Processing of Noni Liquor based on Response Surface Methodology

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Abstract

Noni (*Morinda citrifolia* L.) is widely used as a health food and medicine because it is rich in polysaccharides, polyphenols, and flavonoids; it is precisely because noni is rich in these substances that people usually like to soak it in liquor to drink. This study sought to maximize the main active constituents (polysaccharides, polyphenols, and flavonoids) dissolved in liquor and spirits soaked with noni fruit, using ethanol as the solvent to optimize the extraction conditions using response surface methodology. The highest polysaccharide yield of 16.35% was found at 60°C for 3.5 h, a liquid-solid ratio of 52 mL/g, and an ethanol concentration of 25%. The optimal extraction conditions for polyphenols were 75°C for 1.5 h, a liquid-solid ratio of 90 mL/g, and an ethanol concentration of 50%, resulting in a 10.37% yield. The optimum extraction conditions for flavonoids were 75°C for 1 h, a liquid-solid ratio of 70 mL/g, and an ethanol concentration of 100%, with 1.35% yield. Many active ingredients, including polysaccharides, polyphenols, and flavonoids, were obtained via alcohol extraction of noni fruit, suggesting that liquor and spirits soaked with noni fruit are novel and promising types. This study provides a technical reference for the production of high-quality noni liquor. It is recommended to use the

31 best conditions for the extract of polysaccharides, polyphenols, and flavonoids, and blending

32 them to obtain the desired degree of alcohol.

33

34 **Subjects** Liquor, Food Science, Extracting Method

35 **Keywords** noni · polysaccharides · polyphenols · flavonoids · ethanol extraction

36

Introduction

38 Noni (*Morinda citrifolia* L.) is a perennial green tree species belonging to Rubiaceae. It is

39 distributed in some tropical and subtropical coastal regions of South Pacific islands, Hawaii,

40 Southeast Asia, West India, the Caribbean, and South America (*Wu et al., 2019*). As a

41 multifunctional plant, it is widely used as herbal medicine, cosmetic, nutraceutical food, animal

42 feed, dyestuff, and lignum. Each part of noni such as flower, fruit, seed, bark, stem, leave, and

43 root has a unique efficacy and value (*Arunachalam, 2018*). Notably, the noni fruit is an abundant

44 source of diverse and valuable phytochemicals. The polysaccharides, polyphenols, and

45 flavonoids fractions in noni fruit are of interest due to the superior medicinal value. The

46 polysaccharides of noni fruit exhibit significant antitumor, anti-inflammatory, and

47 antinociceptive activities (*Jin et al., 2019*). Polyphenols are the most active and standard

48 pharmacological component of noni fruit with antioxidant, free radical-scavenging, anti-

49 inflammatory, and antibacterial properties (*Krishnaiah et al., 2015; Thomaz et al., 2018*). In

50 addition, the hepatoprotective (*Nayak et al., 2011*), immunomodulatory (*Lohani and Madhukar,*

51 *2010*), and antioxidant properties (*Dussossoy et al., 2011*) of noni fruit are attributed to its

52 flavonoid content. The active ingredients extracted from noni are utilized in different fields such

53 as medicine, toiletry, nutritional and health products.

54   Liquor and spirits can be used to effectively deliver active ingredients. Responsible

55 drinking practices are another approach to maintain good health. Liquor and spirits are used

56 universally as solvents to soak active ingredients derived from plants, animals, and insects dating

57 back to ancient China. They are regarded as traditional preparations including medicinal liquors

58 or tonic wines for healing and prophylactic applications (*Nikolova et al., 2018*), similar to

59 tinctures used in western medicine. The medicinal liquors, such as notoginseng (*Panax*

60 *notoginseng*) (*Liao et al., 2017*) and ginseng (*Panax ginseng*) (*Li et al., 2019*), occupy an

61 important position in Chinese medicine and culture, and play an integral role in daily medical

62 care in China or the Asia. For instance, individuals living in peat swamps in Narathivas province,

63 Thailand, still use their unique tropical plants soaked in liquor for drinking or smearing on the

64 diseased areas as febrifuges, antidiarrheas, tonics, and to treat skin injury. (*Kitirattrakarn and*

65 *Anantachoke, 2003*). Medicinal liquors derived from ginseng, *Schisandrae fructus*, *Angelica*

66 *sinensis*, and *Chrysanthemum indicum* are popular in Korea (*Kim et al., 2014*). Additionally, in

67 Poland, the bison grass was soaked in vodka used for flavoring alcohols, which represents the

68 origin of Zubrowka Bison Brand Vodka (*Bączek et al., 2014*).

69 Medicinal liquor is very popular in China due to its simple preparation, low cost, and

70 curative effect. With the introduction of noni in China, the knowledge and awareness of its

71 health function have led to preparation of medicinal liquor with dried noni slices. However,

72 improper preparation methods can diminish the efficacy of noni, which can prevent large-scale,

73 standardized production. Ramamoorthy and Bono (*2007*) generated high content of total phenols

74 and flavonoids by comparing different methods of high-pressure and ultrasonic extraction.

75 Sasmito *et al* (*2015*) evaluated the fractionation of noni polysaccharides using the precipitation

76 method, and Jin *et al*. (*2019*) extracted polysaccharides of noni fruit with hot water. Nonetheless,

77 few studies have investigated the technological conditions of noni fruit soaked in alcohol.

78 Therefore, the appropriate conditions for extracting polysaccharides, polyphenols, and flavonoids

79 from noni fruit should be investigated.

80 Bai *et al* (*2011*) used the response surface method (RSM) to optimize supercritical CO_2

81 extraction of noni seed oil. In addition, they optimized and developed a simple and rapid

82 blitzkrieg extraction procedure using RSM to increase the yield of hexane extraction from noni

83 seed (*Bai et al., 2011*). Wang *et al* (*2021*) optimized the extraction parameters of noni fruit tea

84 polysaccharides and polyphenols using RSM. In this study, we investigated the temperature,

85 extraction time, the concentration of ethanol, and the liquid-solid ratio using RSM based on

86 single-factor experiments to determine the optimal conditions for polysaccharides, polyphenols,

87 and flavonoids derived from ethanol soaked with a dry piece of noni fruit. These results facilitate

88 the screening and processing of noni fruit liquor containing the most active ingredients, and

89 promote the development of noni and medicinal liquor industry.

