Food Bioprocess Technol (2010) 3:300–307
DOI 10.1007/s11947-009-0212-0

COMMUNICATION

Screening the Experimental Domain for the Microwave-Assisted Extraction of Secoisolariciresinol Diglucoside from Flaxseed Prior to Optimization Procedures

Simona M. Nemes · Valérie Orsat

Received: 20 January 2009 / Accepted: 4 May 2009 / Published online: 15 May 2009

Abstract This paper presents the first part of a study that aims at developing an optimized microwave-assisted method for extracting the lignan secoisolariciresinol diglucoside from defatted flaxseed meal. Two-level fractional factorial designs were used for screening the following factors: the microwave power level (30–360 W), the time of residence in the microwave cavity (1–25 min), the concentration of NaOH (0.25–1 M), and the mode of microwave power application (power on 30 and 60 s/min). The experimental domain had to be adjusted after each screening in order to focus the future optimization study within the factors' ranges likely to contain the optimal extraction conditions.

Keywords MAE · SDG · Flaxseed · Lignan · Nutraceutical · Phytoestrogen · Screening design · Fractional factorial design

Introduction

Recently, there has been increasing demand for new extraction techniques that are environmentally friendlier, faster, and more efficient than traditional extraction methods. Microwave-assisted extraction (MAE) has emerged as an efficient method for the extraction of nutraceuticals (Kaufmann and Christen 2002; Wang and Weller 2006). Many studies proved that, by applying microwave energy, it was possible to reduce the extraction time of natural compounds from plant matrices tremendously. Furthermore, it helped achieve extraction yields higher or at least comparable with those achieved by traditional methods (Alfaro et al. 2003; Brachet et al. 2002; Dai et al. 2001; Ganzler et al. 1990; Williams et al. 2004). Two recent studies reported MAE methods for extracting secoisolariciresinol diglucoside (SDG) from flaxseed hull (Zhang and Xu 2007) and pressed flaxseed cake (Beejmohun et al. 2007). Both studies found that MAE of SDG was more efficient than conventional methods such as stirring extraction and Soxhlet extraction.

The physical principle behind microwave-assisted processes is based on the dielectric properties of materials and their interaction with the microwave energy (Boyacı et al. 2008; Liao et al. 2001; Vadivambal and Jayas 2008). When microwaves impinge on a material, the polar molecules and the ionic species in the material polarize. This phenomenon results in fast volumetric heating through dipolar polarization and ionic conduction (Meda et al. 2005) and leads to enhanced reaction rates in microwave-assisted chemistry (Kappe 2004). Dedicated laboratory microwave extraction systems are commercially available. They operate at 2,450 MHz frequency and use monomode (also called focused) and multimode cavities. Monomode systems have a well-defined spatial distribution of the microwave field strength inside the cavity and are recommended for precise research purposes (Orsat et al. 2005).

SDG is the main lignan found in flaxseed; it naturally occurs as part of complex structures in which it is covalently bound via ester linkages to 3-hydroxy-3-methyl glutaric acid. SDG can be released from these complex structures by hydrolytic cleavage of the ester linkages (Davin and Lewis 2003, 2005; Ford et al. 2001). It has been shown that SDG offers protection against breast,

S. M. Nemes (✉) · V. Orsat
Bioresource Engineering Department,
Macdonald Campus of McGill University,
Macdonald–Stewart Building, 21111 Lakeshore Road,
Sainte-Anne-de-Bellevue, H9X 3V9 QC, Canada
e-mail: Simona.nemes@mail.mcgill.ca

colon, prostate, and thyroid cancers (Westcott and Muir 2003); it has strong antioxidant properties (Kitts et al. 1999; Prasad 2000a), and therefore, it has the potential to reduce the risk of diabetes (Prasad 2000b, 2002) and the levels of low-density lipoprotein cholesterol in the blood (Prasad 1999).

There is no standardized methodology for extracting SDG or other lignans from plant or food matrices. Most of the published methods are time-consuming (duration can vary from several hours up to 2 or 3 days) and require multiple manipulations of the extracts (Nemes 2007). The need for standardized methodologies for phytoestrogens analysis was recently acknowledged (Oomah 2002). This paper presents the first part of a study aiming at developing an optimized MAE analytical method for extracting SDG from defatted flaxseed meal (DFM). The objective of this paper is to find the experimental domain to be used in future optimization response surface experimentation. Screening studies are used for achieving this objective.

