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# **Optimization of ultrasound-assisted extraction of crude** polysaccharides and polyphenols from passion fruit peels

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## ABSTRACT

Introduction: This study aimed to optimize the conditions of ultrasound-assisted extraction to simultaneously obtain the highest yields of polysaccharides and polyphenols from passion fruit (Passiflora edulis) peels. Methods: Box–Behnken design (BBD) and response surface methodology were employed for the optimization. The factors and their levels studied in BBD included a solventto-solid ratio ( $X_1$ ) of 30-70 mL/g, an ultrasonic temperature ( $X_2$ ) of 40-70°C and an ultrasonic duration ( $X_3$ ) of 40-70 min. The results revealed that the optimal conditions were an  $X_1$  of 53.9 mL/g, an X<sub>2</sub> of 57.6°C, and an X<sub>3</sub> of 57.0 min. Under these optimized conditions, the predicted yields of polysaccharides and polyphenols were 36.46% and 48.35 mg gallic acid equivalent (GAE)/g, respectively. The experimental data, which were 35.76  $\pm$  1.54% and 47.51  $\pm$  1.77 mg GAE/g, respectively, agreed well with the predicted data and hence validated the good fit of the models. Conclusion: This study demonstrated that the ultrasound-assisted extraction method could be effective and ecologically benign for extracting bioactive compounds and natural ingredients from agricultural sources.

Key words: ultrasound, passion fruit, polysaccharides, polyphenols, extraction, optimization

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**INTRODUCTION** 

International University, Quarter 6, Linh 2 Passion fruit (Passiflora edulis) originates from the <sup>3</sup> American tropics and is introduced to and grown in 4 most subtropical and tropical parts of the world. Ac-<sup>5</sup> cording to Morton (1987)<sup>1</sup>, passion fruit has natural-6 ized and spread throughout the tropics and subtrop-7 ics, including Southeast Asia. Considering the cur-8 rent research trends on passion fruit, its peels are re-9 ceiving the attention of researchers because they con-<sup>10</sup> stitute approximately 50–60% of the fruit weight<sup>2</sup> 11 and are the main waste from juice processing. Passion fruit peels contain a significant amount of bioac-12 13 tive compounds such as polyphenols and functional 14 compounds such as polysaccharides<sup>3</sup>. Both bioac-15 tive polyphenols and polysaccharides have been re-16 ported to have biological effects on the body, to pro-17 tect against degenerative and chronic diseases, and to 18 inhibit mutagenesis and carcinogenesis. These sub-19 stances have also been linked to antiviral, antiallergic, <sup>20</sup> antiplatelet, and anti-inflammatory properties<sup>4</sup>.

> 21 Extraction is the most essential step for the isolation 22 and identification of polysaccharides and polyphenols. Alternative extraction techniques have evolved 24 over the last few decades as a result of their time-25 saving and environmentally benign characteristics, as 26 well as their cost-effective output of high-quality ex-<sup>27</sup> tracts<sup>5</sup>. Ultrasound-assisted extraction (UAE) is a

novel approach that has been successfully used to 28 extract a variety of substances with various advan-29 tages. Its application minimizes extraction time, re-30 duces solvent usage, and provides great repeatability. Previous investigations have demonstrated that 32 this process is a green and cost-effective alternative to 33 traditional procedures for food and natural products, 34 such as maceration, Soxhlet extraction, and Clevenger 35 distillation <sup>6–9</sup>. Due to cell disruption caused by cavi-36 tation, the use of ultrasonic energy can also aid in the extraction of plant components<sup>10</sup>. Although UAE has 38 been used to extract certain bioactive compounds or 39 polysaccharides from passion fruit peels, these sub-40 stances can be extracted individually<sup>11,12</sup>. Therefore, this study aimed to employ UAE for the simultane-42 ous extraction of both components. In addition, opti-43 mization using response surface methodology (RSM) 44 in conjunction with Box–Behnken design (BBD) was 45 also applied to determine the optimal process condi-46 tions and formulate models describing the process. 47

