Talanta 115 (2013) 300–307

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Pressurised liquid extraction of volatile compounds in coffee bean

Mun-Wai Cheong [a], Alena Ann-Ann Tan [a], Shao-Quan Liu [a], Philip Curran [b], Bin Yu [b,*]

[a] Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore
[b] Firmenich Asia Pte. Ltd., 10 Tuas West Road, Singapore 638377, Singapore

ARTICLE INFO

Article history:
Received 10 January 2013
Received in revised form
5 April 2013
Accepted 12 April 2013
Available online 18 April 2013

Keywords:
Coffee
Volatile
Pressurised liquid extraction
Response surface methodology
Descriptive sensory analysis

ABSTRACT

In this work, we reported a novel application of pressurised liquid extraction (PLE) on coffee bean. The condition of PLE was carefully optimised with the aid of response surface methodology (RSM) including adjustment of experimental parameters (solvent type and sample to hydromatrix ratio) and other operating parameters (i.e. temperature (50–100 °C), pressure (1000–2000 psi) and static extraction time (5–15 min)). The coffee extracts obtained under three different extraction conditions were evaluated through descriptive sensory analysis. Then, the results showed that those targeted compounds obtained from PLE were nearly three times higher (1473 ppm) than conventional solvent extraction (571 ppm). Thus, PLE demonstrated the feasibility of producing a series of coffee extracts under controllable extraction conditions in correlation with desirable sensory attributes. This approach has not previously reported to characterise the aroma of coffee bean.

1. Introduction

Recent advancement in developing rapid and sensitive extraction techniques has become increasingly important [1–6]. Pressurised liquid extraction (PLE), also known as accelerated solvent extraction (ASE), is developed based on Soxhlet extraction except the solvents used close to their supercritical region [4]. At elevated pressure and temperature, the solubility and diffusion rate of solvents is enhanced, and thereby improves the mass transfer of analytes [5,6]. With this improvement, extraction yield from complex matrices could be significantly increased with the decrease of extraction time. Moreover, extraction procedure becomes versatile through tuning different operating parameters (e.g. temperature, pressure, time, extraction cycles and solvent) [7]. Another detailed optimisation indicated the possibility of manipulating the composition of the extract by adjusting the extraction parameters [8]. On the other hand, even minor adjustments of PLE parameters could affect the composition of extracted compounds.

Multivariate statistical approach, namely response surface methodology was applied effectively to determine optimum extraction parameters [5,9–11]. With the linear or square polynomial functions obtained from RSM, the significant effects of main factors as well as their interaction effects were identified and predicted. In order to comprehend the interactions among PLE parameters, multiple responses of targeted compounds with different scaling can be transformed into a desirability function [10,12]. Furthermore, under elevated temperature and pressure during PLE process, the composition of delicate aroma is easily distorted majorly due to thermal degradation of labile compounds and some side-reactions. Hence, besides the optimisation of operating parameters, a thorough sniffing is required to identify and monitor the desirable flavour profiles of different extracted products that are concomitant to the analytical work [13,14]. In contrast to other common PLE applications on environmental aspects [6,11,15–20], PLE has been found very limited reports in flavour isolation [7,8]. For example, PLE was applied to study volatile compounds in turmeric leaves, in which simultaneous optimisation of several responses was carried out based on the desirability function and evaluated the flavour intensity of turmeric leave extracts [8]. However, high extraction yields as well as desirable flavour profiles are still challenging, especially dealing with complex food matrices.

Underlying the unique aroma of coffee is a profound complexity that involves more than 800 different chemical compounds inherited in roasted coffee beans [21–24]. Although the extensive studies have been carried out for decades, the determination of volatiles in roasted coffee bean is still a challenging task as many of the important odorants are present in trace amounts and/or are reactive and unstable [3,23,25,26]. To the best of our knowledge, there is only one study reported on the PLE of polycyclic aromatic hydrocarbon from coffee [20], and has yet to be applied on any other coffee volatiles.

In this work, the objective was to extract volatiles in the coffee bean using PLE. Initially, the feasibility of PLE on extracting coffee volatiles was evaluated through comparison to solvent extraction. Furthermore, the factors affecting PLE of volatiles in coffee beans were systematically optimised using RSM. Finally, the aromatic profiles of these coffee extracts were evaluated through descriptive sensory analysis.

* Corresponding author. Tel.: +65 63472888; fax: +65 68619640.
E-mail address: gsgpbiy@gmail.com (B. Yu).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.04.034

2. Materials and methods

2.1. Coffee bean and chemicals

In this study, Boncafé International Pte. Ltd. (Singapore) provided the roasted Sumatra Mandheling coffee (*Coffea arabica* L. cv. Catimor), which underwent a roasting process for 14 min with an initial temperature of 160 °C and discharged at 223 °C. Coffee beans were grounded (Café Select Classic KMM 30, Braun, Germany) and sieved (Coffee Grind Sizer, Coffee Chemistry, CA, USA) into the size range of 1.77–2.36 mm. The coffee powder was sealed in the aluminium pouch and stored at −20 °C until use.

Acetone, dichloromethane, hexane and methanol from Fisher Scientific UK Limited (Loughborough, UK) were of analytical grade. Anhydrous sodium sulphate purchased from Merck (Darmstadt, Germany) was used as a drying agent while hydro super gel diatomaceous earth (hydromatrix) obtained from Sigma Aldrich (Missouri, USA) was applied as drying agent and dispersing agent during extraction.

All standard compounds used in the identification of the volatiles were obtained from the Firmenich Asia Pte. Ltd., Singapore.

2.2. PLE procedure

Ten grams of coffee powder was evenly mixed with 5 g of diatomaceous earth, and then packed into a 40-mL stainless steel cell secured with a neoprene filtration end cap. Extraction was performed with a Power-Prep PLE (Fluid Management Systems, MA, USA). The automated extraction cycle was operated using DMS6000 software as follows: the cell containing coffee powder was prefilled with extraction solvent (i.e. methanol, hexane, and dichloromethane), pressurised and heated for a static period (see Table 1). The cell was then flushed with fresh extraction solvent and purged with a flow of nitrogen gas and the extracts were eluted out of the extraction cell into the collection bottle placed in an ice bath. The extract was cooled for 30 min and dried by 10 g of anhydrous sodium sulphate before being concentrated to 1.0 ml using TurboVap II (Caliper Life Science, Massachusetts, USA). Finally, the extract was transferred to a 2-ml vial and stored at −30 °C until further analysis by GC–MS/FID and sensory evaluation.