90

91 **Materials & Methods**

92 **Chemicals and reagents**

93   The chemicals used in this study were purchased from Sigma-Aldrich, including Folin-Ciocalteu

94   reagent, tea polyphenols, and rutin standard. Ethanol, sulfuric acid, phenol, sodium nitrite,

95   glucose, sodium carbonate, aluminum nitrate, sodium hydroxide, aluminum chloride, and sodium

96   acetate were analytical grades.

97   **Plant material**

98   The ripe noni fruit was harvested from Yuanjiang, Yunnan Province, China, on August 17, 2019.

99   The fruit was cut into slices of about 8 mm and dried in a 60℃ oven until a constant weight was

100   achieved. The dry noni slices were pulverized with an automatic pulverizer and sieved through

101   an 80-mesh. The noni powders were stored in the dark at room temperature with silica-gel

102   desiccant until used.

103   **Extraction procedures**

104   A single-factor design was used to identify the primary range of extraction process variables.

105   The noni powders (0.5 g) were extracted in an ethanol solvent at different liquid-solid ratios (10

106   mL/g, 20 mL/g, 30 mL/g, 40 mL/g, 50 mL/g, 60 mL/g, 70 mL/g, 80 mL/g, and 90 mL/g) and

107   concentrations (25%, 50%, 60%, 75%, and 100%) for varying times (0.5 h, 1 h, 1.5 h, 2 h, 2.5 h,

108   and 3 h) at specific temperatures (30 ℃, 40 ℃, 50 ℃, 60 ℃, and 70 ℃), and then sonicated (45

109   kHz) for 12 min. The crude extracts were separated from the residue via centrifugation (4000

110   rpm, 10 min) and then filtered through Whatman No. 1 filter paper. The extraction of the residue

111   was repeated under the same conditions.

112   **Experimental design**

113   The extraction optimization of noni polysaccharides, polyphenols, and flavonoids was carried

114   out under three operational parameters of Box–Behnken design (BBD): liquid-solid ratio,

115   extraction time, and extraction temperature. The response factors coded as X_1, X_2, and X_3, were

116   evaluated at three levels coded as -1, 0, and 1. The range of parameters was confirmed via single-

117   factor experiments, and the ethanol concentration was fixed at 25%, 50%, and 100% for

118   polysaccharides, polyphenols, and flavonoids, respectively, resulting in the highest yield. Table 1

119   shows the ranges and medians of the three operational parameters based on the above

120   experimental results.

121   **Determination of extraction yield**

122   The yield of polysaccharides, polyphenols, and flavonoids (%) was calculated using the

123   following formula:

124 $$\text{Extraction yield (\%)} = \frac{C \times N \times V}{W} \times 100\%$$ (Eq.1)

125   In the formula, C is the concentration of polysaccharides, polyphenols, and flavonoids

126 calculated using the calibrated regression equation (μg/mL); N denotes the dilution ratio; V

127 represents the total volume of crude extract solution (mL), and W is the weight of noni powders

128 (g).

129 **Determination of total polysaccharide content**

130 The total polysaccharide content of noni extract was determined via slight modification of

131 phenol–sulfuric acid method (*Han et al., 2016*), using glucose as the standard. Each 0.1 mL

132 sample was mixed with 0.9 mL of distilled water and 1 mL of 5% phenol after rapid mixing with

133 3.5 mL of concentrated sulfuric acid. The mixture was heated at 100℃ for 15 min, and then

134 cooled down to room temperature. Next, the UV absorption at 490 nm was monitored, and the

135 straight line was represented by the equation Y=0.012X+0.0213, R^2=0.9953.

136 **Determination of total polyphenol content**

137 The total polyphenol content of noni extract was determined using a slightly modified Folin-

138 Ciocalteu method (*Hannachi et al., 2019*), taking tea polyphenols as the standard. Each 0.5 mL

139 sample was mixed with 0.5 mL of distilled water and 1 mL of Folin-Ciocalteu reagent, and

140 allowed to stand for 5 min. Next, 2 mL of 10% sodium carbonate solution was added and left in

141 the dark at room temperature for about 60 min. Finally, the UV absorption at 760 nm was

142 monitored, and the straight line was represented by the equation Y=0.0044X+0.09, R^2=0.9991.

143 **Determination of total flavonoid content**

144 The total flavonoid content was determined using a slightly modified colorimetric method

145 (*Zheng et al., 2019*), and rutin as the standard. Each 5 mL sample was mixed with 0.3 mL of 5%

146 sodium nitrite solution and maintained for 6 min, and subsequently mixed with 0.3 mL of 10%

147 aluminum chloride solution followed by the addition of 2 mL of 4% sodium hydroxide solution

148 after 6 min. The solution was mixed well and allowed to sit for 10 min. The UV absorption at

149 504 nm was monitored, and the straight line was represented by the equation

150 Y=0.0041X+0.0629, R^2=0.9979.

151 **Statistical analysis**

152 Statistical analysis was conducted using IBM SPSS Statistics (Version 22) to determine the

153 effect of individual factors on the yield of polysaccharides, polyphenols, and flavonoids (single-

154 factor experiment). Design-Expert software (Version 8.0.6) was used to construct Box–Behnken

155 design and assess the effect of linear and quadratic interactions of the operational parameters on

156 the extraction of polysaccharides, polyphenols, and flavonoids. All tests were performed in

157 triplicate.

158

Results and Discussion

Results of one-factor experiment

161 In this single-factor experiment, the polysaccharide extraction yield was decreased by the

162 increase in ethanol concentration (Fig. 1 a). This is consistent with the study of Samavati that is

163 as the concentration of alcohol increases, the viscosity of the alcohol solution increases, and the

164 extraction rate of polysaccharides decreases. (*2013*). The extraction time was 3 h when the

165 ethanol concentration was 25%, and the extraction temperature was 50℃. The polysaccharide

166 extraction rate was the highest at a liquid-solid ratio of 40 mL/g (Fig. 1 b, c, d).

