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Pressurized liquid extraction of ginger (*Zingiber officinale* Roscoe) with bioethanol: An efficient and sustainable approach

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ABSTRACT

To develop an efficient green extraction approach for recovery of bioactive compounds from natural plants, we examined the potential of pressurized liquid extraction (PLE) of ginger (*Zingiber officinale* Roscoe) with bioethanol/water as solvents. The advantages of PLE over other extraction approaches, in addition to reduced time/solvent cost, the extract of PLE showed a distinct constituent profile from that of Soxhlet extraction, with significantly improved recovery of diarylheptanoids, etc. Among the pure solvents tested for PLE, bioethanol yield the highest efficiency for recovering most constituents of gingerol-related compounds; while for a broad concentration spectrum of ethanol aqueous solutions, 70% ethanol gave the best performance in terms of yield of total extract, complete constituent profile and recovery of most gingerol-related components. PLE with 70% bioethanol operated at 1500 psi and 100 °C for 20 min (static extraction time: 5 min) is recommended as optimized extraction conditions, achieving 106.8%, 109.3% and 108.0% yield of [6]-, [8]- and [10]-gingerol relative to the yield of corresponding constituent obtained by 8 h Soxhlet extraction (absolute ethanol as extraction solvent).

1. Introduction

Ginger (*Zingiber officinale* Roscoe) is the root from a kind of monocotyledonous plant that belongs to the Zingiberaceae family. Ginger and ginger extracts are used extensively in the food, beverage, and confectionary industries in the fabrication of products such as marmalade, pickles, chutney, ginger beer, ginger wine, liquors, and biscuits [1]. Ginger has also been shown to have a number of pharmacological activities such as antiemetic, antitussive, analgesic, anti-inflammatory, cardiotonic, anticancer, antihepatotoxic and antifungal [2–4]. Therefore, ginger is widely used as both traditional and contemporary natural medicine, which has been included in the pharmacopoeias in UK, Europe, China and Japan [1].

The class and content of bioactive constituents in different gingers depend on variable sources, climate and cultivation conditions [1,5]. However, the central pungent ingredients that exhibit the biological activities have been structurally classified as gingerol-related compounds and diarylheptanoids [3]. Fig. 1. presents some representative structures. Many studies have also demonstrated that gingerols are easily converted into the corresponding shogaols (thermodynamically stable α,β-unsaturated ketone) through thermal dehydration by variable factors [3,6,7].

Natural product extraction forms the basis for a growing nutraceutical industry. Traditionally, bioactive compounds from plant materials are obtained through steam distillation or solvent extraction. Steam distillation is inefficient for those compounds that are unable to form an azeotropic mixture with water. The traditional solvent extraction techniques, such as Soxhlet extraction, are generally time-consuming, laborious, and have low selectivity. Larger volumes of expensive and toxic organic solvents have to be used in many cases, which is not applicable for food industry and solvent disposal is expensive. In recent years, continuous efforts have been made in order to reduce the amount of solvent required and operation time, and improve efficiency and selectivity [8–10], among which clean, efficient and sustainable represent some of the criteria for the success of a new approach [10,11].

Pressurized liquid extraction (PLE) is not a very new technology for extraction but with significant advantages because it requires only small volumes of solvents, and allows faster extraction than classical methods. PLE is similar in principle to Soxhlet extraction, except that elevated temperatures and pressures are used in enclosed vessels, which allows extraction by a small amount of solvent to be completed in a very short time [10,12]. It was acknowledged that hot and pressurized solvents were able to more effectively dissolve the compounds and penetrate the

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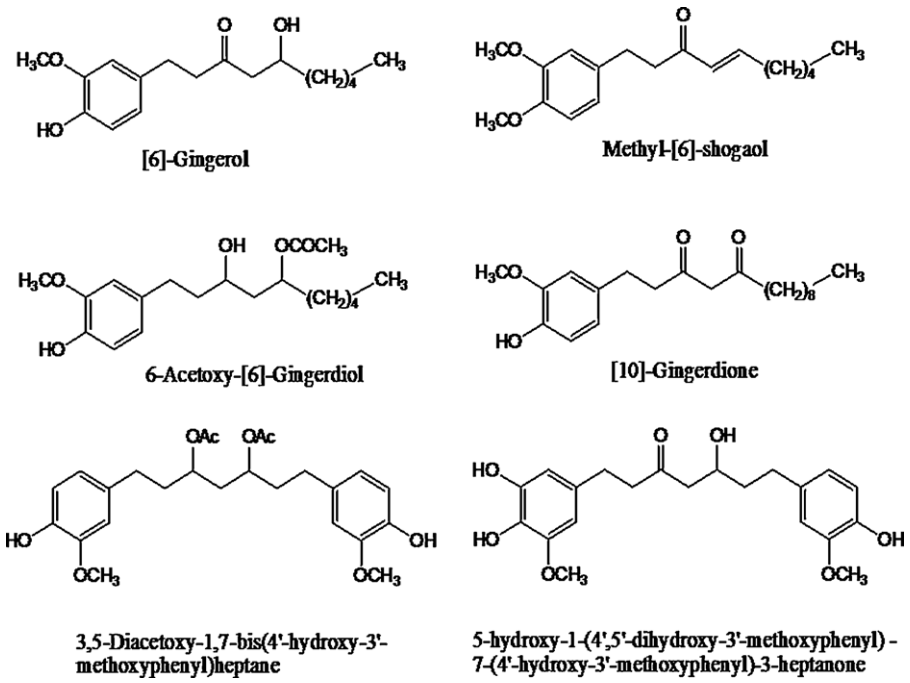

Fig. 1. Chemical structures of some representative gingerol-related compounds and diarylheptanoids identified in dried ginger 70% ethanol extract with PLE.

sample matrixes [12–15]. With continuous interests and investment in biofuel industry, annual production of ethanol has amounted up to 20 billion gallons and it has become the cheapest solvent after water [16]. Different from industrial ethanol synthesized from petroleum, bioethanol is the fermentation product of glucose from renewable biomass (edible starch and non-edible cellulose, etc.), thus, bioethanol is "safe", clean, "green" and sustainable. However, bioethanol also has some property that water does not have, for example, to dissolve less-polar compounds. Therefore, ethanol represents one of the promising future solvents [17]. Even though different methods have been used for ginger extraction with organic solvents and supercritical fluids [11,18], surprisingly no systematic work using PLE technique for ginger extraction has been reported. Most studies focused on evaluating the extraction efficiency by measuring gingerol and shogaol yields, while few reports investigate the effect of solvent property on the extraction of diarylhepanoids which represent an important group of pungent compounds but are difficult to extract. Hence, this work attempted to investigate the efficiency and potential of PLE for ginger extraction. More focus was placed on examination of the correspondence between solvent polarity and extracted constituent essentially based on the sustainable solvent ethanol at different aqueous concentrations. High-performance liquid chromatography (HPLC) and HPLC coupled with time-of-flight mass spectrometry (HPLC–TOF-MS) were applied for structural identification and extract quantification. Optimized extraction conditions with desired constituent profile and efficiency by PLE were thus determined.

