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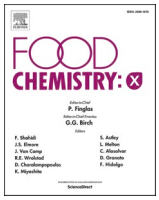

Economical and eco-friendly isolation of anthocyanins from grape pomace with higher efficiency

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ABSTRACT

Isolating anthocyanins from grape pomace, byproduct of red wine, becomes attracting for the multiple health beneficial effects of anthocyanins. Here in the ultrasound assisted anthocyanin isolation, parameters of time, ethanol concentration and pH, as well as temperature were individually optimized first. Then, surface response methodology was employed to further optimize the interactive and synergistic effect of these parameters. Optimal isolation condition was identified as the following: at the material liquid ratio of 1:15, 78.9 % of ethanol of pH 7.0 was utilized to extract at 63.8 °C for ~48 min. Experimental yield with the optimal isolation conditions was 193.547 mg/100 g anthocyanin from grape pomace, almost twice as much as previously reported. Two more anthocyanins, delphinidin-acetylglucoside and cyanidin-coumaroylglucoside, were identified in the extract. With ethanol as the only organic solvent used, this isolation method is an economical, eco-friendly and more efficient, anthocyanin preparation method with simpler instrument setups.

1. Introduction

Anthocyanins are often chemicals composed of an aglycone (the so-called anthocyanidin), sugar(s), and usually acyl group(s) (Liang et al., 2021). They naturally present in almost all the plant tissues including flowers, fruits, leaves, stems, and roots, and endow cyanic colors to these tissues as pigments. Many parts of our daily diet are good sources of anthocyanins, and their health beneficial effects were studied in different aspects including reducing the risks of diseases such as cancer (Kocic et al., 2011), atherosclerosis (Aboonabi & Singh, 2015), cardiovascular diseases (Cassidy, 2018), and type II diabetes (Guo & Ling, 2015). Due to their many health benefiting effects, supplements of anthocyanins has become a major portion of the nutrition market (Zhang et al., 2021). Thus, studies have been performed to isolate anthocyanins from many plants including black currant fruits (Matsumoto et al., 2001), bilberry (Du et al., 2004), red kiwi fruit (Comeskey et al., 2009), blueberry (Wang et al., 2014), wild blueberry (Chorfa et al., 2016), mulberry (He et al., 2018), red cabbage (Chen et al., 2018), and black rice (Yi et al., 2021). On the other hand, anthocyanin isolation from agri-byproducts are attracting more and more attentions for the full utilization of these precious molecules (S et al., 2020).

Red wines are good sources of anthocyanins (Mateus et al., 2003), and the byproduct of red wine-grape pomace also contains considerable amount of anthocyanins. In a previous study, methanol and formic acid were used in the extraction of anthocyanins from grape pomace (Zhao et al., 2020). Formic acid was used to obtain the flavylium cation form of anthocyanins (Tena & Asuero, 2022). However, methanol was harmful to human health and their residual presence in the anthocyanin product may not meet the criteria of food grade production. Besides, anthocyanin isolation with column chromatography as stated in this study is not readily applicable and economical to industrial preparation in many circumstances.

Here in this research, a bio-safer and eco-friendlier, anthocyanin isolation method with simpler and more economical setups were studied. The ultrasound assisted anthocyanin isolation conditions were optimized, and the extraction yield almost doubled with two more anthocyanins identified in the end product.

2. Materials and methods

2.1. Materials

The Beibinghong grape (*Vitis vinifera* × *Vitis amurensis*) pomace was kindly provided by Dr. Yibin Lan in the College of Food Science and

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Nutritional Engineering, China Agricultural University (Lan et al., 2016). Acetic acid and absolute ethanol were purchased from Modern Oriental Fine Chemistry (Beijing, China). Potassium chloride and sodium acetate were purchased from XiLong Scientific (Shantou, Guangdong Province, China). Hydrochloric acid was purchased from Sinopharm Chemical Reagent Co., ltd. (Beijing, China).

2.2. Ultrasound assisted anthocyanin extraction

For each extraction, around 1 g grape pomace was weighted, mixed with ethanol with a material liquid ratio of 1:15. The isolation parameters, including time, pH, ethanol concentration, temperature, were according to the single parameter optimization or surface response methodology optimized values. The extract solution was centrifuged at 4000 rpm for 15 min at room temperature to remove precipitates, and anthocyanin content in the supernatant was measured with the following described method.