Materials and Methods

Flaxseed

Brown flaxseed, coarsely ground and vacuum-packaged in 425 g bags (Puresource PP, Guelph, ON, Canada), was used for all experiments. A sample of 100 g flaxseed was defatted twice for 1 h with hexane in the proportion of 1:6 (*w*/*v*, in grams per milliliter) under magnetic stirring, filtered, and kept in the fume hood overnight to allow the residual hexane to evaporate. Since hexane is not miscible with the mobile phase used in high-performance liquid chromatography (HPLC), this should not be found in the extracts subjected to chromatographic analysis. The DFM was milled with a coffee grinder for 5 min to reduce the particle size to 0.25–1 mm. The moisture content (4.88% wet basis [wb], SD=0.08) was determined in triplicate by drying 0.5 g DFM at 105°C for 17 h (Eliasson et al. 2003). For reference purposes, the SDG content of the DFM was determined in five replicates by hydrolyzing samples of 1 g DFM with 50 mL of 1 M NaOH for 1 h at room temperature under magnetic stirring (Eliasson et al. 2003), and it was found to be 20.22 mg SDG/g DFM (SD= 1.08).

Standard, Solvents, and Reagents

Pure SDG standard was purchased from ChromaDex (Santa Ana, CA, USA). Acetonitrile and methanol of HPLC grade were obtained from Fisher Scientific (Ottawa, ON, Canada). Sodium hydroxide, sulfuric acid, phosphoric acid, and dipotassium phosphate were obtained from Sigma-Aldrich (Oakville, ON, Canada).

MAE System

All MAE experiments were carried out with a monomode (focused) open-vessel microwave system (Star System 2, CEM, Matthews, USA) operating at 600 W maximum power and 2,450 MHz frequency. The extractions were carried out in batch mode. Samples and solvents were placed in a Pyrex vessel with a volume of 250 mL that was then placed inside the microwave cavity and fitted with a Graham type condenser and a fiber optic temperature sensor (FISO Technologies, QC, Canada).

Sample Preparation for Chromatography

All samples were prepared for chromatography as follows. Methanol was added to hydrolyzed samples (2:1, *v*/*v*) to precipitate the proteins and carbohydrates under magnetic stirring for 10 min, followed by 5 min without stirring to allow the solid phase to settle down. The liquid phase was acidified to pH 3 by titration with 2 M H_2SO_4; the pH was measured with an Accumet 25 pH-meter (Fisher Scientific). The acidified hydrolyzate was centrifuged for 5 min at 3,000 rpm using a Spinette centrifuge (Needham Heights, USA), then filtered through 0.22 µm 12 mL Cameo syringe filters (Sigma-Aldrich) into 2 mL capped vials and analyzed by HPLC.

HPLC Analysis of SDG

The HPLC analysis was performed in five replicates per sample with an Agilent 1100 series instrument equipped with a variable wavelength detector. The Chemstation software was used for chromatographic data analysis. The separation was carried out at 25°C on a reversed-phase Discovery column (Supelco) RP C18 (5 µm, 25 cm× 4.6 mm), fitted with a Supelguard cartridge (Discovery), C18 (5 µm, 2 cm×4 mm). The injection volume was 10 µL, and the flow rate was 1 mL/min. The separation was carried out using the gradient reported by Johnsson et al. (2000) and Eliasson et al. (2003). The mobile phase consisted of 5% acetonitrile in 0.01 M phosphate buffer, pH 2.8 (solvent A) and acetonitrile (solvent B). The gradient was as follows: A–B: 0 min (100:0, *v*/*v*), 30 min (70:30, *v*/*v*), and 32 min (30:70, *v*/*v*). The calibration curve was obtained by using concentrations of 20, 30, 40, 50, 70, 100, and 200 µg/mL of SDG standard. The detector was set at 280 nm. The concentration of SDG in the extracts was calculated using peak areas and the calibration curve (R^2=0.99996, Area= 3.8289×Amount−2.6156). A typical chromatogram of flaxseed extract is presented in Fig. 1; SDG eluted at

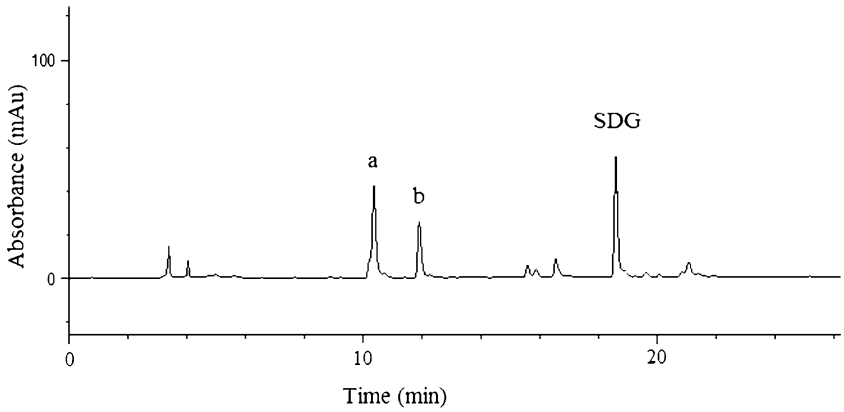

Fig. 1 Typical HPLC chromatogram of flaxseed extract (280 nm): SDG eluted at approximately 18.6 min, peaks *a* (approximately 10.3 min) and *b* (approximately 11.8 min) were tentatively identified as *p*-coumaric acid glucoside and ferulic acid glucoside, respectively, based on the resemblance with chromatograms presented by Johnsson et al. (2000) and Eliasson et al. (2003)