2.3. Solvent extraction

Coffee extract was prepared from 10 g coffee powder with a volume of 40 mL dichloromethane. The suspension was stirred by vortex shaker (Heidolph Rotamax 120, Schwabach, Germany) at 200 rpm for 1 h. The extract was then filtered from the ground coffee beans and dried over anhydrous sodium sulphate. The solvent was removed under purified nitrogen stream using TurboVap II until the volume of sample was reduced to 1 mL. The experiment was performed in triplicate and stored at −30 °C until used for analysis.

Table 1
Face-centered central composite design (CCD).

Factor	Low (-)	High (+)	Centre
Temperature, x_1	50	100	75
Pressure, x_2	1000	2000	1500
Static extraction time, x_3	5	15	10

The design was a two-level full factorial design with 8 cube points, 6 centre points in cube, 6 axial points and alpha value 1.

2.4. GC–MS/FID analysis

GC–MS/FID analysis was carried out using Agilent 6890 N GC coupled with FID and a 5975 inert MS (Agilent Technologies, Palo Alto, CA, USA). In order to quantify the volatiles in the coffee bean extracts, 199 μL of the sample extracts were spiked with 1 μL of 20,000 ppm 5-methyl-2-hexanone (Sigma-Aldrich, Missouri, USA) as internal standard. One μL of the spiked extract was directly injected by an auto sampler (Gerstel Multi Purpose Sampler MPS, Mülheim an der Ruhr, Germany) into the GC injector under splitless mode, which was connected to a fused silica capillary column (60 m × 0.25 mm × 0.25 μm DB-FFAP, Agilent Technologies, Woodbridge, USA) coated with 0.25 μm film thickness of nitroterephthalic acid modified with polyethylene glycol. The injector temperature was 250 °C. The GC oven temperature was programmed from initially 50 °C for 5 min, then was raised to 230 °C at 5 °C/min and held for 30 min. FID temperature was set at 250 °C. Helium was used as carrier gas at a flow rate of 1.2 mL/min. The ionisation mode in the MS was electron impact (EI) mode at the ionisation energy of 70 eV, with turning performed using perfluorotributylamine. Identification of the eluted compounds was achieved by matching the mass spectra against NIST commercial library (Scientific Instrument Services, USA). Linear Retention Indices (LRI) values on a DB-FFAP column were determined by using a series of alkanes (C8–C40) (Fluka, Missouri, USA) run under identical conditions. LRI were further confirmed with the values of standard compounds and those reported in the literature [27–29].

The concentration of the compounds was expressed as parts per million (ppm) based on the relative FID peak area of each compound against internal standard with the response factor, which was previously determined with standard compounds under the same conditions. The relative response factors (RRFs) were calculated as: $RRF = (M_{compound} \times A_{IS})/(M_{IS} \times A_{compound})$, where $M_{compound}$ and $A_{compound}$ are the mass and corresponding GC peak area of the compounds, M_{IS} and A_{IS} are the mass and GC peak area of the internal standard. For the commercially unavailable compounds, the RRFs were assumed to be 1.00.

2.5. RSM and statistical analysis

A face-centered central composite design (CCD) was constructed and analysed using the Design Expert Version 6.0.10 software (Stat-Ease, MN, USA), where the effects of three independent parameters on the selected key odorants [13,23–26] were taken as the response variables. Three main factors were selected as reported in the literature [16], i.e. temperature (x_1, 50–100 °C), pressure (x_2, 1000–2000 psi) and static extraction time (x_3, 5- 15 min) (Table 1). The 20 runs were in triplicate in order to calculate the averages and standard deviations (Table 1).

The generalised response surface model to describe the variations in response variables is given as follows [12]:

$$y = \beta_0 + \sum_{j=1}^{q} \beta_j x_j + \sum_{i=1}^{q} \beta_{jj} x_j^2 + \sum\sum_{i<j} \beta_{ij} x_j x_j$$

where y is the predicted response; β_0 is a constant; β_j is the linear regression coefficient; β_{jj} is the quadratic coefficient, β_{ij} is the interaction coefficient; and x_i and x_j are independent parameters. The adequacy of the model was determined by evaluating the coefficient of determination (R^2) and lack-of-fit tests obtained from the analysis of variance (ANOVA). Statistical significance of the model and model terms were determined at 95% confidence level [12]. The terms found to be non-significant ($p > 0.05$) were dropped from the initial model and refitted with the significant ($p < 0.05$) independent parameters in order to obtain the final reduced model. However, some insignificant linear terms were retained in the model if a quadratic or interaction term containing this parameter was significant [30].

Three dimensional response surface plots were generated by keeping one factor at its optimal level and plotting that against two independent parameters.

2.6. Optimisation and validation procedures

Selective optimisation based on individual desirability to response variables was obtained through an objective function. With the objective function, individual desirability of each response variable was combined [10], as follow:

$$d_i = \begin{cases} 0 & y_i \le y_{i\,\min} \\ \left[\frac{y_i - y_{i\,\min}}{y_{i\,\max} - y_{i\,\min}}\right] & y_i < y_{i\,\min} < y_{i\,\max}, \text{ for } i = 1,\ 2\ldots,\ 14 \\ 1 & y_i \ge y_i \end{cases}$$

$$D = \left(\prod_{i=1}^{n} d_i^{w_i}\right)^{1/\sum w_i} = \left(\prod_{i=1}^{n} d_i\right)^{1/n}$$

where d_i is the individual desirability value of ith response, the value of y_{imin} and y_{imax} are the minimum and maximum acceptable value of y_i, overall desirability (D) with n is the total number of responses and w_i, is the individual response importance, in our case $w_i = 1$ as desirability function was set as linear.