2. Experimental

2.1. Chemicals and reagents

Bioethanol (99.8% purity) (claimed as fermentation product with purification process) was purchased from VERBIO Ethanol Zörbig GmbH & Co. KG (Leipzig, Germany). HPLC-grade acetonitrile, methanol, hexane, ethyl acetate, chloroform were procured from Sigma–Aldrich (Steinheim, Germany). Standards of 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol were purchased from LGC Standards, Ltd. (Boraas, Sweden). Ultrapure water (resistivity > 18.2 MΩ cm) obtained from a Milli-Q water-purification system (Millipore Corp., Bedford, MA, USA) was used throughout the work.

2.2. Plant material and sample preparation

Fresh ginger (*Zingiber officinale* Rosc.) was purchased from a local supermarket (originally from China). Before preparation of the samples for extraction the ginger was first washed and cut into small piece. The dried samples were in powder form obtained after oven drying (55 ± 1 °C) and grinding.

2.2.1. Pressurized liquid extraction

Extractions were performed using a Dionex ASE 150 (Sunnyvale, CA, USA) accelerated solvent extraction system at 1500 psi with 5 min static extraction time and a 100 s purge for a total of two cycles. Dionex ASE 150 is a programmed operation system, which the static time is the extracting time at the set parameters (the time counting started when the system reaches the set parameters, and ended when the system started cooling, de-pressuring and flushing by fresh solvents). One operation is called a static cycle. From loading sample to reaching a static condition takes 3–5 min; 2 cycles of solvent purging of extraction cell take 3 min, and cooling, depressuring and unloading sample 6–7 min. Thus, plus 5 min static extraction time the total operation time for one static cycle is about 5 + 5 + 3 + 7 = 20 min. A cellulose filter (Dionex Corp.) was placed at the bottom of the extraction cell prior to being loaded with sample. One gram of ginger powder was mixed with DE (diatomaceous earth) (Dionex P/N 062819) and placed in a 22 mL stainless steel cell, then extracted using different solvents. The extractions were normally done at 100 °C except for temperature comparison at 60, 80, 100, 115 and 130 °C. Examination of the effects of pressures was performed on Dionex ASE 200 (Sunnyvale, CA, USA) using the same size of extraction cell as on ASE 150, with the operation condition at 100 °C with 70% ethanol and the pressure varied at 1000, 1500 and

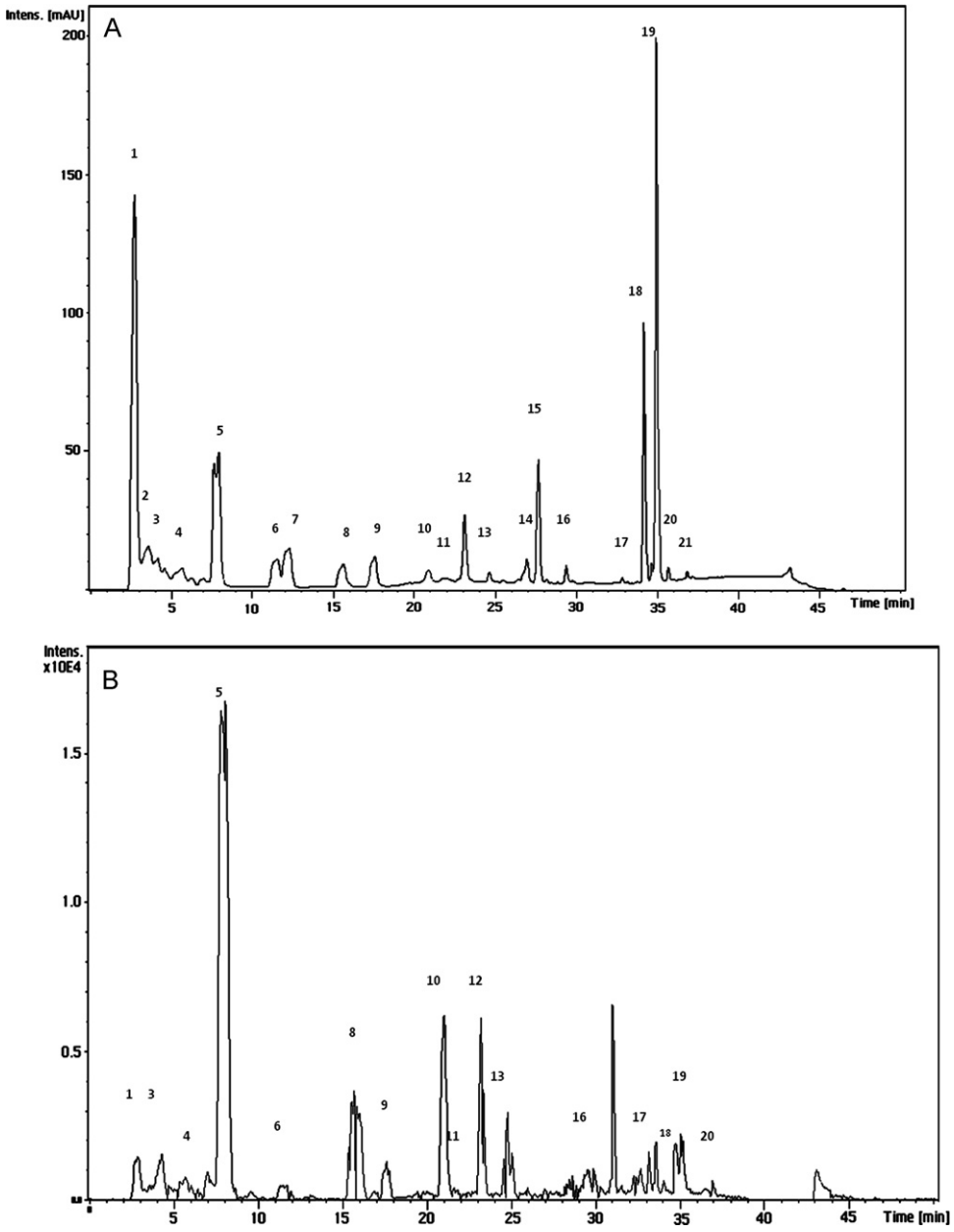

Fig. 2. Coinstantaneous HPLC-UV (A) and HPLC–ESI-MS (B) chromatograms of dried ginger 70% ethanol extract performed in pressurized liquid extraction (PLE) at 1500 psi and 100 °C with static time 5 and 15 min washing. Peak assignments are shown in Table 1.