Initial evaluation of the isolation yield with and without ultrasound assistance was performed by extraction with 80 % ethanol and a material liquid ratio of 1:15, at 37 °C for 20, 30, 40, 50, and 60 min, respectively. Ultrasound was applied with a VGT-1730QTD ultrasonic water bath (GT Sonic, Meizhou, Guangdong Province, China).

2.3. Single parameter optimization

2.3.1. Extraction time

Grape pomace with 80 % ethanol at a material liquid ratio of 1:15, incubated in the ultrasound water bath at 55 °C for 20 min, 25 min, 30 min, 35 min, 40 min, 45 min, and 50 min, respectively. The anthocyanin yield was measured as described in the following.

2.3.2. Extraction pH

Grape pomace with 80 % ethanol at a material liquid ratio of 1:15, incubated in the ultrasound water bath at 55 °C, and different pH of 3, 4, 5, 6, 7, respectively. The anthocyanin yield was measured as described in the following.

2.3.3. Extraction ethanol concentration

Grape pomace with ethanol at a material liquid ratio of 1:15, incubated in the ultrasound water bath at 53 °C for 30 min, and different ethanol concentration of 50 %, 60 %,70 %, 80 %, 85 %, and 90 %, respectively. The anthocyanin yield was measured as described in the following.

2.3.4. Extraction temperature

Grape pomace with 80 % ethanol at a material liquid ratio of 1:15, incubated in the ultrasound water bath for 30 min, and different temperature of 35 °C, 45 °C, 55 °C, 63 °C, 65 °C, 68 °C, 75 °C, respectively. The anthocyanin yield was measured as described in the following.

2.4. Surface response methodology optimization

The optimal mixture design consisting variables of ethanol concentration, temperature and time was used in this study via the Box-Behnken design, and a second order regression yielded 17 formulations with the Design-Expert 12 software (Stat-Ease Inc., Minneapolis, MN, USA) as in the previous study (Guo et al., 2021). 3D response curves were generated with anthocyanin extraction yield as the output and ethanol concentration, temperature and time as the input. Analysis of variance (ANOVA) was performed to evaluate any significant differences between independent variables.

2.5. Anthocyanin content and composition determination

Anthocyanin content and composition in each extraction was determined as described previously (Wang et al., 2021). Roughly, the extracts were filtered with a 0.45 μM PTFE microfiltration membrane and then analyzed with a Zorbax SB-C18 column on a 1290 series liquid chromatography system (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with diode array detector.

2.6. Statistical analysis

All measurements were performed in duplicates. One-way ANOVA was applied to the data with the Duncan test for significance analysis using SPSS statistics (version 17, IBM Corp., Armonk, NY, USA). The results were plotted with GraphPad Prism (version 8.0, GraphPad Software Inc, La Jolla CA, USA).

3. Results and discussion

3.1. Effect of ultrasound assisted extraction

Ultrasound application to bioactive compounds extraction with organic solvent often not only increases the yield, but also reduces energy consumption (Cassiana Frohlich et al., 2022). Additionally, it allows the use of low temperature and thus conserves the heat-sensitive materials, such as anthocyanins (Wei et al., 2016). In the initial extraction experiment where other parameters were not optimized yet, the effectiveness of ultrasound assisted extraction was assessed first. Grape pomace was mixed with 80 % ethanol at a material liquid ratio of 1:15, and extracted at 37 °C for 20 min, 30 min, 40 min, 50 min, and 60 min, respectively. It was observed that the extraction yield with the presence of ultrasound assistance was significantly higher than that without ultrasound assistance at all time scales (Fig. 1, A). In the absence of ultrasound assistance, the extraction yield increased first when the incubation time extended from 20 to 50 min, and then decreased when the incubation time was beyond 50 min. On the other hand, with the presence of ultrasound assistance, the extraction yield kept increasing from 20 min to 50 min, indicating that ultrasound assistance contributes positively to the extraction yield of anthocyanins. The increase of anthocyanin extraction yield in the presence of ultrasound assistance is due to the release of anthocyanin in the disruption of plant tissue caused by physical forces during acoustic cavitation (Kumar et al., 2021).

