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Article

Optimization of the Manufacturing Process for Black Ginseng

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Black ginseng is derived from a repeated steaming/drying process; however, the black ginseng manufacturing process is not well established. To determine the steaming and drying optimum conditions for producing high levels of biologically active substances such as acidic polysaccharides, ginsenoside Rg3, and polyphenols and low levels of benzopyrene, response surface methodology was used with temperature and time as independent variables. As steaming temperature/time increased, acid polysaccharide, polyphenol, and benzopyrene content increased; ginsenoside Rg3 levels increased with steaming time at temperatures ≥100°C. As drying temperature/time increased, ginsenoside Rg3, benzopyrene, and acidic polysaccharide content also increased. These substances showed a nonlinear canonical form, whereas the phenolic component showed a linear canonical form. Optimum conditions were determined to be steaming at 113.04°C for 18 h and drying at 100°C for 8.03 h, resulting in 0.75 mg/g ginsenoside Rg3, 13.72 mg% acidic polysaccharide, 0.26 ppb benzopyrene, and 3.24 mg% polyphenol.

Key words: acidic polysaccharide, benzopyrene, black ginseng, ginsenoside Rg3, optimization process, response surface methodology

Ginseng (*Panax ginseng* C. A. Meyer) is a perennial plant that belongs to the Araliaceae family, genus Panax, and has been widely used in Asian medicine for thousands of years [Park *et al*., 2003]. Because saponin is a typical ingredient among other physiological active ingredients in ginseng, it can be used as an indicator for judging the quality of ginseng and ginseng-functional products [Choi *et al*., 1989; Jeon *et al*., 1991]. Acidic polysaccharide as a nonsaponin ingredient has antihyperglycemic effects and regulates immune function [Konno *et al*., 1984; Gao *et al*., 1991; Chang *et al*., 2007]. The phenol ingredient includes an active aging-control substance and shows antioxidant effects on lipid oxidation [Wee *et al*., 1998].

Red ginseng is obtained through a process of steaming and drying from white ginseng. During this process, the ingredients of fresh ginseng are changed, and new physiological ingredients, which are not present in fresh ginseng, are generated, and their content increased. Recently, a black ginseng product produced by a new process that uses a principle derived from Chinese herbal medicine called "kujeungkupo" (nine times steaming, nine times drying) is being developed [Roh and Park, 2008]. Black ginseng contains more ginsenoside Rg3, an anticancer medicine, than red ginseng [Han *et al*., 2005]. However, a standard process of kujeungkupo for the production of black ginseng has not yet been established, and this ad-hoc process has been occasionally found to produce excessive benzopyrene which has recently been classified as a carcinogen.

Therefore, the aim of this study was to standardize and optimize the steaming and drying manufacturing process for manufacturing the black ginseng using response surface methodology in order to reduce the content of benzopyrene and enhance the content of acidic polysaccharide, ginsenoside Rg3, and polyphenol.

Materials and Methods

Materials. The dried ginseng used in this experiment was a 4-year-old certified white ginseng that had been produced in Geumsan district in Korea. Acetonitrile and isopropyl alcohol were purchased from JT Baker (Phillipsburg, NJ) for use in extraction and fractionation.

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Carbazole (Sigma, Steinheim, Germany), Folin-Ciocalteu phenol reagent (Sigma, St. Louis, MO), and sodium carbonate (Yakuri Pure Chemical Co. Ltd., Kyoto, Japan) were used as reagents.

Production and extraction of black ginseng. Ten-gram batches of white ginseng were steamed using a steaming machine (T-30, Jinghong Tech., Ilsan, Korea) at different temperatures (60, 80, and 100°C) and times (6, 12, and 18 h). The drying process was performed using a drying machine (HK-D0135, Hankook Jonghabgigi Co., Hwasung, Korea) at different temperatures (60, 80, and 100°C) and times (3, 6, and 9 h). All experiments were repeated three times. The resultant black ginseng was homogenized with a homogenator (ULTRA-TURRAX T-25, IKA-Labortechnik, Staufen, Germany) and extracted with 80% methanol (100 mL). Finally, the black ginseng extract was filtered and concentrated to a constant volume of 50 mL.