2000 psi, respectively. The extracts were collected in pre-cleaned 60 mL glass vials.

All extracts with ethanol or methanol were centrifuged at 4000 rpm, 25 °C for 20 min (ROTINA, 46 R, Hettich, Zentrifugen, Germany) to remove any particulate material, then filtered with a 0.22 μm Polypropylene syringe filter (OSMONICS INC., Denmark) and transferred to a 50 mL volumetric flask, stored at 4 ± 1 °C for HPLC analysis. Extracts with hexane or ethyl acetate or chloroform were concentrated in a rotary evaporator at less than 80 °C under vacuum, then dried by purging nitrogen and redissolved in ethanol for analysis.

2.2.2. Soxhlet extraction

One gram of ginger powder was accurately weighed and extracted with 150 mL of ethanol for 8 h in a water bath at 85 °C on a Soxhlet extraction apparatus (Sigma–Aldrich Denmark A/S, Brøndby, Denmark), and then the ethanol extract was concentrated in a rotary evaporator at less than 85 °C under vacuum.

2.2.3. Heat reflux extraction and ultrasonication-assisted extraction

One gram of ginger powder was added to 250 mL round bottom flask containing 120 mL ethanol placed in a water bath, and the heat reflux extraction was performed at 85 °C for 8 h with magnetic agitation. After extraction, the resulting mixture was centrifuged and filtered to remove the solid. The ethanol extract was concentrated for HPLC analysis and calculation of total extraction yield.

Ultrasonication-assisted extraction (1 g ginger powder dispersed in 50 mL ethanol in a glass baker) was carried out in a 2510E-DTH Bransonic Ultrasonic Bath (Branson Ultrasonics Corporation, Bilston, UK). The extraction was performed at 25–30 °C (temperature control by changing water in ultrasonication bath) at 40 kHz for 60 min. The resulting preparation was centrifuged and

Table 1
Peak assignment [M+H]+, [M+Na]+ and [2M+Na]+ are found by HPLC–ESI-MS.

Peak	RT (min)	Measured $[M+H]^+$ (m/z)	Theoretical $[M+H]^+$ (m/z)	Error (ppm)	$[M+Na]^+$ (m/z)	$[2M+Na]^+$ (m/z)	UV λ_{max} (nm)	Identification (proposed formula)
1	2.9						281	n.d.
2	3.6						281	n.d.
3	4.3						280	n.d.
4	5.8						280	n.d.
5	7.8	295.1919	295.1914	−1.6938	317.1678	611.3378	229, 280	[6]-Gingerol ($C_{17}H_{26}O_4$)
6	11.6	–	309.2060	–	331.1869	–	243, 308	Methyl-[6]-gingerol ($C_{18}H_{28}O_4$)
7	12.1	–	339.2166	–	361.1957		241	6-Acetoxy-[6]-gingerdiol ($C_{19}H_{30}O_5$)
8	15.6	323.2216	323.2217	0.3094	345.2036	667.3963	229, 283	[8]-Gingerol ($C_{19}H_{30}O_4$)
9	17.6	277.1791	277.1798	2.5254	299.1589	575.3192	229, 283	[6]-Shogaol ($C_{17}H_{24}O_3$)
10	21.0	–	381.2999	–	403.2093	–	229, 283	[12]-Gingediol ($C_{23}H_{40}O_4$)
11	21.6	291.1967	291.1955	−4.1209	313.1379	–	229, 283	Methyl-[6]-shogaol ($C_{18}H_{26}O_3$)
12	23.2	351.3529	351.3530	0.2846	373.2349	723.4537	229, 283	[10]-Gingerol ($C_{21}H_{34}O_4$)
13	24.6	–	395.3156	–	417.2259	–	229, 283	Methyl-[12]-gingediol ($C_{24}H_{42}O_4$)
14	26.9	–	349.2373	–	371.2191		229, 283	[10]-Gingerdione ($C_{21}H_{32}O_4$)
15	27.7	425.2671			447.2481	871.5162	280	n.d.
16	29.4	333.2407	333.2424	5.1014	356.2305	–	283	[10]-Shogaol ($C_{21}H_{32}O_3$)
17	32.8	455.3151		2.1963	477.2945	–	288	n.d.
18	34.2	382.2728			404.3168	784.5583	232, 287	n.d.
19	34.9	–	461.2170	–	483.3440	–	232, 282	3,5-Diacetoxy-1,7-bis(4′-hydroxy-3′-methoxy phenyl) heptanes ($C_{25}H_{32}O_8$)
20	35.7	–			482.3573	–	229, 263	n.d.
21	36.9	391.1748	391.1751	0.7669	413.2686	803.5144	238, 283	5-Hydroxy-1-(4′,5′-dihydroxy-3′-methoxyphenyl) -7-(4′-hydroxy-3′-methoxyphenyl)-3-heptanone ($C_{21}H_{26}O_7$)

n.d., not determined.

filtered to remove the solid, and the ethanol extract solution was concentrated for analysis.

2.3. HPLC analysis

2.3.1. Preparation of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol standard solutions

The standards of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol were used as received for preparing standard solutions. Sufficient HPLC-grade methanol was added to each standard to produce a stock standard of 5.0 mg/mL. Various standard solutions were prepared from the stock solution by dilution with methanol. For establishing standard curves, the solutions were prepared containing 10.0, 20.0, 40.0, 60.0, 80.0 and 100.0 μg/mL, respectively. All ginger standards were capped and stored at −20 °C until used.

