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Optimization of Water Based-extraction Methods for the Preparation of Bioactive-rich Ginger Extract Using Response Surface Methodology

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Authors' contributions

This work was carried out in collaboration between all authors. Author HPVR was the principle investigator of this study. Author KDPPG performed the research and statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Evaluation of three extraction methods to prepare bioactive-rich ginger extract for incorporation into a functional beverage.
Study Design: Response surface methodology.
Methodology: For the preparation of bioactive-rich ginger extract with water, conventional hot water extraction, ultrasonic-assisted extraction and high pressure homogenization-assisted extraction were evaluated. Response surface methodology was employed to optimize the extraction conditions of each method with respect to the highest polyphenols, antioxidant capacity (ferric reducing antioxidant power; FRAP) and percent inhibition of low density lipoprotein (LDL) cholesterol oxidation.
Results: Multiple response optimizations revealed that the optimum extraction conditions for each extraction method were 60min extraction time under 55°C for hot water extraction, 15min ultrasonication under 52°C for ultrasonic-assisted extraction and 62°C under 140MPa homogenization pressure for high pressure homogenization-assisted

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extraction.
Conclusion: The extract prepared from the ultrasonic-assisted extraction method exhibited the highest polyphenol recovery and antioxidant activity, compared to the extracts prepared from other two methods.

Keywords: Ginger; Zingiber officinale; hot water extraction; high pressure homogenization; ultrasonication; polyphenols; LDL oxidation; response surface methodology.

1. INTRODUCTION

Ginger (*Zingiber officinale*) has been demonstrated to have various pharmacological benefits, such as anticancer, antioxidant, anti-platelet, anti diabetes and cardio-protective properties [1,2]. It has been demonstrated that the active principles of ginger are gingerols, a homologous series of polyphenols, and they are believed to be the most pharmacologically active component in ginger [3-5]. Research findings demonstrated that the ginger extracts possess antioxidant properties including the inhibition of human LDL oxidation (*in vitro*), which is considered as one of the pathophysiologically important step in the progression of development of atherosclerosis [6-8]. Recently, the demand for ginger has grown in North America, because of its health benefits, especially anti-hypertensive [9] and anti-atherogenic properties [1,10]. Therefore, there are possibilities of incorporating ginger as a value-added food ingredient for the formulation of functional foods containing multiple classes of bioactive molecules to obtain health benefits.

Incorporation of bioactive-rich ginger extracts as value-added ingredients in functional foods can be achieved by identifying the appropriate extraction methods. Consequently, novel extraction techniques, such as ultrasonic-assisted extraction [11], microwave-assisted extraction [12], high pressure homogenization-assisted extraction [13], have been introduced to enhance bioactive recovery in water-extraction and to replace solvent-based bioactive extractions. Ultrasonic-assisted extraction is a good alternative extraction method when compared to classical and conventional extraction techniques because of its high efficiency and low energy requirement [14]. Ultrasonication is not only a rapid, efficient and reliable alternative to enhance the quality of food, but also has potential use in developing innovative new products with unique functionality [15]. Homogenization-based extraction has been used to extract many different chemical substances from different food materials and proven to be a more efficient practice [16]. Use of hot water for the extraction of bioactives is a conventional technique and employed for the extraction of polyphenols from ginger by Kishk [17]. However, according to Rupasinghe [18], most of the reported ultrasonic-assisted bioactive extraction protocols are based on “substandard processes” without optimization for specific bioactive constituents of interest. Therefore, optimization and standardization of bioactive extraction procedures from various plant sources are necessary for the development of natural health products. The aim of this study was to evaluate three different water-based bioactive extraction methods for fresh ginger. These methods include ultrasonic-assisted extraction, high pressure homogenization-assisted extraction and hot water extraction. The optimum extraction conditions of each method, which yielded the greatest phenolic phytochemicals and antioxidant activity in terms of FRAP and percent LDL oxidation inhibition were estimated and compared using response surface methodology.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

LDL isolated from human plasma (in 150mMNaCl, 0.01% ethylene diamine tetra acetic acid (EDTA), pH7.4) was purchased from EMD chemicals Inc. (Gibbstown, NJ, USA). Other chemicals used were of analytical grade and obtained from Sigma-Aldrich, Oakville, ON, Canada.

