored at 4 °C. Working standard solutions were prepared by diluting with Milli-Q ultrapure water (Millipore, Bedford, MA, USA) daily. Ethanol, NaOH, HCl, H_2SO_4, sodium carbonate (Na_2CO_3), Sodium bicarbonate ($NaHCO_3$) and acetonitrile were purchased from Merck (Darmstadt, Germany).

2.2. Instrumentation and operating condition

Separation of two flavonoids was performed using a Waters HPLC System with a 1525 Binary LC Pump and 2489 UV– VIS detector equipped with a C_{18} column (150 mm, 4.6 mm I.D. 5 mm particle size). The separations were carried out using a mobile phase of methanol: % 0.3 phosphoric acid in water: THF (40:59.7:0.3, v/v/v) with a flow rate of 1.0 mL min^{-1} by isocratic elution at ambient temperature. Detection of both flavonoids was conducted at the wavelength of 275 nm.

2.3. Sample preparation

All samples, black and green tea, fruit samples (apple, orange and cherry) and vegetable sample (onion) were purchased from a local market (Tehran, Iran). 2.0 g aliquots of tea samples were added to 100 mL of boiling water for 10 min, followed by cooling down to the room temperature and separation of the infusions. Fruit samples were initially washed with distilled water, homogenized in a juice extractor and centrifuged at 3500 rpm for 5 min and the juices were then passed through a filter. The onion sample preparation was the same, except peeling off before washing.

2.4. In-situ-CO_2-SFA-ME-SFO microextraction procedure

For 10 mL of the sample solution containing 50 μg L^{-1} of quercetin and morin in 15 mL conical glass test tubes, 150 μL of nonanoic acid was added as the extraction solvent, followed with ultrasonication (1 min). Then, the effervescence process was performed via the addition of 400 μL of Na_2CO_3 (2 mol L^{-1}). Upon Na_2CO_3 addition and increasing the pH, a clear solution was observed due to the conversion of the water-insoluble fatty acid to water-soluble aggregations. Then, 600 μL of H_2SO_4 (3 mol L^{-1}) was added to the tube, and the effervescence happened in situ followed by CO_2 bubbles generated, resulting in a dispersion of extractant and then phase separation. Therefore, in situ microextraction was accomplished and separation was performed without centrifuging step. The resulting mixture was kept on an ice bath for 2 min. Subsequently, the solidified extraction phase containing analytes was formed at the surface of the aqueous sample and transferred into a clean conical vial and melted (~95 μL) at room