2.7. Sensory evaluation

In the present study, descriptive sensory analysis was adopted as qualitative responses in rating the intensity of prescribed flavour attributes. Sensory profiles of the coffee extracts were evaluated by experienced flavourists from Firmenich Asia Pte. Ltd., Singapore. After reaching a consensus among the flavourists, the appropriate descriptive sensory attributes were established for the coffee extracts. The corresponding descriptive attributes were ashy, beany, berry-like, burnt, caramellic, earthy, nutty, roasted, smoky/phenolic and sulphury. A 6-point scale was used with '0' indicating the uncharacterised attribute intensity and '5' indicating the strongest attribute intensity. A set of coded smelling strips was presented to the panellists, and the intensity of the attributes was rated after sniffing each of the coffee extracts. The results were averaged for each of the descriptive attributes and expressed in a plotted web diagram.

3. Results and discussion

PLE is conducted under elevated temperature and pressure within short time period [4]. Several factors, which may be interrelated, significantly influence PLE, mainly solvent, temperature, pressure and static extraction time [5,16,31]. Generally, pressure helps to force liquid into the pores and maintain the solvent in liquid or condensed state at operating temperatures. Temperature can enhance the solvent wetting of the sample, permit the analytes to dissolve faster into the solvent, and then achieve an improved extraction process [5]. With an improved diffusion rate and mass transfer process, it allows shorter extraction time and reduces the risk of degradation in the process of PLE. On the other hand, the selectivity of extraction could decrease at higher temperature due to the co-extraction of interfering matrix components such as fatty acids [32]. In addition, degradation or evaporation of volatile components might occur at elevated temperatures. Therefore, a systematic assessment of the interrelated factors mentioned above is crucial.

3.1. Selection of extraction solvent, ratio of hydromatrix to sample and extraction cycle

Optimisation of an extraction process commonly begins with an appropriate choice of the extraction solvent, which is able to solubilise the analytes of interest and minimise the co-extraction of other matrix components [16]. The main classes of volatile compounds identified in the coffee bean were acids, furans, phenols, pyrazines, pyridines and sulphur-containing compounds. Among three selected solvents (i.e. hexane, dichloromethane and methanol), it is observed that methanol was more effective to extract polar compounds such as pyridine, acetic acid (Table 2). With regard to the total extracted amount, dichloromethane provided the highest. In addition, some key volatile compounds (i.e. furfury mercaptan and furaneol) were only detected in dichloromethane extract. Therefore, for better extraction, dichloromethane is the preferable among three solvents. Subsequently, PLE was compared with conventional solvent extraction, of which both extracts shared similar volatile profiles but total extracted amount in the extracts of PLE was nearly 3 times higher (Table 2). The suitability of PLE for the volatile analysis in coffee powders was also justified with the efficiency in the extraction time. Other experimental parameters were further optimised.

Hydromatrix, serving not only as a dispersing agent but also as a dehydrating agent might alter the extraction efficiency [31]. Different ratios of sample to hydromatrix (i.e. 1:2, 2:1 and 1:1) were compared in order to understand its effect on the extraction efficiency. There was a slightly higher yield of volatiles extracted at a sample to hydromatrix ratio of 2:1 compared to the ratio of 1:2 and 1:1. It implied that a good approximation of phase ratio was required in order to aid the extraction process (data not shown here).

All the above discussion was based on one extraction cycle. For comparison, we also studied the number of extraction cycle as an affecting factor. In general, an increment in the number of extraction cycle allows the exposure of the matrix to fresh solvents and favours the solvent-to-sample equilibrium, thereby improving compound partition into the solvent phase [15,33]. The percentage yield was calculated by dividing the concentration obtained in a particular cycle by the total concentration obtained in all successive cycles. Under the present condition, the first extraction cycle was able to extract more than 60% of the compounds, and the percentage yield of the compounds decreased as the number of extraction cycle increased. It was also noted that the aromatic profiles of extracts in subsequent cycles significantly changed and became undesirable (data not shown here). In the following experiments, the number of extraction cycle was set as one.

3.2. Face-centered central composite design

Considerations of the interrelated factors are crucial to the efficiency of PLE on volatile extraction. Fourteen compounds, reported as key volatiles in coffee bean [13,23–26], were selected for further optimisation (Table 3). These response variables were assessed as a function of main, quadratic and interaction effects of temperature (x_1), pressure (x_2) and static extraction time (x_3). For regression coefficients, positive values indicate that the yield of the compounds is favoured toward the increasing values of the respective parameters within the range studied, while negative coefficients indicate the decrease of yield toward the increasing value of studied range [12].

3.2.1. Effect of PLE operating parameters

Table 3 summarises the predicted functions, coefficient of determination (R^2), along with the corresponding lack-of-fit test (F-and p-Values) and individual probability of the independent parameters in the final reduced models. The results suggested that the final reduced models were significantly ($p < 0.05$) fitted for 14 response variables studied with relatively high R^2, ranging from 0.646 to 0.929. Moreover, extraction temperature was the most critical factor on PLE where most of the compounds were significantly affected ($p < 0.05$). This further emphasised the volatile nature of these compounds as a slight change in extraction temperature affected their amount, which

Table 2
Identification of volatiles and their concentrations (ppm) in coffee beans extracted using hexane, dichloromethane and methanol.