2.2 Sample Preparation

Fresh rhizomes of Chinese ginger were purchased from a local market in Truro, Nova Scotia, Canada. The rhizomes were cut into thin slices of 1-2mm. Twenty five grams of sliced ginger was used to make a homogeneous mass with 100mL of deionized water at room temperature, using a food processor (Bead Beater; BioSpec Products, Inc. Bartlesville, OK, USA), at a maximum speed for 2min; then the homogenates were transferred into 200mL conical flasks. Homogenates were subjected to subsequent extraction based on the experimental design using three extraction methods: hot water extraction, ultrasonic-assisted extraction and high pressure homogenization-assisted extraction. The extraction conditions used in each extraction method were based on previously reported findings [14,17,19,20].

2.3 Hot Water Extraction

Homogenized ginger in 200mL conical flasks were placed in a shaking water bath (Shel Lab, Model: 23E GeneQinc, Montreal, QC, Canada) at different time-temperature combinations Table 1 for the extraction of phenolic bioactive constituents into the solution. The extracts were filtered with an 11cm diameter glass fibre filter (G6), under reduced pressure, to remove the solids and subsequently stored at -20°C for further analysis.

2.4 Ultrasonic-assisted Extraction

The process for polyphenols extraction from ginger was performed in an ultrasonic bath (Model 4HT-1524-12, Crest Ultrasonic Corp., Trenton, NJ, USA). The frequency and the power of the ultrasonic were 40 kHz and 150 W, respectively. Homogenized ginger in conical flasks was sonicated for different time periods at a specific temperature Table 2. The extracts were filtered with an 11cm diameter glass fibre filter (G6), under reduced pressure, to remove the solids, and they were stored at -20°C for further analysis.

2.5 High Pressure Homogenization-assisted Extraction

An EmulsiFlex-C3 high pressure homogenizer (Avestin Inc., Ottawa, ON, Canada) was used for the bioactive extraction from ginger. The filtered ginger extracts were pre-heated to the selected temperature in a water bath (Isotemp Model: 205, Fisher Scientific, Ubuque, IA, USA). Then, the preheated samples were loaded into a funnel and the flow rate was maintained at 3L/hr. Each sample was run at each treated pressure Table 3. for 3 cycles.

Table 1. Response values for given levels of variables in hot water extraction of ginger root in RSM

Experiment No.	Coded		Uncoded		Polyphenols (mg GAE/L)	FRAP value (mg TE/L)	% LDL oxidation inhibition
	Temp. (°C)	Time (min)	Temp. (°C)	Time (min)			
1	-1	-1	45	20	363.7	219.9	31.2
2	-1	+1	45	60	446.9	232.4	55.4
3	+1	-1	100	20	405.0	314.8	36.2
4	+1	+1	100	60	427.2	229.9	42.3
5	0	0	72.5	40	443.7	217.4	39.8
6	0	0	72.5	40	428.4	234.9	45.3
7	0	0	72.5	40	435.8	212.4	33.6
8	0	0	72.5	40	435.3	197.4	40.8
9	0	0	72.5	40	420.6	242.4	41.0
10	+1.41	0	100	40	418.5	259.9	41.5
11	-1.41	0	34	40	383.8	217.4	37.5
12	0	+1.41	72.5	68	454.5	292.4	34.7
13	0	-1.41	72.5	12	404.9	187.4	32.1

Table 2. Response values for given levels of variables in ultrasonic-assisted extraction of ginger root in RSM