167 High levels of ethanol concentration decreased the polyphenol yield. A similar phenomenon was

168 reported during the extraction of polyphenols from olive (*Martínez-Patiño et al., 2019*) and

169 sunflower seed cake (*Zardo et al., 2019*) under the optimal conditions of 50% (Fig. 1 a). The

170 optimal extraction temperature, extraction time, and liquid-solid ratios were 70℃, 2 h, and 80

171 mL/g, respectively (Fig. 1 b, c, d).

172 The extraction yield of flavonoids was significantly elevated at the highest ethanol concentration

173 (Fig. 1 a). Compared with a study by Yusof *et al* (*2019*) who reported that the flavonoid yield

174 increased with the ethanol concentration and reduced after reaching a concentration of 80%, the

175 disparity could be explained by the difference in material. Likewise, when the extraction

176 temperature was at its highest, the extraction rate was also at its peak (Fig. 1 b). The optimal

177 extraction time and liquid-solid ratio were 1.5 h and 80 mL/g, respectively (Fig. 1 c, d).

178 These data were employed as fixed parameters in the optimization experiment.

179

Optimization of polysaccharide conditions

181 The extraction yield of polysaccharides was considered as a dependent variable in the Box–

182 Behnken design. Under the experimental conditions of 15 runs, the results from polysaccharides

183 varied in the range of 14.42% to 16.25% (Table 2). The regression equation of the predicted

184 response Y for polysaccharides yield was obtained as follows:

$$Y = 15.50 + 0.041X1 + 0.15X2 + 0.0025X3 + 0.24X1X2 - 0.048X1X3$$
$$+ 0.78X2X3 - 0.75X1^2 - 0.022X2^2 - 0.060X3^2 \qquad \text{(Eq.2)}$$

185

186   The significance and fit of the model were interpreted via analysis of variance (Table 3).

187 Statistical significance ($p < 0.05$) suggested the construction of the final predictive model

188 (*Mojerlou and Elhamirad, 2018*). The RSM analysis indicated that the model was significant (p

189 < 0.05) for polysaccharides, although the lack-of-fit of the model was not significant ($p > 0.05$),

190 which suggested that the experiment and the response surface quadratic model fit satisfactorily,

191 and the extraction yield of polysaccharides could be analyzed and predicted using this model

192 within the design range. The coefficient of determination was 0.9628, which was closer to 1,

193 meaning a better association between the observed and predicted values (*Pan et al., 2012*).

194 Further, the model performance was also evaluated using the coefficient of variation, which was

195 1.3% (C.V. < 5%), suggesting that the experimental values were highly accurate and reliable.

196 The terms X2X3 and X1^2 were highly significant ($p < 0.001$), indicating that the interaction of

197 temperature with time played a key role in polysaccharide extraction and the quadratic term of

198 liquid-solid ratio dominated the exponential change in the extraction yield.

199   The 3D response plot showed that the polysaccharides yield increased rapidly with the

200 increase in the liquid-solid ratio of 40-50 mL/g, and the polysaccharide yield decreased above

201 the liquid-solid level of 50 mL/g (Fig. 2 a, b). In addition, the polysaccharide yield along with

202 the temperature increased under 3 h at a liquid-solid ratio of 50-60 mL/g (Fig. 2 a). Temperature

203 positively affects the transfer efficiency of the water-soluble polysaccharides from cells (*Qu et*

204 *al., 2016*). The liquid-solid ratio of 50 mL/g and the extraction time of 2.5 h contributed to the

205 high polysaccharides yield of 16.15% when the temperature was reduced to 40°C (Fig. 2 c).

206 Conversely, the liquid-solid ratio of 50 mL/g and the temperature of 60°C contributed to the

207 highest polysaccharide yield of 16.25% when the extraction time was increased to 3.5 h. This

208 result suggested that the interaction between temperature and time significantly affected

209 polysaccharide extraction. An increase in temperature and duration of extraction enhanced the

210 polysaccharide yield as reported by Chaiwut *et al* (*2019*).

211 **Optimization of polyphenol conditions**

212 The polyphenol levels changed from 8.21% to10.09% (Table 2). A second-order polynomial

213 equation of the predicted response Y for polyphenol yield was as follows:

$$Y = 8.55 + 0.58X1 + 0.24X2 - 0.23X3 - 0.030X1X2 - 0.27X1X3$$
$$- 0.18X2X3 + 0.13X1^2 - 0.057X2^2 + 0.29X3^2 \qquad \text{(Eq.3)}$$

214

215   This model was significant ($p < 0.05$) for polyphenols, and the lack of fit of the model

216 was not significant ($p > 0.05$) (Table 3). The coefficient of determination was 0.9607, and the

217 coefficient of variation was 2.17% (C.V. < 5%). In addition, the terms X1, X2, X3, X1X3, and

218 $X3^2$ were significant, indicating that the role of the three-factor variables (liquid-solid ratio,

219 temperature, and time) was vital in polyphenol extraction.

220   The effects of extraction temperature and liquid-solid ratio on extraction rate showed a

221 similar trend (Fig. 3 a). The extraction time showed a significant interaction with the liquid-solid

222 ratio; in the response surface, the polyphenol extraction was enhanced by a higher liquid-solid

223 percentage under time. An increase in liquid-solid ratio from 70 mL/g to 90 mL/g, or a decrease

224 in extraction time from 2.5 h to 1.5 h boosted the polyphenol yield with a maximum recovery of

225 10.09% at 70℃ (Fig. 3 b). Yusof *et al* (*2019*) defined a region of low response variability as an

226 optimal region, which was supposed to be within the liquid-solid ratio above 80 mL/g, a

227 temperature higher than 70°C and between 1.5 and 2 h. Azahar *et al* (*2017*) reported a similar

228 maximum yield of phenolics at the higher temperature of 75-80°C.

