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Studies on statistical optimization of sulforaphane production from broccoli seed

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Abstract

Background: Natural sulforaphane (SF) has been of increasing interest for nutraceutical and pharmaceutical industries due to its anti-cancer effect. The main objective of the present work was to optimize the production of SF from broccoli seed using response surface methodology.
Results: Three major factors (hydrolysis time, water volume and ethyl acetate volume) were screened out through Plackett-Burman (PB) factorial design. The methods of steepest ascent combined with central composite design (CCD) were employed for optimization of the SF production process. The optimal extraction conditions for SF production were a hydrolysis time of 13 min, a hydrolysis volume/weight ratio of 2.9:1 (v/g) and an extraction volume/weight ratio of 17.5:1 (v/g). The maximum SF yield was 14.8 ± 0.1 mg/g, a value that was in perfect agreement with the actual experimental value (14.8 mg/g).
Conclusions: These results suggested that PB design combined with CCD were proved effective in screening and optimization of the parameters of SF production.

Keywords: broccoli seed; central composite design; Plackett-Burman design; production; sulforaphane.

INTRODUCTION

Cruciferous plants such as broccoli (*Brassica oleracea* var. *italica*) contain glucoraphanin (4-methylsulfinyl-3-butyl glucosinolates), secondary metabolites that are part of an elegant herbivore defense mechanism (Bones and Rossiter, 1996). When the tissue of broccoli is damaged, glucoraphanin is hydrolyzed by cytosolic myrosinase (EC 3.2.3.1) and/or cofactors to sulforaphane (4-methylsulfinylbutyl isothiocyanate, SF) or sulforaphane nitrile, as shown in Figure 1 (Matusheski et al. 2004). SF is of interest since it primarily modulates activities of phase II enzymes that convert carcinogens to inactive metabolites thereby preventing them from interacting with DNA (Zhang et al. 1994). SF does boost Phase II enzymes that trigger ongoing and long-lasting antioxidant activity (Fahey and Talalay, 1999; Gao et al. 2001). In addition, SF has recently been shown to produce altered signal transduction through covalent modification of cell target proteins (Mi et al. 2008; Cross et al. 2009). Consumption of cruciferous vegetables such as broccoli and Brussels sprouts, which can provide SF, is linked to reduced cancer risk (Jeffery and Araya, 2009). A means to effectively extract SF from its source, hence the ability to process it further, is therefore desirable.

To date, many reports have focused on SF detection, screening and purification. Sivakumar et al. (2007) analyzed 14 different important European Brassica species, and the new natural sources high in SF were selected. Liang et al. (2006) studied the effects of metal ions on the formation of SF in broccoli

seed and found that zinc ion was beneficial to the formation of SF. Mild heat treatment of broccoli and broccoli sprouts, such as steaming for 1 to 3 min could increase the formation of SF (Matusheski et al. 2004; Wang et al. 2012), and addition of powdered mustard seeds that can provide natural source of myrosinase enzyme to the heat processed broccoli could also significantly increased the formation of SF (Ghawi et al. 2013). Tanongkankit et al. (2013) studied the microwave-assisted extraction of SF from white cabbages. The results showed that microwave-assisted extraction was more effective than the conventional extraction led to higher yield of SF in a much shorter extraction time. SF can be purified by macroporous resins adsorption (Li et al. 2008a), low pressure column chromatography (Liang et al. 2005), preparative HPLC (Matusheski et al. 2001; Kore et al. 1993; Bertelli et al. 1998) or high-speed countercurrent chromatography (HSCCC) (Liang et al. 2008) from broccoli seed meal. However, there are few reports on the optimization of the SF production process. SF production from broccoli seeds involves three steps: de-fatting, hydrolysis and extraction, each step is dependent on a number of parameters that can affect the yield of SF, thus a great number of experiments should be simultaneously run, and their possible interactions also should be studied. For this reason, design of appropriate hydrolysis and extraction processes are of crucial importance. Plackett-Burman (PB) design and response surface methodology (RSM) can collectively optimize all the affected parameters to eliminate the limitations of a single-factor optimization process, and therefore these statistical experimental designs were employed in this work. Plackett-Burman (PB) design involve a two level fractional factorial saturated design that uses only k+1 treatment combinations to estimate the main effects of k factors independently, and assuming that all interactions are negligible (Plackett and Burman, 1946). PB design provides a fast and effective way of screening a large number of factors and identifying the significant ones, thereby, saving time and maintaining convincing information on each component (Li et al. 2008b). After the screening to obtain the significant variables, RSM is often applied to explore the relationship between a response and a set of design variables (Jiang et al. 2011).

In this work, PB design combined with central composite design was applied to optimize the extraction conditions for SF production. The PB design was employed to screen critical factors from a number of process variables, then apply the steepest ascent method to approach the experimental design space, and finally central composite design (CCD) was used to further optimize the factors that have significant effects in order to reach desirable responses.

MATERIALS AND METHODS

Materials and chemicals

Broccoli seed was kindly provided by Taizhou Academy of Agricultural Science. Distilled water was used throughout the study. Methanol (TEDIA, USA) was HPLC grade, and all other reagents were of analytical reagent grade and were purchased from Huadong Medicine Co. Ltd. (Hangzhou, China).

