Separation/preconcentration and determination of quercetin in food samples by dispersive liquid–liquid microextraction based on solidification of floating organic drop -flow injection spectrophotometry

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Abstract A new dispersive liquid–liquid microextraction based on solidification of floating organic drop (DLLME-SFOD)-flow injection spectrophotometry (FI) method for the separation and preconcentration of trace amounts of quercetin was developed. 1-Undecanol and methanol was used as the extraction and disperser solvent, respectively. The factors influencing the extraction by DLLME-SFOD such as the volume of the extraction and disperser solvents, pH and concentration of salt were optimized. The optimal conditions were found to be; volume of the extraction solvent, 80 μL ; the volume of the disperser solvent, 100 μL ; and the pH of the sample, 3. The linear dynamic range and detection limit were 5.0×10^{-8} – 5.0×10^{-7} mol L^{-1} and 1×10^{-8} mol L^{-1} , respectively. The relative standard deviation (R.S.D.) at 6.1×10^{-8} mol L^{-1} level of quercetin ($n=10$) was found to be 2.8 %. The method was successfully applied to the determination of quercetin in the apple, grape, onion and tomato samples.

Keywords Quercetin · Dispersive liquid–liquid microextraction based on solidification of floating organic drop · Spectrophotometry · Separation and preconcentration

Introduction

Flavonoids are a large family of over 4,000 ubiquitous secondary plant metabolites, which can be further divided into

subclasses of flavonols, flavones, anthocyanins, catechins and flavonones (Wang and Huang 2004). Flavonoids are polyphenolic compounds which play an important role as natural potent antioxidants, exhibiting various physiological and biological activities, such as anti-inflammatory, anti-allergic and anti-carcinogenic activities, in the human metabolic system (Verma et al. 1988; Kawaii et al. 1999). The antioxidant activity of flavonoids is related to their reactivity in the free radical scavenging process. Epidemiological studies have indicated that their consumption assured a reduction in the risk of cancer and cardiovascular disease (Wei et al. 1990). Quercetin 3,3',4',5,7-pentahydroxyflavones, is one of the most abundant flavonoids present in fruits and vegetables. Food rich in quercetin include black and green tea, apple, onion, red grape, tomato, broccoli and other leafy green vegetables (Neustadt 2007; Aherne and O'Brien 2002). Quercetin is weakly soluble in water and its solubility increase in lipids and proteins (Chebil et al. 2007). Quercetin shows high affinity toward reaction with metal ions, it can acts as a free radical scavenging in treatment with different bioorganic materials (Bravo and Anaconda 2001; Meda et al. 2005) and its antioxidant effect is implied to be helpful for human health. Thus, due to the unique properties of quercetin, its determination in food samples is attractive.

High performance liquid chromatography (HPLC) is the most widely used technique for the determination of quercetin in different matrices (Nuengchamnong et al. 2004; Wang et al. 2003; Careri et al. 2003). Other techniques used for the separation and determination of quercetin include capillary electrophoresis (Chen et al. 2000; Khalili Zanjani et al. 2007), gas chromatography-mass spectrometry (Watson and Oliveira 1999), derivative spectrophotometry and spectrophotometry. Among these methods, spectrophotometry

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provides the use of a relatively inexpensive and easy handling instrument, which is available in most laboratories. However, determination of quercetin usually required a pre-separation/enrichment step. Solid phase extraction (SPE) (Watson and Oliveira 1999; Molinelli et al. 2002), hot solvent extraction (Numata and Tanaka 2011), supercritical water extraction (SWE) (Ko et al. 2011) and solid phase microextraction (SPME) (Kumar et al. 2009) had been used for the separation and preconcentration of quercetin prior to its determination. However, to the best of our knowledge, the report on the use of liquid-liquid microextraction (LLME) for the separation and preconcentration of quercetin is limited (Ranjbari et al. 2012; Viñas et al. 2011). LLME technique has received a growing amount of attention due to its simplicity, low consumption of the organic solvent, low cost, ease of the operation and the possibility of obtaining high enrichment factor. Dispersive liquid-liquid microextraction (DLLME) (Rezaee et al. 2006) and solidification of floating organic drop microextraction (SFODME) (Khalili Zanjani et al. 2007) are the new microextraction techniques used for the extraction of organic compounds and metal ions (Shah et al. 2012). DLLME is based on a ternary solvent system in which a dispersion of fine droplets is produced by injection of a mixture solution of the extraction and dispersive solvents into the sample which cause formation of a cloudy solution. In this system a vast contact area produces, which quickly reached equilibrium and resulted in a fast mass transfer and short extraction time. In SFODME method a droplet of an extraction solvent with a melting point of 10–30 °C is floated on the surface of an aqueous sample containing the analytes and the mixture is agitated during the extraction period. The sample vial is then placed in an ice bath to solidify the droplet which is easily removed and allowed to melt for the analysis.