229   At the same time, the polyphenols extraction time was reduced to 80-100 min. Additional

230 extraction time does not necessarily lead to higher extraction rate (Fig. 3 c), which might be

231 attributed to the change of adsorption surface and the diffusion of additional polyphenols into the

232 solvent with high liquid-to-solid ratio (*Hannachi et al., 2019*). The high temperature might soften

233 the cell wall, hydrolyze polyphenols, increase the diffusion coefficient and enhance the solubility

234 of polyphenols. The short extraction time prevents the degradation of polyphenols and reduces

235 the types of polyphenols extracted (*Sai-Ut et al., 2015*).

236 **Optimization of flavonoid conditions**

237 The extraction ratio of flavonoids was in the range of 0.89-1.09% (Table 2). The mathematical

238 models were used to predict flavonoid yield using the following second-order polynomial

239 equation:

$$Y = 1.04 - 0.010X1 + 0.065X2 - 0.020X3 - 0.075X1X2 + 0.055X1X3$$
$$- 0.095X2X3 - 0.014X1^2 - 0.014X2^2 + 0.005833X3^2 \qquad \text{(Eq.4)}$$

240

241   In addition to the lack of fit ($p > 0.05$), a determination coefficient of 0.9147 and

242 coefficient of variation (C.V. < 5%) fully demonstrated the reliability of the model. The

243   interaction between liquid-solid ratio with temperature (X1X2) and temperature with time
244   (X2X3) had a significant impact on flavonoid extraction ($p < 0.05$). The interaction between
245   liquid-solid ratio and time has little effect on the extraction rate (Fig .4 b). The temperature
246   showed a significant effect on the extraction yield of flavonoids. Increase in temperature from 65
247   to 75°C (for an extraction time of 1 h under liquid-solid ratio 80 mL/g) increased the flavonoid
248   yield from 0.92% to 1.23%, or the increase in temperature from 65 to 75°C under an extraction
249   time of 1.5 h and a liquid-solid ratio of 70 mL/g, with the flavonoid yield increasing from 0.89%
250   to 1.13% (Fig .4 a, c). With other conditions unchanged, the increase in temperature led to an
251   increase in the yield. Mohd-Setapar et al (*2014*) reported that the higher temperature had a
252   positive effect on the diffusion of the solute, the viscosity and surface tension of the solution, the
253   solubility of the target compound in the solvent, and the extraction efficiency. In addition, the
254   extraction time and the liquid-solid ratio were not linearly correlated with the extraction yield of
255   flavonoids. The 3D response plot indicated that a liquid-solid ratio below 80 mL/g, a temperature
256   higher than 70°C and extraction time ranging between 1 and 1.5 h were the optimal parameters.
257   **Experimental validation of optimal conditions**
258   The optimal conditions for the extraction of polysaccharides, polyphenols, and flavonoids
259   including the liquid-solid ratio, extraction temperature, and extraction time, were determined
260   from the constructed model. To determine the validity of the quadratic model for predicting the
261   yield of polysaccharides, polyphenols, and flavonoids, experimental verification was performed
262   under optimal conditions, and the yields were confirmed. The predicted extraction yield of
263   polysaccharides was 16.37% with 25% ethanol at 60°C for 3.5 h, and the liquid-solid ratio was
264   51.88 mL/g. Considering the actual production conditions, the liquid-solid ratio was revised to
265   52 mL/g, resulting in an experimental yield of 16.35±0.05%. The predicted extraction yield of
266   polyphenols was 10.39% with 50% ethanol at 75°C for 1.5 h, and the liquid-solid ratio was 90
267   mL/g. The experimental yield of polyphenols was $10.37 \pm 0.05\%$. Further, the predicted
268   extraction yield of flavonoids was 1.34% with 100% ethanol at 75°C for 1 h, and the liquid-solid
269   ratio was 70 mL/g. The experimental yield of flavonoids was $1.35 \pm 0.02\%$. All the predicted
270   results were consistent with the experimental results, suggesting model reliability for
271   optimization of extraction.
272   **Lack of experimentation**

273 In the model constructed in this study (Table 3), the Adj R^2 values were less than 0.9 for

274 polysaccharides, polyphenols, and flavonoids. The Pred R² values were also not in reasonable

275 agreement with the Adj R², in which the difference was greater than 0.2, although the R² values

276 were closer to 1. Anomalies in these values suggest model inadequacy (*Koocheki et al., 2009;*

277 *Sulaiman et al., 2017*), which may be due to experimental error. The dependent variable in the

278 quadratic regression model was the mean value of the experimental data, and there were no

279 repeated groups. Using stepwise regression and after deleting non-significant terms, in case of

280 polysaccharides, the value of R^2 was 0.9582; the value of Adj R^2 was 0.9269, and the value of

281 Pred R² was 0.8657. Likewise, in polyphenols and flavonoids, the values tended to be normal.

282 The values of Adj R^2 was also similar to the values of Pred R² after deletion of insignificant

283 terms, suggesting that the model was reasonable. However, the reduced regression quadratic

284 model failed to display a few interactions. Therefore, we still preferred to use the original model

285 for response surface analysis.

286 **Conclusions**

287 The overall results indicated the feasibility and success of RSM for optimal extraction of active

288 ingredients in noni via RSM, which was accurate and reliable in identifying the optimal

289 parameters for the extraction yield of polysaccharides, polyphenols, and flavonoids from noni

290 fruit. Ethanol concentration, extraction temperature, time, and liquid-solid ratio were essential

291 parameters influencing extraction when noni fruit soaked in liquor was used to obtain

292 polysaccharides, polyphenols, and flavonoids. The use of noni powders was recommended for

293 rapid preparation of noni fruit liquor. To obtain noni fruit liquor with high levels of

294 polysaccharides, polyphenols, and flavonoids, we recommend the best extraction conditions

295 according to the 3D response plot, followed by a combination of the active ingredients. Finally,

296 the mixture was adjusted to the appropriate alcohol content (ethyl alcohol number of degrees).