Experiment No.	Coded		Uncoded		Polyphenols (mg GAE/L)	FRAP value (mg TE/L)	% LDL oxidation inhibition
	Temp. (°C)	Time (min)	Temp. (°C)	Time (min)			
1	-1	-1	30	20	432.7	217.9	38.5
2	-1	+1	30	60	449.2	275.5	49.6
3	+1	-1	60	20	386.8	246.9	35.9
4	+1	+1	60	60	454.5	231.9	42.1
5	0	0	45	40	475.3	210.2	43.7
6	0	0	45	40	454.6	230.2	38.5
7	0	0	45	40	488.9	211.2	51.7
8	0	0	45	40	476.8	230.7	50.7
9	0	0	45	40	458.3	243.2	42.2
10	+1.41	0	66	40	454.2	173.8	25.2
11	-1.41	0	24	40	390.7	203.2	50.7
12	0	+1.41	45	68	485.5	231.9	21.6
13	0	-1.41	45	12	393.3	200.2	33.7

Table 3. Response values for given levels of variables in high pressure homogenization-assisted extraction of ginger root in RSM

Experiment No.	Coded		Uncoded		Polyphenols (mgGAE/L)	FRAP value (mg TE/L)	% LDL oxidation inhibition
	Temp. (°C)	Press MPa	Temp. (°C)	Press MPa			
1	-1	-1	30	100	461.6	200.2	12.9
2	-1	+1	30	200	457.8	225.4	32.2
3	+1	-1	60	100	448.4	225.9	37.6
4	+1	+1	60	200	482.2	225.1	32.3
5	0	0	45	150	470.4	225.7	32.6
6	0	0	45	150	475.0	275.9	36.6
7	0	0	45	150	448.0	300.4	44.8
8	0	0	45	150	438.9	225.3	32.1
9	0	0	45	150	473.7	200.8	41.1
10	+1.41	0	66	150	482.3	275.1	39.4
11	-1.41	0	24	150	453.9	175.0	42.4
12	0	+1.41	45	220	478.7	325.1	17.1
13	0	-1.41	45	80	448.9	225.7	32.3

2.6 Determination of Total Phenolic Content

The total phenolic content was determined using Folin-Ciocalteu reagents assay [21] with some modification, as described by Rupasinghe [22]. Twenty µL of extract was added into the wells of the 96-well plate and then 100µL of the Folin–Ciocalteu phenol reagent was also added. After 5 min, 80 µL of 7.5% sodium carbonate was added to the mixture. After 2 hours in darkness, the absorbance at 750nm was measured using the FLU Ostar OPTIMA plate reader (BMG Labtech, Durham, NC, USA). The concentration of total phenols was expressed as mg gallic acid equivalent (GAE)/L of extract. The linear range used for the calibration was 10-250mg GAE/L.

2.7 FRAP (Ferric Reducing Antioxidant Power) Assay

Antioxidant capacity of ginger extracts were measured using FRAP assay, according to the Benzie and Strain [23] method with some modifications as described by Rupasinghe [22]. Briefly, the reaction was carried out in a 96-well microplate. The antioxidant capacities of the standards/extracts were estimated by the increase of absorbance caused by the generated ferrous ions. The working FRAP reagent contained 300mM acetate buffer (pH 3.6), 10mM 2, 4, 6-tripyridyl-s-triazine (TPTZ), 40mMHCl and 20mM $FeCl_3$. $6H_2O$ in the ratio of 10:1:1. Freshly prepared FRAP working reagent was incubated to 37°C. One hundred and eighty µL of this working solution was dispensed to each well of the microplate. Then, addition of 20µL diluted extracts initiated the reaction and absorbance was read after 10 min. FLU Ostar OPTIMA plate reader (BMG Labtech, Durham, NC, USA) was utilized to read the absorbance at 593nm. Trolox was the standard and all measurements were performed in triplicate.