Compounds	*LRI*		Solvent extraction	PLE			Identification
	FFAP	Ref	Dichloromethane	Hexane	Dichloromethane	Methanol	
Acids							
Acetic acid [d]	1444	1468	22.73 ± 2.51	10.20 ± 5.05	34.17 ± 7.81	152.93 ± 47.82	MS, *LRI*[a], STD
3-Methylbutanoic acid [d]	1657	1687	13.18 ± 0.46	20.81 ± 8.21	41.31 ± 13.22	–	MS, *LRI*[a], STD
trans-2-butenoic acid	1764	–	–	–	4.17 ± 1.64	–	MS, STD
Furans							
Furfural [d]	1473	1473	4.99 ± 0.48	5.00 ± 2.34	11.19 ± 3.74	5.93 ± 1.96	MS, *LRI*[b], STD
Furfuryl acetate [d]	1538	1507	27.50 ± 3.82	40.35 ± 17.10	62.97 ± 18.42	14.01 ± 9.29	MS, *LRI*[b], STD
Furfuryl alcohol	1653	1671	141.47 ± 20.05	106.26 ± 14.36	415.80 ± 99.83	244.75 ± 119.30	MS, *LRI*[b], STD
2–Acetyl-5-methylfuran [d]	1623	1653	6.33 ± 3.77	4.16 ± 2.41	3.52 ± 1.42	0.78 ± 0.24	MS, *LRI*[c]
Furfuryl ether	1987	1996	9.12 ± 1.03	10.91 ± 5.95	19.18 ± 7.68	2.40 ± 1.03	MS, *LRI*[b]
Phenols							
Guaiacol [d,e,f,g]	1865	1886	9.28 ± 2.04	13.12 ± 6.19	25.49 ± 12.50	6.14 ± 4.67	MS, *LRI*[a], STD
Phenol [d]	2004	2030	12.33 ± 1.08	9.53 ± 5.05	26.98 ± 12.67	9.66 ± 6.37	MS. *LRI*[a], STD
4-Ethylguaiacol [d,e,f,g]	2035	2065	17.00 ± 3.85	14.36 ± 7.43	28.23 ± 12.55	2.79 ± 1.44	MS. *LRI*[c], STD
p-Cresol [d]	2090	–	–	2.03 ± 1.45	8.80 ± 7.93	0.57 ± 0.21	MS, STD
p-Vinylguaiacol [d,f,g]	2206	2225	34.02 ± 5.20	46.03 ± 27.72	86.73 ± 29.30	12.02 ± 7.33	MS, *LRI*[a], STD
Pyrazines							
2-Methylpyrazine [d]	1256	1267	21.35 ± 2.34	9.72 ± 2.54	32.24 ± 8.55	14.03 ± 5.74	MS, *LRI*[b]. STD
2,5-Dimethylpyrazine [d]	1308	1324	7.55 ± 1.23	12.09 ± 4.52	21.76 ± 8.51	5.07 ± 1.08	MS. *LRI*[b], STD
2,6-Dimethylpyrazine [d]	1314	1330	9.60 ± 1.51	–	18.46 ± 5.23	5.99 ± 2.85	MS. *LRI*[b], STD
2,3-Dimethylpyrazine	1339	1348	2.45 ± 0.42	5.00 ± 2.96	4.60 ± 1.63	1.46 ± 0.96	MS, *LRI*[b]
2-Ethyl-6-methylpyrazine [d]	1383	1388	5.91 ± 0.99	10.87 ± 5.27	10.03 ± 5.57	–	MS, *LRI*[b]
2-Ethyl-5-methylpyrazine [d]	1392	1394	2.81 ± 0.49	5.55 ± 2.82	4.01 ± 3.70	2.10 ± 2.85	MS, *LRI*[b]
2,3,5-Trimethylpyrazine [d,e,f,g]	1407	1429	5.41 ± 0.41	9.27 ± 4.83	9.27 ± 3.11	2.22 ± 1.43	MS. *LRI*[a], STD
Pyridines							
Pyridine [d]	1176	1182	34.75 ± 3.90	1.66 ± 0.38	63.42 ± 13.59	63.50 ± 15.76	MS, *LRI*[b], STD
2-acetylpyridine [d]	1613	–	–	–	–	0.28 ± 0.49	MS, STD
Sulphur-containing compounds							
Furfuryl mercaptan [d, e, f, g]	1438	–	0.58 ± 0.35	–	2.31 ± 2.43	–	MS, STD
Furfuryl methyl sulphide [d]	1496	1506	3.05 ± 0.60	2.90 ± 1.42	2.55 ± 2.01	–	MS, *LRI*[a], STD
Miscellaneous							
2,3-Pentanedione [d, e, f, g]	1070	1067	2.6 ± 0.56	–	3.14 ± 0.52	–	MS, *LRI*[b]
Acetoin[d]	1270	1291	4.74 ± 0.44	–	14.64 ± 5.89	6.73 ± 2.42	MS, *LRI*[b], STD
γ-Butyrolactone[d]	1645	1637	43.45 ± 7.95	19.11 ± 7.04	115.65 ± 32.42	59.58 ± 28.73	MS, *LRI*[b], STD
Maple lactone [d]	1830	1857	14.61 ± 3.32	–	25.37 ± 5.89	7.74 ± 3.03	MS, *LRI*[a]
Maltol [a,g]	1975	2004	16.52 ± 4.45	12.50 ± 3.50	37.57 ± 23.45	13.36 ± 10.89	MS, *LRI*[a], STD
2-Acetylpyrrole [d]	1977	1983	21.70 ± 3.14	24.16 ± 9.57	27.66 ± 15.91	15.15 ± 8.48	MS, *LRI*[b], STD
Furaneol [d,e,f,g]	2028	2062	4.94 ± 1.46	–	9.59 ± 2.50	–	MS, *LRI*[a], STD
2-Pyrrolidinone	2055	–	9.59 ± 1.20	5.05 ± 3.28	45.19 ± 35.84	8.61 ± 6.91	MS
Methyl palmitate [d]	2228	–	31.65 ± 1.95	39.52 ± 23.66	77.58 ± 33.76	74.16 ± 44.90	MS
3-Pyridinol [d]	2426	–	33.96 ± 1.77	10.74 ± 7.87	161.31 ± 66.88	72.28 ± 25.43	MS
Total concentration			571.42	465.81	1473.80	806.93	

[a] *LRI* refers to the values in Ref. [26].
[b] *LRI* refers to the values in Ref. [12] and.
[c] *LRI* refers to the values in Ref. [11]; "-", not detected.
[d] Compounds reported in [6]
[e] Compounds reported in Ref. [7].
[f] Compounds reported in Ref. [8].
[g] Compounds reported in Ref. [33]. Identification method: MS=mass spectrum; LRI=Linear Retention Indices obtained from references or literature values and STD=standards.

was especially significant ($p < 0.0001$) for furfuryl mercaptan, furfural, furfuryl alcohol and maltol. Detailing the coefficient of the linear temperature factor (x_1) with a negative sign indicated that the amount of furfuryl mercaptan and furaneol decreased as extraction temperature increased. This suggested that temperature must be restricted for thermally labile volatile components.