2.3.2. HPLC chromatographic analysis

The standards and ginger extracts were analyzed on a HPLC system consisting of a Thermo Finnigan Surveyor with a photodiode array detector (PDA) set at 280 nm (for signal A) and 230 nm (for signal B) and a Thermo Finnigan AutoSampler (Thermo Fisher Scientific, Copenhagen, Denmark). UV spectra were taken in the region of 200–500 nm. Chromatographic analyses were performed on a 150 mm × 3.0 mm Phenomenex Kinetex 2.6 μm C_{18} 100 R chromatographic column (Phenomenex, Inc., Torrance, CA, USA). The HPLC operating parameters were according to He et al. [6] with some modifications: injection volume, 10 μL; mobile phases flow rate, 0.2 mL/min; chromatographic run time, 50.0 min; eluents: (A) water and (B) acetonitrile. The gradient elution had the following profile: 0–8 min, 50% B; 8–17 min, 65% B; 17–32 min, 100% B; 32–38 min, 100% B; 38–40 min, 45% B; 40–50 min, 45% B, and the column temperature, 30 °C.

The 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol peak identifications in ginger extracts were based on comparison of their retention time with that of the corresponding standards. The quantification of the concentrations of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol in each sample was calculated by comparing their response with the corresponding standard curves.

Noting the extracts with different methods have different constituents, we thus obtained recovery yield of total extracted compounds; namely, the extract solution after removal of solvent was dried to a constant by purging nitrogen and used for estimation of recovery yield of extract.

2.3.3. Statistical analysis

All extraction operations were conducted in two replicates in this work. Means and standard deviation of the data were reported. The data sets were analyzed statistically at the significant level of $P < 0.05$ with SAS version 8.2 using the Generalized Linear Model procedures to determine if there were differences between treatments (SAS (2002) SAS/STAT User's Guide, version 8.2; SAS Institute: Cary, NC).

2.4. HPLC–ESI-MS analysis

HPLC–ESI-MS analyses were performed with an electrospray ionization (ESI) inlet coupled to a quadrupole time-of-flight mass spectrometer (Bruker micrOTOF-Q, Bremen, Germany). The column and chromatographic conditions for the HPLC part were the same as for HPLC analysis with UV absorbance recorded at 230 nm. Ionisation was performed in the positive mode with capillary voltage of 4.5 kV and an 8 L/min nitrogen flow, 0.8 bar nebuliser pressure and a temperature of 190 °C. Scan range was from 50 to 1000 m/z. The structure identification of [6]-, [8]- and [10]- gingerol as well as [6]-shogaol in extract was determined by comparing its ESI MS spectrum with the ESI-MS spectrum of the corresponding standard. For other constituents of the extract without authentic standards available, the structural identification was based on the molecular ion peak and diagnostic ion peaks with reference to previously reported identification [3,6,19].

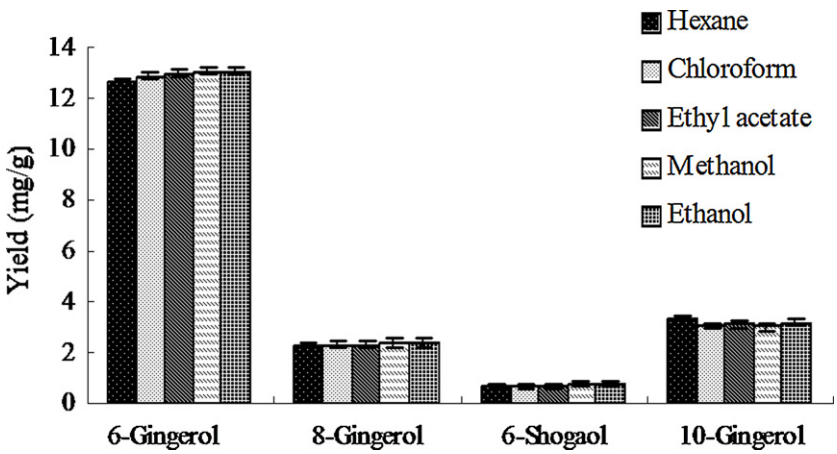

Fig. 3. Effects of organic solvents on extraction yield of 6-, 8-, 10-gingerol and 6-shogaol. The extractions were on PLE with pressure at 1500 psi and 100 °C.

3. Results and discussion

3.1. Characterization of PLE extracts

Sesquiterpene derivatives (aldehydes and ketones), diarylheptanoids and gingerol-related compounds have been identified as major classes of constituents of ginger extract [20]. Fig. 2 showed the simultaneous HPLC-UV (A) and HPLC–ESI-MS (B) chromatograms of dried ginger 70% ethanol PLE extract (performed at 1500 psi and 100 °C for 20 min). As can be seen, the MS total ion chromatogram (TIC) responses for Peaks 1–4 and Peaks 18–20 are much lower than their UV signals, indicating that these compounds may contain functional groups that exhibit high absorption at 230 nm or are not ionized as easily by ESI.

The corresponding peaks have been assigned in Table 1. Based on the retention time, the chromatogram of ginger extract may be classified into 3 groups. The peaks eluting before 6 min were probably smaller aromatic components that were not well separated and difficult to identify by only ESI-MS. The peaks between 6 and 35 min are gingerol- and shogaol-related, which have been well characterized [6,21]. For example, the MS of Peak 8 (retention time 15.6 min), identified as [8]-gingerol, displaying the highest ion signal intensity at m/z 345.2036 $[M+Na]^+$ and relatively high signal at m/z 667.3963 $[2M+Na]^+$, and diagnostic ion m/z 305.2085 which is $[M-H_2O+H]^+$ (dehydrated [8]-gingerol). The characteristic UV spectra of gingerol compounds, absorption maximum at 283 and a shoulder at 229 nm were also observed (Table 1). Other peaks were identified accordingly. Diarylheptanoids have relatively larger molecular sizes and are eluted after 35 min. Jiang et al. [3] has characterized diarylheptanoids through LC/ESI-MS/MS. We were also able to identify some of the peaks, for example Peak 21 was identified as 5-hydroxy-1-(4′,5′-dihydroxy-3′-methoxyphenyl) -7-(4′-hydroxy-3′-methoxyphenyl)-3-heptanone, from the ions at m/z 413.2686 $[M+Na]^+$, 391.1748 $[M+H]^+$ and $[2M+Na]^+$ 803.5144. The identified peaks shown in Fig. 2 are summarized in Table 1. As we see, many peaks could not be identified just based on ESI-MS. Characterization of all eluted peaks would demand further work deploying more analytical methods, which is not the focus of this work. The current results illustrate the constituent profile of the extract, which is sufficient for evaluation of the effects of different extraction methods, conditions and solvents.