Dispersive liquid-liquid microextraction based on the solidification of floating organic drop (DLLME-SFOD) is one of the LLME methods which were introduced in 2008 (Leong and Huang 2008). It is based on the principle of DLLME and SFODME, i.e. a mixed solution of the extraction solvent (with density less than water and a melting point close to room temperature) and the dispersive solvent is rapidly injected into the sample containing the analyte of interest which produce a cloudy solution with a vast contact area between the extraction solvent and the sample; consequently, the equilibrium is reached quickly. The mixture is centrifuged and the droplet of the organic phase containing the analyte is floated on the aqueous phase. The mixture is then placed in an ice bath until the drop is solidified. Then the drop is easily removed and is allowed to melt for quantification of analyte. DLLME-SFOD has the advantages of simplicity; low cost, rapidity, simple apparatus and consumption of very small amount of low-toxic organic solvent. DLLME-SFOD had been used for the determination of various organic and inorganic analytes (Dadfarnia et al. 2009;

Mohadesi and Falahnejad 2012). In this study, the possibility of separation and preconcentration of quercetin by DLLME-SFOD was considered and a simple, reliable and low cost method for its spectrophotometric determination was developed. The method was finally applied to the determination of quercetin in several categories of food samples.

Experimental

Reagents and chemicals

All chemicals were of reagent grade from Merck company (Darmstadt, Germany) and used without further purification. A stock solution (4×10^{-4} mol L⁻¹) of quercetin was prepared by dissolving an accurate mass of 0.0304 g of quercetin into a minimum amount of methanol in a 250 mL volumetric flask and was diluted to the mark with doubly distilled water. Standard solutions were prepared daily from the stock solution by serial dilutions with water. Doubly distilled water was used throughout the sample preparation and all the solutions were stored in pre-cleaned polypropylene (Nalgene, Lima, OH, USA) containers.

Apparatus and software

Spectral data were obtained by using an Avantes photodiode array spectrophotometer model AvaSpec-2048 with a source model of AvaLight-DH-S-BAL and a micro flow cell (The micro flow cell was a Z-cell that was easily coupled to the flow injection system through the 1.5 mm PTFE tubing with 0.5 mm inner diameter for on-line absorption measurements). The pH measurements were carried out with a Metrohm pH meter (model 691, Switzerland) using a combined glass calomel electrode. The centrifuge (Hettic, Universal 320, Tuttlingen, Germany) was used for centrifuging. The flow injection manifold consisted of a peristaltic pump (Ismatic, MS-REGLO 8–100, Switzerland) and a rotary injection valve (Rheodyne, California, USA) with a sampling loop of 100 µL was used.

Procedure

The pH of 10 mL of sample or standard solution was adjusted to ~3 using 0.1 mol L⁻¹ hydrochloric acid solution. The solutions was placed in a screw cap glass tube and a mixture of 100 µL of methanol (disperser solvent) and 80 µL of 1-undecanol (extraction phase) was rapidly injected into the solution. A cloudy solution containing a variety of dispersed fine droplets of 1-undecanol was formed. The cloudy solution was stable for a long time. At this stage the quercetin was extracted from the aqueous phase into the fine droplets of 1-undecanol. The mixture was then centrifuged for 3 min at 2,000 rpm and the organic solvent droplet was floated on the

surface of the aqueous solution due to its low density. The vial was then transferred into an ice bath, the organic solvent was solidified after 5 min and the solidified solvent was transferred into a conical vial where it melted immediately. In order to decrease the viscosity of the organic phase, it was diluted with ethanol (1:1) and was introduced via the sample loop of injection valve into a quartz micro flow-cell of spectrophotometer, the pump was then stopped for 2 s and absorbance was measured at 375 nm against the reagent blank.