3.2. Single parameter optimization on ultrasound assisted extraction

There are couple of parameters when ultrasound assisted anthocyanin extraction is applied in industrial preparation, including time, pH and composition of organic solvent, temperature (Bamba et al., 2018; Carrera et al., 2021; Görgüç et al., 2019; Setyaningsih et al., 2019; Zhu et al., 2017). As stated in the above section, with the assistance of ultrasound, the extraction yield increased as the incubation time extended. Here the impact of incubation time was assessed, where the grape pomace and 80 % ethanol was mixed at a material liquid ratio of 1:15, and incubated at 55 °C for different time scales. It was observed that the extraction yield increased initially from 20 to 45 min, and then decreased significantly at 50 min, while the extraction yields between 40 and 45 were not significantly different (Fig. 1, B). The decrease of anthocyanin yield is highly likely due to its degradation by oxidation, hydrolysis, and polymerization at such a temperature for longer time (Benvenutti et al., 2022).

Next, the pH of the extraction solution was assessed for its impact on the extraction yield. The grape pomace was mixed with 80 % ethanol at a material liquid ratio of 1:15, and incubated at 55 °C with different pH in the range of 3–7. It is observed that the extraction yield increased significantly as the pH increased from 3 to 7 (Fig. 1, C). Extraction yield at higher pH than 7 was not investigated, since basic solution deprotonates anthocyanins and lead to its degradation (Peanparkdee et al., 2020). Though the material liquid ratio of 1:15 was used, ethanol concentration could be optimized. The grape pomace was mixed with 50 %, 60 %, 70 %, 80 %, 85 %, and 90 % ethanol, and incubated at 53 °C for 30