3.2. Effect of solvent property on the constituent profile of extracts

The purpose of this work is to examine the effects of solvents and extraction conditions. We first examined the effects of solvents with variable polarity, namely hexane, chloroform, ethyl acetate, methanol and ethanol, covering polar and nonpolar, protic and aprotic solvents (Fig. 3). The results showed a pronounced connection between the polarity of solvents and the polarity of constituents (Fig. 3). For example, [10]-gingerol is a more hydrophobic compound than [6]-gingerol, which could explain why hydrophobic hexane achieves an improved relative yield (recovery) of [10]-gingerol compared to more polar solvents (Fig. 3). Among the 4 compounds evaluated, [6]-gingerol may be the most polar one and has hydroxyl groups, which might explain why extraction with methanol and ethanol obtain the highest yields (recovery) (Fig. 3). An overall inspection of Fig. 3 reveals that both ethanol and methanol obtain almost the same yields of 6-, 8-gingerol and 6-shogaol and also higher than other solvents. Ethanol achieved a somewhat higher yield of [10]-gingerol than methanol but slightly lower than hexane. Overall, ethanol may be treated as the most efficient solvent. As mentioned earlier, ethanol has achieved a price advantage compared to other solvents, and unlike other solvents ethanol is also permitted in food processes, therefore, ethanol as a solvent for pressurized extraction has the potential deserving for further investigation.

3.3. Comparison of efficiency and constituent profiles between PLE and other extraction approaches

Fig. 4 showed HPLC chromatograms of the extracts by Soxhlet and PLE extraction with all other conditions being identical. Before we are going to quantify the extraction efficiency, it is clear that the PLE displays distinct constituent profiles different from Soxhlet extract. Specifically for the peaks with retention time between 35 and 40 min which have been identified as different diarylheptanoids, PLE showed much higher extraction efficiency than Soxhlet (Fig. 4). Unfortunately, without the related standards this difference is difficult to quantify. Fig. 4 also showed that, the extraction by PLE with different concentrations of ethanol can be significantly different, especially for the constituents appearing before 6 min and after 35 min, as indicated by differing intensities of the peaks. It seems that 70% ethanol was especially efficient for extracting those compounds (see detailed discussion in Section 3.4).

Table 2 presented the yields of [6]-, [8]- and [10]- gingerol and [6]-shogaol by Soxhlet, heat reflux and ultrasonication extraction with absolute ethanol, and PLE with absolute ethanol (1 and 5 cycles) and with 70% ethanol. As depicted in Table 2, the significance of statistical difference depends on extraction operations and/or properties of target compounds. Even though ultrasonication-assisted extraction has somehow advantages in easy operation and relatively shorter operation time and solvent cost in comparison with Soxhlet and heat reflux extraction, among all operations it

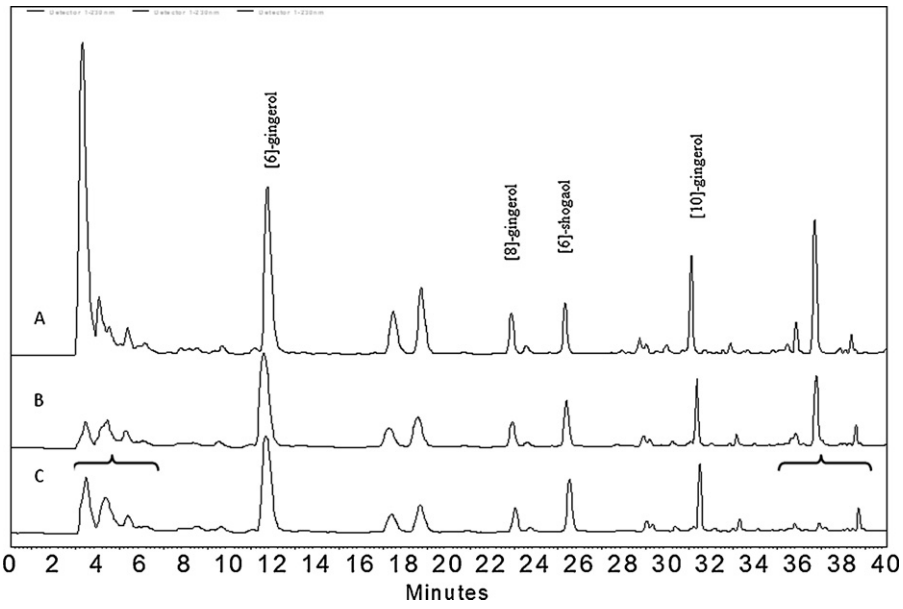

Fig. 4. Comparison of the HPLC-UV (280 nm) chromatograms of ginger extracts by different extraction methods. PLE extractions were performed with 70% ethanol solution (A) and absolute ethanol (B) for one times (5 min static time). Soxhlet extraction (C) was performed with absolute ethanol at refluxed condition for 8 h.