approximately 18.6 min, the peaks a (approximately 10.8 min) and b (approximately 12.3 min) were tentatively identified as *p*-coumaric acid glucoside and ferulic acid glucoside, respectively, based on the resemblance with HPLC chromatograms presented by Johnsson et al. (2000) and Eliasson et al. (2003).

Experimental Design

Two-level factorial screening designs are largely used in industrial research at the beginning of response surface studies (Myers and Montgomery 2002a, b). Two screening studies were carried out for assessing the direction and the magnitude of factors' effects on the yield of MAE of SDG and for identifying the experimental domain for future response surface optimization studies. For this purpose, two-level, four-factor, half fraction factorial designs of resolution 4 were used. The four factors investigated were: the microwave power level (in watts) labeled "Power," the time of residence in the microwave cavity (in minutes) labeled "Time," the molar concentration of NaOH (in molars) labeled "Molarity," and the mode of microwave power application, that is, intermittent and continuous, expressed as power on seconds per minute, labeled "PMode." The purpose of using screening designs is to be economical, that is, to generate valuable information while reducing the consumption of chemicals and saving time. Therefore, saturated (unreplicated) screening designs were used. Their statistical analysis was done according to Myers and Montgomery (2002a, b). The screening designs were generated and analyzed with the ADX interface of SAS (version 9.1 TS1M3 Institute, Cary, NC, USA). The significance level for the statistical tests was α=0.05. The factors tested were considered significant when the p value (labeled Pr>F) was smaller than 0.05, that is, the null hypothesis that all regression coefficients were equal to zero was rejected.

Results and Discussion

Determination of a Practical Liquid to Solid Ratio

Flaxseed contains about 8% (wb) of polysaccharidic mucilage that forms a gel in aqueous solutions. The viscosity of the gel is affected by pH; it is high for pH values ranging from 6 to 9 and decreases for pH values smaller than 6 (Chen et al. 2006; Mazza and Biliaderis 1989). In order to identify the liquid to solid ratio that would facilitate the preparation of samples for chromatography, samples of 1 g DFM were hydrolyzed with 10, 20, 30, 40, and 50 mL of 1 M NaOH at 120 W for 20 min. It was observed that the viscosity of extraction mixtures, due to the release of mucilage in the solution, was too high when less than 50 mL NaOH were used for hydrolysis. For the samples hydrolyzed with 10, 20, 30, and 40 mL NaOH, the addition of methanol to the hydrolyzates in the proportion of 2:1 (v/v) and their acidification to pH 3 did not cause the viscosity to decrease sufficiently. This led to the clogging of the 0.22-μm syringe filters, thus preventing the injection of the samples into the HPLC. Therefore, the liquid to solid ratio of 50:1 (in milliliters per gram, v/w) was used for all subsequent experiments.

The Identification of Experimental Domain is Required

A recent literature review on the MAE of natural compounds from plant matrices revealed a great diversity of methodologies. Besides the fact that microwaves enhanced the extraction yields and reduced the extraction times, there was no pattern or rule regarding the microwave power level or the time of irradiation (Nemes 2007). Consequently, methodology development for MAE of SDG requires the identification of the experimental domain (the low and high levels of the investigated factors) within

Table 1 The first screening design

Run	Power (W)		Time (min)		Molarity (M NaOH)		PMode (power on s/min)		SDG[a] (mg/g DFM)
	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded	
1	−1	30	−1	1	−1	0.25	−1	30	17.8475
2	1	120	−1	1	−1	0.25	1	60	20.0155
3	−1	30	1	5	−1	0.25	1	60	19.7564
4	1	120	1	5	−1	0.25	−1	30	20.7795
5	−1	30	−1	1	1	1	1	60	18.8787
6	1	120	−1	1	1	1	−1	30	20.4696
7	−1	30	1	5	1	1	−1	30	19.3621
8	1	120	1	5	1	1	1	60	19.1720

[a] The SDG extraction yield was calculated on a wb; the DFM had a moisture content of 4.88% (wb)

which the optimum combination of factors that would maximize the extraction yield of SDG is expected to occur (Nemes 2007).