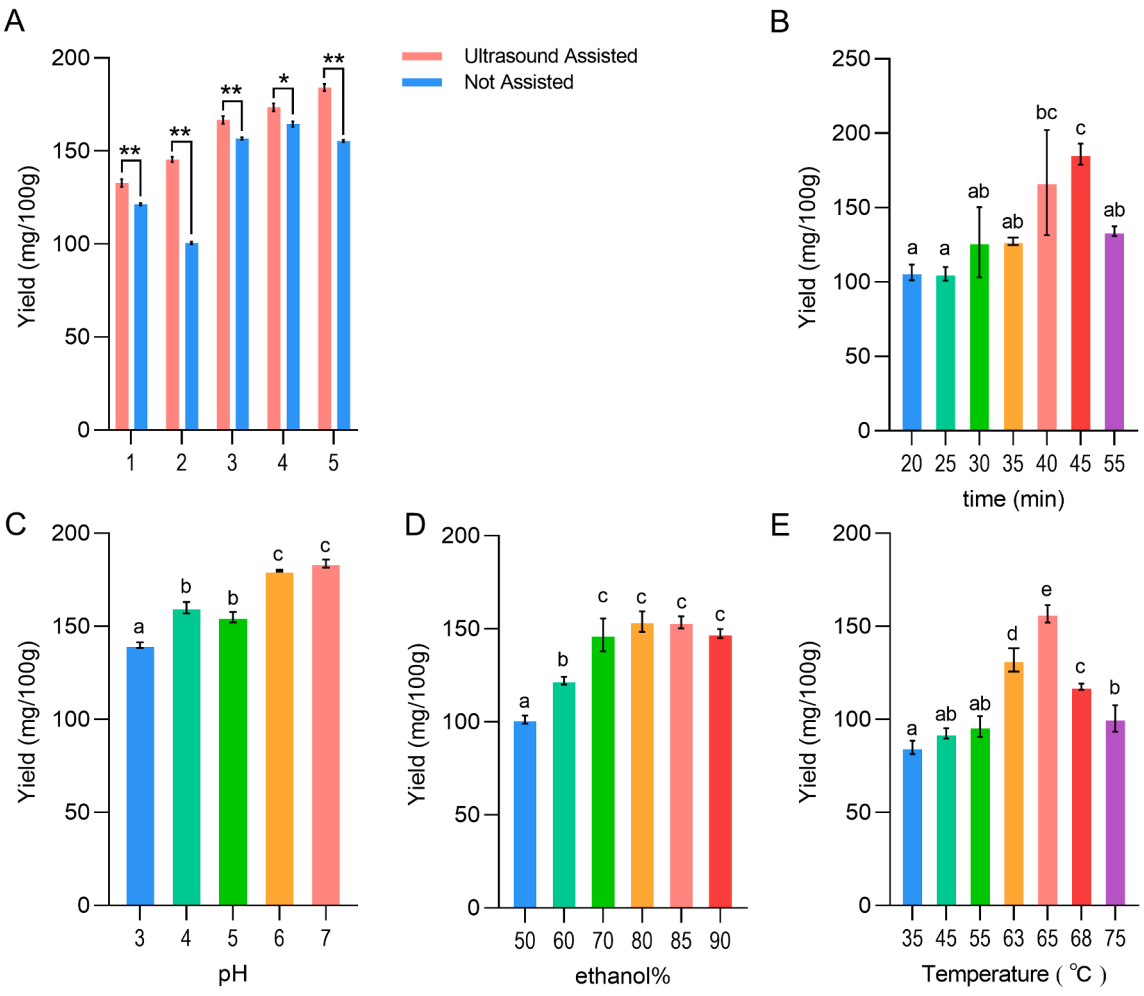

Fig. 1. Optimization of single parameter on anthocyanin isolation from grape pomace. (A) Comparison between the ultrasound assisted and not assisted anthocyanin isolation yield at a material liquid ratio of 1:15, 80 % ethanol, 37 °C, and different time of 1 (20 min), 2 (30 min), 3 (40 min), 4 (50 min), 5 (60 min), respectively. (B) Anthocyanin isolation yield at a material liquid ratio of 1:15, 80 % ethanol, 55 °C, and different time of 20, 25, 30, 35, 40, 45, 55 min, respectively. (C) Anthocyanin isolation yield at a material liquid ratio of 1:15, 80 % ethanol, 55 °C, and different pH of 3, 4, 5, 6, 7, respectively. (D) Anthocyanin isolation yield at a material liquid ratio of 1:15, 53 °C for 30 min, and different ethanol content of 50 %, 60 %,70 %, 80 %, 85 %, and 90 %, respectively. (E) Anthocyanin isolation yield at a material liquid ratio of 1:15, 80 % ethanol for 30 min, and at different temperature of 35, 45, 55, 63, 65, 68, 75 °C, respectively.

min. It was observed that when the ethanol concentration increased from 50 % to 70 %, the extraction yield increased significantly (Fig. 1, D). However, when the ethanol concentration further increased from 70 % to 90 %, there was no significant difference between the extraction yields. This is likely due to higher concentration of ethanol enhanced permeability of solvent while similar polarity of solvent to anthocyanins permits their dissolve (Zhou et al., 2022).

Finally, the extraction temperature as examined. Grape pomace was mixed with 80 % ethanol at a material liquid ratio of 1:15, and incubated at 35 °C, 45 °C, 55 °C, 63 °C, 65 °C, 68 °C, 75 °C for 30 min, respectively. Initially as the temperature increased from 35 °C to 65 °C, the extraction yield increased significantly (Fig. 1, E). When the temperature further increased from 65 °C, the extraction yield decreased significantly. This is highly likely due to the low stability of anthocyanins under high temperatures (Fernandes et al., 2020; Wang et al., 2021).

3.3. Surface response methodology optimization on ultrasound assisted extraction

Since the extraction yield at neutral pH reached its maximal, ultrasound assisted extraction experimental parameters of time, ethanol concentration, and temperature could be further optimized. The interaction and synergistic effect between these three parameters could be revealed with the surface response methodology (Guo et al., 2021). Three levels of each input parameter were utilized. For ethanol concentration, 70 %, 77.5 %, and 80 % were investigated. For temperature, 60 °C, 64 °C, and 68 °C were studied. For incubation time, 40 min, 47.5 min, and 55 min were utilized. The Box-Behnken design for this 3^3-factorial set up yielded 17 experimental setups, and anthocyanin extraction was performed following each condition. The 3D surface response surface with the resulting extraction yield was generated, and fitting of extraction yield to the quadratic equation yielded an F value of 419.59 (Table 1, $p < 0.0001$, significant), indicating the 3D surface as an adequate modeling of extraction yield to the input variables.