Extraction methods

Seeds were ground in a Chinese herbal medicine grinder to produce seed meal, and was heated in 50ºC for 10 min to inactivate the epithiospecifier protein (Matusheski et al. 2004) and then immediately cooled on ice. 3 g of each seed meal was subsequently de-fatted with hexane in an incubator shaker for a set amount of time, after which the residual seed meal was allowed to dry overnight in a fume hood. De-fatted seed meal was mixed with potassium phosphate buffer (0.05 mol/L), the mixture was placed in an incubator shaker to autolyze and then ethyl acetate was added for the extraction. Following this, sodium chloride and anhydrous sodium sulphate were added and mixed thoroughly. The ethyl acetate layer was filtered and the residual paste was extracted with equal volumes of ethyl acetate again, which was combined and dried in a vacuum rotary evaporator (the volume in these next experiments was the total volume). The residue was filtered through a 0.45 μm membrane for HPLC analysis.

PB design

The PB design was used to screen the most important factors that significantly influenced SF production. The experimental design with the name, symbol code, and level of the variables is shown

in Table 1. The upper and lower limits of each variable were chosen according to the previous one-factor-at-a-time test, which are denoted by (+) and (-), respectively (Singh and Tripathi, 2008). Three dummy variables were studied in the experiments to calculate the standard error. The rows in Table 1 represent the 16 different trials and each column represents a different variable. SF extraction was carried out in triplicate and the average value was taken as the response.

Table 1. **The PB design for screening variables in SF production.**

Code	Term	Variable Levels	
		Low (-)	High (+)
A	De-fat temperature (°C)	24	36
B	De-fat time (hr)	16	24
C	De-fat volume-weight ratio (w/v)	1:8	1:12
D	Dummy	…	…
E	Hydrolysis temperature (°C)	24	36
F	Hydrolysis time (hr)	0[a]	3
G	Mesh	30	45
H	Dummy	…	…
I	Hydrolysis volume-weight ratio (w/v)	1:2	1:3
J	Hydrolysis pH	5.8	7.2
K	Extraction temperature (°C)	24	36
L	Dummy	…	…
M	Extraction time (hr)	3	5
N	Extraction volume-weight ratio (w/v)	1:4	1:6
O	Dummy	…	…

[a] means PBS and ethyl acetate were added simultaneously.

The path of steepest ascent

To find the neighbourhood of the optimum condition quickly, the method of the steepest ascent was employed. The experiments were applied to determine a suitable direction by increasing or decreasing the variables according to the results obtained from the PB design (Gheshlaghi et al. 2005).

Central composite designs (CCD) and response surface methodology

To describe the nature of the response surface in the optimum region, a CCD was performed. The central composite design (CCD) is a common method to design experiments for building a second order (quadratic) model in RSM with response variables (Jin et al. 2011). The five coded levels of CCD were designated as -1.682, -1, 0, 1, and 1.682. All experimental designs were repeated at least three times.

Statistical analysis

Minitab 16.0 (Minitab Inc., Pennsylvania, USA) was used for the experimental design and subsequent regression analysis of the experimental data. The fitting quality of the second-order model equation was expressed by the determination coefficient R^2, and its statistical significance was tested by regression coefficients, *T*-test values and *P*-values. For each variable, the quadratic model was represented as the response surface.

HPLC

The content of SF was analyzed on a Waters e2695 HPLC system by the method of Wu et al. (2013). The column employed in our experiment was a ZORBAX Eclipse XDB-C_{18} (4.6 x 250 mm, 5 μm). The mobile phase consisted of 20% methanol in water, changing linearly over 10 min to 60% methanol, then increasing to 100% in 2 min and maintained for 2 min to purge the column. The column oven temperature was set at 25ºC, the flow rate was 1.0 ml/min, and 10 μl samples were injected onto the column. SF was detected using a Waters 2489 detector at 241 nm.

RESULTS AND DISCUSSION

Optimization by PB experimental design

Suitable sources of plant materials for the isolation of SF are chosen based on their content of specific parent glucoraphanin. Researchers interested in isolating glucoraphanin should consider using seeds as the preferred staring material (West et al. 2002), because non germinated seeds have the highest glucoraphanin levels, which decline as sprouts germinate and develop (Pereira et al. 2002). In a separate study, we found that glucoraphanin content in broccoli seed is much higher than in the vegetable itself (data not show). This finding is consistent with that of Tookey (1980), who reported that crucifer seeds contained approximately 10 times the total glucosinolate concentration in the edible portion of the vegetable. For these reasons, seeds were chosen as the most concentrated plant source of SF.