Extraction of crude saponin. Crude saponin content was determined according to the Korean Food Code and was subsequently used to analyze ginsenoside. Crude saponin was concentrated by centrifugation, and the sample was extracted twice using n-butanol at 70-80°C for 1 h. Fat was removed by heating the concentrated sample with ethyl ether for 30 min. The amount of crude saponin was calculated according to the following formula:

$$\text{Crude saponin content (mg/g)} = A/S$$

where A is the weight of the dried saturated butanol layer, and S is the amount of collected sample (g).

Analysis of acidic polysaccharide content. As acidic polysaccharide is primarily composed of galacturonic acid, polysaccharide content was measured by the carbazole-sulfuric acid method according to Bitter and Muir [1962]. After adding 0.25 mL of carbazole and 3 mL conc. H_2SO_4 into 0.5 mL of black ginseng extract at 85°C and stirring for 5 min, the mixture was cooled at room temperature for 15 min. The content of acidic polysaccharide was calculated by absorbance at 525 nm using a spectrophotometer (SP-830 Plus; Barnstead/Thermolyne, Dubuque, IA).

Analysis of black ginseng saponin. The concentration and components of black ginseng saponin were analyzed by applying the conditions of Ko *et al*. [2005] to compare crude saponin with a standard product. The standard product used was a ginsenoside (Wako Chemical, Inc., Osaka, Japan) with a 99% or higher degree of purity.

Analysis of saponin was performed using HPLC equipment (HPLC system Model 627; Alltech Associates, Inc., Deerfield, IL) and Prevail Carbohydrates ES Column (Alltech Associates, Inc., IL). Acetonitrile (ACN) and isopropyl alcohol (IPA) (HPLC grade) were used for the mobile phase. Solvent A was ACN:water: IPA=80:5:15 and solvent B was ACN: water:IPA=67: 21:12; the proportion of solvent B was changed, step-by-step, at 10, 85, 80, 75, 90, 100, and 25%. The proportion was finally set at 10% at room temperature with a flow rate of 0.8 mL/min. The chromatogram was detected using an Evaporative Light Scattering Detector (Alltech Associates, Inc.).

Benzopyrene analysis of black ginseng. A sample of black ginseng (10 g) was added to 50 mL of hexane according to the method of Hu *et al*. [2008]. The sample was extracted twice by cooling and refluxing it in a bath at 69°C for 1 h and was concentrated after adding 15 g of sodium sulfate anhydrous. Analysis of benzopyrene was performed using HPLC system Model 627 and an LC-polyaromatic hydrocarbons column (25×4.6 mm, particle size 5 mm).

Analysis of total polyphenol content of black ginseng. The total polyphenol content of black ginseng was analyzed using Folin-Ciocalteu phenol reagent. A mixture of 200 μL of black ginseng extract, 2.6 mL of distilled water, and 200 μL of Folin-Ciocalteu phenol reagent was stirred for 6 min. Then, 200 μL of 7% Na_2CO_3 was added to the black ginseng mixture and stirred at room temperature for 90 min. The absorbance of the mixture was measured at 750 nm and compared with a gallic acid standard curve. Total polyphenol content was expressed as gallic acid equivalent (GAE)/100 g sample.

Design and optimization of the black ginseng manufacturing process. The response surface methodology for experimental design and optimization was used to determine the optimum black ginseng manufacturing process. The Design-Expert 7 program (Stat-Ease, Inc., Minneapolis, MN) was used. To optimize each steaming and drying manufacturing process, steaming temperature and time along with drying temperature and time were set as factor variables for establishing the experimental design. The response variables included acidic polysaccharide content, ginsenoside Rg3 content, benzopyrene content, and polyphenol content. Each experimental group was selected by central composition, and steaming time was selected in the range of 6 (-1), 12 (0), and 18 (1) h by coding as -1, 0, and +1 data points. Steaming temperature was selected with the range of 60 (-1)°C, 80 (0)°C, and 100 (+1)°C. To evaluate benzopyrene content increase as a function of steaming temperature, a temperature of 120 (+2)°C was also selected. The range of drying time was 3 (-1), 6 (0), and 9 (+1) h, and the range of drying temperature was 60 (-1)°C, 80 (0)°C, and 100 (+1)°C.