2.8 Percent LDL Oxidation Inhibition

2.8.1 LDL preparation

LDL were dialyzed extensively against phosphate buffer saline (PBS) containing 138mMNaCl and 27mMKCl (pH7.4) to remove inherent antioxidants using cellulose dialysis tubing (Thermo Fisher Scientific Inc., Ottawa, ON, Canada) at 4°C for 24 hours. The buffer was changed every six hours. The dialyzed LDL was immediately stored at -80°C and used within two weeks. Protein content of the dialyzed-LDL was measured by the Lowry's method [24], using bovine serum albumen as the standard.

2.8.2 Measurement of percent LDL oxidation inhibition as TBARS

Briefly, 180µL LDL (50µg protein/mL) was incubated, with 20µL ginger extracts or 20µL blank, for 4 hours at 37°C in 50mM phosphate buffer saline (PBS) at pH 7.4, for a total volume of 200µL. The reaction was initiated with the addition of 10µM $CuSO_4$. The experimental units consisted of a blank, a positive control (induction with 10µM $CuSO_4$, but without the antioxidant treatment), a negative control (without induction or treatment) and water extracts of ginger. Oxidation was terminated by adding 50µL of 5mM solution of EDTA to have 1mM final concentration of EDTA in the 250µL solution mixture. The LDL oxidation was determined by spectrophotometrically measuring the amount of TBARS using the method described by Xu [25], with minor modifications. Briefly, after terminating the LDL oxidation, TBA reagent (0.67% thiobarbituric acid and 20% trichloroacetic acid (TCA) in 0.2 M NaOH) was added to the reaction mixture. Then the mixture was incubated at 95°C for 30 min to develop a pink chromogen. The samples were placed in the refrigerator for 10min to cool down to room temperature. Then the tubes were centrifuged at 1500g for 15 min and absorbance was measured at 532nm using the FLU Ostar OPTIMA plate reader. TBARS activity was determined as the percent inhibition of LDL oxidation with comparison of positive control.

$$\text{Percent inhibition (\%)} = \frac{\text{Absorbance (positive control)} - \text{Absorbance (sample)}}{\text{Absorbance (positive control)}} * 100$$

2.9 Experimental Design for the Optimization of Ginger Bioactive Extraction

A two factor and three levels (-1, 0 and +1) central composite design was used for each of the three extraction techniques to achieve maximal information about the process from a minimum number of possible experiments. For the hot water extraction and the ultrasonic-assisted extraction, the independent variables were extraction temperature and extraction time, for the high pressure homogenization-assisted method, the extraction temperature and extraction pressure, were the independent variables. The dependent variables were total polyphenols content (mg GAE/L), antioxidant capacity (mg TE/L) and percent inhibition of LDL oxidation and each variable was coded at three levels, -1, 0 and +1 Table 1, 2 and 3. This experiment was carried out separately for each extraction technique to identify the optimum extraction conditions. For data analysis, RSREG procedures of SAS software (SAS, 9.2, Cary, NC), as well as Minitab16 software, were used. Canonical analysis was performed to optimize the independent variables using SAS procedure. The assumptions of normality and constant variance were checked using Anderson-Darling test and confirmed. The fitness of the model was determined by evaluating the Fisher test value (F-value), and the coefficient of determination (R^2) was obtained from an analysis of variance (ANOVA).

The central composite design uses least square regression to fit the experimental data to a quadratic model and the regression coefficients for linear, quadratic and interactions terms are shown in Table 4. Adequacy of the model was determined by the ANOVA. Ridge analysis was performed to compute the ridge of the optimum response when the results showed a saddle point in the response surfaces [26]. The contour plots and overlaid contour plots were generated using the Minitab software.