11 variables and the upper and lower limits of each variable were chosen according to the previous one-factor-at-a-time test (data not show). The results of PB design with respect to the SF yield after statistical analysis are shown in Table 2. The results indicated that there was a wide variation of SF yield from 7.4 ± 0.03 mg/g to 11.7 ± 0.08 mg/g in the 16 trials. Results of t-tests showed that hexane had a negative effect on SF yield (Figure 2), implying that the hexane may decrease the activity of myrosinase. Hydrolysis time, hydrolysis volume-weight ratio and pH values also showed negative effects on the yield. It is not entirely clear why a longer hydrolysis time would cause a decrease in the amount of SF extracted. The possible explanation however, is that glucoraphanin produces not only SF, but also sulforaphane nitrile and other substances when it is hydrolyzed by myrosinase and residue epithiospecifier protein (Matusheski et al. 2004), a longer hydrolysis time would result in the production of other substances except for SF, and the activity of residue epithiospecifier protein may decrease in the presence of hexane and ethyl acetate, thus the analysis of myrosinase and epithiospecifier protein activity on glucosinolates hydrolysis during organic solvent treatment is certainly required for future work. On the other hand, optimal myrosinase activity was recorded in phosphate buffer at pH 6-6.5 (Jwanny et al. 1995), and SF is stable at a lower pH value (Wu et al. 2010; Wu et al. 2013). Therefore, a reduction in hydrolysis time, a reduction in pH and an increase in the extraction time would be beneficial. Particle size also has an effect on the extraction yield (Chiang and Ciou, 2010; Hu et al. 2012, it was noteworthy that mesh had a negative effect on the yield, implying that smaller particles may lead to filtration and/or centrifugation difficulties, thus decreasing the yield of SF. It is as expected that extraction time and extraction volume-weight ratio showed positive effect, implying that increase in extraction time and volume of ethyl acetate were generally beneficial in obtaining a higher yield of SF from the seeds of broccoli.

Upon analysis of regression coefficients and the t-value of 11 variable factors, the factors having the greatest positive impacts on the production of SF were identified as the extraction volume/weight ratio while factors such as hydrolysis time and hydrolysis volume/weight ratio had the greatest negative effects (Figure 2). Thus the variables selected for further optimization were extraction volume/weight ratio, hydrolysis time and hydrolysis volume/weight ratio.

Optimization by the path of steepest ascent

Based on PB experimental design, the path of the steepest ascent was employed to find the proper direction of changing variables (Gheshlaghi et al. 2005). Extraction volume-weight ratio (w/v) had a significant positive effect on SF production while the hydrolysis volume-weight ratio (w/v) and hydrolysis time (hr) had a negative effect. To move away from the base point (No. 1 in Table 3) along the path of the steepest ascent, we move 0.5, 2 and 10 in hydrolysis time (hr), water volume (ml) and ethyl acetate volume (ml), respectively, the yield of SF increased along the path from No. 1 to 3 reaching a peak of 14.2 ± 0.3 mg/g and then decreased in No. 4, indicating the optimal level was close to that in No. 3. Using the experimental conditions suggested by No. 3, the yield of SF remarkably improved, implying the steepest ascent method was an effective technique for approaching the optimal level. Nevertheless, the optimum values of the three variables are still unknown and need to be confirmed by the subsequent CCD.

Table 2. PB design for 15 variables with coded values along with the observed results for SF production.

Number	Variable levels															SF yield (mg/g)
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
1	+	+	+	+	-	+	-	+	+	-	-	+	-	-	-	7.4 ± 0.03
2	-	+	-	-	-	+	+	+	+	-	+	-	+	+	-	8.6 ± 0.3
3	-	-	+	-	-	-	+	+	+	+	-	+	-	+	+	9.1 ± 0.3
4	+	+	+	-	+	-	+	+	-	-	+	-	-	-	+	8.3 ± 0.1
5	+	-	+	-	+	+	-	-	+	-	-	-	+	+	+	9.2 ± 0.4
6	+	-	-	+	-	-	-	+	+	+	+	-	+	-	+	8.0 ± 0.4
7	+	+	-	+	-	+	+	-	-	+	-	-	-	+	+	8.1 ± 0.9
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.9 ± 0.6
9	-	-	+	+	+	+	-	+	-	+	+	-	-	+	-	10.4 ± 1.1
10	+	-	+	+	-	-	+	-	-	-	+	+	+	+	-	10.7 ± 0.4
11	-	-	-	+	+	+	+	-	+	-	+	+	-	-	+	7.7 ± 0.5
12	-	+	+	-	-	+	-	-	-	+	+	+	+	-	+	8.9 ± 0.6
13	+	-	-	-	+	+	+	+	-	+	-	+	+	-	-	7.8 ± 0.03
14	-	+	+	+	+	-	+	-	+	+	-	-	+	-	-	7.8 ± 0.4
15	+	+	-	-	+	-	-	-	+	+	+	+	-	+	-	8.9 ± 0.4
16	-	+	-	+	+	-	-	+	-	-	-	+	+	+	+	11.7 ± 1.0

Table 3. The steepest ascent experiment and its results.