Results and Discussion

Steaming conditions. To establish the optimum steaming conditions for black ginseng, a central composite design was selected by setting steaming temperature and time as independent variables. The resulting contents of acidic polysaccharide, ginsenoside Rg3, benzopyrene, and polyphenol of black ginseng obtained from 12 experimental steaming conditions are shown in Table 1.

The least acidic polysaccharide content (10.13 mg%) of ginseng steamed was obtained at 60°C for 6 h steaming condition, while the greatest content (37.52 mg%) was obtained at 120°C for 18 h. Do *et al.* [1993] reported that acidic polysaccharide was extracted more efficiently by the red ginseng manufacturing process. In this study, as the steaming temperature increased and steaming time was extended, more acidic polysaccharides were solubilized, and extractable content increased.

Ginsenoside is a key element for quality evaluation of ginseng and is the primary medicinal element of ginseng [An *et al.*, 2002]. Ginsenoside Rg3 was not detected when steaming temperature was below 80°C. However, when black ginseng was steamed at 100°C for 6 h, 0.027 mg/g of ginsenoside Rg3 was detected. Further steamed at 100°C for 18 h, more ginsenoside Rg3 was generated (0.436 mg/g), indicated that ginsenoside Rg3 does not exist in white ginseng, but it can be generated through a steaming process. It has been reported that ginsenoside Rg3 content derived from the nine-time steaming process increased more than 45-fold as compared to that in red ginseng [Song *et al.*, 2006]. Kim *et al.* [2008] reported that when black ginseng was steamed at 95°C, 0.25 mg/g of ginsenoside Rg3 was obtained.

Benzopyrene is a polycyclic aromatic hydrocarbon that has recently been classified as a carcinogen. Benzopyrene was not detectable under the low-temperature steaming condition of 60°C, and a small amount (0.002-0.005 ppb) was detected under the steaming condition of 100°C. At 120°C, benzopyrene content dramatically increased to 0.204-0.511 ppb, which is an increase of more than 100-fold compared with steaming at 100°C. It has been reported that benzopyrene is generally not detected at 98°C, which is the temperature used in the red ginseng manufacturing process [Hu *et al.*, 2008]. However, when ginseng was steamed at 60°C for 18 h, benzopyrene was detected. Thus, both steaming time and steaming temperature are important factors in the induction of benzopyrene generation.

When ginseng was steamed at 60°C for 6 h, 0.81 mg% of polyphenol was detected but steaming at 120°C for 18 hours greatly increased polyphenol content to 4.16 mg%. This increase was assumed that insoluble phenol compounds are converted into free-type polyphenol that can be easily extracted and decomposed into detached phenol compounds through the steaming process [Yang *et al.*, 2006].

Drying conditions. The composition method was selected to establish the optimum drying condition for black ginseng while setting drying temperature and time as independent variables. The resulting contents of acidic polysaccharide, ginsenoside Rg3, benzopyrene, and polyphenol of black ginseng obtained from nine experimental drying conditions are shown in Table 2.

Acidic polysaccharide content of black ginseng dried at 60°C for 3 h was 11.73 mg%, while the content after drying at 100°C for 9 h was 15.45 mg%; this increase was not significant. As shown by the study of Yoon *et al.*

Table 1. Experimental data on ginsenoside Rg3, benzopyrene, acidic polysaccharide, and phenolic content under different steaming conditions based on a central composite design for response surface analysis

No.	Steaming condition		Acidic polysaccharide (mg%)	Ginsenoside Rg3 (mg/g)	Benzopyrene (ppb)	Phenolics content (mg%)
	Temp. (°C)	Time (h)				
1	120	6	28.56^{c}*	2.04^{b}	0.204^{c}	1.26^{c}
2	120	12	33.75^{b}	2.24^{b}	0.307^{b}	1.72^{b}
3	120	18	37.52^{a}	3.52^{a}	0.511^{a}	4.16^{a}
4	100	6	21.24^{d}	0.03^{d}	0.002^{ef}	0.92^{e}
5	100	12	22.64^{d}	0.25^{c}	0.001^{e}	1.7^{b}
6	100	18	31.24^{bc}	0.44^{c}	0.005^{d}	4^{a}
7	80	6	15.72^{e}	0^{e}	0^{f}	0.69^{f}
8	80	12	15.71^{e}	0^{e}	0^{f}	1.01^{de}
9	80	18	19.91^{d}	0^{e}	0.004^{d}	1.6^{b}
10	60	6	10.13^{f}	0^{e}	0^{f}	0.81^{ef}
11	60	12	10.76^{f}	0^{e}	0.001^{de}	0.81^{ef}
12	60	18	12.5^{ef}	0^{e}	0.002^{de}	1.18^{cd}