# **MATERIALS AND METHODS**

### Materials and chemicals

Fresh passion fruit peels were collected at a juice shop 50 in Thu Duc city, transferred to the laboratory, washed 51 and dried on the same day at 60°C overnight so that 52 the sample moisture was less than 10%. Afterward, 53

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- 54 the dried samples were ground and sieved through
- 55 500  $\mu$ m mesh to obtain a uniform powder. The pas-
- <sup>56</sup> sion fruit peel powder (PFPP) was collected, sealed in
- $_{\rm 57}\,$  small bags (50 g each), and stored in a refrigerator for
- 58 further use. Chemicals of analytical grade were used
- <sup>59</sup> for extraction and analyses.

## 60 Ultrasound-assisted extraction

The ultrasound-assisted extraction was carried out by adapting the approach of Ahmad et al.  $(2015)^{13}$ . In 62 detail, various amounts of PFPP were mixed with 20 63 mL of sodium acetate buffer (pH 5) to achieve different solvent-to-solid ratios ranging from 30-70 mL/g. 65 The mixtures were then treated in an ultrasonic bath (WUC-A10H, South Korea) at a frequency of 40 kHz 67 <sup>68</sup> in the temperature range of 40-70°C for 40-70 min. After treatment, the mixtures were quickly cooled to 69 ambient temperature and centrifuged (Z326K, Ger-70 many) for 15 minutes at 4°C and 4000 rpm. The su-71 pernatants were collected, mixed with 96% ethanol at 72 a ratio of 1:10 (v/v) and kept overnight in a refrigera-<sup>74</sup> tor for complete precipitation. The precipitated crude 75 polysaccharides were obtained by filtration, and the 76 filtrates were collected for polyphenol recovery.

# 77 Box–Behnken design and regression anal-78 ysis

<sup>79</sup> A Box–Behnken factorial design (BBD) was em<sup>80</sup> ployed for the optimization of UAE with three vari<sup>81</sup> ables: the solvent-to-solid ratio, ultrasonication tem<sup>82</sup> perature and duration. Table 1 presents the symbols,
<sup>83</sup> units, and coded and true levels of these three vari<sup>84</sup> ables. The design included 12 factorial points (1, +1)
<sup>85</sup> and 5 central points (0), while the entire set of tests
<sup>86</sup> comprised 17 runs, which were conducted in a ran<sup>87</sup> dom order with three replicates.

<sup>88</sup> The obtained data were fitted to a second-order poly-

- <sup>89</sup> nomial equation (quadratic model) as described in
- 90 Eq. (1) to correlate the relationships between the in-
- <sup>91</sup> dependent variables and the response:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \beta_{ij} x_i x_j$$
(1)

<sup>92</sup> where *Y* is the response for either PS or TPC;  $\beta$  sym-<sup>93</sup> bolizes the coefficients; and *x* represents the coded in-<sup>94</sup> dependent variables.

<sup>95</sup> To assess the statistical significance of the developed <sup>96</sup> model, the *F* value, *p* value, coefficient of determi-<sup>97</sup> nation ( $R^2$ ), adjusted  $R^2$  ( $R^2_{adj}$ ), and predicted  $R^2$ <sup>98</sup> ( $R^2_{pred}$ ) were used. The information was then used to <sup>99</sup> create a 3-D response surface. The desirability func-<sup>100</sup> tion methodology was utilized to estimate the optimal <sup>101</sup> extraction conditions.

### Analytical methods

## **PS yield determination**

After filtration, the collected crude polysaccharides 104 were dried (UNE 700, Germany) at 130°C until a constant weight was reached to determine the dry solid 106 content. The PS yield was then calculated based on 107 the weight of the obtained polysaccharides divided by 108 the initial weight of PFPP relative to dry matter. 109

## **TPC** determination

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The remaining solution after filtration was used to determine the total phenolic content (TPC) following the method of Kupina et al. (2018)<sup>14</sup> with some modifications. Specifically, 0.5 mL of the polyphenol solution was mixed with 0.5 mL of 10% (v/v) Folin-Ciocalteu solution and 3 mL of distilled water, along with 0.5 mL of sodium carbonate. After thoroughly shaking the tubes for a homogeneous mixture, each tube was wrapped in aluminum foil and placed at room temperature for 45 minutes before being analyzed with a spectrometer (V730, Japan) at 765 nm. The results are expressed as mg gallic acid equivalent per gram dry matter of PFPP (mg GAE/g).