Sample preparation

Onion, grape, tomato and apple samples were prepared according to the procedure given in the literature (Suryavanshi et al. 2007; Zhang et al. 2008), i.e. the samples were cut into small pieces, were dried to constant weight at 60 °C for 4 h in an oven and were gently pulverized. Then a proper amount of each powder sample was accurately weighed and was placed in a stopper tube, 10 mL of methanol was added and the mixture was left over night. The solution was placed in an ultrasonic bath for 40 min and then the content of the tube were filtered through a Whatman filter paper No. 41 (Macherey-Nagel, Germany). Finally, the clear supernatant solution was diluted to 50 mL upon addition of distilled water and was treated according to the given procedure.

Results and discussion

Preliminary experiments revealed that when the mixture of 1-undecanol and ethanol was injected into the aqueous sample, the quercetin was extracted into the 1-undecanol and the color of organic phase was changed to yellow. The quercetin absorbance spectrum in 1-undecanol (Fig. 1) shows a maximum absorbance at 375 nm. Then, the effect of various parameters

Fig. 1 Absorption spectra of quercetin

Table 1 Properties of common extraction solvents for the SFO-DLLME method

Extraction solvent	Density (g mL ⁻¹)	Boiling point (°C)	Melting point (°C)
1-Undecanol	0.83	243	13–15
1-Dodecanol	0.83	259	22–24
1,10-Dichlorodecane	0.99	140–142	14–16
n-Hexadecane	0.77	287	18

such as pH, nature and volume of the extraction solvent, nature and volume of the dispersive solvent and ionic strength on the extraction was considered using the univariable method.

Optimization of the extraction conditions

Effects of the extraction solvent and its volume

The selection of an appropriate extraction solvent is of major importance in the optimization of the DLLME-SFOD. The extraction solvent must fulfill several requirements. It must have low volatility, a density less than water, low toxicity, low solubility in water, high solubility of the desired analyte and a melting point near the room temperature (in the range of 10–30 °C). Accordingly, the organic solvents most commonly used as extractant in DLLME-SFOD are 1-undecanol, 1-dodecanol, 1,10-dichlorodecane and n-hexadecane. The properties of these solvents are given in Table 1. The extraction was performed with a mixture of 80 µL of each of the extracting solvent and 200 µL of ethanol as the dispersive solvent. 1-undecanol was found to give the best extraction efficiency and was chosen as the extracting solvent for the further experiments (Fig. 2). The melting point of 1-dodecanol

Fig. 2 Effect of the type of extraction solvent. Extraction conditions: amount of quercetin 2.5×10^{-7} mol L⁻¹ aqueous volume 10 mL; Organic volume 80 µL; ethanol volume 200 µL; pH~3

is close to room temperature and under experimental conditions it was relatively viscous and was not properly dispersed in the aqueous phase; consequently, the contact area between the two phases and the extraction efficiency decrease. N-hexadecane was ruled out as it has low solubility in dispersing solvent, however, the low extraction efficiency of 1,10-dichlorodecane can be related to its higher solubility in water.

In order to select the optimum volume of the extraction solvent, several experiments were performed using 200 μL of ethanol and different volumes of 1-undecanol. The results of this study (Fig. 3) revealed that when the volume of 1-undecanol is greater than 50 μL the recovery of quercetin is constant. An optimum volume of 80 μL of 1-undecanol was chosen in the subsequent studies to insure complete extraction of the analyte and yet to have high enrichment factor. It should be noted that the decrease in absorbance at volume greater than 50 μL is proportional to the increase in the extraction volume.

Effects of type and volume of the dispersive solvent

The dispersive solvent in DLLME-SFOD must be miscible with both water and the extraction solvent. In this study, four types of dispersive solvents, acetonitrile, acetone, ethanol and methanol were investigated. The result showed that the absorbance signal with methanol was greater than with other dispersive solvents. Thus, methanol was selected as the most suitable dispersive solvent. The higher absorbance signal in methanol and ethanol can be related to the higher ability of these dispersive solvent in competition with the extraction solvent in extraction of the analyte from the liquid sample. However, the slight decrease in the extraction efficiency with ethanol is due to the increase in solubility of quercetin in water in the presence of ethanol in compare to methanol.