Run	Hydrolysis time (hr)	Water volume (ml)	Ethyl acetate volume (ml)	SF yield (mg/g)
1	2.0	10	20	10.9 ± 0.9
2	1.5	8	30	11.2 ± 0.2
3	1.0	6	40	14.2 ± 0.3
4	0.5	4	50	13.8 ± 0.3

Optimization by central composite designs (CCD)

The response of the yield of SF, as a function of hydrolysis time (hr), water volume (ml) and ethyl acetate volume (ml), was evaluated in CCD. According to the results of the path of steepest ascent, the center point was chosen as a hydrolysis time of 1 hr, a water volume of 4.5 ml and an ethyl acetate volume of 50 ml. The design and results of CCD are shown in Table 4 and Table 5.

Minitab 16.0 was used to determine a quadratic mathematical model for SF production. By applying multiple regression analysis to the experimental data, regression coefficients, *T*-values, and *P*-values for the model of SF yield are presented in Table 6. By removing the statistically non-significant terms (X_1X_2), the following second-order polynomial equation was generated, representing the SF production as a function of the variables:

$$Y = 14.139 - 0.717X_1 + 0.051X_2 + 0.145X_3 - 0.327X_1^2 - 0.330X_2^2 - 0.578X_3^2 - 0.245X_1X_3 + 0.675X_2X_3$$

where Y is the predicted SF yield; X_1, X_2 and X_3 are the coded values of hydrolysis time, water volume and ethyl acetate volume, respectively.

Table 4. Levels of factors chosen for the experimental design.

Factors	Variable	Coded level					
		-1.682	-1	0	1	1.682	Δ*X*
Hydrolysis time	X_1	0.16	0.5	1	1.5	1.8	0.5
Water volume	X_2	2.6	4	6	8	9.4	2
Ethyl acetate volume	X_3	23.2	30	40	50	56.8	10

Table 5. CCD for the experimental design and predicted results.

Hydrolysis time	Water volume	Ethyl acetate volume	Results (mg/g)	Predicted (mg/g)
1	1	1	12.6 ± 0.3	12.8
1	1	-1	11.1 ± 0.6	11.7
1	-1	1	10.8 ± 0.6	11.4
1	-1	-1	12.9 ± 0.1	12.9
-1	1	1	14.4 ± 0.8	14.7
-1	1	-1	12.7 ± 0.8	12.6
-1	-1	1	13.7 ± 0.7	13.3
-1	-1	-1	14.0 ± 0.1	13.9
-1.682	0	0	14.1 ± 0.2	14.4
1.682	0	0	12.7 ± 0.3	12.0
0	-1.682	0	13.0 ± 1.0	13.1
0	1.682	0	13.8 ± 0.8	13.3
0	0	-1.682	12.4 ± 0.4	12.3
0	0	1.682	13.1 ± 0.4	12.8
0	0	0	14.0 ± 0.3	14.1
0	0	0	14.3 ± 0.2	14.1
0	0	0	14.5 ± 0.04	14.1
0	0	0	13.8 ± 0.2	14.1
0	0	0	14.3 ± 0.3	14.1
0	0	0	13.9 ± 0.1	14.1

Table 6. Regression coefficients and their significance for response surface model.

Term	Coefficients estimated	*T*-value	*P*-value
Constant	14.139	68.913	0.000
X_1	-0.717	-5.265	0.000
X_2	0.051	0.372	0.718
X_3	0.145	1.067	0.311
X_1^2	-0.327	-2.467	0.033
X_2^2	-0.330	-2.494	0.032
X_3^2	-0.578	-4.361	0.001
X_1X_2	0.068	0.380	0.712
X_1X_3	-0.245	-1.378	0.198
X_2X_3	0.675	3.795	0.004

The statistical significance of the second-order model equation was evaluated using ANOVA (Table 7), one can observe that the mathematical model was very significant, with a *P*-value less than 0.0001, and the lack-of-fit of the simplified model was non-significant ($P > 0.05$). In addition, the goodness of fit of the quadratic model was checked using the coefficient of determination (R^2 = 87.7%), indicating that 87.7% of the total variation was explained by the model. This confirms that the accuracy and suitability of the quadratic model was good, and analysis of the associated response trends was reasonable.

Table 7. ANOVA of regression model.

Source	DF	Seq. SS	Adj. MS	*F*	*P*
Regression	8	18.24	2.28	9.77	0.000
Linear	3	7.34	2.45	10.48	0.001
Quadratic	3	6.78	2.26	9.68	0.002
Interactions	2	4.13	2.06	8.84	0.005
Residual error	11	2.57	0.23		
Lack of fit	6	2.12	0.35	3.98	0.076
Pure error	5	0.44	0.09		
Total	19	20.81			

To study the interactions of variables and analyze the SF yield, three-dimensional response surfaces were plotted in Figure 3. All possible combinations of the three variables were studied. In the response surface curves, two factors were varied at a time while the other factor remained at a fixed level (zero level). As shown in Figure 3, the higher SF concentration could be obtained with hydrolysis time, water volume and ethyl acetate volume close to a zero level.

The prediction ability of the model is of key importance in the final optimization step. Therefore, the RSM developed for modeling the SF yield was used to identify the optimal set of input conditions that yielded a maximum output. On the basis of SF production optimization, the quadratic model predicted that the maximum production of SF was 14.83 mg/g, when the X_1 was -1.563, X_2 was 1.351 and X_3 was 1.246, which represented 13 min hydrolysis time, 9 ml water volume and 52 ml ethyl acetate, *i.e.* 2.9:1 (v/g) hydrolysis volume/weight ratio and 17.5:1 (v/g) extraction volume/weight ratio. To verify the predicted result of the second-order polynomial equation, the experiment was performed under the optimal conditions.