#### Statistical analysis

Each experiment was performed in triplicate, and the the experimental data are expressed as the mean  $\pm$  standard deviation. Design-Expert software (Trial version, Stat-Ease Inc., USA) was used for ANOVA and the optimization.

## RESULTS AND DISCUSSIONS

# Box–Behnken design and regression analy- 131 sis 132

Table 2 presents the experimental data for the BBD 133 matrix with PS yield and TPC as the response. Af- 134 ter 17 runs, the PS vield ranged from 8.68 to 36.13%, 135 while the TPC varied between 11.55 and 47.51 mg 136 GAE/g. To investigate the combined effects of inde- 137 pendent variables (i.e., the solvent-to-solid ratio, ul- 138 trasonic temperature and duration) on the PS yield 139 and TPC from PFPP, quadratic models were con- 140 structed with the linear and quadratic terms of each 141 variable and their interactions. Table 3 provides the 142 ANOVA results used to evaluate the models. Both 143 models for PS yield and TPC were highly significant, 144 with p values of < 0.0001 and a nonsignificant lack of 145 fit (p values > 0.05), showing the adequacy of pure er- $_{146}$ ror. The coefficients of determination (R<sup>2</sup>) were de- 147 termined to be > 0.998, indicating that the formulated 148 models could explain more than 99.8% of the vari- 149 ability. Furthermore, the predicted  $R^2$  values > 0.97 150

#### Table 1: Levels of factors tested in Box–Behnken design (BBD)

		-			
Factors	Symbol	Units	Coded level		
			-1	0	1
Solvent-to-solid ratio	X <sub>1</sub>	mL/g	30	50	70
Ultrasonic temperature	X <sub>2</sub>	°С	40	55	70
Ultrasonic duration	X <sub>3</sub>	minute	40	55	70

# Table 2: Box–Behnken design of factors (in coded levels) with the polysaccharide yield (PS) and total phenolic content (TPC) as the response

No.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	PS yield (%)			TPC (mg GAE/g)
				Experimental value	Predicted value	Experimental value	Predicted value
1	-1	-1	0	$8.68 \pm 1.24$	8.48	$11.55\pm2.18$	10.94
2	1	-1	0	$\textbf{27.60} \pm \textbf{2.44}$	26.77	$39.57 \pm 1.24$	39.33
3	-1	1	0	$16.85\pm1.08$	17.26	$19.62\pm3.01$	19.69
4	1	1	0	$23.17 \pm 1.26$	23.42	$\textbf{36.57} \pm \textbf{2.43}$	37.19
5	-1	0	-1	$12.85\pm2.17$	12.82	$12.52\pm1.11$	13.22
6	1	0	-1	$24.62 \pm 1.26$	24.75	$35.61 \pm 2.05$	35.78
7	-1	0	1	$17.96 \pm 0.99$	17.69	$19.46 \pm 2.15$	19.28
8	1	0	1	$25.19 \pm 1.95$	25.09	$\textbf{37.49} \pm \textbf{2.51}$	36.76
9	0	-1	-1	$20.71 \pm 1.91$	21.06	$\textbf{30.19} \pm \textbf{1.65}$	30.13
10	0	1	-1	$25.85\pm2.13$	25.41	$38.23 \pm 2.19$	37.45
11	0	-1	1	$22.17\pm0.99$	22.75	$33.91 \pm 0.98$	34.73
12	0	1	1	$29.16 \pm 1.82$	28.95	$39.77 \pm 1.43$	39.87
13	0	0	0	$35.28 \pm 2.44$	35.67	$\textbf{46.18} \pm \textbf{1.93}$	46.66
14	0	0	0	$35.42 \pm 0.54$	35.67	$46.56 \pm 1.67$	46.66
15	0	0	0	$\textbf{36.13} \pm \textbf{3.14}$	35.67	$\textbf{46.69} \pm \textbf{3.13}$	46.66
16	0	0	0	$\textbf{35.76} \pm \textbf{1.54}$	35.67	$47.51 \pm 1.77$	46.66
17	0	0	0	$35.49 \pm 1.85$	35.67	$46.27\pm2.33$	46.66