The influence of the volume of methanol in the range of 50–400 μL on the extraction was examined while the volume of 1-undecanol was fixed at 80 μL . At the volume of methanol

Fig. 3 Effect of the volume of 1-undecanol. Extraction conditions: amount of quercetin $2.5 \times 10^{-7} \text{ mol L}^{-1}$ aqueous volume 10 mL; methanol volume 200 μL ; pH~3

Fig. 4 Effect of volume of disperser solvent on extraction of quercetin by DLLME-SFOD. Extraction conditions: amount of quercetin $2.5 \times 10^{-7} \text{ mol L}^{-1}$ aqueous volume 10 mL; Organic volume 80 μL of 1-undecanol; pH~3

less than 100 μL the extracting solvent was not properly dispersed and the extraction efficiency was low. The absorbance signal remained constant and was maximized at the volume of 100–250 μL (Fig. 4). However, when the volume of methanol was greater than 250 μL , the extraction efficiency was decreased due to the increase in solubility of the quercetin in aqueous phase in the presence of higher amount of methanol. Thus, for the formation of a stable cloudy solution and high recovery of quercetin, 100 μL of methanol was selected as the optimum volume of the dispersive solvent.

Effect of sample pH

Aqueous phase pH plays a significant role in the extraction of quercetin by DLLME-SFOD, as quercetin is only extracted in the pH range in which it is not ionized and has sufficient hydrophobicity for extraction into small volume of 1-undecanol. The effect of pH on the extraction of quercetin

Fig. 5 Effect of pH on extraction of quercetin by DLLME-SFO. Extraction conditions: amount of quercetin $2.5 \times 10^{-7} \text{ mol L}^{-1}$ aqueous volume 10 mL; Organic volume 80 μL of 1-undecanol; methanol volume 100 μL

was studied within the range of 1.0–8.0. The results (Fig. 5) show that the absorbance is maximum and nearly constant in the pH range of 3.0–4.0. The decrease in absorbance at pH less than 3 may be related to the protonation of quercetin, and at pH greater than 4 is related to the formation of phenoxide anion which prevents its extraction into the organic phase. Therefore, in the further experiments a pH of ~3 was chosen as an optimum value for the extraction of quercetin.

Salt effect

In order to study the effect of ionic strength on the DLLME-SFOD performance, several experiments were carried out with different NaCl concentration (0.0–0.5 mol L⁻¹) while keeping other experimental parameters constant. The results showed that the salt addition has no significant effect on the extraction efficiency of the quercetin. This observation suggests the possibility of applying this method for the separation of quercetin from saline solutions.

Interference study

The sensitivity and utility of the DLLME-SFOD in the extraction of quercetin in the presence of potential interfering ions in the food samples (ions which could form complex with quercetin) at an initial mole ratio of 1,000 (ion/quercetin) was examined. When interference was observed the concentration of interfering ion was lowered. A relative error of less than 5 % was considered to be within the range of the experimental error. The results (Table 2) indicated that the presence of cations at the given mole ratio has no significant influence on the extraction of the quercetin under the

Table 3 Parameters of linear calibration graph for quercetin (number of samples =6)

Parameter	Quercetin
Linear range (mol L ⁻¹)	5×10^{-8} – 5×10^{-7}
Slope	24.898
Correlation coefficient	0.9996
Enrichment factor	55.6
Detection limit (mol L ⁻¹)	1×10^{-8}

optimum conditions. Furthermore, the effect of some molecular compounds such as rutin and ascorbic acid which usually exist with quercetin in the food samples and may interfere in the extraction and determination of quercetin was considered. The results revealed that at the given mol ratio, these compounds has no significant effect in the determination of quercetin. It should be also noted that the ascorbic acid in 1-undecanol has a maximum absorbance in UV region far from the absorbance of quercetin, thus even though it may extract into organic phase under the experimental conditions, it has no significant effect on the determination of quercetin. On the other hand, rutin has a low lipophilicity (log p (octanol/water) = -1.13) and so it does not extract significantly into the organic phase.