Initial Screening Design

The first screening design investigated the four factors described in the experimental design section as follows: Power=30 and 120 W, Time=1 and 5 min, Molarity=0.25 and 1 M NaOH, and PMode=power on 30 and 60 s/min. The design comprises eight experiments or runs resulting from the combinations of the four factors at the low (−1) and high (1) levels as presented in Table 1. The eight MAE runs were carried out in random order with 1 g DFM and 50 mL NaOH. The results were recorded as milligrams of SDG per gram of DFM (wb). The analysis of variance (ANOVA) of the first screening design is presented in Table 2. Overall, the model was not significant at α=0.05. The variability in the design was explained in the proportion of 93.48% (R^2) by the model. The eight response observations had a mean value of 19.53 mg SDG/g DFM and a standard deviation of 0.94. The predictive model for the first screening design using coded (−1, 1) levels is presented in Eq. 1:

$$\begin{aligned}\mathrm{SDG} = {} & 19.535 + 0.574 \times \mathrm{Power} + 0.232 \times \mathrm{Time} \\ & - 0.064 \times \mathrm{Molarity} - 0.079 \times \mathrm{PMode} \\ & - 0.366 \times \mathrm{Power} \times \mathrm{Time} - 0.436 \times \mathrm{Power} \\ & \times \mathrm{PMode}. \end{aligned} \quad (1)$$

The interpretation of the predictive model shows that the value of the response (SDG) can be slightly increased from the intercept, 19.535 (the intercept is the mean of all response observations), by increasing the levels of the factors Power and Time from −1 to 1. Although this increase is not significant, it points in the direction of the factors' effects. Moreover, it is clear that the factors Molarity and PMode have almost no influence on the response as the absolute values of their regression coefficients are very close to zero. Similarly, the plot showing the main effects of the four factors (Fig. 2) indicates that, while increasing the levels of the factors Power and Time, it

Table 2 The ANOVA for the first screening design

Source of variation	*df*	SS	MS	F	Pr>*F*
Power	1	2.6356	2.6356	6.5791	0.2366
Time	1	0.4318	0.4318	1.0779	0.4881
Molarity	1	0.0333	0.0333	0.0832	0.8212
PMode	1	0.0506	0.0506	0.1263	0.7826
Power × Time + Molarity × PMode	1	1.0702	1.0702	2.6714	0.3495
Power × Molarity + Time × PMode[a]	–				
Power × PMode + Time × Molarity	1	1.5199	1.5199	3.7940	0.3019
Model	6	5.7414	0.9569	2.3887	0.4584
Error	1	0.4006	0.406		
Total	7	6.1420			

The two-factor interactions are aliased with each other

[a] Interaction discarded from the model in order to gain 1 *df*

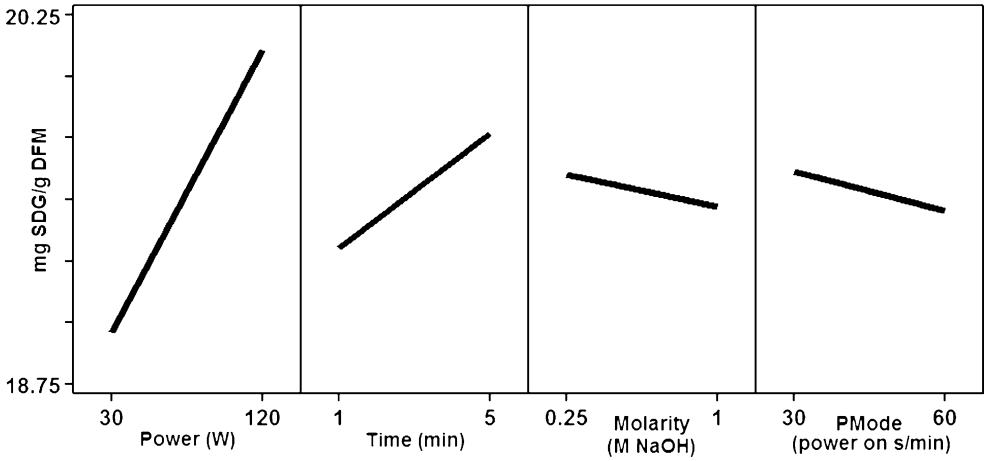

Fig. 2 First screening design—plot of the effects of the factors Power, Time, Molarity, and PMode on the extraction yield of SDG. A greater slope indicates a greater effect of a given factor on the extraction yield of SDG

enhances the extraction yield of SDG slightly; the effects of changing the levels of the factors PMode and Molarity are almost nonexistent.