<sup>151</sup> were reasonably consistent with the adjusted  $\mathbb{R}^2$  value

<sup>152</sup> of 0.99. Desirable Adeq. Precision values greater
<sup>153</sup> than 4 also indicated appropriate signals for the mod<sup>154</sup> els. Second-order polynomial models representing
<sup>155</sup> the correlation between the three independent vari<sup>156</sup> ables (in their coded levels) and responses were pro<sup>157</sup> duced in Equations (2) and (3) as follows:

$$Y_1(\%) = 35.8 + 1.93X_1 - 1.14X_2 - 0.9702X_3$$
  
-0.5849X\_1X\_2 - 0.3785X\_1X\_3 + 0.2053X\_2X\_3  
-2.8X\_1^2 - 3X\_2^2 - 1.94X\_3^2

 $Y_2(mg \ GAE/g) = 46.98 + 4.58X_1$ 

 $-0.7229X_2 - 1.21X_3 - 0.4218X_1X_2$  $-0.4227X_1X_3 - 0.2427X_2X_3 - 3.83X_1^2$  $-2.68X_2^2 - 2.26X_3^2$ (3)

where  $Y_1$  and  $Y_2$  are the responses (PS yield and 158 TPC, respectively), and  $X_1$ ,  $X_2$  and  $X_3$  are the independent variables, i.e., the solvent-to-solid ratio, ultrasonic temperature and duration, respectively. 161

(2) The predicted data of the responses obtained from the 162 two models are presented in Table 2 for comparison 163

		PS			TPC		
Source	DF	Coefficient Esti- mate	F Value	P Value	Coefficient Estimate	F Value	P Value
Model	9	35.8000	430.0400	< 0.0001	46.9800	414.5200	< 0.0001
$X_1$	1	1.9300	276.5300	< 0.0001	4.5800	746.4100	< 0.0001
$X_2$	1	-1.1400	38.3100	0.0004	-0.7229	7.3700	0.0300
X <sub>3</sub>	1	-0.9702	27.3600	0.0012	-1.2100	20.5100	0.0027
$X_1X_2$	1	-0.5849	30.1300	0.0009	-0.4218	7.5300	0.0288
$X_1X_3$	1	-0.3785	17.4800	0.0041	-0.4227	10.4700	0.0143
$X_2X_3$	1	0.2053	2.8900	0.1327	-0.2427	1.9400	0.2060
$X_1^2$	1	-2.8000	1490.9000	< 0.0001	-3.8300	1335.0400	< 0.0001
$X_2^2$	1	-3.0000	642.4100	< 0.0001	-2.6800	245.3000	< 0.0001
$X_3^2$	1	-1.9400	262.6900	< 0.0001	-2.2600	171.1800	< 0.0001
Lack of Fit	3		4.6900	0.0848		3.7800	0.1159
$\mathbb{R}^2$		0.9982			0.9981		
Adjusted 1	R <sup>2</sup>	0.9959			0.9957		
Predicted	R <sup>2</sup>	0.9767			0.9749		
Adeq. Pre	cision	65.2546			59.4188		
C.V. %		2.1300			2.2700		