The average SF yield from five experiments was 14.8 ± 0.1 mg/g compared with 14.8 mg/g predicted by RSM, which verified the model validation and existence of an optimal point. The PB design together with path of steepest ascent and CCD applied in the present investigation have been successfully used in many natural products production for optimization of fermentation technologies (Gao et al. 2009; Zhang et al. 2012). However, to the best our knowledge, there are no reports of optimization of

hydrolysis and extraction of SF and other isothiocyanates production by organic solvent using these statistical experimental design.

CONCLUDING REMARKS

PB design and RSM were employed for the statistical optimization of SF production. According to the PB design, three major factors (hydrolysis time, hydrolysis volume/weight ratio and extraction volume/weight ratio) contributed the most to improve SF yield during the de-fatting, hydrolysis and extraction procedures. CCD and regression analysis were used to identify the optimized hydrolysis time, hydrolysis volume/weight ratio and extraction volume/weight ratio. To confirm the applicability of the model, the SF yield at the suggested optimum conditions was determined. Under the optimum conditions, the model predicted a SF yield of 14.8 mg/g, the experimental SF yield of 14.8 ± 0.1 mg/g confirmed the accuracy of the model. The results suggested that the PB design and RSM were successfully applied for the rapid screening and optimization of the parameters of SF production.

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REFERENCES

BERTELLI, D.; PLESSI, M.; BRAGHIROLI, D. and MONZANI, A. (1998). Separation by solid phase extraction and quantification by reverse phase HPLC of sulforaphane in broccoli. *Food Chemistry*, vol. 63, no. 3, p. 417-421. **[CrossRef]**

BONES, A.M. and ROSSITER, J.T. (1996). The myrosinase-glucosinolate system, its organisation and biochemistry. *Physiologia Plantarum*, vol. 97, no. 1, p. 194-208. **[CrossRef]**

CHIANG, P.Y. and CIOU, J.Y. (2010). Effect of pulverization on the antioxidant activity of water caltrop (*Trapa taiwanensis Nakai*) pericarps. *LWT - Food Science and Technology*, vol. 43, no. 2, p. 361-365. **[CrossRef]**

CROSS, J.V.; RADY, J.M.; FOSS, F.W.; LYONS, C.E.; MACDONALD, T.L. and TEMPLETON, D.J. (2009). Nutrient isothiocyanates covalently modify and inhibit the inflammatory cytokine macrophage migration inhibitory factor (MIF). *Biochemical Journal*, vol. 423, no. 3, p. 315-321. **[CrossRef]**

FAHEY, J.W. and TALALAY, P. (1999). Antioxidant functions of sulforaphane: A potent inducer of phase II detoxication enzymes. *Food and Chemical Toxicology*, vol. 37, no. 9-10, p. 973-979. **[CrossRef]**

GAO, X.; DINKOVA-KOSTOVA, A.T. and TALALAY, P. (2001). Powerful and prolonged protection of human retinal pigment epithelial cells, keratinocytes, and mouse leukemia cells against oxidative damage: The indirect antioxidant effects of sulforaphane. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 26, p. 15221-15226. **[CrossRef]**

GAO, H.; LIU, M.; LIU, J.; DAI, H.; ZHOU, X.; LIU, X.; ZHOU, Y.; ZHANG, W. and ZHANG, L. (2009). Medium optimization for the production of avermectin B1a by *Streptomyces avermitilis* 14-12A using response surface methodology. *Bioresource Technology*, vol. 100, no. 17, p. 4012-4016. **[CrossRef]**

GHAWI, S.K.; METHVEN, L. and NIRANJAN, K. (2013). The potential to intensify sulforaphane formation in cooked broccoli (*Brassica oleracea var. italica*) using mustard seeds (*Sinapis alba*). *Food Chemistry*, vol. 138, no. 2-3, p. 1734-1741. **[CrossRef]**

GHESHLAGHI, R.; SCHARER, J.M.; MOO-YOUNG, M. and DOUGLAS, P.L. (2005). Medium optimization for hen egg white lysozyme production by recombinant *Aspergillus niger* using statistical methods. *Biotechnology and Bioengineering*, vol. 90, no. 6, p. 754-760. **[CrossRef]**

HU, J.; CHEN, Y. and NI, D. (2012). Effect of superfine grinding on quality and antioxidant property of fine green tea powders. *LWT - Food Science and Technology*, vol. 45, no. 1, p. 8-12. **[CrossRef]**

JEFFERY, E.H. and ARAYA, M. (2009). Physiological effects of broccoli consumption. *Phytochemistry Reviews*, vol. 8, no. 1, p. 283-298. **[CrossRef]**