297 The alcohol content, polysaccharides, polyphenols, and flavonoids in the mixture is clearly

298 visible. The optimized extraction processes reported in the study can be used to manufacture

299 noni fruit liquor commercially.

300

301 **ADDITIONAL INFORMATION AND DECLARATIONS**

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307

Conflict of interest

309 We declare that we have no conflict of interest.

310

Author Contributions

312 Fei Yang and Qingfen Wang experimented, Shusen Gong analyzed the data, wrote the
313 manuscript, and approved the final draft.
314 Tian Wu designed the experiment, modified the manuscript, and approved the final draft.

315

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318

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443

Figure captions

Table 1 The levels of parameters of BBD for extraction experimental.

Table 2 Design and results of RSM experiment for extraction yield of polysaccharide, polyphenol and flavonoid.

Table 3 Variance analysis for fitting quadratic model of extraction yield of polysaccharide, polyphenol and flavonoid.

Fig. 1 The results of a one-way experiment(a: Relationship between Ethanol Concentration and Extraction rate; b: The relationship between extraction temperature and extraction rate; c: The relationship between extraction time and extraction rate; d: The relationship between Liquid to Solid Ratio and extraction rate).

Fig. 2 The 3D response surface plot and contour plot of polysaccharide extraction yield (a: Liquid-solid ratio & Temperature, while time was constant at 3 h; b: Liquid-solid ratio & Time, while temperature was constant at 50 ℃; c: Temperature & Time, while liquid-solid ratio was constant at 50 mL/g).

Fig. 3 The 3D response surface plot and contour plot of polyphenol extraction yield (a: Liquid-solid ratio & Temperature, while time was constant at 2 h; b: Liquid-solid ratio & Time, while temperature was constant at 70 ℃; c: Temperature & Time, while liquid-solid ratio was constant at 80 mL/g).

Fig. 4 The 3D response surface plot and contour plot of flavonoid extraction yield (a: Liquid-solid ratio & Temperature, while time was constant at 1.5 h; b: Liquid-solid ratio & Time, while temperature was constant at 70 ℃; c: Temperature & Time, while liquid-solid ratio was constant at 80 mL/g).

Figure 1

The results of a one-way experiment.

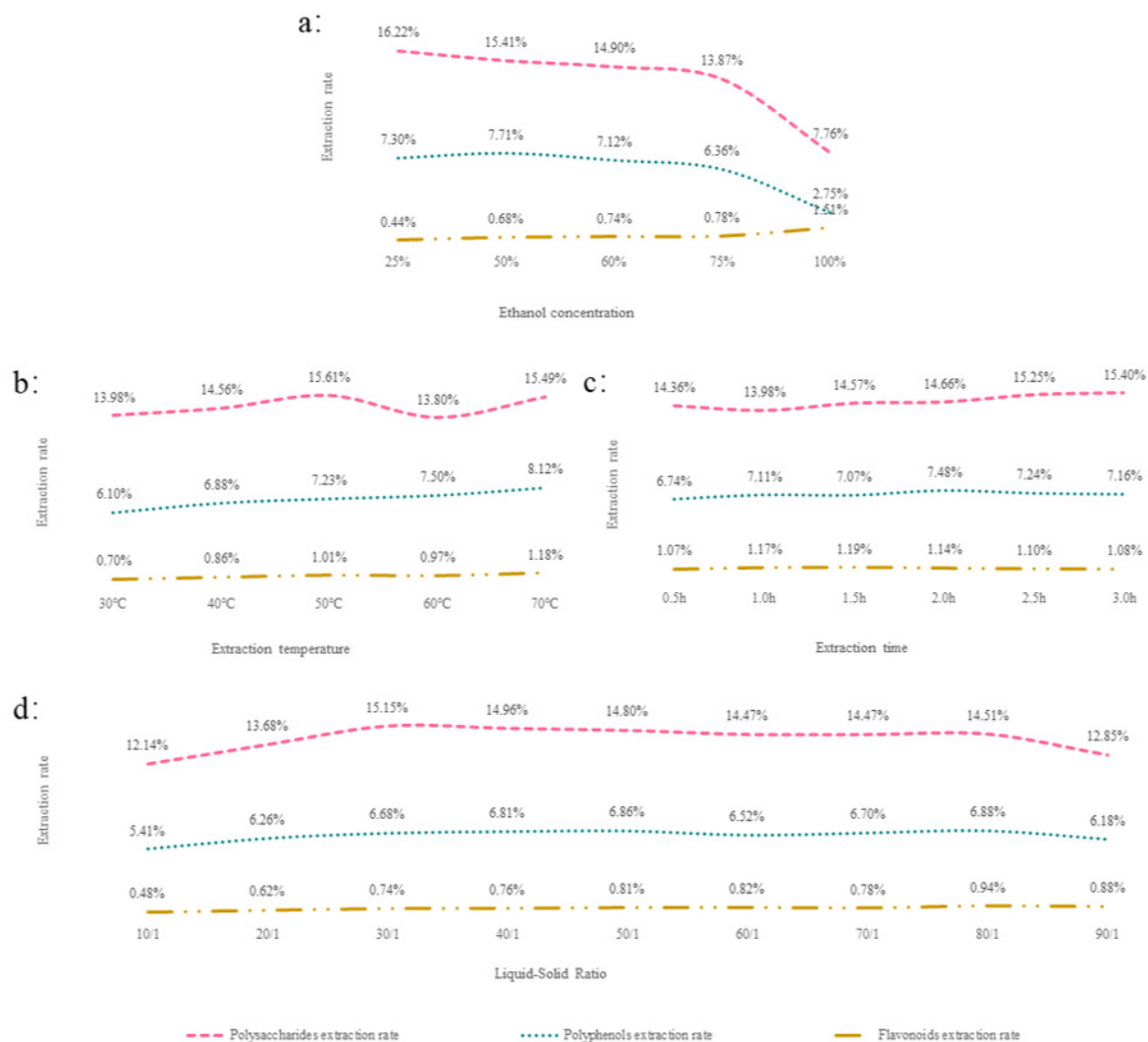



Figure 2

The 3D response surface plot and contour plot of polysaccharide extraction yield.