Table 3: ANOVA for Box-Behnken Design for PS and TPC as the response

	Table 4: Predicted and (	experimental res	ponses under o	ptimal conditions
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	Predicted	Experimental
Solvent-to-solid ratio (mL/g)	53.9	54
Ultrasonic temperature (°C)	57.6	58
Ultrasonic duration (min)	57.0	57
PS yield (%)	36.46	$35.76 \pm 1.54$
TPC (mg GAE/g)	48.35	$47.51 \pm 1.77$

<sup>164</sup> with their experimental values. The results in Table 3 <sup>165</sup> indicate that all three variables had significant effects <sup>166</sup> on both responses at their linear and quadratic levels <sup>167</sup> (X and X<sup>2</sup>), with p values < 0.05. On the other hand, <sup>168</sup> the interaction effects of X<sub>1</sub>X<sub>2</sub> and X<sub>1</sub>X<sub>3</sub> were sig-<sup>169</sup> nificant (p < 0.05), and those of X<sub>2</sub>X<sub>3</sub> were negligible <sup>170</sup> (p > 0.05) for both responses.

### 171 3D-surface responses

172 To further understand the interaction of variables,
173 3D response surface graphs (Figure 1) were gener174 ated by plotting the response against two independent
175 variables while holding the third constant at its zero

level. The images illustrated that both the PS yield and176TPC were low at the lowest solvent-to-solid ratio (30177mL/g). These responses markedly increased with in-178creasing solvent-to-solid ratio but slightly decreased179at the highest concentration of 70 mL/g. These obser-180vations align well with the principles of mass trans-181fer, which suggest that the concentration gradient be-182tween the solid and the solvent drives the transfer of183gradient, accelerating the diffusion rate of chemicals185from the solid material into the solvent. However, it186also prolongs the time needed to achieve equilibrium.187The solvent-to-solid ratio can profoundly influence188



Figure 1: The effects of two process variables, namely ultrasonic temperature and solvent-to-solid ratio, ultrasonic duration and solvent-to-solid ratio, and ultrasonic duration and ultrasonic temperature on PS yield (upper row) and TPC (lower row)

the equilibrium constant, revealing a relationship between yield and solvent consumption characterized
by an exponential increase followed by a plateau as the
maximum yield approaches <sup>16</sup>.

Similar trends were also observed for the effects of 193 ultrasonic temperature and duration. A lower ul-194 195 trasonic temperature could reduce the solubility of the target compounds in the solvent, leading to in-196 sufficient extraction efficiency<sup>17</sup>. Furthermore, some 197 plant materials may require higher temperatures to ef-198 fectively breakdown cell walls for the release of their 199 internal substances. However, at elevated tempera-200 tures (higher than 60°C in this study), both responses 201 decreased with increasing temperature. This may 202 be due to membrane denaturation at high temper-203 atures, causing difficulty in substance diffusion into 204 the solvent, or due to the instability of phenolic com-205 pounds at high temperatures 18. On the other hand, 206 increasing the ultrasonication duration to less than 60 207 min could improve the extraction yield by softening 208 plant tissues, weakening cell wall integrity, and hy-209 drolyzing phenolic-protein, polysaccharide-protein, 210 211 and phenolic-polysaccharide complex bonds, as well as increasing the solubility of target compounds in the 212 solvent<sup>19</sup>. In contrast, extending sonication beyond 213 60 minutes resulted in a lower extraction efficiency for 214 PS yield and TPC. This could be attributed to struc-215 tural alterations in polyphenols<sup>20</sup> or polymeric break-216 <sup>217</sup> down of polysaccharides<sup>21</sup>.