Analytical performance

The figures of merit of the proposed method are summarized in Table 3. A calibration curve was constructed by extracting quercetin from 10 mL of the standard solutions. The calibration curve was linear in the range of 5.0×10^{-8} – 5.0×10^{-7} mol L⁻¹ with the equation of $A = 24.898 C + 0.001$ ($R^2 = 0.9996$) (where A is the absorbance and C is the concentration of quercetin in

Table 2 Effect of diverse species on the recovery of quercetin

Coexisting species	Molar ratio	Recovery (%)
Na ⁺	1,000	98±2
K ⁺	1,000	99±3
Ca ²⁺	1,000	102±5
Mg ²⁺	1,000	101±2
Cr ³⁺	1,000	98±2
Cd ³⁺	700	102±4
Fe ³⁺	500	97±2
Zn ²⁺	1,000	100±4
Cl ⁻	1,000	98±2
Ascorbic acid	500	97±1
Rutin	300	96±2

Table 4 Determination of quercetin in real samples

Sample	Added (μg g ⁻¹)	Found (μg g ⁻¹)	Recovery (%)
Onion	0	2000.0±0.1	-
	100	2097.0±0.2	97.0
Tomato	0	155.10±0.01	-
	0.2	155.31±0.05	105.0
	0.4	155.48±0.02	95.0
Grape	0	9.21±0.04	-
	0.5	9.70±0.03	98.0
	0.7	9.90±0.02	98.6
Apple	0	95.50±0.04	-
	0.5	96.01±0.03	103.3

Table 5 Characteristic performance data obtained by using the proposed method and other reported methods for determination of quercetin

Method	Detection limit	Linear dynamic range	Reference
SPE-GC-MS	10 pg	—	(Goto et al. 1977)
RP-HPLC-UV	45 ng mL ⁻¹	1–100 µg mL ⁻¹	(Watanabe and Tanaka 1978)
HPLC	0.01 µM	0.1–1.0 µM	(Chen and Teo 2001)
SPME-HPLC-UV	0.76 pg mL ⁻¹	10–1,000 ng mL ⁻¹	(Supriyanto and Simon 2005)
SWE-HPLC	0.00982 mg mL ⁻¹	50–2,000 µg mL ⁻¹	(Silva et al. 2009)
IDLLME-HPLC-UV	0.26 ng mL ⁻¹	0.5–1,000 ng mL ⁻¹	(Ranjbari et al. 2012)
DSLLME-GC-MS	0.011–0.13 ng mL ⁻¹	0.05–500 ng mL ⁻¹	(Viñas et al. 2011)
DLLME-SFO-UV–vis	0.01 µM	0.05–0.5 µM	This work

the aqueous phase (mol L⁻¹). The limit of detection (LOD) defined as the ratio of three times of the standard deviation of the blank signals to the slope of the calibration curve, was found to be 1.0×10^{-8} mol L⁻¹. The relative standard deviations at 6.1×10^{-8} mol L⁻¹ ($n=10$) was found to be 2.8 %. The enhancement factor determined as the slope ratio of the calibration curve with and without preconcentration was found to be 55.6.

Application

The reliability of the recommended procedure was examined through the determination of quercetin in onion, grape, tomato, and apple samples. The accuracy of the method was verified by the analysis of the samples spiked with the different known amounts of quercetin. The results of this study (Table 4), demonstrated that the recoveries of added quercetin are good (95–105 %), indicating that the matrices of the samples type examined had no significant effect on the determination of quercetin. Thus the method is reliable for the determination of quercetin in a variety of food samples.

Conclusion

In this study it was demonstrated that the DLLME-SFOD combined with fiber optic-linear array spectrometers has the capability of extraction and determination of traces of quercetin in different food samples. Furthermore, comparison of the developed method with other reported methods for the determination of quercetin (Table 5) indicated that the detection limit and linear dynamic range of the proposed method is comparable to other reported method. It also have the advantages of the use of common spectrophotometry instrument, minimum use of the toxic organic solvent, rejection of the matrix constituent, simplicity, low cost and ease of the operation.

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