JIANG, F.Y.; WANG, J.M.; JU, L.C.; KALEEM, I.; DAI, D.Z. and LI, C. (2011). Optimization of degumming process for soybean oil by phospholipase B. *Journal of Chemical Technology and Biotechnology*, vol. 86, no. 8, p. 1081-1087. **[CrossRef]**

JIN, E.Y.; LIM, S.; KIM, S.O.; PARK, Y.S.; JANG, J.K.; CHUNG, M.S.; PARK, H.; SHIM, K.S. and CHOI, Y.J. (2011). Optimization of various extraction methods for quercetin from onion skin using response surface methodology. *Food Science and Biotechnology*, vol. 20, no. 6, p. 1727-1733. **[CrossRef]**

JWANNY, E.W.; EL-SAYED, S.T.; RASHAD, M.M.; MAHMOUD, A.E. and ABDALLAH, N.M. (1995). Myrosinase from roots of *Raphanus sativus*. *Phytochemistry*, vol. 39, no. 6, p. 1301-1303. **[CrossRef]**

KORE, A.M.; SPENCER, G.F. and WALLIG, M.A. (1993). Purification of the ω-(methylsulfinyl) alkyl glucosinolate hydrolysis products: 1-isothiocyanato-3-(methylsulfinyl) propane, 1-isothiocyanato-4-(methylsulfinyl) butane, 4-(methylsulfinyl) butanenitrile, and 5-(methylsulfinyl) pentanenitrile from broccoli and *Lesquerella fendleri*. *Journal of Agricultural and Food Chemistry*, vol. 41, no. 1, p. 89-95. **[CrossRef]**

LI, C.; LIANG, H.; YUAN, Q. and HOU, X. (2008a). Optimization of sulforaphane separation from broccoli seeds by macroporous resins. *Separation Science and Technology*, vol. 43, no. 3, p. 609-623. **[CrossRef]**

LI, Y.; JIANG, H.; XU, Y. and ZHANG, X. (2008b). Optimization of nutrient components for enhanced phenazine-1-carboxylic acid production by *gacA*-inactivated *Pseudomonas* sp. M18G using response surface method. *Applied Microbiology and Biotechnology*, vol. 77, no. 6, p. 1207-1217. **[CrossRef]**

LIANG, H.; YUAN, Q. and XIAO, Q. (2005). Purification of sulforaphane from *Brassica oleracea* seed meal using low-pressure column chromatography. *Journal of Chromatography B*, vol. 828, no. 1-2, p. 91-96. **[CrossRef]**

LIANG, H.; YUAN, Q. and XIAO, Q. (2006). Effects of metal ions on myrosinase activity and the formation of sulforaphane in broccoli seed. *Journal of Molecular Catalysis B: Enzymatic*, vol. 43, no. 1-4, p. 19-22. **[CrossRef]**

LIANG, H.; LI, C.; YUAN, Q. and VRIESEKOOP, F. (2008). Application of high-speed countercurrent chromatography for the isolation of sulforaphane from broccoli seed meal. *Journal of Agricultural and Food Chemistry*, vol. 56, no. 17, p. 7746-7749. **[CrossRef]**

MATUSHESKI, N.V.; WALLIG, M.A.; JUVIK, J.A.; KLEIN, B.P.; KUSHAD, M.M. and JEFFERY, E.H. (2001). Preparative HPLC method for the purification of sulforaphane and sulforaphane nitrile from *Brassica oleracea*. *Journal of Agricultural and Food Chemistry*, vol. 49, no. 4, p. 1867-1872. **[CrossRef]**

MATUSHESKI, N.V.; JUVIK, J.A. and JEFFERY, E.H. (2004). Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli. *Phytochemistry*, vol. 65, no. 9, p. 1273-1281. **[CrossRef]**

MI, L.; XIAO, Z.; HOOD, B.L.; DAKSHANAMURTHY, S.; WANG, X.; GOVIND, S.; CONRADS, T.P.; VEENSTRA, T.D. and CHUNG, F.L. (2008). Covalent binding to tubulin by isothiocyanates. A mechanism of cell growth arrest and apoptosis. *The Journal of Biological Chemistry*, vol. 283, no. 32, p. 22136-22146. **[CrossRef]**

PEREIRA, F.M.V.; ROSA, E.; FAHEY, J.W.; STEPHENSON, K.K.; CARVALHO, R. and AIRES, A. (2002). Influence of temperature and ontogeny on the levels of glucosinolates in broccoli (*Brassica oleracea var. italica*) sprouts and their effect on the induction of mammalian phase 2 enzymes. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 21, p. 6239-6244. **[CrossRef]**

PLACKETT, R.L. and BURMAN, J.P. (1946). The design of optimum multifactorial experiments. *Biometrika*, vol. 33, no. 4, p. 305-325. **[CrossRef]**

SINGH, V. and TRIPATHI, C.K.M. (2008). Production and statistical optimization of a novel olivanic acid by *Streptomyces olivaceus* MTCC 6820. *Process Biochemistry*, vol. 43, no. 11, p. 1313-1317. **[CrossRef]**

SIVAKUMAR, G.; ALIBONI, A. and BACCHETTA, L. (2007). HPLC screening of anti-cancer sulforaphane from important European *Brassica species*. *Food Chemistry*, vol. 104, no. 4, p. 1761-1764. **[CrossRef]**