For ethanol concentration, initial increasing from 70 % to 79 % increased the anthocyanin extraction yield while further increasing of ethanol concentration from 79 % to 85 % decreased the extraction yield (Fig. 2, A, B). For extraction temperature, initial increasing from 60 °C to 64 °C enhanced the anthocyanin extraction yield and further temperature increasing from 64 °C to 68 °C undermined the yield (Fig. 2, A, C). For the extraction time, initial extension from 40 min to 48 min increased the extraction yield while further extension from 48 min to 55 min decreased the yield (Fig. 2, B, C). In the quadratic equation modeling anthocyanin extraction yield, the regression coefficients for ethanol concentration, temperature, time, and ethanol concentration × time are significant (Table 1, $p < 0.05$), indicating that these parameters

Table 1
ANOVA of response surface quadratic model for anthocyanin isolation.

Source	Sum of Squares	Mean Square	F value	*p*-value Prob > F
Model	15145.71	1682.86	419.59	<0.0001
A-ethanol conc (%)	68.63	68.63	17.11	0.0044
B-Temperature (°C)	316.46	316.46	78.9	<0.0001
C-time (min)	48.9	48.9	12.19	0.0101
AB	18.17	18.17	4.53	0.0708
AC	38.03	38.03	9.48	0.0178
BC	0.53	0.53	0.1321	0.727
A^2	364.04	364.04	90.77	<0.0001
B^2	12679.88	12679.88	3161.5	<0.0001
C^2	858.04	858.04	213.94	<0.0001
Residual	28.07	4.01		
Lack of Fit	21.44	7.15	4.31	0.096

were all playing significant roles in anthocyanin extraction while a strong interaction present between ethanol concentration and time. The surface response methodology yielded a mathematical maximal extraction yield of 193.108 mg/100 g grape pomace, provided that 78.9 % ethanol was applied at 63.8 °C for 48.3 min.

3.4. Composition analysis and quantification of anthocyanins in the optimized extract

With the conclusion in the above section, we performed anthocyanin extraction with 78.9 % ethanol (pH 7.0) at a material liquid ratio of 1:15, extracted at 63.8 °C for ~ 48 min, and yielded 193.547 mg/100 g anthocyanin from grape pomace. Our experimental yield is slightly higher than the predicated numerical value. Previous study obtained 112.3 mg/100 g anthocyanin from grape pomace, while our optimized isolation yield is ~ 1.72-folds of that in the previously reported (Zhao et al., 2020).

The composition of the above isolated anthocyanins was analyzed with the HPLC-MS/MS method (Supplementary Information Table S1). It was found that the glucoside-conjugated anthocyanidins are the major composition, while there are also acetylglucoside- and coumaroylglucoside-conjugated anthocyanidins (Fig. 3, A). Malvidin-glucoside is the richest content which takes about 48.9 % of all the anthocyanins, followed by malvidin-acetylglucoside (11.0 %), malvidin-

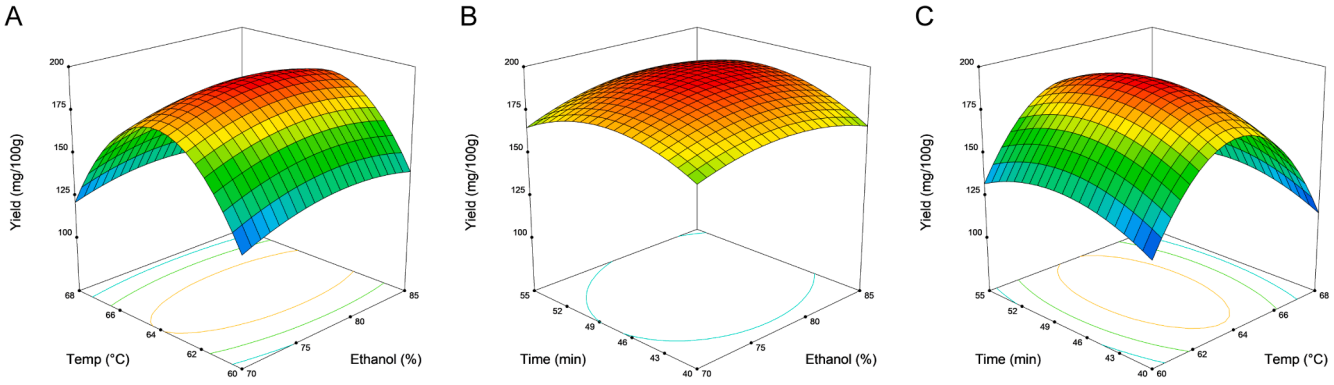

Fig. 2. Response surface plots of different parameters on anthocyanin isolation from grape pomace. Response surface plots to anthocyanin isolation yield to the input of temperature and ethanol concentration (A), time and ethanol concentration (B), time and temperature (C).