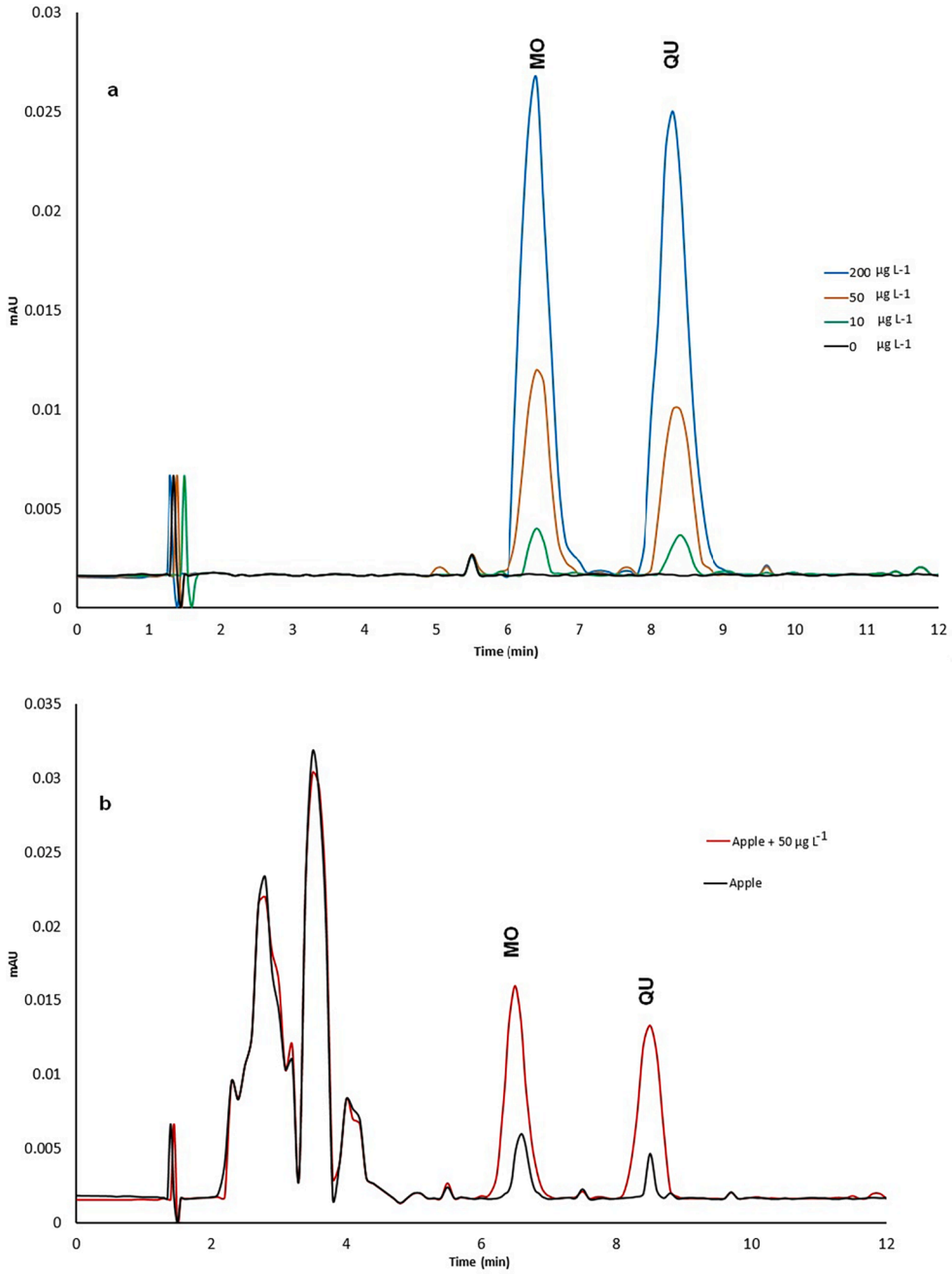

Fig. 6. (a) Typical HPLC-UV chromatograms of standard solutions (0, 10, 50 and 200 µg L^{-1}) of quercetin and morin, (b) the extracted flavonoids from the apple juice sample, (c) onion sample, and (d) black tea sample spiked at 0 and 50 µgL^{-1} of quercetin and morin.

temperature. Finally, 20 µL of the extractant was introduced into the sample loop of HPLC-UV for quercetin and morin detection.

The preconcentration factor (PF) was calculated from dividing the aqueous phase volume (V_{aq}) to the final volume (V_f)

$$PF = \frac{V_{aq}}{V_f} \quad (1)$$

Relative recovery (RR%) was estimated by equation (2):

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (2)$$

Where C_{found}, C_{real}, and C_{added} demonstrate the founded concentration of the analyte after adding a known amount of standard to real samples, the concentration of the analyte in the real sample and a standard solution spiked in the real sample respectively.