Table 4. Estimated regression coefficients for predicted models and analysis of ANOVA for three extraction methods

Extraction methods	Regression coefficient	Polyphenol content (mg GAE/L)	FRAP value (mg TE/L)	% LDL oxidation inhibition
Hot water extraction	β_0	156.364	149.34	6.401
	β_1	4.688	0.278	0.144
	β_2	3.645	1.341	1.234
	β_{11}	-0.022	0.0150	0.001
	β_{12}	-0.007	-0.044	-0.005
	β_{22}	0.028	0.029	-0.008
	(p-values)			
	Lack-of-fit	0.389	0.0722	0.1118
Ultrasonication-assisted extraction	β_0	194.807	64.186	14.795
	β_1	8.382	5.244	0.391
	β_2	2.569	2.138	1.521
	β_{11}	-0.108	-0.036	-0.007
	β_{12}	0.043	-0.061	0.004
	β_{22}	-0.039	0.014	-0.017
	(p-values)			
	Lack-of-fit	0.109	0.0629	0.107
High pressure homogenization-assisted extraction (HPH)	β_0	123.279	416.321	-66.229
	β_1	6.160	-0.628	-0.406
	β_2	2.471	-2.067	1.521
	β_{11}	0.061	-0.021	0.030
	β_{12}	-0.067	0.019	-0.014
	β_{22}	0.002	0.003	-0.003
	(p-values)			
	Lack-of-fit	0.903	0.076	0.141

β_0; intercept, β_1; linear (temp.), β_2; linear (time; pressure for HPH), β_{11}& β_{22}; quadratic, β_{12}; interaction

2.10 Statistical Analysis

All data from the study were presented as mean±SD of three replications, and means were compared using analysis of variance (ANOVA). Acquired data were manipulated to calculate statistical values such as mean and standard deviation (SD) using Microsoft Excel (Microsoft Inc., Redmond, WA, USA). The assumptions of normality and constant variance were tested using Anderson-Darling test and examining residual versus fits.

3. RESULTS AND DISCUSSION

3.1 Hot Water Extraction

Hot water extraction is a conventional technique used to isolate bioactives from plant sources. The combined effects of temperature and time during hot water extraction of ginger root on total polyphenols, FRAP value and percent inhibition of LDL oxidation *in vitro*, were investigated. The results of the central composite design are presented in Table 1. According to the extraction conditions used in the experiment, the polyphenol yield varied between 364 to 455 mg GAE/L, the FRAP ranged from 187 to 314mg TE/L and % LDL oxidation inhibition ranged from 31% to 55%. The statistical software SAS and Minitab were used to fit contour plots for the response variables. Regression coefficients of predicted models for the responses of polyphenols and antioxidant activities are shown in Table 4. Contour plots of polyphenols (mg GAE/L) (a), FRAP (mg TE/L) (b) and % LDL oxidation inhibition (c) of hot water extraction of ginger is shown in Fig. 1.

Contour plots demonstrated the saddle points for all predicted responses for hot water extraction and the estimated surfaces did not have unique optima. Therefore, ridge analysis was performed to determine the estimated ridge of the maximum response (data not shown). The ridge analysis indicated that maximum polyphenol content can be extracted at above 60°C for more than 60min. However, according to the Ranilla [27], extractable polyphenol content decreases at longer thermal treatment period may be due to breakdown of polyphenols at higher temperature. The maximum FRAP value in the extract can be predicted to be obtained at 63°C temperature and 26 minutes. Similar antioxidant activities were reported by Kishk and Sheshetawy [17] and they have found that the optimum water-based extraction temperature and time with maximum radical scavenging activity for dried ginger powder were 56°C for 21min. Maximum LDL oxidation inhibition can be achieved at a relatively low temperature (about 44°C) over a longer time (55 min). Analysis of contour plots Fig. 1 of polyphenols, FRAP and percent LDL oxidation inhibition allows one to conclude that the high extraction temperature and shorter extraction time leads to an extract which is high in phenolic content together with high antioxidant activities, FRAP and LDL oxidation inhibition. Some researchers indicated that the thermal processing enhanced the antioxidant potential due to improving the antioxidant activities of naturally occurring compounds [17,28].