The application of high pressure in PLE allows the use of extraction temperature above the boiling point of the solvent while maintaining the solvent in its liquid state. High pressure could also improve the solvent accessibility to the analytes that are bound within the matrix pores [6]. However, single-factor pressure term was insignificant for most compounds except for furfuryl mercaptan ($p > 0.05$). Under the present condition, pressure seemed to play a minimum role, as long as the solvent was maintained in a condensed phase. This result was in good agreement with previous work on essential oil extracted from herbal plants where the effect of pressure on the amount of most substances was almost negligible [34,35].

In PLE, static extraction time refers to the duration of the heat and maintenance of pressure step in the extraction cycle. It determines the time for the solute to equilibrate and partition between sample matrix and extraction solvent. Table 3 shows that the effect of static extraction time was significant for some compounds such as pyridine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine and furfuryl alcohol ($p < 0.05$). Thus, static extraction time to a certain extent affected the extraction of target compounds, but was less pronounced than temperature.

Table 3
Odour description, polynomial equation, R^2, probability values, lack-of-fit and significance probability of regression coefficients in the final reduced models.

Compound	Odour description	Second order Polynomial equation	R^2	Regression	Lack-of-Fit		Factors	
				p-Value	*F*-Value	*p*-Value		*p*-Value
Furfural	Bread-like, caramellic, sweet	$Y=-22.38+0.52x_1+0.25x_3$	0.871	0.0021	1.80	0.2677	x_1	**< 0.0001**
							x_3	**0.0046**
Furfuryl alcohol	Caramellic, fruity, sweet	$Y=1077.22+20.37x_1+28.02x_3$	0.898	0.0007	2.21	0.2026	x_1	**< 0.0001**
							x_3	**0.0022**
Guaicol	Phenolic, spicy, vamilla	$Y=-16.53+0.31x_1$	0.646	0.1433	5.52	0.0421	x_1	**0.0117**
Phenol	Phenolic, plastic, rubber	$Y=-14.08+0.088x_1-0.26\times{}_3-+0.02x_{13}$	0.830	0.0071	4.50	0.0623	x_1	**0.0003**
							x_3	**0.0091**
							x_{13}	**0.0406**
2-methylpyrazine	Cocoa, nutty, roasted	$Y=-72.29+1.56x_1+1.28x_3$	0.784	0.0203	1.21	0.4184	x_1	**0.0014**
							x_3	**0.0192**
2,5-dimethylpyrazine	Cocoa, roasted nut-like	$Y=-17.15+0.49x_1+0.50x_3$	0.674	0.1060	1.73	0.2802	x_1	**0.0260**
							x_3	**0.0469**
2,6-dimethylpyrazine	Cocoa, nutty, roasted	$Y=-22.32+0.70x_1+0.42x_3$	0.709	0.0690	1.06	0.4741	x_1	**0.0148**
							x_3	**0.0385**
Pyridine	Amine-like, fishy	$Y=-175.49+3.47x_1+5.90x_3$	0.863	0.0027	1.42	0.3540	x_1	**0.0001**
							x_3	**0.0037**
Furfuryl mercaptan	Coffee-like, roasted, sulphury	$Y=0.79-0.02x_1+0.0006x_2-0.04x_3+0.0002x_1{}^2-0.000004x_{12}-0.00001x_{23}$	0.863	< 0.0001	–	–	x_1	**< 0.0001**
							x_2	**0.0230**
							x_3	**0.0346**
							$x_1{}^2$	**0.0041**
							x_{12}	**0.0134**
							x_{23}	**0.0314**
Acetoin	Caramellic, toasted grain-like	$Y=-19.68+0.33x_1+0.006x_2+1.07x_3-0.0008\times x_{23}$	0.794	0.0167	4.38	0.0654	x_1	**0.0002**
							x_2	0.3223
							x_3	0.3452
							x_{23}	**0.0472**
Maltol	Caramellic, fruity, sweet	$Y=-60.61+0.61x_1+0.01x_3+0.06x_{13}$	0.929	0.0001	5.81	0.0381	x_1	**< 0.0001**
							x_3	**0.0006**
							x_{13}	**0.0175**
2-acetylpyrrole	Musty, sweet, walnut-like	$Y=-17.18+0.53x_1+0.20x_3$	0.783	0.0204	2.92	0.1325	x_1	**0.0007**
							x_3	**0.0303**
Maple lactone	Fruity, maple, sweet caramel	$Y=-38.37+0.96x_1-1.18x_3$	0.840	0.0054	3.44	0.1005	x_1	**0.0002**
							x_3	**0.0070**
Furaneol	Caramellic, fruity, strawberry	$Y=19.51-0.25x_1-0.26x_3+0.02x_{13}$	0.789	0.0184	4.31	0.0675	x_1	**0.0006**
							x_3	0.0796
							x_{13}	**0.0277**

x_1, x_2 and x_3: the main effects of temperature, pressure and static extraction time, respectively. $x_1{}^2$, $x_2{}^2$, $x_3{}^2$: the quadratic effects of temperature, pressure and static extraction time, respectively. x_{12}: the interaction effect of temperature × pressure, x_{13}: the interaction effect of temperature × static extraction time, x_{23}: the interaction effect of pressure × static extraction time. Model terms with statistical significance ($p < 0.05$) are shown in bold.