achieved the lowest yields of [6]-, [8]- and [10]- gingerol and [6]-shogaol. In principle the extraction mechanisms of Soxhlet and heat reflux extraction are much similar; the corresponding yields of 4 constituents is also very close with no significant difference ($P > 0.05$) (Table 2) [22]. Except for [6]-shogaol, 70% ethanol PLE (PLE3) generally achieves significantly higher yield than one cycle 100% ethanol (PLE1) and Soxhlet ($P < 0.05$); while the differences, in terms of the yield of [6]-, [8]- and [10]- gingerol, between one cycle 100% ethanol (PLE1) and Soxhlet approach are not significant ($P > 0.05$). As we observed, in the last one of 5 cycle PLE extraction with absolute ethanol almost no peaks of [6]-, [8]- and [10]-gingerol and [6]-shogaol can be detected, which indicate extraction completed limited by the method capacity. Therefore we can treat 5 cycle PLE extraction as a control representative 100% extraction (recovery 100%) for these four components. For [6]-gingerol one cycle PLE and Soxhlet achieved 96.7% and 98.2%, for [8]-gingerol 1 cycle PLE and Soxhlet extracted 94.7% and 95.0%, and [10]-gingerol 96.5% and 98.5%, respectively. The results indicated that PLE is highly efficient compared to Soxhlet, and in 20 min PLE (only 5 min static extraction time) achieves almost comparable recovery to 8 h Soxhlet. Even with successive operation for 5 cycles, the operation time and solvent consumption are still much less than Soxhlet extraction (Table 2). [6]-Shogaol is an exception; Soxhlet achieved the highest yield compared with all PLE operations. This is possibly because [6]-shogaol has very low solubility in ethanol due to removal of the hydroxyl group from [6]-gingerol structure by dehydration with a result of the loss of H-bonding capacity with ethanol. For a compound with low solubility in a solvent, successive extractions with more cycles will result in a higher total yield, which may explain why Soxhlet obtained higher yield. This also can be seen from the [6]-shogaol yield (recovery) difference of one cycle PLE (0.767 mg/g dried ginger) from 5 cycles of PLE (0.968 mg/g dried ginger, 26.6% improvement of yield), while for the same operation the yield of [6]-gingerol was only improved by (13.445–12.996)/12.996 = 3.4%, which reflects that the polarity matching between solvent and extract significantly influences extraction efficiency.

Fig. 4 has shown 70% ethanol is remarkably efficient than absolute ethanol for extraction of [6]-, [8]- and [10]-gingerols. Table 2 further quantify the difference. One cycle extraction by 70% ethanol achieved 104.9% recovery of [6]-gingerol, 103.8% recovery of [8]-gingerol and 106.4% recovery of [10]-gingerol with 5 cycles of PLE (by absolute ethanol) as a reference (Table 2). Compared to one cycle PLE and Soxhlet with absolute ethanol, 70% ethanol is even more efficient, except for [6]-shogaol the differences of extraction efficiency in terms of [6]-, [8]- and [10]-gingerol are statistically significant (Table 2). For example, if we use 8 h Soxhlet extraction (absolute ethanol as solvent) as a reference the relative yields

Table 2
Comparison of yield of [6]-, [8]-, [10]-gingerol and [6]-shogaol by Soxhlet, ultrasonication-assisted extraction, heat reflux extraction and PLE with different operation conditions.

Methods	Extraction yields† (mg/g dried ginger)				Operation time (min)	Solvent consumption (mL)	Total extract yield (mg/g dried ginger)
	[6]-Gingerol	[8]-Gingerol	[6]-Shogaol	[10]-Gingerol			
PLE1	12.996 ± 0.087^{a}	2.397 ± 0.026^{a}	0.767 ± 0.011^{a}	3.025 ± 0.039^{a}	20	41	174.0 ± 8.7^{a}
PLE2	13.445 ± 0.009^{a}	2.531 ± 0.022^{b}	0.968 ± 0.001^{b}	3.134 ± 0.010^{b}	100	205	246.7 ± 12.1^{b}
PLE3	14.106 ± 0.342^{b}	2.627 ± 0.138^{b}	0.789 ± 0.029^{a}	3.336 ± 0.134^{c}	20	41	364.8 ± 15.4^{c}
Soxhlet	13.203 ± 0.384^{a}	2.404 ± 0.148^{a}	1.151 ± 0.091^{c}	3.088 ± 0.115^{a}	480	150	96.3 ± 4.6^{d}
Ultrasonication	11.317 ± 0.037^{c}	2.013 ± 0.021^{c}	0.072 ± 0.003^{a}	2.137 ± 0.071^{d}	60	50	81.6 ± 2.1^{e}
Heat reflux	13.012 ± 0.279^{a}	2.419 ± 0.083^{a}	1.174 ± 0.021^{c}	2.967 ± 0.024^{a}	480	120	132.1 ± 6.8^{f}

Note: PLE1 is the yield of the extraction on Dionex ASE 150 with absolute ethanol for one time extraction. PLE2 is the total yield for five successive extractions on Dionex ASE 150 with absolute ethanol. PLE3 is the yield of one extraction on Dionex ASE 150 with 70.0% ethanol solution.
^{abcdef}means with the same letters in the same column are not significantly different ($P > 0.05$).
† The tabulated values are the means ± standard deviations of two replicates.

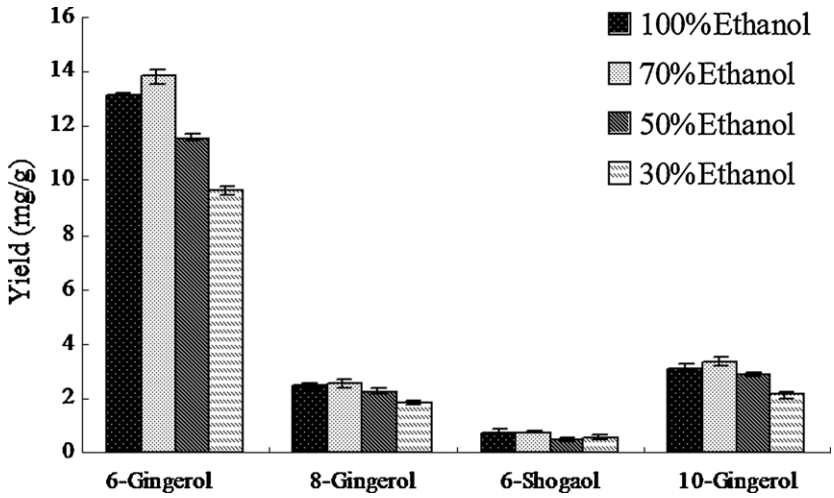

Fig. 5. Effects of different concentrations of ethanol on the yields of 6-, 8-, 10-gingerol and 6-shogaol. The PLE extraction was operation at 1500 psi and 100 °C by one cycle.