$^{a\text{-}f}$Values within a column with different superscript letters are significantly different each other groups at $p<0.05$.

Table 2. Experimental data on ginsenoside Rg3, benzopyrene, acidic polysaccharide, and phenolic content under different drying conditions based on a central composite design for response surface analysis

No.	Drying condition		Acidic polysaccharide (mg%)	Ginsenoside Rg3 (mg/g)	Benzopyrene (ppb)	Phenolics content (mg%)
	Temp. (°C)	Time (h)				
1	100	3	9.71^{de}*	0.521^{b}	0.204^{bc}	3.24^{a}
2	100	6	13.47^{b}	0.823^{a}	0.221^{b}	2.34^{bc}
3	100	9	15.45^{a}	0.854^{a}	0.304^{a}	3.16^{a}
4	80	3	8.85^{e}	0.274^{de}	0.102^{d}	1.43^{e}
5	80	6	10.81^{cd}	0.311^{d}	0.104^{d}	1.99^{cd}
6	80	9	11.87^{c}	0.401^{c}	0.157^{c}	1.63^{de}
7	60	3	11.73^{c}	0.139^{f}	0.01^{f}	1.97^{cd}
8	60	6	9.61^{de}	0.149^{f}	0.021^{ef}	2.57^{b}
9	60	9	11.77^{c}	0.212^{e}	0.043^{e}	1.92^{cd}

$^{a\text{-}f}$Values within a column with different superscript letters are significantly different each other groups at $p<0.05$.

Table 3. Analysis of selected models and regressions using polynomial equations for the response to different steaming conditions

Response	Model	Prob>F	Equation in term of coded factors
Acidic polysaccharide	Linear	0.0001	Y_{AS}= -23.2987+0.3894A+0.7513B
Ginsenoside Rg3	Quadratic	0.0009	Y_{GR}= -10.4612-0.25A-0.2289B+0.002AB+0.0015A^2+0.0036B^2
Benzopyrene	Quadratic	0.0065	Y_{BP}= 1.609-0.0374A-0.03686B+0.00038AB+0.0002A^2+0.000382B^2
Phenolics content	2FI	0.0007	Y_{PC}= 1.65325+0.81705A+0.9065B+0.73305AB

A, steaming temperature; B, steaming time.

[2005], considering that the extracted content of acidic polysaccharide in ginseng processed by heat is lower than that of ginseng processed by steaming, it can be assumed that the acidic polysaccharide extract content of black ginseng is more influenced by the steaming process than by the drying process.

Ginsenoside Rg3 content was found to increase significantly through the drying process. Ginseng dried at 60°C for 3 h produced 0.139 mg/g of ginsenoside Rg3; however, ginseng dried at 60°C for 9 h produced 0.212 mg/g because the structure of ginsenoside was changed in the steaming process. Yoon *et al*. [2005] found that the saponin content of ginseng was more influenced by heating time than by heating temperature.

Benzopyrene was not generated at 60°C in the steaming process, but 0.01-0.043 ppb benzopyrene was generated at that temperature in the drying process, with the content increasing with drying temperature. As ginseng is gelatinized, arginine and maltose are combined due to Maillard reaction. Because the Maillard reaction occurs more easily under acidic conditions and less moisture [Katano, 1988], more benzopyrene content was generated in the drying process, compared to steaming.