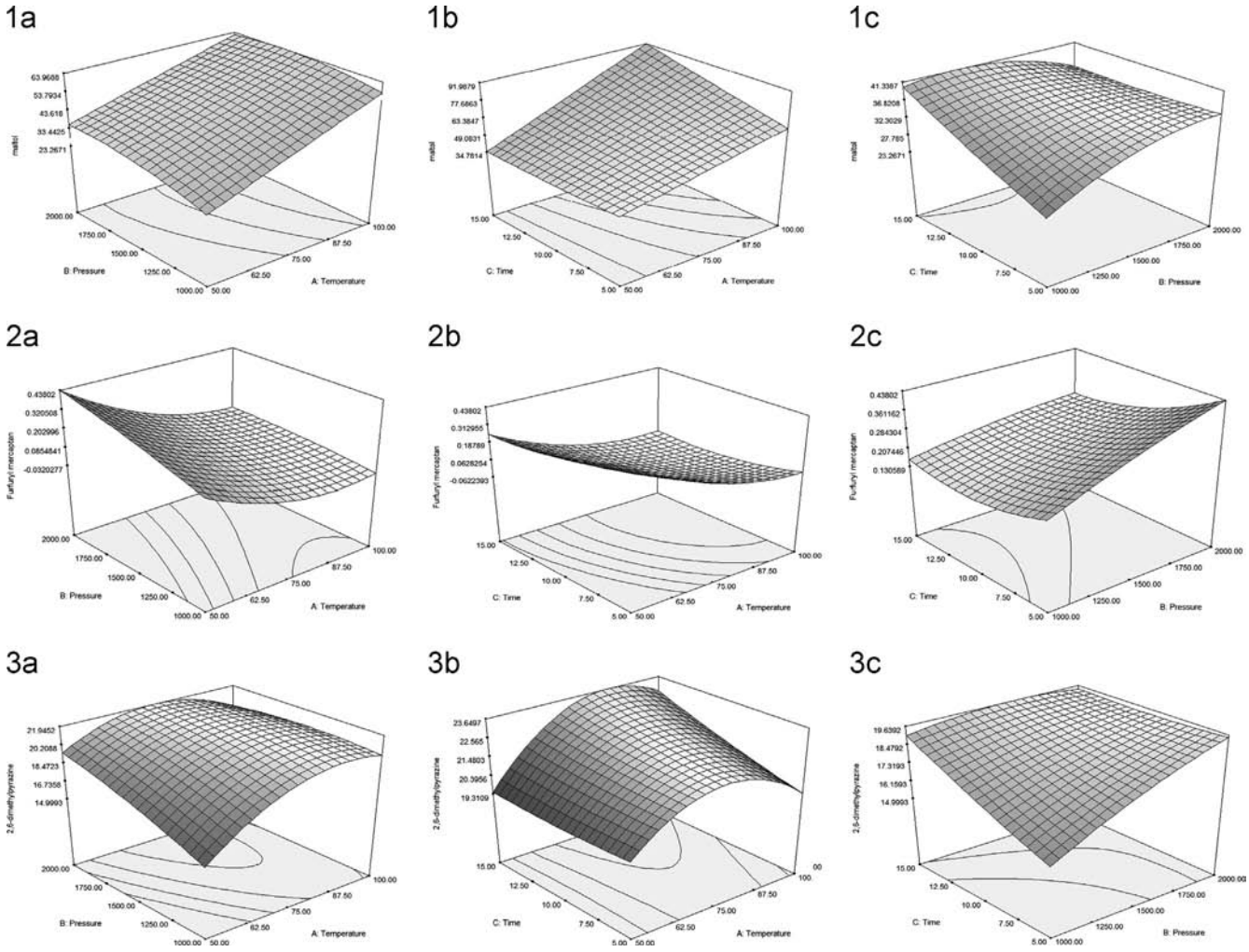

Fig. 1. Response surface plots showing the effects of temperature, pressure and static extraction time of selected compounds: 1. maltol; 2. furfuryl mercaptan; 3. 2,6-dimethylpyrazine. (a) Interaction between temperature and pressure; (b) interaction between temperature and time; and (c) interaction between pressure and time.

3.2.2. Interaction among PLE operating parameters

The interaction of independent parameters is especially important to promote PLE efficiency. For instance, temperature helps to enhance solvent penetration inside sample matrix, pressure can improve the solvent accessibility to the analytes, and static extraction time facilitates equilibration between solvent and matrix. As shown in Table 3, most of the significant interactions existed between temperature and static extraction time. Fig. 1 depicts the response surface plots of interaction effects between factors on the variation of selected responses (amounts extracted). For example, the amount of maltol was found to be a quadratic function of the temperature and time where the amount tended to increase with increasing temperature and time. These results could be associated with the increased ability of the solvent to solubilise maltol in the coffee bean matrix and the reduction of the viscosity of the solvent which allowed more effective penetration into the matrix at higher temperatures and prolonged heating.

In contrast, furfuryl mercaptan was negatively correlated with the increase of temperature and time. By referring to the model terms, the amount of furfuryl mercaptan was significantly influenced by all three linear factors, quadratic effect of temperature and also interaction between *temperature* × *pressure* and *pressure* × *static extraction time*. In a particular study, it was reported that this compound was not stable as 40 day-storage at room temperature lowered its concentration by 81%, even though the coffee sample was vacuum-packed [36].

3.2.3. Optimisation of PLE operating parameters

Under elevated temperature and pressure, interfering substances may be extracted along with desired compounds during PLE process. Moreover, it is worthy to note that no single experimental condition can be found under which the extraction of all volatile compounds is maximised due to the difference in physicochemical properties of the compounds [10]. In order to assess the feasibility of PLE in flavour analysis, we attempted to selectively maximise the target compounds while minimising the interferences, and thus several combinations were obtained through multi-response optimisation to manipulate the compositions of coffee extracts.

With the aim of maximising the total amount extracted, the optimal conditions obtained were 100 °C, 1000 psi and 15-min static extraction time with a desirability function of 0.731. In the second optimisation, the emphasis was placed on compounds that responded significantly in the response surface models. The results gave an optimal point of 75 °C, 1300 psi and 15-min static extraction time with a median desirability factor of 0.40. The optimal conditions and relatively low desirability were different from those obtained previously due to the differences in pre-selection of response goals.

Some thermal labile compounds (e.g. furfuryl mercaptan and furaneol) are key odourants of coffee aroma. The importance of their odour contribution strengthens the necessity to maximise their concentration. Thus, the third optimisation was performed

Table 4
Validation of response surface model.