(recovery) of [6]-, [8]- and [10]-gingerol were 106.8%, 109.3% and 108.0%, respectively. It has to be pointed out that, because of azeotropic phenomena, even 70% ethanol is used, 95% ethanol will be actually extraction solvent for Soxhlet at reflux condition at atmosphere pressure. Importantly, PLE also achieved high recovery of other pungent compounds (diarylheptanoids) and other un-identified constituents as indicated in Fig. 4. It is difficult to quantify them because of lack of standards, however, we obtained isolated extraction yields of PLE and other extraction approaches (Table 2), from which we may estimate the difference of extraction efficiency. As we can see, for all 6 approaches the difference between their isolated extraction yields is significant at a 95% confidence level (Table 2). In terms of crude extraction yield (including all ethanol-soluble compounds), ultrasonication-assisted extraction is the lowest one; while the yield of heat reflux is 1.4 folds of that of Soxhlet, indicating exposure of ginger to higher temperature enhances extraction efficiency (the extraction chamber of Soxhlet apparatus < 85 °C after ethanol vapour condensed back to the chamber). The crude yield of PLE with 70% ethanol was 3.8 times higher than Soxhlet with absolute ethanol, and 2.1 times higher

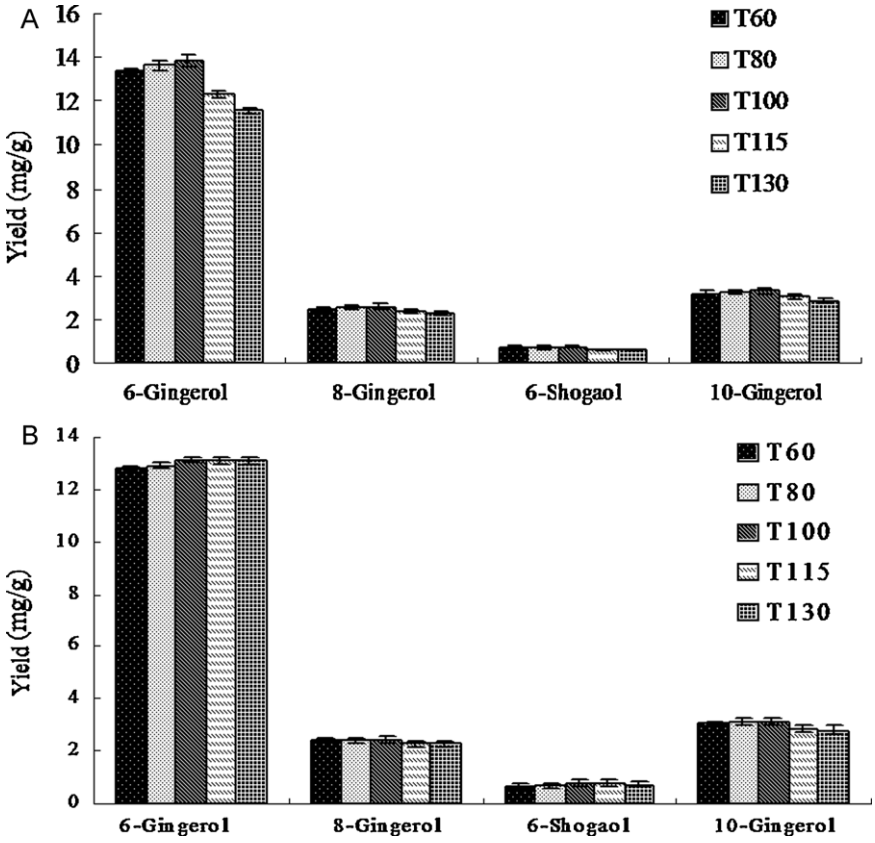

Fig. 6. Effects of extraction temperatures on the yields of 6-, 8-, 10-gingerol and 6-shogaol with 70% ethanol (A) and absolute ethanol (B) as a solvent. The PLE extraction was operation at 1500 psi and different temperature by one cycle.

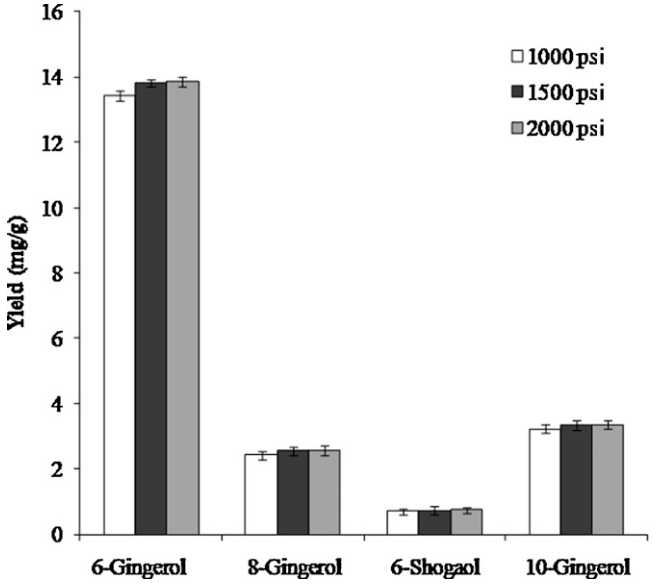

Fig. 7. Effects of pressures on the yields of 6-, 8-, 10-gingerol and 6-shogaol with 70% ethanol. The PLE extraction was operation at 100 °C and different pressures by one cycle.

than PLE with absolute ethanol. There exist different extractable compounds in plant stems and roots where Folin–Ciocalteu assay has been widely used to estimate crude extraction yield [23]. However, this work focused on the compounds of our interests, and no data have been collected in this regard.

3.4. Effects of ethanol concentrations and operation temperatures

Besides economic considerations, other factors inspired us to investigate effects of ethanol concentrations on PLE extraction. One is the promising result from 70% ethanol (Fig. 4 and Table 2); the second one is the progress in pressurized hot water extraction (PHWE) made it as a popular "green" extraction method which is compatible with PLE operation [24]; the last but not least is that ethanol/water represents sustainable green solvents. Fig. 5 displayed PLE extraction yields with ethanol concentrations varied from 30% to 100%. 70% ethanol gave the highest yields (recovery) of [6]-, [8]- and [10]-gingerols, and almost the same yield of [6]-shogaol as absolute ethanol. The decrease in the yield of extracts with 50% and 30% ethanol is very significant (Fig. 5). One hypothesis is that with a decreased ethanol concentration, the solvent becomes much polar and lead to lower solubilities of gingerols and shogaols. In the operation of the PLE extraction with 50% or 30% ethanol, the pores of the membrane on the bottom of extraction cell was found to be blocked by the ginger meal, which influence the rinsing operation (little extract liquid was eluted out). A centrifugation has to be employed in order to get extraction liquid out. This is probably because the matrix of ginger (e.g. cellulose) was degraded by pressurized ethanol aqueous solution, however, there is not a similar problem when absolute and 70% ethanols were employed as solvents.