The conventional direct hydrolysis study reported by Eliasson et al. (2003) and the first screening study presented above led to similar conclusions regarding the effect of NaOH concentration on the SDG extraction yield. The direct hydrolysis study used conventional heating (20, 30, and 40°C) and NaOH concentrations of 0.3, 1, and 1.7 M. The authors observed that the temperature and concentration of NaOH had very little influence on the extraction yield of lignans and recommended the practical concentration of 1 M and room temperature for the direct hydrolysis of lignans. In the case of MAE study, the temperatures increased from room temperature (22–23°C) to 35–40°C; and the concentrations of 0.25 and 1 M NaOH lead to similar results. Although the first MAE screening study did not investigate the effect of temperature, the results confirmed the finding of Eliasson et al. (2003) that the concentration of NaOH had no significant effect on the extraction yield of SDG. The direct hydrolysis method led to very good extraction yields in only 30 min; the highest extraction yields were obtained after 1 h of hydrolysis (Eliasson et al. 2003). In the case of the first MAE screening study, the best results were obtained with run 4 (Table 1). The highest extraction yield of 20.78 mg SDG/g DFM was obtained in only 5 min, when 1 g DFM was hydrolyzed with 50 mL of 0.25 M NaOH at 120 W of microwave power applied intermittently (power on 30 s/min).

The main conclusion of the first MAE screening study is that SDG can be efficiently extracted in a microwave environment, given that the result obtained with run 4 was similar with that obtained with conventional direct hydrolysis (20.78 vs. 20.22 mg SDG/g DFM). It might be tempting to stop the experimentations here, but the question arises whether the MAE of SDG can be further enhanced. Since the statistical model of the first screening design cannot predict how the response (SDG) will be affected outside the studied range of factors, a new screening design is needed for investigating power levels higher than 120 W and times of residence in the microwave cavity longer than 5 min. Regarding the concentration of NaOH, it was surprising that this did not have a significant effect on the SDG extraction yield. This lack of significance could be due to the low range of microwave power levels.

Table 3 The second screening design

Run	Time (min)		Power (W)		PMode (power on s/min)		Molarity (M NaOH)		SDG[a] (mg/g DFM)
	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded	
1	-1	5	-1	120	-1	30	-1	0.5	20.5401
2	1	25	-1	120	-1	30	1	1	20.2949
3	-1	5	1	360	-1	30	1	1	20.5273
4	1	25	1	360	-1	30	-1	0.5	20.7396
5	-1	5	-1	120	1	60	1	1	19.8210
6	1	25	-1	120	1	60	-1	0.5	19.4941
7	-1	5	1	360	1	60	-1	0.5	21.3369
8	1	25	1	360	1	60	1	1	20.2199

[a] The SDG extraction yield was calculated on a wb; the DFM had a moisture content of 4.88% (wb)

Table 4 The ANOVA for the second screening design

Source of variation	*df*	SS	MS	F	Pr>*F*
Time	1	0.2726	0.2726	19.7095	0.1410
Power	1	0.8934	0.8935	64.6056	0.0788
PMode	1	0.1891	0.1891	13.6714	0.1681
Molarity	1	0.1946	0.1946	14.0691	0.1659
Time × Power + PMode × Molarity[a]	–				
Time × PMode + Power × Molarity	1	0.2489	0.2489	17.9983	0.1474
Time × Molarity + Power × PMode	1	0.4095	0.4095	29.6089	0.1157
Model	6	2.2081	0.3680	26.6105	0.1473
Error	1	0.0138	0.0138		
Total	7	2.2219			

The two-factor interactions are aliased with each other

[a] Interaction discarded from the model in order to gain 1 *df*

The Second Screening Design

A second screening design was generated to investigate the four factors as follows: Power=120 and 360 W, Time=5 and 25 min, Molarity=0.5 and 1 M NaOH, and PMode = power on 30 and 60 s/min. The coded and uncoded factors' levels and the results of the second screening experiment are presented in Table 3. Overall, the predictive model of the second screening design was not significant at α=0.05 (Table 4). The variability in the design was explained in a proportion of 99.4% by the predictive model. The predictive model using the coded factor levels (−1, 1) is presented in Eq. 2:

$$\begin{aligned} SDG = {} & 20.372 - 0.184 \times Time + 0.334 \times Power \\ & - 0.154 \times PMode - 0.156 \times Molarity \\ & - 0.176 \times Time \times PMode + 0.226 \times Time \\ & \times Molarity. \end{aligned} \quad (2)$$

The mean value for the eight runs was 20.37 mg SDG/g DFM with a standard deviation of 0.56. This value is higher than that obtained with the previous design (20.37 vs. 19.54 mg SDG/g DFM) and is comparable with the reference value obtained with the direct hydrolysis method (20.37 vs. 20.22 mg SDG/g DFM). The plot of the main effects (Fig. 3) suggests that the extraction yield could be maximized around the following combination of factors: Power=360 W, Time=5 min, Molarity=0.5 M NaOH, and PMode=power on 30 s/min.

The analysis of the predictive models for the first and the second designs, presented in Eqs. 1 and 2, respectively, reveals two similarities. In both cases, the factors with the regression coefficients that had the highest absolute values were: Power, followed by the aliasing structure Power × PMode + Time × Molarity. At this point, it is not possible to distinguish the interaction with the highest effect. It is assumed that a higher power level applied intermittently might have an enhancing effect on the yield of MAE of SDG.