Compounds	Concentration (ppm)							
	75 °C, 1500 psi and 10 min				50 °C, 2000 psi and 15 min			
	Prediction	95% CI* low	95% CI* high	Average	Prediction	95% CI* low	95% CI* high	Average
Pyridine	85.83	76.77	94.89	82.44 ± 5.67	55.88	32.41	79.35	63.86 ± 20.02
Acetoin	12.14	10.08	14.19	11.93 ± 0.98	6.94	1.63	12.26	9.46 ± 3.16
2,5-dimethylpyrazine	16.32	14.95	17.68	15.28 ± 1.33	14.24	10.70	17.78	14.73 ± 4.56
2,6-dimethylpyrazine	22.83	21.08	24.58	23.57 ± 1.79	19.42	14.88	23.95	21.01 ± 4.70
2-methylpyrazine	40.36	36.28	44.43	37.952.81	30.24	19.69	40.80	33.27 ± 9.95
Furfural	14.26	12.83	15.68	13.96 ± 1.05	8.92	5.21	12.62	10.40 ± 2.66
Furfuryl alcohol	552.43	497.71	607.15	533.30 ± 36.33	337.48	195.72	479.24	383.98 ± 113.65
Maple lactone	23.43	20.43	26.43	21.27 ± 3.48	17.06	9.28	24.84	17.79 ± 5.29
Guaiacol	16.47	14.67	18.28	16.26 ± 2.19	13.63	8.96	18.30	14.23 ± 3.51
Maltol	59.47	53.74	65.19	57.63 ± 10.65	35.07	20.24	49.89	37.77 ± 10.70
Phenol	20.95	18.80	23.10	20.98 ± 0.70	14.39	8.82	19.96	14.81 ± 4.52
Furaneol	10.82	9.04	12.61	10.36 ± 2.86	5.90	1.28	10.52	6.26 ± 2.02
2-acetylpyrrole	44.24	39.91	48.58	39.56 ± 2.61	33.98	22.75	45.20	33.68 ± 11.49

* Confidence interval with 95% confidence level.

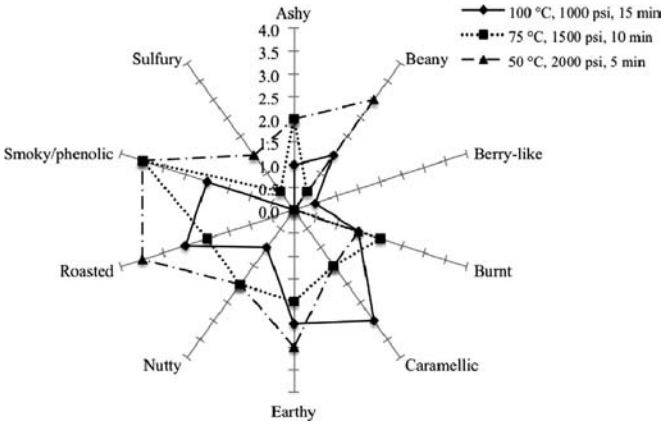

Fig. 2. Sensory profiles of coffee extracts under three optimised extraction conditions.

only for furfuryl mercaptan and furaneol. The optimal conditions derived were 50 °C, 2000 psi and 5 min with a desirability factor of 0.911 to avoid thermally induced decomposition.

3.2.4. Validation of response surface model

In order to assess the long-term variability of the response surface model, intermediate precision was validated by selecting two experimental points within the experimental range. The averaged concentrations of the compounds together with the standard deviations are tabulated in Table 4. The experimental points chosen were 75 °C, 1500 psi and 10 min (centre point) and 50 °C, 2000 psi and 15 min. In this present study, furfuryl mercaptan was not included for the validation due to high variability. From Table 4, the average concentrations of all selected compounds fell well within the predicted response range (confidence interval) at 95% confidence level.

3.3. Sensory evaluation

The varying concentrations of the key odourants in the coffee bean extract give rise to its overall aroma and odour. During the three types of PLE optimisation, different goals were set in order to obtain the optimal extraction condition required to yield coffee extracts with desirable aromatic profiles. Fig. 2 reveals the notable differences in the aromatic profiles of the coffee extracts obtained under different extraction conditions.

As 14 selected compounds possess distinctive attributes, odour descriptions are listed in Table 3. Under the operating condition of 100 °C, 1000 psi and 15 min, all responses were given equal emphasis. The coffee extract obtained exhibited weaker perception, except for the attribute of caramellic (3.0). Thus, this suggested that although most of the target compounds were extracted maximally, the coffee extract lost its genuine aromatic profile especially nutty (1) and sulphury (0) notes. The high score of the caramellic note was possibly attributed to furfuryl alcohol, furfural, maple lactone, maltol and furaneol.

On the other hand, the sensory profile of the coffee extract obtained at 75 °C, 1500 psi and 10 min revealed an average score of 2.0 for ashy, burnt, earthy, nutty, roasted notes. However, a strong smoky/phenolic attribute (3.5) was perceived, which overwhelmed the other attributes. This could be due to the extraction condition being favourable to the extracted compounds that responded significantly to the RSM model, particularly phenol, which are known to be responsible for smoky and phenolic odours in coffee aroma.

Due to the pre-set goal of maximising furfuryl mercaptan and furaneol, the sensory profile of the extract obtained at 50 °C, 2000 psi and 5 min revealed a higher sulphury note (1.5) compared to other sensory profiles. This was in accordance with the aim to develop an extraction condition suitable for furfuryl mercaptan exhibiting coffee-like, roasted and sulphury notes.

4. Conclusions

A novel approach to extraction of volatile compounds in coffee bean by PLE was developed. RSM was applied to optimise the PLE operating conditions (i.e. temperature, pressure and static extraction time). Temperature was clearly found to be the most important factor followed by pressure. Moreover, significant interactions existed between temperature and static extraction time. Through descriptive sensory analysis, the aromatic profiles of the coffee extracts under three different optimum conditions were expressed. Therefore, PLE is a convenient, reliable and flexible technique, which may be potentially useful for flavour isolation.