3. Result and discussion

In-situ-CO_2-SFA-ME-SFO microextraction method was assessed by two sequential steps. At the first step, nonanoic acid was added to the sample and a hydrophilic form of fatty acid was resulted by addition of sodium carbonate solution. Fatty acids can be converted into their anionic form at pH higher than their pKa by at least three pH units and at three (or more) pH units below their pKa, they are in their hydrophobic form [23]. Almost 99.9% of nonanoic acid is converted into its anion form in alkaline sodium carbonate solution (the pKa of nonanoic acid is 4.96). Also, both morin and quercetin are weak acids. They are in

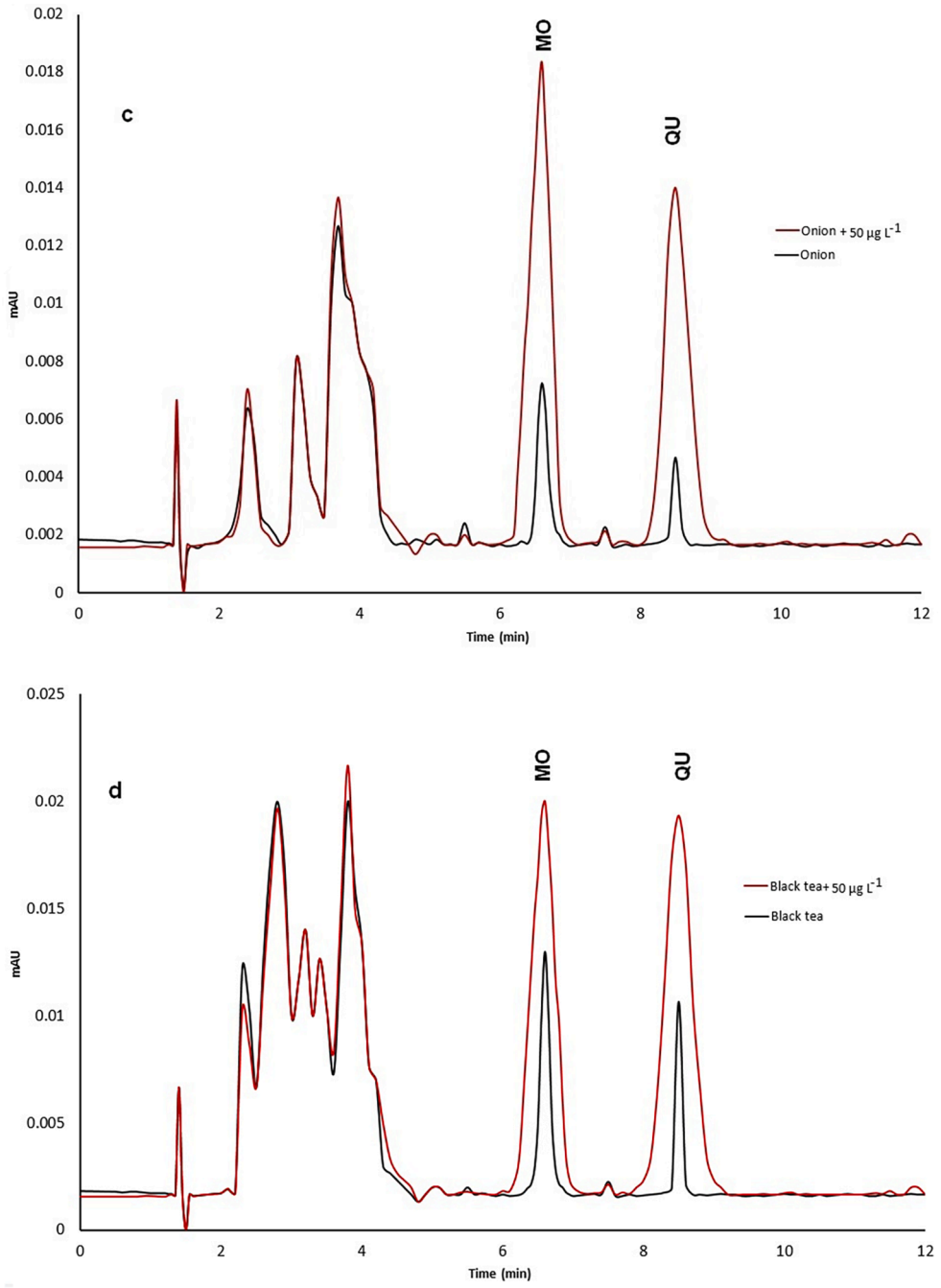

Fig. 6. (*continued*).