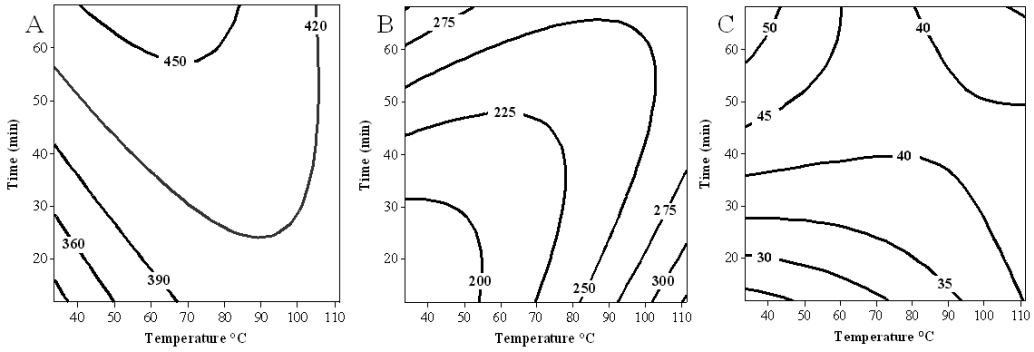

Fig. 1. Contour plots of total polyphenols (mg GAE/L) (A), FRAP value (mg TE/L) (B) and % LDL oxidation inhibition (C) of hot water extraction of ginger

3.2 Ultrasonic-assisted Extraction

Ultrasonication has been identified for potential industrial application in the phyto-pharmaceutical extraction industry for a wide range of herbal extracts [29]. Ultrasonication conditions were investigated to optimize the extraction conditions to prepare a water extract of ginger with high antioxidants activities. Minitab software was used to fit response contour plots for total polyphenols, FRAP and percent LDL oxidation inhibition in ultrasonic-assisted extraction of ginger. The response values obtained using response surface methodology and the central composite design, is given in Table 2. Ultrasonic-assisted extraction condition between 30-60°C and 20-60min time-temperature range yields 387-489 mg GAE/L polyphenols, 174-276mg TE/L antioxidant capacity and 22-52% LDL oxidation inhibition. Results indicated that the phenolic content extracted from ultrasonic-assisted extraction is higher than that of hot water extraction. Vinatoru [30] also published an overview of the ultrasonication-assisted extraction of bioactive principles from herbs and has been reported that the improvement in extractive value by ultrasonication compared with classic methods in water for fennel, marigold and mint was 34%, 2%, and 3%, respectively. Analysis of variance was performed on each response separately Table 4. There was no significant lack of fit ($p>0.05$), indicating that the design of the experiment was enough to determine the effect of independent variables on the responses.

Contour plots obtained for three response variables, with respect to the ultrasonic-assisted extraction, are shown in Fig. 2. Contour plots Fig. 2A and 2C illustrated that the predicted response for polyphenols and percent LDL oxidation inhibition are maximum. Based on the canonical analysis, maximum polyphenol content can be extracted from ginger by ultrasonication at 60°C for 51min. Higher polyphenol extraction from ginger can be achieved under ultrasonication conditions of relatively higher temperature for an extended time duration Fig. 2A. However, predicted response for FRAP value at ultrasonic-assisted extraction of ginger is a saddle point. The contour plot Fig. 2B of the predicted FRAP values confirmed this saddle point clearly. Therefore, ridge analysis was performed to determine the levels of the design variables that would produce maximum response for FRAP value in the extract. According to the ridge analysis (data not shown), maximum FRAP value can be obtained at 35°C for 65 min under ultrasonication conditions. The contour plot Fig. 2C clearly shows that the maximum LDL oxidation inhibition can be achieved by selecting relatively low extraction temperatures for longer time, nearly 44 min in ultrasonic-assisted extraction.