### **Optimization and validation**

The trade-offs among numerous variables were bal- 219 anced to simultaneously optimize two responses, i.e., 220 PS vield and TPC. The results in Table 4 present the 221 optimal conditions, including a solvent-to-solid ra- 222 tio of 53.9 mL/g, an ultrasonication temperature of 223 57.6°C and an ultrasonication duration of 57 min. 224 Meanwhile, the predicted optimal response values 225 were 36.46% and 48.35 mg GAE/g for the PS yield 226 and TPC, respectively. The data obtained from the 227 experiment under the optimal conditions with minor 228 modifications to the variable levels, as shown in Ta- 229 ble 4, aligned well with their predicted values, which 230 were 35.76  $\pm$  1.54% and 47.51  $\pm$  1.77 mg GAE/g for  $_{231}$ PS yield and TPC, respectively. This could confirm 232 the adequacy and significance of the models. Com- 233 pared with the efficiency of individual extraction, the 234 yield of polysaccharides in this study was greater than 235 that previously reported by Pereira et al. (2024)<sup>22</sup> us- 236 ing pressurized solvent extraction, by Vasco-Correa 237 and Zapata (2017)<sup>23</sup> using enzymatic extraction, or 238 by Kulkarni and Vijayanand (2010)<sup>2</sup> using the con- 239 ventional method (28%, 26% and 15%, respectively). 240 Moreover, the TPC in this study seemed to be slightly 241 greater than that recorded by Wang et al. (2021)<sup>24</sup> us- 242 ing the cellulase-assisted extraction method or by Vo 243 et al. (2023)<sup>25</sup> using UAE under milder conditions 244 (liquid-to-solid ratio of 28 mL/g and 20 min) for sin- 245 gle extraction (22.34 mg GAE/g and 39.38 mg GAE/g, 246

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<sup>247</sup> respectively). The difference may be due to variations 248 in the employed methods, extraction conditions and 249 materials. For example, in this study, pH 5 buffer was used as the solvent for the extraction. Although 250 water extraction has been applied as the traditional 251 method for the extraction of natural polysaccharides, 252 acidic environments have been demonstrated to en-253 able more effective cleavage of glycoside bonds, re-254 sulting in higher yields of bioactive low-molecular-255 weight polysaccharides<sup>26-28</sup>. In addition, although 256 various solvents, such as ethanol, methanol, or natural 257 deep eutectic solvents, are commonly used to extract 258 phenolics, acidic conditions have been revealed for 259 their ability to hydrolyze glycoside bonds in phenolic derivatives and transform them into free phenolics for 261 easier release<sup>29-31</sup>. Therefore, the use of acidic buffer 262 may be effective for the coextraction of both polysac-263 charides and polyphenols. In conclusion, these com-264 parisons implied the potential benefits of simultane-265 ous extraction of polysaccharides and polyphenols us-266 ing pH 5 buffer with the UAE method. 267

# 268 CONCLUSION

This study aimed to conduct two-response optimiza-269 tion for the ultrasound-assisted extraction of polysac-270 charides and polyphenols from PFPP using response 271 surface methodology. By using a three-variable, 272 three-level Box-Behnken design (BBD), the optimal 273 extraction conditions to obtain the highest PS yield 274 (36.46%) and TPC (48.35 mg GAE/g) were as follows: 275 53.89 mL/g, 57.62°C, and 56.99 min for the solvent-276 to-solid ratio, ultrasonication temperature and dura-277 tion, respectively. Furthermore, it was discovered that 278 the experimental response values were closely compa-279 rable to the predicted values, indicating that the mod-280 els were good fits and capable of making accurate pre-281 dictions. Future research should focus on compre-282 hensive characterizations of the obtained polysaccha-283 rides and polyphenols for their potential applications. 284 285

## **ABBREVIATIONS**

- 287 BBD : Box-Behnken design
- 288 GAE : gallic acid equivalent
- 289 PBD : Plackett–Burman design
- 290 PFPP : passion fruit peel powder
- 291 PS : polysaccharide
- 292 RSM : response surface methodology
- 293 TPC: total phenolic content
- 294 UAE : ultrasound-assisted extraction

## **AUTHOR CONTRIBUTIONS**

Minh K. Q. Le: Conceptualization, Methodology, 296 Formal analysis, Investigation, Writing - Original 297 Draft; Ngoc Lieu Le: Conceptualization, Validation, 298 Resources, Writing - Review & Editing, Supervision, 299 Project administration, Funding acquisition. 300

## COMPETING INTERESTS

The authors declare that they have no competing interests. 303

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