TANONGKANKIT, Y.; SABLANI, S.S.; CHIEWCHAN, N. and DEVAHASTIN, S. (2013). Microwave-assisted extraction of sulforaphane from white cabbages: Effects of extraction condition, solvent and sample pretreatment. *Journal of Food Engineering*, vol. 117, no. 1, p. 151-157. **[CrossRef]**

TOOKEY, H.L.; DAXENBICHLER, M.E.; VANETTEN, C.H.; KWOLEK, W.F. and WILLIAMS, P.H. (1980). Cabbage glucosinolates: Correspondence of patterns in seeds and leafy heads. *Journal of the American Society for Horticultural Science*, vol. 105, no. 5, p. 714-717.

WANG, G.C.; FARNHAM, M. and JEFFERY, E.H. (2012). Impact of thermal processing on sulforaphane yield from broccoli (*Brassica oleracea* L. ssp. *italica*). *Journal of Agricultural and Food Chemistry*, vol. 60, no. 27, p. 6743-6748. **[CrossRef]**

WEST, L.; TSUI, I. and HAAS, G. (2002). Single column approach for the liquid chromatographic separation of polar and non-polar glucosinolates from broccoli sprouts and seeds. *Journal of Chromatography A*, vol. 966, no. 1-2, p. 227-232. **[CrossRef]**

WU, H.; LIANG, H.; YUAN, Q.; WANG, T. and YAN, X. (2010). Preparation and stability investigation of the inclusion complex of sulforaphane with hydroxypropyl-β-cyclodextrin. *Carbohydrate Polymers*, vol. 82, no. 3, p. 613-617. **[CrossRef]**

WU, Y.; MAO, J.; MEI, L. and LIU, S. (2013). Kinetic studies of the thermal degradation of sulforaphane and its hydroxypropyl-β-cyclodextrin inclusion complex. *Food Research International*, vol. 53, no. 1, p. 529-533. **[CrossRef]**

ZHANG, Y.; KENSLER, T.W.; CHO, C.G.; POSNER, G.H. and TALALAY, P. (1994). Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 8, p. 3147-3150. **[CrossRef]**

ZHANG, L.J.; ZHENG, X.; JIN, Z.H.; HU, S. and HE, M.R. (2012). Optimization of fermentation conditions for pristinamycin production by immobilized *Streptomyces pristinaespiralis* using response surface methodology. *Electronic Journal of Biotechnology*, vol. 15, no. 4. **[CrossRef]**

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Figures

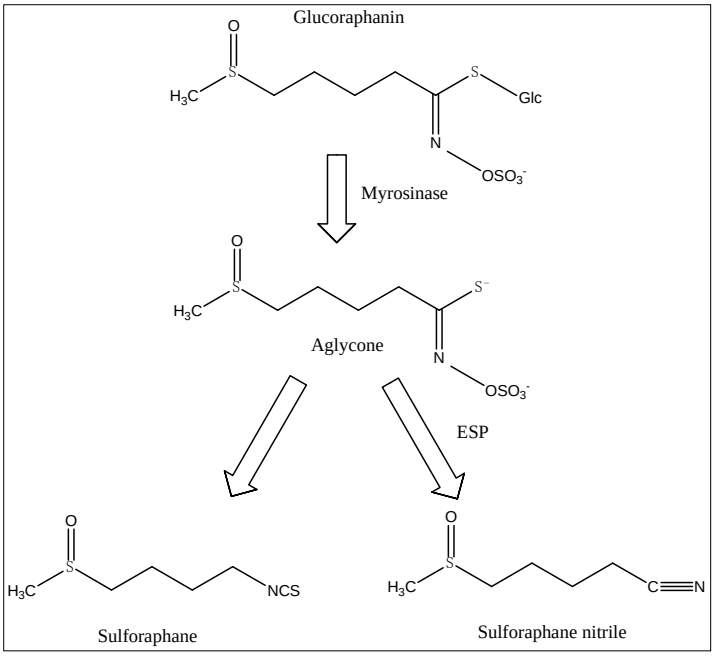

Fig. 1 Model of glucoraphanin hydrolysis. Myrosinase catalysed hydrolysis of glucoraphanin yields unstable aglycones, which spontaneously re-arrange to sulforaphane and sulforaphane nitrile.