With regard to polyphenol, 1.97 mg% was extracted after 3 h under drying conditions of 60°C, and 1.92 mg% was extracted after 9 h at the same temperature. At 100°C, 3.24 and 3.15 mg% were extracted after 3 and 9 h, respectively. Unlike the steaming process, the higher drying process increased polyphenol content, but the extract content did not increase significantly as the drying time was extended. The study of Yoon *et al*. [2005] showed that the polyphenol content of ginseng processed thermally was influenced significantly by heating temperature. Furthermore, Yang *et al*. [2006] showed that polyphenol of ginseng processed at high temperature and high pressure was more influenced by temperature than pressure.

Response surface analysis of black ginseng ingredients. The results of the response surface analysis of acidic polysaccharide, ginsenoside Rg3, benzopyrene, and polyphenols in the steaming process are shown in Table 3. A quadratic model was selected to fit to the experimental data on ginsenoside Rg3 ($p<0.0009$) and benzopyrene content ($p<0.0065$), while the linear model was fitted to the acidic polysaccharide content ($p<0.0001$), and 2FI model was suitable for polyphenols content ($p<0.0007$). Black ginseng ingredients such as ginsenoside Rg3, benzopyrene, and polyphenols were influenced by temperature and time complex variation. With regard to acidic polysaccharide content, the steaming temperature and time influenced the incease in its content independently.

Table 4. Analysis of selected models and regressions using polynomial equations for the response of different drying conditions

Response	Model	Prob>F	Equation in term of coded factors
Acidic polysaccharide	Linear	0.0094	Y_{AS}= 2.6377+0.0627A+0.5998B
Ginsenoside Rg3	Linear	0.0011	Y_{GR}= -0.9003+0.0142A+0.0296B
Benzopyrene	Linear	0.0001	Y_{BP}= -0.3698+0.0056A+0.0104B
Phenolics content	Quadratic	0.0431	Y_{PC}= 11.9793-0.2989A+0.0929B-0.0002AB+0.0021A^2-0.0062B^2

A, drying temperature, B, drying time.

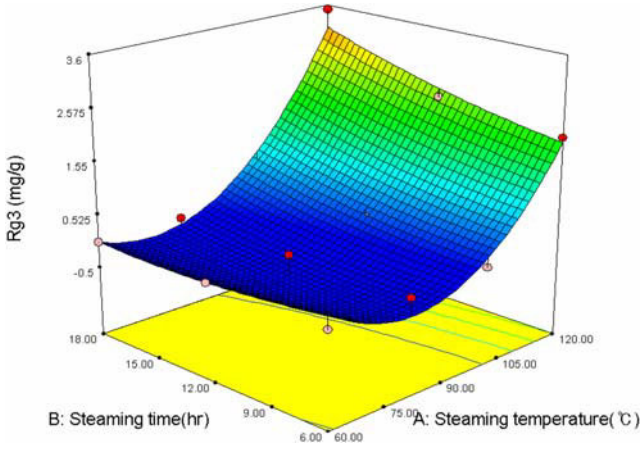

Fig. 1. Response surface for ginsenoside Rg3 of black ginseng depending on steaming temperature and time.

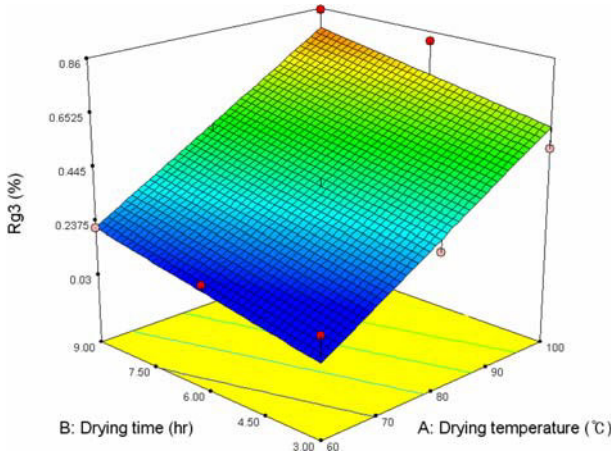

Fig. 2. Response surface for ginsenoside Rg3 of black ginseng depending on drying temperature and time.