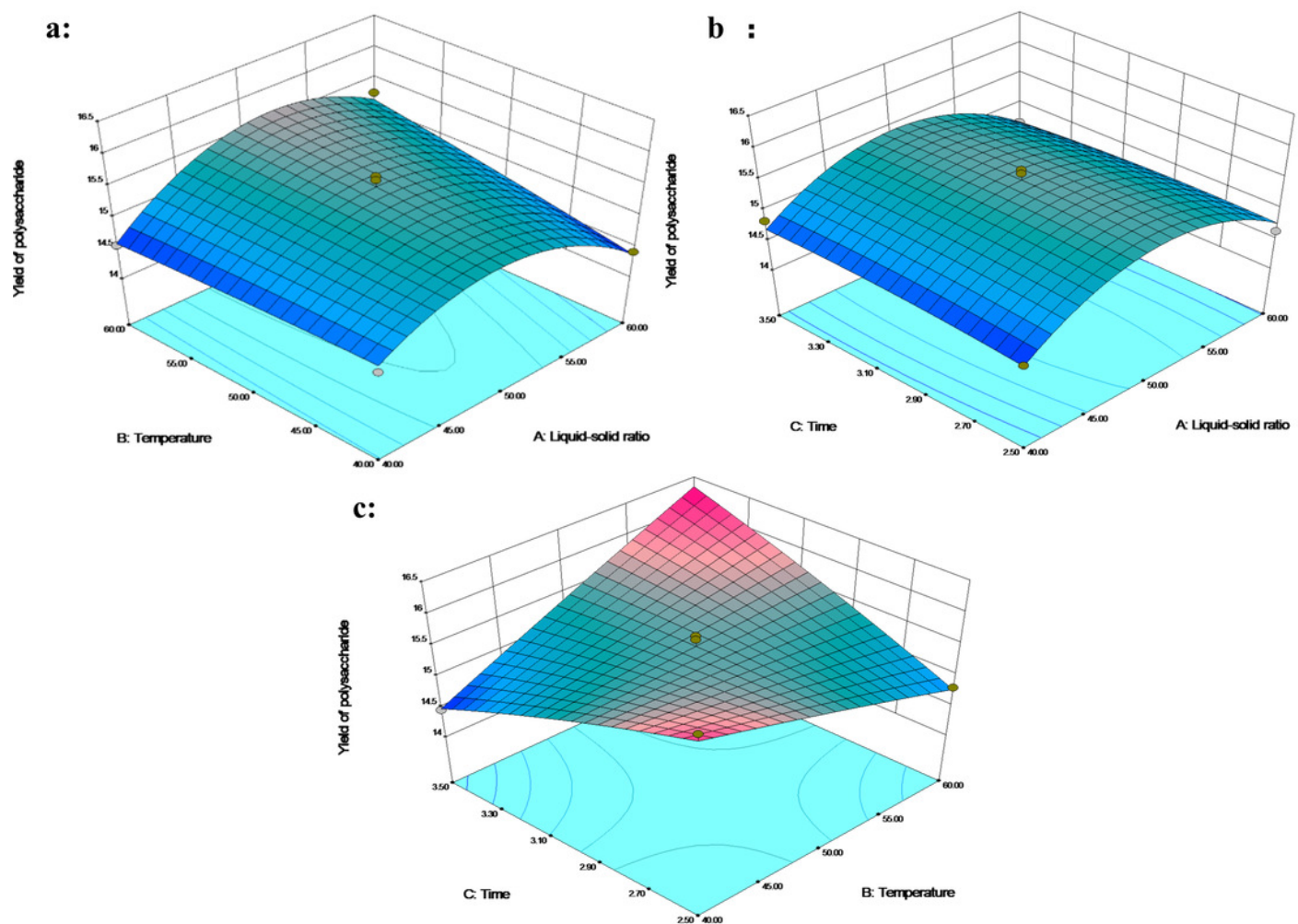



Figure 3

The 3D response surface plot and contour plot of polyphenol extraction yield.