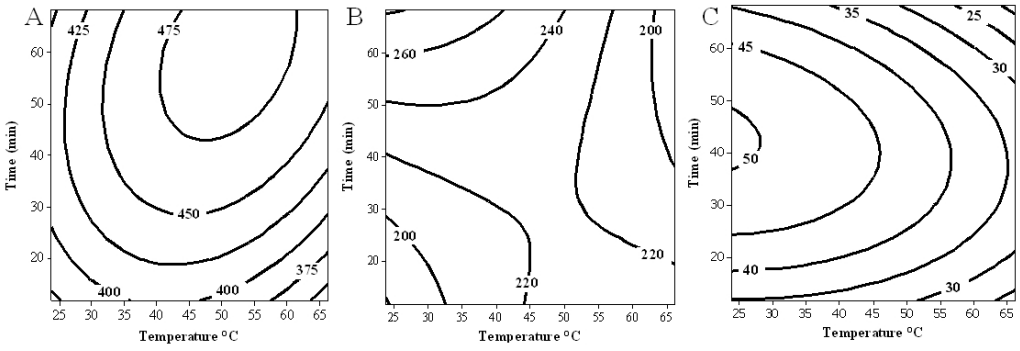

Fig. 2. Contour plots of polyphenols (mg GAE/L) (A), FRAP (mg TE/L) (B) and % LDL oxidation inhibition (C) of ultrasonic-assisted extraction of ginger

3.3 High Pressure Homogenization-assisted Extraction

Total polyphenols, FRAP and percent LDL oxidation inhibition obtained at different extraction pressures and temperatures during the high pressure homogenization assisted-extraction method are given in Table 3. Ginger extracts subjected to high pressure homogenization showed total phenolic content in the range of 412-510mg GAE/L, antioxidant capacity from 186 to 308mg TE/L and the LDL oxidation inhibition from 17 to 62%. Testing of model adequacy, ANOVA and regression analysis for the predicted models are shown in Table 4.

Contour plots obtained for three response variables with respect to the high pressure homogenization-assisted extraction, are shown in Fig. 3. Analysis of contour plots of polyphenols Fig. 3A. FRAP Fig. 3B and percent LDL oxidation inhibition Fig. 3C demonstrated saddle points for all predicted responses during high pressure homogenization-assisted extraction of ginger. Therefore, the estimated surfaces of phenolic content, FRAP and LDL oxidation inhibition did not have unique optimum. Therefore, ridge analysis was performed to determine the estimated ridge of the optimum response. According to the ridge analysis (data not shown), the maximum phenolic content can be extracted at more than 108°C and 62MPa homogenization pressure, whereas it can be predicted that the maximum FRAP value in the extract can be obtained at 40°C for 82MPa homogenization pressure. Maximum LDL oxidation inhibition can be achieved at 65°C temperature and 126MPa homogenization pressure.

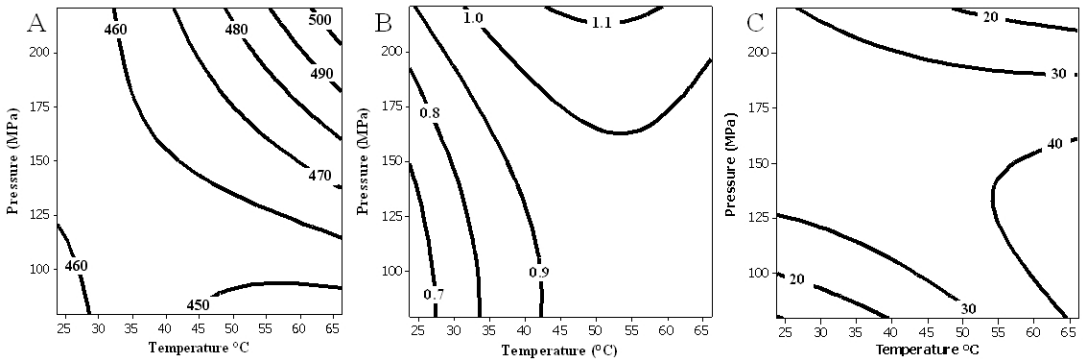

Fig. 3. Contour plots of % polyphenols (mg GAE/L) (A), FRAP (mg TE/L) (B), % LDL oxidation inhibition (C) of high pressure homogenization assisted extraction of ginger

3.4 Optimization of Multiple Responses

To optimize the multiple responses of each extraction method, overlaid contour plots of polyphenols, FRAP and percent LDL oxidation inhibition were generated using Minitab software Fig. 4. The summary of the optimum extraction conditions for each extraction method, obtained from establishing overlaid contour plots are shown in Table 5.