The results of the response surface analysis of acidic polysaccharide, ginsenoside Rg3, benzopyrene, and polyphenols in the drying process are shown in Table 4. The linear model was the most suitable as a regression formula for ingredients except polyphenol. The *P*-values of the model were 0.0094, 0.0011, 0.0001 for acidic polysaccharide, ginsenoside Rg3, and benzopyrene respectively, and the significance level was suitable at 99% or more. With regard to the regression formula for polyphenol, as drying time extended, polyphenol content did not increase, and a nonlinear quadratic model was selected (p<0.0431).

According to the result of the response surface analysis for black ginseng ingredients, in the steaming process, which had more moisture than the drying process, the independent variables such as temperature and time influenced the resulting component concentrations. Especially, the ginsenoside Rg3 content increased sharply at higher temperatures and for long steaming times (Fig. 1), and the model could be identified easily as ginsenoside Rg3 content showed a planar response surface for drying temperature and time (Fig. 2). This suggests that enhancement of the black ginseng's primary ingredients can be made easily in the steaming process through higher moisture contents.

Table 5. Optimum constraint name values for steaming conditions constraint name and predicted score of responses without benzopyrene

Constraints name	Goal	Numerical optimization solution
Steaming Temperature	is in range	120°C
Steaming Time	is in range	18 hr
Acidic poly saccharide	maximize	36.95 mg%
Ginsenoside Rg3	maximize	3.15 mg/g
Polyphenol contents	maximize	4.11 mg%
Desirablity		0.953

Establishment of optimum process condition for black ginseng production. To establish the optimum manufacturing process parameters for black ginseng, the content of acidic polysaccharide, ginsenoside Rg3, and polyphenol were maximized without considering the content of benzopyrene (Table 5). The results showed the optimum steaming temperature and time to be 120°C and 18 h. This was the highest temperature and longest time of our experimental data, and all dependent variables, including acidic polysaccharide, ginsenoside Rg3, polyphenol, and benzopyrene content, had a tendency to increase in proportion to steaming temperature and time. These steaming conditions produced a ginsenoside Rg3

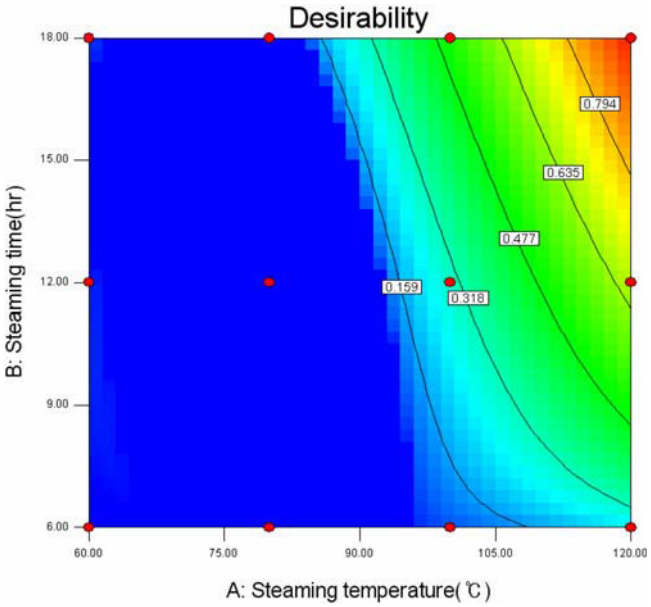

Fig. 3. Contour plot of desirability of the optimum steaming conditions without benzopyrene.

Table 6. Optimum constraint name values for steaming conditions constraint name and predicted score of responses with benzopyrene

Constraints name	Goal	Numerical optimization solution
Steaming Temperature	is in range	113.04°C
Steaming Time	is in range	18 hr
Acidic poly saccharide	maximize	34.24 mg%
Ginsenoside Rg3	maximize	2.24 mg/g
Polyphenol contents	maximize	3.75 mg%
Benzopyrene	minimize	0.31 ppb
Desirablity		0.67

content of 3.15 mg/g, polyphenol content of 4.11 mg%, and acidic polysaccharide content of 36.95 mg% (Table 5). The desirability figure-of-merit for optimization suggests that the higher the desirability value, the more appropriate the proposed parameters and desired results become [Corzo and Gomez, 2004]. As all ingredients tended to increase when steaming temperature was higher and steaming time was longer, the optimum process was selected at the 0.953 desirability level, i.e., the upper right corner of the plot (Fig. 3).