molecular forms at pH lower than their pKa, while at an alkaline solution, they are converted to their ionic forms. Thus, a homogeneous solution was formed. In the second step, by addition of H_2SO_4 solution, microextraction was performed due to two simultaneous chemical processes. With the decrease of the sample solution pH, the hydrophobic nonanoic acid was formed and hydrophobic analytes were transferred to organic phase. Moreover, the effervescence was accomplished due to the reaction of H_2SO_4 with excess sodium carbonate, and CO_2 bubbles generated in situ and enhanced the extraction process and final phase separation. Thus, the total microextraction fulfilled by changing of pH value.

3.1. Selection of fatty acid

The extraction solvent choice plays a key role in the efficiency of LPME procedures. For an effective LPME, the extractant requires having several characteristics, such as low solubility in water, high affinity for analytes and lower density than water. Medium-chain fatty acids can be considered as SHS in the SHS-ME methods because they are able to switch between hydrophobic and hydrophilic forms upon pH modification. In this study, hexanoic acid, heptanoic acid, octanoic acid and nonanoic acid have been studied as the extractants. The results in Fig. 1 showed that the quantitative extraction recoveries were obtained using nonanoic acid. This could be explained by special physicochemical properties of the nonanoic acid when compared with the other studied fatty acids. The melting points for hexanoic acid, heptanoic acid, octanoic acid andnonanoic acid are − 4, −7.5, 16 and 12.5 respectively.

We found out only nonanoic acid was able to coagulate rapidly at an ice bath and provided the sufficient volume of organic phase (95 µL) for separation while other acids formed stable emulsions and effective

Table 3
Inter-day and intra-day reproducibility (n = 5) as standard deviations for determination of two flavonoid in distillated water spiked at different concentrations using In situ-CO_2-SFA-ME-SFO method.

Intra-day test (n = 5)(Morin)			
Concentration (μg L^{-1})	Found (μg L^{-1})[a]	Precision (%)	Recovery (%)
20	19.3 ± 1.1	5.7	96.4
100,500	97.9 ± 3.7	3.8	97.9
	486.5 ± 26.3	5.4	97.2
Inter-day test (n = 5)(Quercetin)			
Concentration (μg L^{-1})	Found (μg L^{-1})	Precision (%)	Recovery (%)
20	19.2 ± 1.0	5.4	96.2
100,500	97.3 ± 5.6	5.8	97.3
	484.5 ± 20.8	4.3	96.9

[a] Mean ± SD (n = 3)

phase separation was not observed. This could be attributed to lower solubility of nonanoic acid in the sample solution in comparison to other acids. Hence, the capability of nonanoic acid to change between hydrophilic and hydrophobic forms with changing of pH and the ability to transform from the liquid to a solidified floating state immediately made it a proper choice as a switchable solvent-microextraction in comparison to other fatty acids. Therefore, nonanoic acid was selected as the best fatty acid for the extraction of the two flavonoids.

3.2. Effect of SHS volume

To improve the efficacy of extraction, various volumes (50 to 300 μL) of fatty acid were tested (Fig. 2). An increasing trend in the extraction recovery (up to 90%) was found by enhancing the volume of nonanoic acid from 50 to 150 μL. However, further increment in the volume resulted in a slight decrease on the extraction recovery. By increasing the volume of nonanoic acid, the volume of the floated phase increased and preconcentration factor decreased. Hence, 150 μL of nonanoic acid was selected as the optimum volume of extractant solvent to obtain the highest recovery.