By looking at the predictive models and the plots of the main effects of both designs, a general direction for the factors' effects can be established. Good results can be achieved by keeping the factor Power at the high levels (1) and the factors PMode and Molarity at the low levels (−1). Interestingly, the results can be improved by increasing the level of factor Time from 1 to 5 min for the first design, but

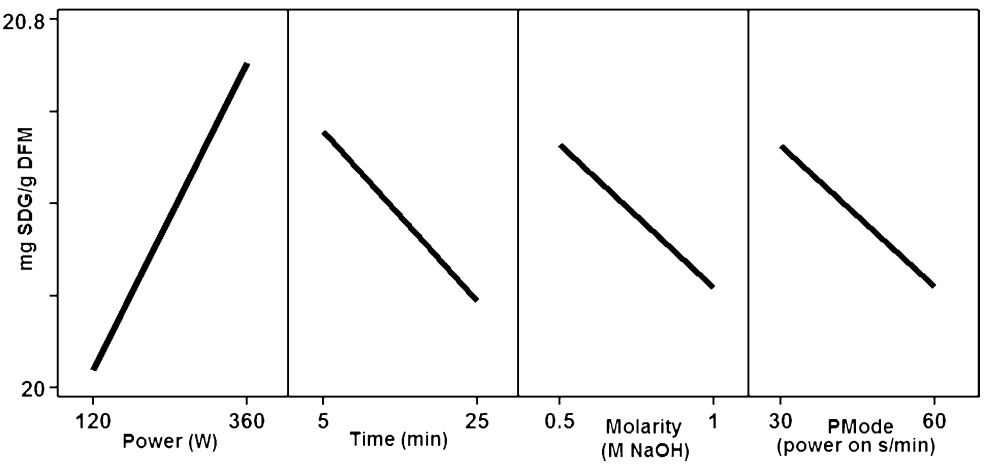

Fig. 3 Second screening design—plot of the effects of the factors Power, Time, Molarity, and PMode on the extraction yield of SDG. A greater slope indicates a greater effect of a given factor on the extraction yield of SDG

the response factor (SDG) is negatively affected by increasing the level of Time from 5 to 25 min for the second design. Long extraction time in combination with high concentration of NaOH and high levels for the other two factors caused the extraction mixtures to boil, foam, expand outside the zone of exposure to microwave, and move up into the coil of the condenser. The decrease in SDG extraction yield caused by these conditions could be explained by the degradation of SDG and/or incomplete recovery of the compound from the condenser.

The main conclusion of the second MAE screening study is that, overall, it was more efficient than the first study, as it led to a higher mean value for SDG (20.37> 19.35 mg SDG/g DFM), which was very similar with that obtained with the conventional direct hydrolysis method (20.37 vs. 20.22 mg SDG/g DFM).

The time scale for the hydrolysis of flaxseed meal was reduced tremendously under microwave conditions as opposed to conventional hydrolysis. This accelerating effect is caused by the way the microwaves interact with the NaOH–DFM mixtures. On one hand, the DFM matrix is made up of a variety of complex molecules that interact differently with the microwave energy. It is possible that the only component of DFM that interacts strongly with microwaves is water, which is found in a small quantity (4.88% wb). On the other hand, the solutions of 0.25–1 M NaOH are completely ionic (Na^{+}+OH^{-}) and they strongly interact with microwaves (Nemes 2007). Therefore, it could be assumed that the enhancing effect is due mostly to the microwave–NaOH interaction (coupling). The ions in the NaOH solution oscillate and collide with the DFM particles dispersed in the reaction media thus enhancing the hydrolysis kinetics, which results in efficient extraction yields of SDG at reduced time scales (around 5 min). When the microwaves impinge on the NaOH–DFM mixtures, some or all the microwave energy is dissipated in the reaction media as heat. If the microwave power is too low (e.g., 30 W for the first screening design; Table 1), the extraction yields are low too. There is too little energy to dissipate as heat. Although the temperature does not play an important role in conventional hydrolysis of flaxseed meal (Eliasson et al. 2003), it is important to have enough microwave energy dissipated as heat, as the effects of ionic conduction and dipolar polarization increase with the temperature. If the power level is too high, energy is wasted, and it may lead to variation as the hydrolysis mixture foams and expands outside the irradiation zone.