Desirable results in terms of high polyphenol content, together with high antioxidant activities, antioxidant capacity and LDL oxidation inhibition, are described for the ginger extract to be incorporated into a functional food. Overlaying these three responses provided the visual output required to select optimum extraction conditions. The optimum extraction conditions which yielded the greatest polyphenols, FRAP and LDL oxidation inhibitions of hot water extraction, ultrasonic-assisted extraction and high pressure homogenization-assisted

extraction were 55°C for 60min Fig. 4A. 52°C for 15min Fig. 4B and 140MPa homogenization pressure at 55°C Fig. 4C respectively. Based on the results, the highest polyphenol content and antioxidant activity can be achieved with relatively at lower extraction conditions using ultrasonication compared with the other two extraction methods. Ultrasonication-assisted extraction is highly effective because it produces higher yield of polyphenol constituents and antioxidant activity while taking less time [18,31]. The ultrasonication extraction exerts two types of physical phenomena: diffusion of the extractant through the cell walls and washing out the cell content through ruptured cell walls [23]. Homogenization pressure and temperature had significant effect on stability of nanoemulsion of bioactives in the extract [19] and it improved the extractibility of bioactives as well [13]. However, the predicted polyphenol content and antioxidant activities associated with the high pressure homogenization-assisted extraction is lower than that of the ultrasonic-assisted extraction, but higher than the hot water extraction.

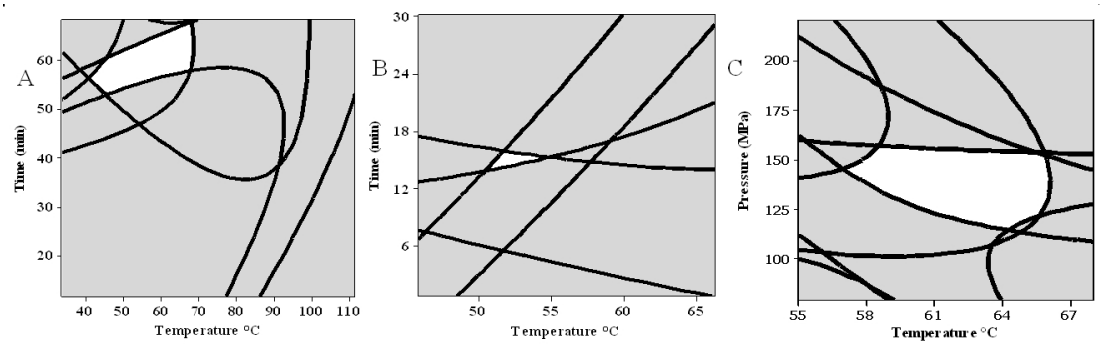

Fig. 4. Overlaid contour plots of polyphenols, FRAP and LDL oxidation inhibition of hot water extraction (A), ultrasonic-assisted extraction (B) and high pressure homogenization-assisted extraction (C) of ginger

Table 5. Optimum extraction conditions drawn from overlaid plots of different extraction methods

Extraction method	Optimum extraction conditions
Hot water extraction	Temperature:55°C Time:60min
Ultrasonic-assisted extraction	Temperature:52°C Time:15min
High pressure homogenization-assisted extraction	Temperature:62°C Pressure:140MPa

4. CONCLUSION

The response surface methodology was used to determine the optimum extraction conditions for three extraction methods which give the highest polyphenol content and antioxidant activities. Based on the results, ultrasonication-assisted extractions yielded the highest predicted polyphenols and antioxidant activities at lower extractiontemperatures, 52°C for 15min, compared with other two extraction methods tested.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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