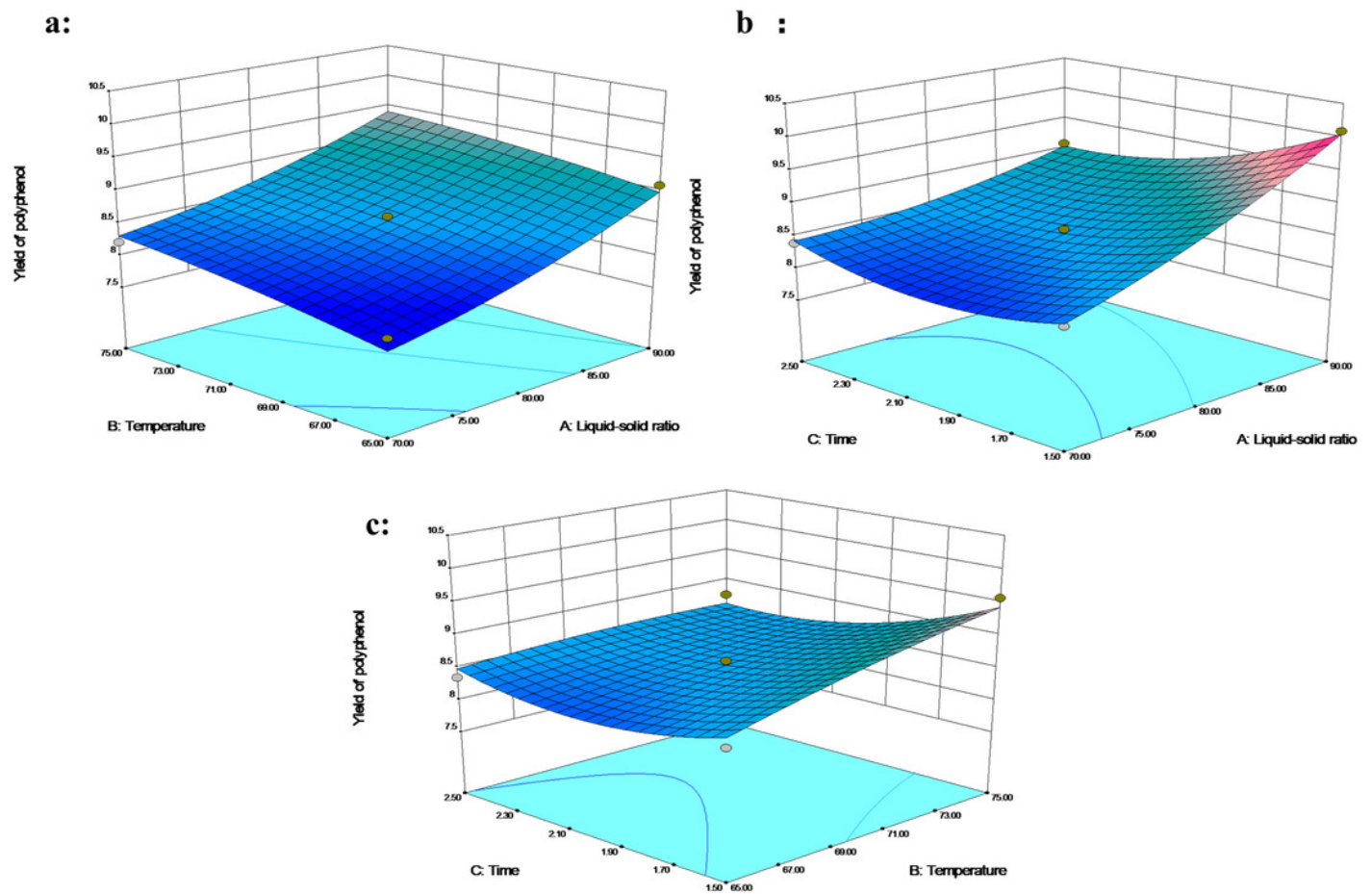
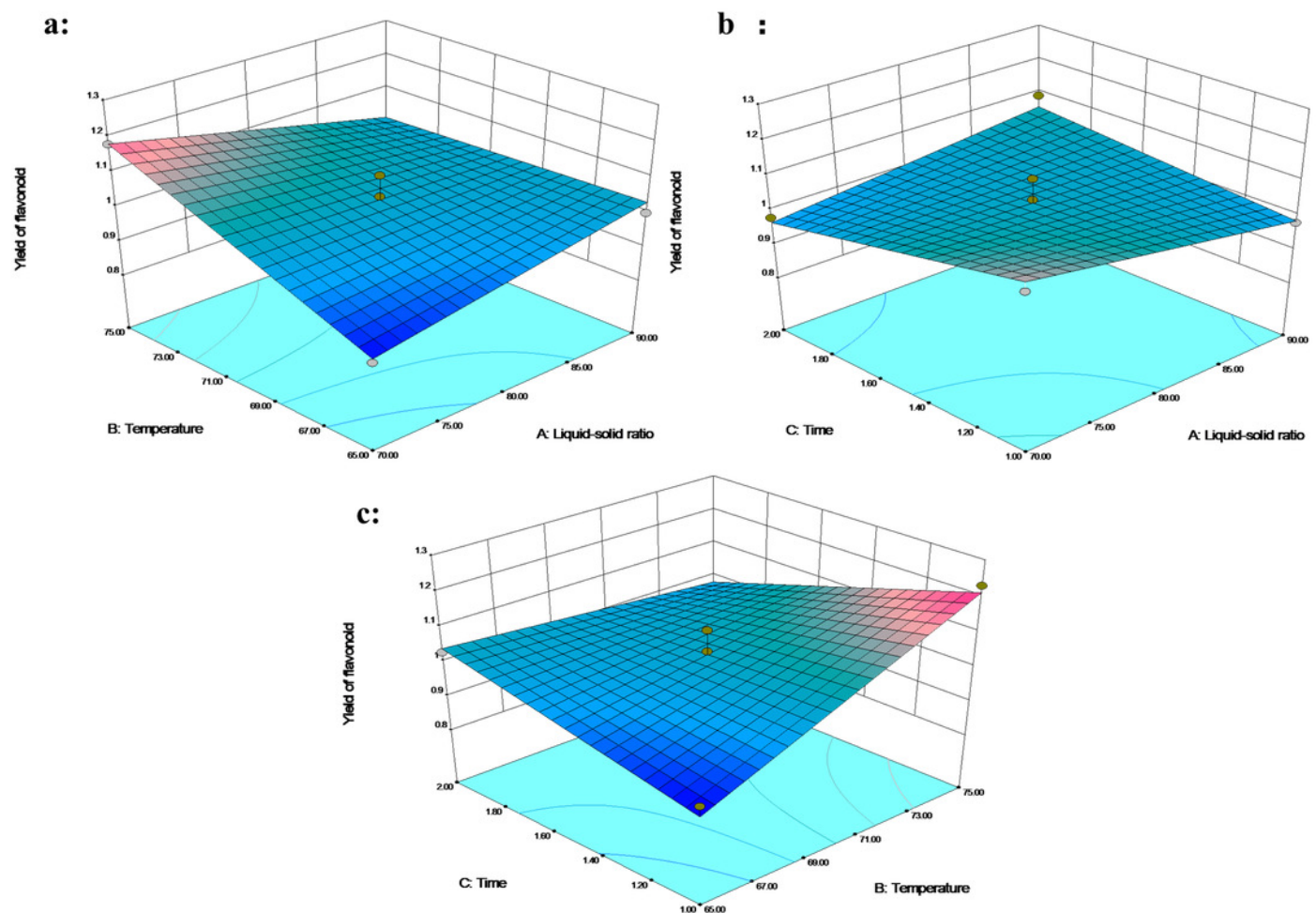



Figure 4

The 3D response surface plot and contour plot of flavonoid extraction yield.

The levels of parameters of BBD for extraction experimental.