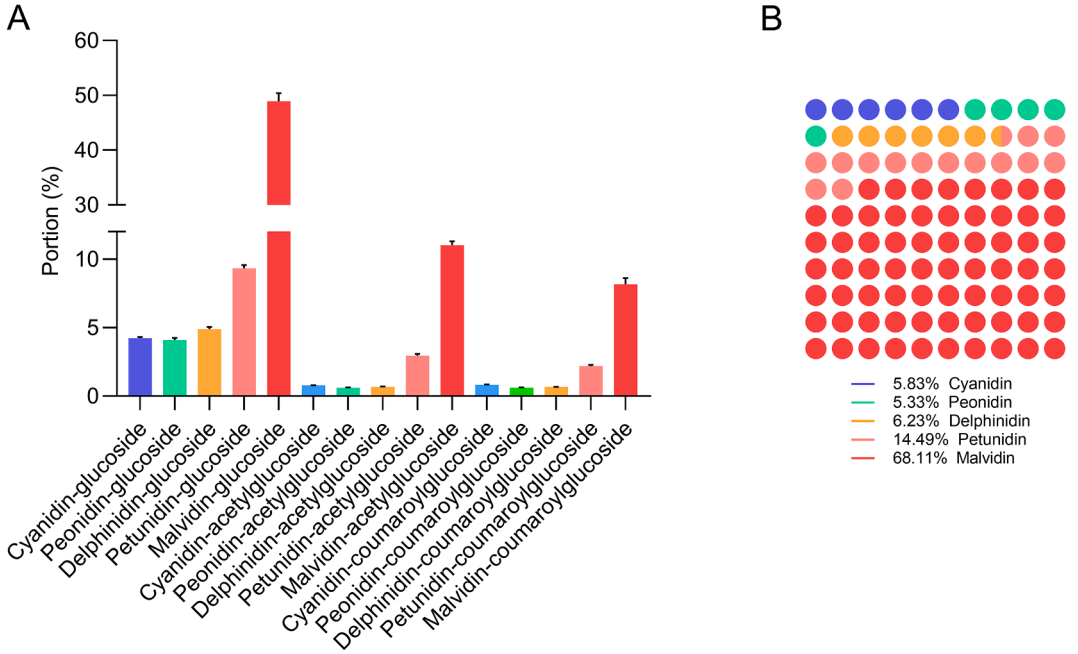

Fig. 3. Composition analysis and quantification of anthocyanins isolated from grape pomace. The portion of anthocyanins (A) and anthocyanidins (B) obtained with the optimized isolation method.

coumaroylglucoside (8.2 %), and petunidin-glucoside (9.3 %). Among these three anthocyanidin derivatives, malvidin derivatives are the major species that takes 68.11 %, followed by petunidin (14.49 %), and delphinidin (6.23 %, Fig. 3, B). Cyanidin and peonidin derivatives were taking about the same proportion. Compare with Zhao et al. (2020), delphinidin-acetylglucoside and cyanidin-coumaroylglucoside were identified in our extract, while malvidin-caffeoylglucoside was not identified.

There are studies extracting anthocyanins using acetone, butanol, (Zuleta-Correa et al., 2020), and eutectic solvents including chloride-citric acid-glucose (Guo et al., 2019). However, these solvents are harmful to human health, and their residual presence in the extracted anthocyanin put consumers' safety at risk. Here, ethanol as the only organic solvent was used in the extraction process, which is safe and evaporates before anthocyanin reached the consumers.

4. Conclusion

Ultrasound is identified as a positive factor to the extraction of anthocyanins from grape pomace. The optimal ethanol concentration and pH, extraction temperature and time were optimized with the response surface methodology, which exhibits almost twice as much yield of anthocyanin. Two more anthocyanins, delphinidin-acetylglucoside and cyanidin-coumaroylglucoside, were identified in the extract. This optimized method utilizes harmless ethanol as the only organic solvent with simple extraction instruments, thus it is an economical to industry and eco-friendly procedure with food grade end product.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100419.

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