3.3. Effect of effervescent composition

To induce an effervescent process, enhance the dispersion of extractant, and fulfill the microextraction process, an effervescency agent (Na_2CO_3, $NaHCO_3$, etc.) and a proton donor agent (mineral acids such as H_2SO_4, HCl, etc.) are necessary. Herein, Na_2CO_3 and $NaHCO_3$ were studied as a bivalent effervescency and HCl and H_2SO_4 were considered as proton donor agents. As shown in Fig. 3, the extraction recoveries for combinations of Na_2CO_3-H_2SO_4 and Na_2CO_3-HCl were higher than $NaHCO_3$-H_2SO_4 or $NaHCO_3$-HCl. It can be explained that the thermal stability of carbonate is more than that of bicarbonate. Also, Na_2CO_3 can excrete carbon dioxide slowly causing longer effervescence time for an increase in the transfer efficiency [24]. On the other hand, a mineral acid was used to convert ionic form of fatty acid into hydrophobic form and to provide effervescence (the reaction with excess Na_2CO_3). Thus, Na_2CO_3 and H_2SO_4 were chosen for the in situ-CO_2-SFA-ME-SFO method.

3.4. Effect of sodium carbonate and sulfuric acid concentrations and their volumes

To achieve the highest extraction recovery, it is necessary to determine the optimum concentrations of H_2SO_4 and Na_2CO_3 in the effervesce process. A proper concentration of effervescent agents can generate more carbon dioxide to enable the dispersion of extraction solvent and enhance the transfer of the target analytes in the extractant but an excessive amount of each agent can decrease the extraction recoveries due to the increase of the solution viscosity [25]. To this end, a range of H_2SO_4 and Na_2CO_3 concentrations (0.5 to 5 mol L^{-1}) were studied on the extraction recovery. Our data showed that quantitative extraction recoveries of two flavonoids were obtained at 2 mol L^{-1} of Na_2CO_3 and 3 mol L^{-1} of H_2SO_4.

Moreover, the effect of different volumes of effervescent agent was investigated in the range of 100–600 μL (Fig. 4). The highest recovery was achieved at 400 μL of Na_2CO_3. As seen in Fig. 4, the extraction recovery enhanced with the increase of Na_2CO_3 up 400 μL and after that, the extraction recovery decreased. The reason for the enhanced recovery at higher volumes of Na_2CO_3 can be due to the more effective formation of CO_2 bubbles and better dispersion of the extractant solvent. However, with increasing Na_2CO_3 volume more than optimum volume (400 μL), higher volume of the proton donor agent is required to form an effective effervescence and enhance dispersion of the organic solvent in the sample solutions. Thus, it can lead to provide a large volume of aqueous phase versus extractant solvent and lower extraction recovery. As a result, there are not enough extraction solvent generated to extract all analytes from the sample solution. Therefore, 400 μL of Na_2CO_3 was selected. Also, to find the optimum volume of H_2SO_4, various volumes of

Table 4
Comparison of In situ-CO_2-SFA-ME-SFO method with other methods in determination of morin and quercetin.

Method	Analyte	Sample	LOD (μg L^{-1})	LR (μg L^{-1})	RSD %	PF	ER(%)	Extraction time time(min)	Reference
SBME-DES-HPLC-UV[a]	Morin	Apple, orange, pineapple, onion	0.2	1–500	2.2	361,377	90.3		[26]
	Quercetin		2.6	10-500	2.5		94.4	40	
VAIL-DLLM-Spectrophotomet[b]	Morin	Tea, fruit, wines	–	–	–	–	–	–	
	Quercetin		12.2	35–750	2.9–3.5	150	95.5	0.5	[27]
D- μSPE-DES-HPLC-UV[c]	Morin	Dark tea, onion dark chocolate,	0.2	1–500	2.9–3.3	–	93–97.4		[28]
	Quercetin		0.3	1-500	2.8–4	-	92.8–99.8	7.1	
HF-LPME-HPLC UV[d]	Morin	pineapple, orange echinophora platyloba DC. and	1.5	5–500	3.2	293,165	80		[29]
	Quercetin	Mentha piperita	4	10-500	4.6		45	80	
IL-CPE- HPLC- UV[e]	Morin	Apple, gapes juice	–		–	–	–	–	[30]
	Quercetin		2	1000–20000	3.76	20	92.5	20	
This work	Morin	Tea. Fruit juice, vegetable	0.5	1.6–500	2.8	105,105	96.7	3	This work
	Quercetin		1.3	4.3–500	3.1		95.9		

[a] Solvent bar microextraction based on a deep eutectic solvent
[b] Vortex assisted-ionic liquid dispersive liquid–liquid microextraction
[c] Dispersive micro-solid phase extraction based on deep eutectic solvent
[d] Hollow fiber-liquid phase microextraction
[e] Ionic liquid-cloud point extraction.