By considering the generation of benzopyrene in the optimization process, the optimal steaming temperature (113.04°C) and steaming time (18 h) could be determined (Table 6). When benzopyrene content was included in the optimization process, the optimum steaming temperature was significantly reduced by about 6.96°C, but there was no difference in steaming time. A desirability contour map of the optimum steaming conditions with the

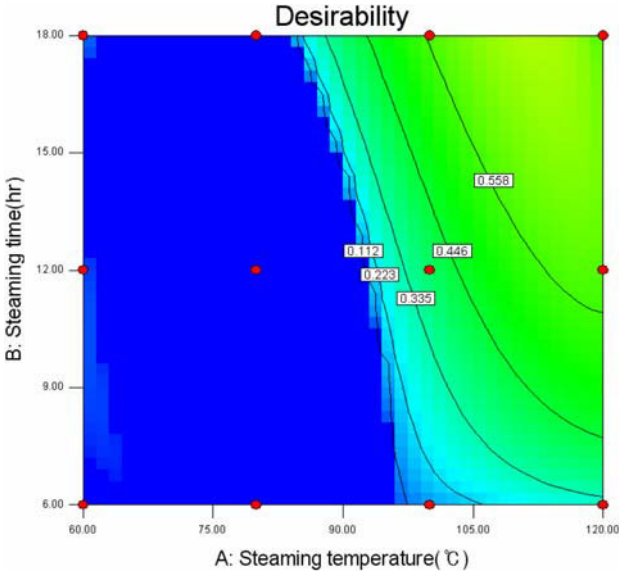

Fig. 4. Contour plot of desirability of the optimum steaming conditions with benzopyrene included.

Table 7. Optimum constraint name values for drying conditions constraint name and predicted score of responses

Constraints name	Goal	Numerical optimization solution
Drying Temperature.	is in range	100°C
Drying Time	is in range	8.03 hr
Acidic poly saccharide	maximize	13.72 mg%
Ginsenoside Rg3	maximize	0.75 mg/g
Polyphenol contents	maximize	3.24 mg%
Benzopyrene	minimize	0.26 ppb
Desirability		0.617

inclusion of benzopyrene led to selection of the steaming conditions at a desirability value of 0.67 (Fig. 4). The optimal drying process used a drying temperature of 100°C and a drying time of 8.03 h to produce acidic polysaccharide content of 13.72 mg%, ginsenoside Rg3 content of 0.75 mg/g, benzopyrene content of 0.26 ppb, and polyphenol content of 3.24 mg% (Table 7). Under these optimum drying conditions, benzopyrene content increased significantly in the drying process rather than in the steaming process, indicating that benzopyrene was affected mainly by drying time. These parameters were selected at a desirability value of 0.617 (Fig. 5).

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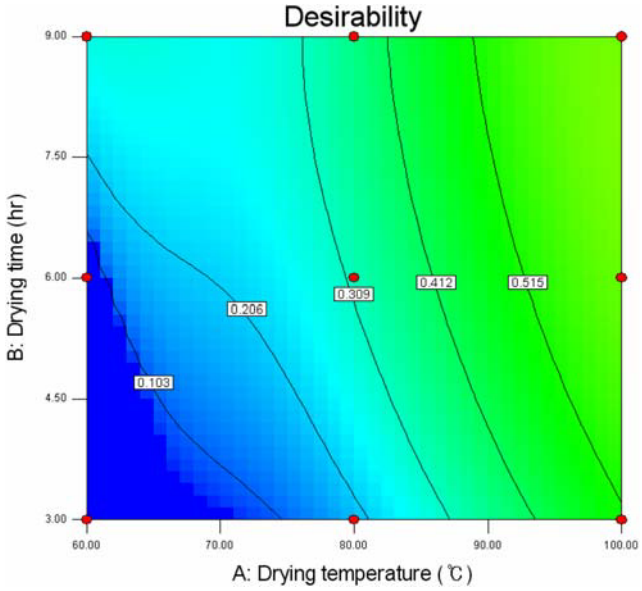

Fig. 5. Contour plot of desirability of the optimum drying conditions.

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