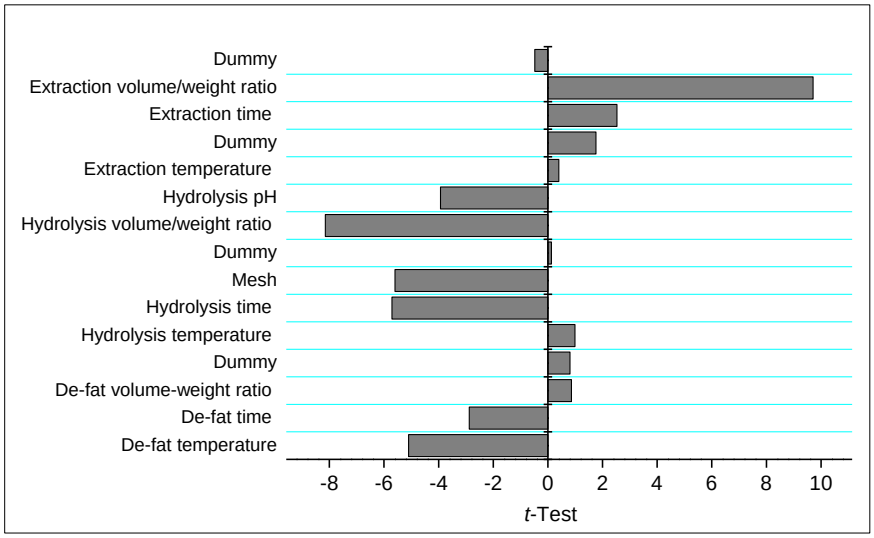

Fig. 2 Statistical analysis of PB design.

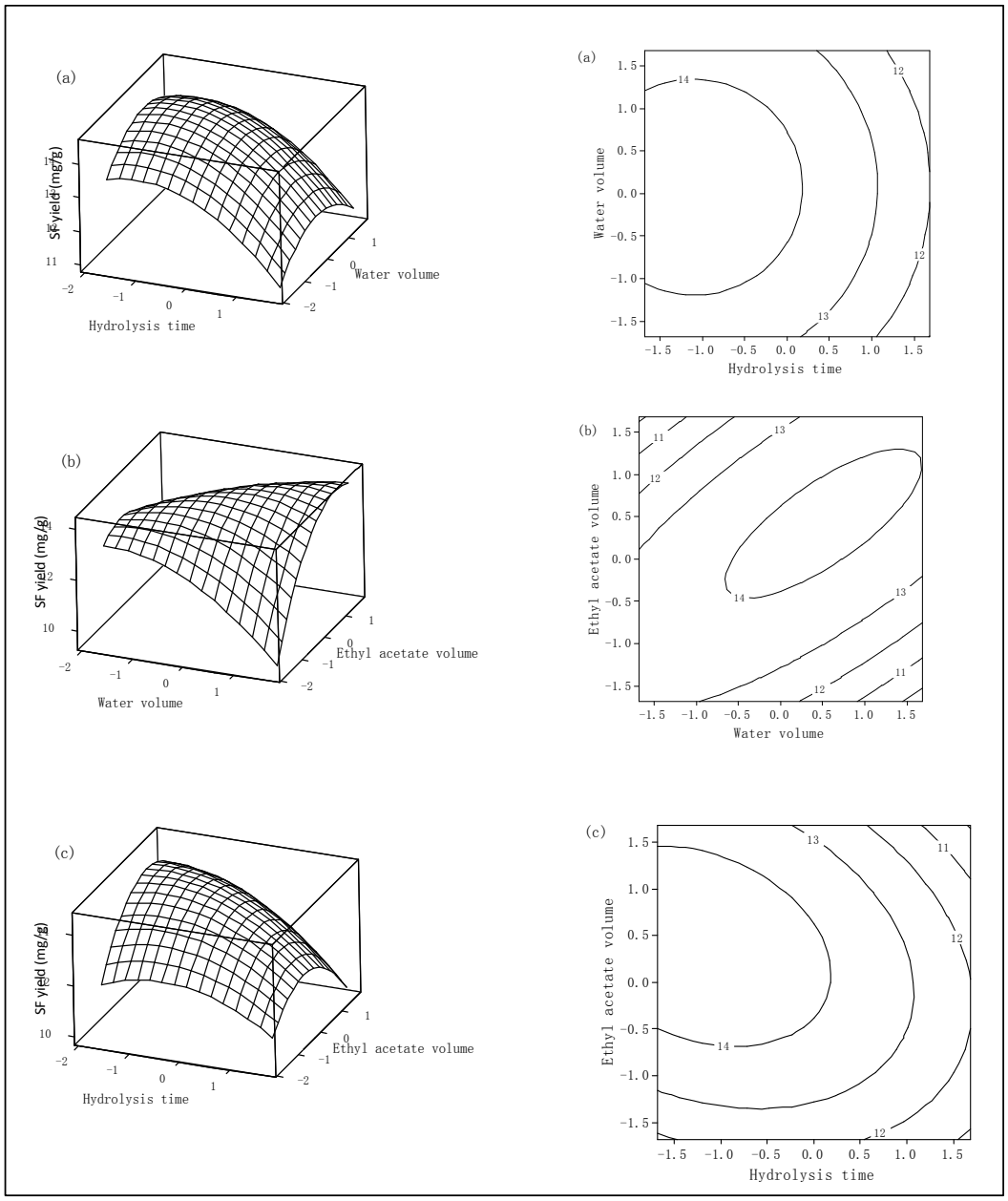

Fig. 3 3D surface graph and its contour plot for: (a) effect of water volume and hydrolysis time, and their mutual interaction; (b) effect of water volume and ethyl acetate volume, and their mutual interaction; (c) effect of ethyl acetate volume and hydrolysis time, and their mutual interaction.