H_2SO_4 were tested in the range from 100 to 800 μL. The results indicted (Fig. 5) that by increasing the volume of H_2SO_4 from 100 to 600 μL, more extraction solvent and more CO_2 bubbles were produced, which provided the high extraction recovery. However, in the more acidic solution a decrease in the extraction recovery was indicated. This can be related to protonation of the carbonyl groups of flavonoids. Therefore, a sufficient volume of H_2SO_4 should be used in the effervesce process. So, 600 μL of H_2SO_4 was selected as optimal for the rest of the experiments.

3.5. Analytical performance

The performance of the analytical procedure was evaluated by preconcentration factor (PF), extraction recovery (ER%), limit of detections (LOD), limit of quantifications (LOQ), the precisions (RSD), linearity range (LR) and the determination coefficient (R^2) (the data are summarized in Table 1). The calibration curves showed a linear regression over the range of 4.3–500 μg L^{-1} for quercetin and 1.6–500 μg L^{-1} for morin with correlation coefficients higher than 0.9960. LODs and LOQs in terms of three and ten times the standard deviation of the blank signal were 1.3 and 0.5 μg L^{-1} and 4.3 and 1.6 μg L^{-1} for quercetin and morin respectively. The relative standard deviations (R.S.Ds%) were 3.1 and 2.8 % for 6 replicate measurements of 50 μg L^{-1} quercetin and morin, respectively. PFs and ERs were obtained 105 and 95.9–96.7% respectively.

3.6. Analysis of real samples

To evaluate the method, the analysis of the two flavonoids in different samples including vegetables, fruit juices and tea samples was performed (Table 2). Fig. 6(a-d) shows typical chromatograms obtained by HPLC-UV from the standard solutions (0, 10, 50 and 200 μgL^{-1}) of morin and quercetin, as well as the extracted flavonoids from the apple juice, onion, and black tea samples spiked at 0 and 50 μg L^{-1} of morin and quercetin. The relative recoveries for the spiked samples were 94.9 % −103.4% for quercetin and 94.0% −102.3% for morin. Also, the precision of the method was assessed by the intra-day precision (one day) (n = 5) and inter-day precision (three different days) (n = 5) in distillated water at different levels of concentration (20–500 μg L^{-1}) (Table. 3). The results for intra-day and inter-day precisions were in the acceptable range of 3.8–5.7% and 4.3–5.8% respectively. Also, the proposed method was compared with other extraction methods and the results are shown in Table.4. Collectively, we developed and evaluated a green in-situ-CO_2-SFA-ME-SFO method for quantification of quercetin and morin in real samples with low LOD, high PF and recovery, short extraction time along with low consumption of hydrophobic extraction solvent.

4. Conclusion

In this study, an in-situ-produced CO_2 switchable fatty acid microextraction based on solidification of floating organic droplet was investigated for microextraction of two widely consumed antioxidant flavonoids (morin and quercetin) in tea, fruit juice and vegetable samples. The proposed method benefits from using a fatty acid as the switchable solvent, using the reaction of an acid and base to induce in-situ generation of CO_2 bubbles as a dispersing agent along with effective phase separation and solidification of floating organic drop. The combination of the developed effervescence-assisted microextraction and solidified floating solvent microextraction include good extraction recovery in a short extraction process (3 min) as well as low consumption of the extraction solvent. In conclusion, we developed a simple, cost-effective and environmentally friendly extraction method for quercetin and morin from real samples. We believe this method can be a good extraction procedure for other analytes in fruit juice and vegetable samples.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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