The optimum amount of microwave power and time of exposure have to be determined experimentally for the given ratio of NaOH to DFM of 50:1 (in milliliters per gram, *v*/*w*). However, before carrying out a response surface optimization study, the experimental domain has to be adjusted. Given that the mean extraction yield of the second screening design was higher than that of the first screening design and the factors Power and Time might affect the MAE of SDG, the experimental domain of the second screening design was modified as follows. The Power range was enlarged by decreasing the low level value from 120 to 60 W, as the first screening indicated that the SDG yield could be increased somewhere around 120 W and 5 min of MAE. However, the Time range had to be corrected as 25 min was clearly causing SDG yields to decrease. Therefore, the Time range was narrowed in order to assess how MAE performs around 5 min by decreasing the values of the low level from 5 to 3 min and decreasing the high level from 25 to 9 min. Further response surface investigations will be carried out for the following experimental domain: Power=60 and 360 W, Time=3 and 9 min, PMode=power on 30 and 60 s/min, and Molarity=0.5 and 1 M NaOH.

Conclusions

Microwave conditions speed up the hydrolysis of DFM tremendously and effectively. Two screening designs revealed that several MAE conditions could be used for rapid and efficient quantification of SDG in flaxseed. However, the lack of statistical significance of the two screening models indicates that possibly influential quadratic effects might occur within the studied screening domains. Therefore, response surface experimentation is required in order to assess the quadratic effects and to optimize the MAE of SDG. The optimization study constitutes the second part of the MAE method development, and it will be presented in a future publication.

Acknowledgements The authors thank the Natural Sciences and Engineering Research Council of Canada (NSERC), the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT), and the Canada Foundation for Innovation (CFI) for the financial support.

References

Alfaro, M. J., Bélanger, J. M. R., Padilla, F. C., & Paré, J. R. (2003). Influence of solvent, matrix dielectric properties, and applied power on the liquid-phase microwave-assisted processes (MAP(TM)) extraction of ginger (*Zingiber officinale*). *Food Research International, 36*, 499–504. doi:10.1016/S0963-9969(02)00198-9.

Beejmohun, V., Fliniaux, O., Grand, É., Lamblin, F., Bensaddek, L., Christen, P., et al. (2007). Microwave-assisted extraction of the main phenolic compounds in flaxseed. *Phytochemical Analysis, 18*, 275–282. doi:10.1002/pca.973.

Boyacı, İ., Sumnu, G., & Sakiyan, O. (2008). Estimation of dielectric properties of cakes based on porosity, moisture content, and formulations using statistical methods and artificial neural networks. *Food and Bioprocess Technology*, in press. doi:10.1007/s11947-008-0064-z.

Brachet, A. P., Christen, P., & Veuthey, J. L. (2002). Focused microwave-assisted extraction of cocaine and benzoylecgonine from coca leaves. *Phytochemical Analysis, 13*, 162–169. doi:10.1002/pca.637.

Chen, H. H., Xu, S. Y., & Wang, Z. (2006). Gelation properties of flaxseed gum. *Journal of Food Engineering, 77*, 295–303. doi:10.1016/j.jfoodeng.2005.06.033.

Dai, J., Yaylayan, V. A., & Raghavan, G. S. V. (2001). Influence of operating parameters on the use of the microwave-assisted process (MAP) for the extraction of azadirachtin-related limonoids from neem (*Azadirachta indica*) under atmospheric pressure conditions. *Journal of Agricultural and Food Chemistry, 49*, 4584–4588. doi:10.1021/jf010592k.

Davin, L. B., & Lewis, N. G. (2003). An historical perspective on lignan biosynthesis: monolignol, allylphenol, and hydroxycinnamic acid coupling and downstream metabolism. *Phytochemistry Reviews, 2*, 257–288. doi:10.1023/B:PHYT.0000046175.83729.b5.

Davin, L. B., & Lewis, N. G. (2005). Dirigent phenoxy radical coupling: advances and challenges. *Current Opinion in Biotechnology, 16*, 398–406. doi:10.1016/j.copbio.2005.06.010.

Eliasson, C., Kamal-Eldin, A., Andersson, R., & Aman, P. (2003). High-performance liquid chromatographic analysis of secoisolariciresinol diglucoside and hydroxycinnamic acid glucosides in flaxseed by alkaline extraction. *Journal of Chromatography A, 1012*, 151–159. doi:10.1016/S0021-9673(03)01136-1.

Ford, J. D., Huang, K. S., Wang, H. B., Davin, L. B., & Lewis, N. G. (2001). Biosynthetic pathway to the cancer chemopreventive secoisolariciresinol diglucoside-hydroxymethyl glutaryl ester-linked lignan oligomers in flax (*Linum usitatissimum*) seed. *Journal of Natural Products, 64*, 1388–1397. doi:10.1021/np010367x.

Ganzler, K., Szinai, I., & Salgo, A. (1990). Effective sample preparation method for extracting biologically active compounds from different matrices by a microwave technique. *Journal of Chromatography A, 520*, 257–262. doi:10.1016/0021-9673(90)85109-9.