In principle, the increasing temperature improves the penetration of solvent into sample matrix and mass transfer [24]; however, a negative effect that may occur is degradation of matrix at high temperature and high pressure, especially when aqueous solvent solution was employed. This phenomenon was observed when 70% ethanol was used as a solvent at higher temperature (Fig. 6A). From 60 to 100 °C the yield of [6]-, [8]-, [10]-gingerol and 6-shogaol increased with increasing temperature, while further increase of extraction temperature led to a decrease of the yields of all 4 constituents but the decrease of yield of [6]-gingerol is mostly significant.

For the PLE extraction with absolute ethanol as solvent, although the maximum yields were also achieved at 100 °C, the decrease of extraction yield, with a continuous increase of temperature higher than 100 °C, was not as significant as the PLE extraction with 70% ethanol (Fig. 6B). This could possibly be ascribed to less degradation of sample matrix by absolute ethanol than by 70% ethanol because high water content promotes hydrolysis of many natural polymers, which has been validated by experimental observations. No significant blocking of the membrane pores of the bottom cell was observed during extraction with absolute ethanol at all tested temperatures, however, blocking was observed for the extractions with 70% ethanol at 115 and 130 °C.

The effects of pressures on PLE extraction were depicted in Fig. 7. A marginal increase of [6]-gingerol yield was observed when the extraction pressure was increased from 1000 to 1500 psi, and further increased to 2000 psi almost no observable increase of the yield of [6]-gingerol was obtained. A statistical analysis demonstrated that the difference of the yield of [6]-gingerol at 1000 psi from those at 1500 and 2000 psi ($P < 0.05$), however, the difference between 1500 and 2000 psi is not significant ($P > 0.05$). The statistical analysis indicated that the difference in terms of the yields of [8]-, [10]-gingerol and 6-shogaol obtained at different pressure is not significant ($P > 0.05$, data not shown), which agreed well with some reported observations in other systems [25]. This probably can be ascribed to that there is a pressure threshold in a specific PLE system with a specific solvent, further higher pressures than this threshold do not further contribute to the penetration of solvent into material matrix and result in similar recovery of analytes.

In conclusion, this work demonstrated a highly time-solvent efficient approach for accelerated extraction of bioactive pungent compounds using sustainable bioethanol as solvent. 70% ethanol aqueous solution proved to be the most effective solvent combination in achieving high yield of total extract, complete constituent profile and recovery of most gingerol-related components.

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References

[1] H. Wohlmuth, D.N. Leach, M.K. Smith, S.P. Myers, J. Agric. Food Chem. 53 (2005) 5772.
[2] I. Khan, P. Pandotra, A.P. Gupta, R. Sharma, B.D. Gupta, J.K. Dhar, G. Ram, Y.S. Bedi, S. Gupta, J. Sep. Sci. 33 (2010) 558.
[3] H. Jiang, B.N. Timmermann, D.R. Gang, Rapid Commun. Mass Spectrom. 21 (2007) 509.
[4] R.D. Hiserodt, S.G. Franzblau, R.T. Rosen, J. Agric. Food Chem. 46 (1998) 2504.
[5] Y.A. Bailey-Shaw, L.A.D. Williams, G.O. Junor, C.E. Green, S.L. Hibbert, C.N.A. Salmon, A.M. Smith, J. Agric. Food Chem. 56 (2008) 5564.
[6] X. He, M.W. Bernart, L. Lian, L. Lin, J. Chromatogr. A 796 (1998) 327.
[7] S. Bhattarai, V.H. Tran, C.C. Duke, J. Pharm. Sci. 90 (2001) 1658.
[8] T.A. van Beek, J. Chromatogr. A 967 (2002) 21.
[9] L. Dinan, J. Harmatha, R. Lafont, J. Chromatogr. A 935 (2001) 105.
[10] J. Shen, X. Shao, Anal. Bioanal. Chem. 383 (2005) 1003.
[11] K.C. Zancan, M.O.M. Marques, A.J. Petenate, M.A.A. Meireles, J. Supercrit. Fluids 24 (2002) 57.
[12] B.E. Richter, B.A. Jones, J.L. Ezzell, N.L. Porter, N. Avdalovic, C. Pohl, Anal. Chem. 68 (1996) 1033.
[13] I. Windal, D.J. Miller, De.E. Pauw, S.B. Hawthorne, Anal. Chem. 72 (2000) 3916.
[14] M. Schnatz, J.J. Nicols, S.A. Wise, Anal. Chem. 69 (1997) 4210.
[15] S.J. Macnaughton, T.L. Jenkins, M.H. Wimpee, M.R. Cormier, D.C. White, J. Microbiol. Methods 31 (1997) 19.
[16] A. Demirbas, Appl. Energy 86 (2009) S108.

[17] J.H. Clark, F.E.I. Deswarte, T.J. Farmer, Biofuels Bioprod. Bioref. 3 (2009) 72.
[18] J.P. Bartley, P. Foley, J. Sci. Food Agric. 66 (1994) 365.
[19] S.D. Jolad, R.C. Lantz, G.J. Chen, R.B. Bates, B.N. Timmermann, Phytochemistry 66 (2005) 1614.
[20] M. Afzal, D. Al-Hadidi, M. Menon, J. Pesek, M.S. Dhami, Drug Metabol. Drug Interact. 18 (2001) 159.
[21] Y. Tao, W. Li, W. Liang, R.B. Vanbreemen, J. Agric. Food Chem. 57 (2009) 10014.
[22] O. Chienthavorn, T. Poonsukcharoen, T. Pathrakorn, Sep. Sci. Technol. 46 (2011) 616.
[23] M. Ikawa, T.D. Schaper, C.A. Dollard, J.J. Sasner, J. Agric. Food Chem. 58 (2010) 8139.
[24] C.C. Teo, S.N. Tan, J.W.H. Yong, C.S. Hew, E.S. Ong, J. Chromatogr. A 1217 (2010) 2484.
[25] K. Kitamura, Y. Takazawa, S. Hashimoto, J.-W. Choi, H. Ito, M. Morita, Anal. Chim. Acta 512 (2004) 27.