Acknowledgements

The authors are grateful to Firmenich Asia Pte. Ltd. for providing its technical assistance and funding during the study.

References

[1] A. Müller, E. Björklund, C. Von Holst, J. Chromatogr. A 925 (2001) 197–205.
[2] A.L. Oliveira, E.S. Kamimura, J.A. Rabi, Innovative Food Sci. Emerging Technol. 10 (2009) 189–194.
[3] C. Sarrazin, J.-L. Le Quéré, C. Gretsch, R. Liardon, Food Chem. 70 (2000) 99–106.
[4] B.E. Richter, B.A. Jones, J.L. Ezzell, N.L. Porter, N. Avdalovic, C. Pohl, Anal. Chem. 68 (1996) 1033–1039.
[5] M.B. Hossain, C. Barry-Ryan, A.B. Martin-Diana, N.P. Brunton, Food Chem. 126 (2011) 339–346.
[6] B.E. Richter, J.L. Ezzell, D.E. Knowles, F. Hoefler, A.K.R. Mattulat, M. Scheutwinkel, D.S. Waddell, T. Gobran, V. Khurana, Chemosphere 34 (1997) 975–987.
[7] E. Cicchetti, A. Chaintreau, J. Sep. Sci. 32 (2009) 1957–1964.
[8] A.H. Zaibunnisa, S. Norashikin, S. Mamot, H. Osman, LWT—Food Sci. Technol. 42 (2009) 233–238.
[9] J. Cescut, E. Severac, C. Molina-Jouve, J.L. Uribelarrea, J. Chromatogr. A 1218 (2011) 373–379.
[10] J.S. Ribeiro, R.F. Teofilo, F. Augusto, M.M.C. Ferreira, Chem. Intell. Lab. Syst. 102 (2010) 45–52.
[11] J.-B. Baugros, C. Cren-Olivé, B. Giroud, J.-Y. Gauvrit, P. Lantéri, M.-F. Grenier-Loustalot, J. Chromatogr. A 1216 (2009) 4941–4949.
[12] R.H. Myers, D.C. Montgomery, Response Surface Methodology: Process and Product Optimization using Designed Experiments, 2nd ed., Wiley-Interscience, New York, 2002.
[13] R.J. Cannon, L. Trinnaman, B. Grainger, A. Trail, The Key Odorants of Coffee from Various Geographical Locations, American Chemical Society, USA, 2010.
[14] T. Aishima, J. Chromatogr. A 1054 (2004) 39–46.
[15] D.R. Baker, B. Kasprzyk-Hordern, J. Chromatogr. A 1218 (2011) 7901–7913.
[16] R. Carabias-Martínez, E. Rodríguez-Gonzalo, P. Revilla-Ruiz, J. Hernández-Méndez, J. Chromatogr. A 1089 (2005) 1–17.
[17] M. Okihashi, H. Obana, S. Hori, Analyst 123 (1998) 711–714.
[18] M. Jánská, M. Tomaniová, J. Hajšlová, V.ı. Kocourek, Anal. Chim. Acta 520 (2004) 93–103.
[19] R. Ghosh, K.J. Hageman, E. Björklund, J. Chromatogr. A 1218 (2011) 7242–7247.
[20] S.B. Hawthorne, C.B. Grabanski, E. Martin, D.J. Miller, J. Chromatogr. A 892 (2000) 421–433.
[21] F. Mayer, M. Czerny, W. Frosch, Eur. Food Res. Technol. 211 (2000) 272–276.
[22] C.A.B. De Maria, L.C. Trugo, F.R. Aquino Neto, R.F.A. Moreira, C.S. Alviano, Food Chem. 55 (1996) 203–207.
[23] S. Oestreich-Janzen, Chemistry of coffee, in: M. Lew, L. Hung-Wen (Eds.), Comprehensive Natural Products II, Elsevier, Oxford, 2010, pp. 1085–1117.
[24] I. Flament, Coffee Flavour Chemistry, John Wiley & Sons, Ltd., New York, 2002.
[25] I. Blank, A. Sen, W. Grosch, Zeitschrift für Lebensmittel-Untersuchung und -Forschung 195 (1992) 239–245.
[26] I. Blank, A. Sen, W. Grosch, Aroma Impact Compounds of Arabica and Robusta Coffee. Qualitative and Quantitative Investigations, in: Proceedings 14th International Scientific Colloquium on Coffee, ASIC, San Francisco, 1991, pp. 117–129.
[27] L. Mondello, R. Costa, P.Q. Tranchida, P. Dugo, M.L. Presti, S. Festa, A. Fazio, G. Dugo, J. Sep. Sci. 28 (2005) 1101–1109.
[28] J.K. Moon, T. Shibamoto, J. Agric. Food Chem. 57 (2009) 5823–5831.
[29] E. Nebesny, G. Budryn, J. Kula, T. Majda, Eur. Food Res. Technol. 225 (2007) 9–19.
[30] K.W. Cheong, C.P. Tan, H. Mirhosseini, N.S. Abdul Hamid, A. Osman, M. Basri, Food Res. Int. 43 (2010) 1267–1276.
[31] A. Mustafa, C. Turner, Anal. Chim. Acta 703 (2011) 8–18.
[32] M.J. Martín, F. Pablos, A.G. González, M.S. Valdenebro, M. León-Camacho, Talanta 54 (2001) 291–297.
[33] D. Chen, Y. Tao, H. Zhang, Y. Pan, Z. Liu, L. Huang, Y. Wang, D. Peng, X. Wang, M. Dai, Z. Yuan, J. Chromatogr. B 879 (2011) 1659–1667.
[34] M.P.K. Choi, K.K.C. Chan, H.W. Leung, C.W. Huie, J. Chromatogr. A 983 (2003) 153–162.
[35] C. Deng, N. Li, X. Zhang, J. Chromatogr. A 1059 (2004) 149–155.
[36] R.A. Buffo, C. Cardelli-Freire, Flavour Fragrance J. 19 (2004) 99–104.