Johnsson, P., Kamal-Eldin, A., Lundgren, L. N., & Aman, P. (2000). HPLC method for analysis of secoisolariciresinol diglucoside in flaxseeds. *Journal of Agricultural and Food Chemistry, 48*, 5216–5219. doi:10.1021/jf0005871.

Kappe, C. O. (2004). Controlled microwave heating in modern organic synthesis. *Angewandte Chemie International Edition, 43*, 6250–6284. doi:10.1002/anie.200400655.

Kaufmann, B., & Christen, P. (2002). Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. *Phytochemical Analysis, 13*, 105–113. doi:10.1002/pca.631.

Kitts, D. D., Yuan, Y. V., Wijewickreme, A. N., & Thompson, L. U. (1999). Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. *Molecular and Cellular Biochemistry, 202*, 91–100. doi:10.1023/A:1007022329660.

Liao, X., Raghavan, G. S. V., Meda, V., & Yaylayan, V. A. (2001). Dielectric properties of supersaturated alpha-D-glucose aqueous solutions at 2450 MHz. *Journal of Microwave Power & Electromagnetic Energy, 36*, 131–138.

Mazza, G., & Biliaderis, C. G. (1989). Functional properties of flax seed mucilage. *Journal of Food Science, 54*, 1302–1305. doi:10.1111/j.1365-2621.1989.tb05978.x.

Meda, V., Orsat, V., & Raghavan, G. S. V. (2005). Microwave heating and the dielectric properties of foods. In H. Schubert & M. Regier (Eds.), *The microwave processing of foods* (pp. 61–75). Cambridge: Woodhead.

Myers, R. H., & Montgomery, D. C. (2002a). Two-level factorial designs. In R. H. Myers & D. C. Montgomery (Eds.), *Response surface methodology—process and product optimization using designed experiments* (pp. 85–154). New York: John Wiley & Sons.

Myers, R. H., & Montgomery, D. C. (2002b). Two-level fractional factorial designs. In R. H. Myers & D. C. Montgomery (Eds.), *Response surface methodology—process and product optimization using designed experiments* (pp. 155–196). New York: John Wiley & Sons.

Nemes, S. M. (2007). Microwave-assisted extraction (MAE) of secoisolariciresinol diglucoside (SDG) from flaxseed. M.Sc. Thesis, Bioresource Engineering Department, McGill University, Ste-Anne de Bellevue, QC, Canada. Retrieved from http://www.agrenv.mcgill.ca/agreng/theses/.

Oomah, B. D. (2002). Phytoestrogens. In W. J. Hurst (Ed.), *Methods of analysis for functional foods and nutraceuticals* (pp. 1–63). Boca Raton, FL: CRC.

Orsat, V., Raghavan, G. S. V., & Meda, V. (2005). Microwave technology for food processing: an overview. In H. Schubert & M. Regier (Eds.), *The microwave processing of foods* (pp. 105–118). Cambridge: Woodhead.

Prasad, K. (1999). Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed. *Circulation, 99*, 1355–1362.

Prasad, K. (2000a). Antioxidant activity of secoisolariciresinol diglucoside-derived metabolites, secoisolariciresinol, enterodiol, and enterolactone. *International Journal of Angiology, 9*, 220–225. doi:10.1007/BF01623898.

Prasad, K. (2000b). Oxidative stress as a mechanism of diabetes in diabetic BB prone rats: effect of secoisolariciresinol diglucoside (SDG). *Molecular and Cellular Biochemistry, 209*, 89–96. doi:10.1023/A:1007079802459.

Prasad, K. (2002). Suppression of phosphoenolpyruvate carboxykinase gene expression by secoisolariciresinol diglucoside (SDG), a new antidiabetic agent. *International Journal of Angiology, 11*, 107–109. doi:10.1007/BF01616377.

Vadivambal, R., & Jayas, D. (2008). Non-uniform temperature distribution during microwave heating of food materials—a review. *Food and Bioprocess Technology*, in press. doi:10.1007/s11947-008-0136-0.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology, 17*, 300–312. doi:10.1016/j.tifs.2005.12.004.

Westcott, N. D., & Muir, A. D. (2003). Flax seed lignan in disease prevention and health promotion. *Phytochemistry Reviews, 2*, 401–417. doi:10.1023/B:PHYT.0000046174.97809.b6.

Williams, O. J., Raghavan, G. S. V., Orsat, V., & Dai, J. (2004). Microwave-assisted extraction of capsaicinoids from capsicum fruit. *Journal of Food Biochemistry, 28*, 113–122. doi:10.1111/j.1745-4514.2004.tb00059.x.

Zhang, W., & Xu, S. (2007). Microwave-assisted extraction of secoisolariciresinol diglucoside from flaxseed hull. *Journal of the Science of Food and Agriculture, 87*, 1455–1462. doi:10.1002/jsfa.2793.