1 **Table 1:**

2 **The levels of parameters of BBD for extraction experimental.**

Parameters		Codes	Levels		
			-1	0	1
Extraction of polysaccharide	Liquid-solid ratio (mL/g)	X1	40	50	60
	Temperature (℃)	X2	40	50	60
	Time (h)	X3	2.5	3.0	3.5
Extraction of polyphenol	Liquid-solid ratio (mL/g)	X1	70	80	90
	Temperature (℃)	X2	65	70	75
	Time (h)	X3	1.5	2.0	2.5
Extraction of flavonoid	Liquid-solid ratio (mL/g)	X1	70	80	90
	Temperature (℃)	X2	65	70	75
	Time (h)	X3	1.0	1.5	2.0

Design and results of RSM experiment for extraction yield of polysaccharide, polyphenol and flavonoid.

1   **Table 2:**

2   **Design and results of RSM experiment for extraction yield of polysaccharide, polyphenol and**

3   **flavonoid.**

No.	Coded parameter levels			Observed			Predicted		
	X1 (mL/g)	X2 (℃)	X3 (h)	Yield of polysaccharide (%)	Yield of polyphenol (%)	Yield of flavonoid (%)	Yield of polysaccharide (%)	Yield of polyphenol (%)	Yield of flavonoid (%)
1	-1	0	-1	14.61	8.3	1.09	14.6	8.34	1.12
2	0	0	0	15.6	8.49	1.04	15.5	8.55	1.03
3	1	1	0	15.27	9.23	0.99	15.16	9.41	1.01
4	0	0	0	15.24	8.61	1.1	15.5	8.55	1.03
5	-1	-1	0	14.67	7.95	0.89	14.78	7.77	0.9
6	0	0	0	15.66	8.54	0.99	15.5	8.55	1.03
7	0	1	-1	14.81	9.57	1.23	14.78	9.43	1.21
8	1	0	-1	14.64	10.09	0.98	14.78	10.05	0.99
9	-1	0	1	14.84	8.39	0.98	14.7	8.43	0.97
10	1	0	1	14.68	9.08	1.09	14.69	9.04	1.06
11	0	-1	-1	16.15	8.44	0.92	16.05	8.58	0.89
12	-1	1	0	14.56	8.21	1.18	14.6	8.31	1.18
13	0	1	1	16.25	8.75	0.96	16.35	8.61	0.98
14	0	-1	1	14.46	8.35	1.03	14.49	8.49	1.04
15	1	-1	0	14.42	9.09	1	14.38	8.99	1.03

Variance analysis for fitting quadratic model of extraction yield of polysaccharide, polyphenol and flavonoid.

1 **Table 3:**

2 **Variance analysis for fitting quadratic model of extraction yield of polysaccharide, polyphenol and**

3 **flavonoid.**

Source	Sum of Square	Degree of Freedom	Mean Square	F-value	P-value
Extraction of polysaccharide					
Model	4.95	9	0.55	14.39	0.00045**
X1	0.014	1	0.14	0.36	0.5766
X2	0.018	1	0.18	4.63	0.0840
X3	5.000E-005	1	5.000E-005	1.309E-003	0.9725
X1X2	0.23	1	0.23	6.03	0.0575
X1X3	9.025E-003	1	9.025E-003	0.24	0.6475
X2X3	2.45	1	2.45	64.11	0.0005**
X1^2	2.06	1	2.06	54.00	0.0007**
X2^2	1.869E-003	1	1.869E-003	0.049	0.8337
X3^2	0.013	1	0.013	0.35	0.5809
Residual	0.19	5	0.038		
Lack of fit	0.088	3	0.029	0.57	0.6883
Pure error	0.10	2	0.052		
Correlation Total	5.14	14			
R^2	0.9628	Adj R^2	0.8959		
C.V.%	1.30	Pred R^2	0.6813		
PRESS	1.64	Adeq Precision	12.336		
Extraction of polyphenol					
Model	4.39	9	0.49	13.28	0.0052*
X1	2.69	1	2.69	74.88	0.0003**
X2	0.47	1	0.47	12.96	0.0156*
X3	0.42	1	0.42	11.65	0.0190*
X1X2	3.600E-003	1	3.600E-003	0.10	0.7644
X1X3	0.30	1	0.30	8.42	0.0337*
X2X3	0.13	1	0.13	3.71	0.1122
X1^2	0.063	1	0.063	1.75	0.2434
X2^2	0.012	1	0.012	0.33	0.5879
X3^2	0.31	1	0.31	8.52	0.0331*
Residual	0.18	5	0.036		
Lack of fit	0.17	3	0.057	15.82	0.0600*
Pure error	7.267E-003	2	3.633E-003		

Correlation Total	4.57	14			
R^2	0.9607	Adj R^2	0.8900		
C.V.%	2.17	Pred R^2	0.3931		
PRESS	2.78	Adeq Precision	14.730		
Extraction of flavonoid					
Model	0.11	9	0.012	5.96	0.0318*
X_1	8.000E-004	1	8.000E-004	0.39	0.5599
X_2	0.034	1	0.034	16.46	0.0098*
X3	3.200E-003	1	3.200E-003	1.56	0.2672
X1X2	0.022	1	0.022	10.96	0.0212*
X1X3	0.012	1	0.012	5.89	0.0596
X2X3	0.036	1	0.036	17.58	0.0085*
X1²	7.410E-004	1	7.410E-004	0.36	0.5742
X2²	7.410E-004	1	7.410E-004	0.36	0.5742
X3²	1.256E-004	1	1.256E-004	0.061	0.8145
Residual	0.010	5	2.053E-003		
Lack of fit	4.200E-003	3	1.400E-003	0.46	0.7383
Pure error	6.067E-003	2	3.033E-003		
Correlation Total	0.12	14			
R^2	0.9147	Adj R^2	0.7612		
C.V.%	4.39	Pred R^2	0.3283		
PRESS	0.081	Adeq Precision	8.919		

4   * Means significant at $P < 0.05$. **Means significant at $P < 0.001$.