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Impact of Choline Chloride and Organic Acid-Based Deep Eutectic Solvents on the Extraction of Bioactive Compounds from Grape Pomace

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ABSTRACT

The objective of this study was to determine the impact of the extraction conditions on the extraction yield of bioactive compounds from grape pomace using choline chloride and organic acid-based deep eutectic solvents (DES). Three different types of DES were used, including choline chloride-lactic acid, choline chloride-malic acid, and choline chloride-citric acid. The effect of the ratios of hydrogen bond acceptor to hydrogen bond donor (2:1, 1:1, 1:2, 1:3), the water addition in the DES (10–35%), the solid-to-solvent ratio (1/15–1/30 (w/w)), and extraction time (1.5–4 hours) on extraction were also investigated. The results showed that the highest total extraction yield was obtained using a DES based on choline chloride and lactic acid with a ratio of 1:2, 20% water, a solid-to-solvent ratio of 1/25 (w/w), and an extraction time of 2.0 hours. Under the best extraction conditions, the total phenolic content (TPC), total saponin content (TSC), and proanthocyanidin content (PAC) of the extract from grape pomace were found to be 42.53 ± 0.52 mg GAE/g DW, 101.43 ± 1.22 mg AE/g DW, and 17.11 ± 0.18 mg CE/g DW, respectively.

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1. Introduction

The wine industry is growing rapidly, followed by a steady increase in the number of grape pomace, which accounts for about 15–20% of the total weight of grapes (about 7 million tons), including skins and seeds [1]. These grape pomaces are usually unused and discarded. This has created significant economic and environmental concerns. The problem dealing with excess solid waste over the years has caused many environmental problems, such as water pollution, soil degradation, and air pollution. Nowadays, these grape pomaces are frequently used by wineries as fertilizers or animal feeds [2], and occasionally they are sold to biogas plants for the production of renewable energy [3]. Grape pomaces are utilized to make ethanol and organic acids, such as tartaric acid, malic acid, and citric acid. It has been reported that in both red and white grape pomaces, significant amounts of bioactive compounds are retained after winemaking, with an estimated 70% of the phenolic content remaining in the grape pomace [4]. The recovery of these bioactive compounds is also economically viable and is a direction for the sustainable development of wine and juice production systems for countries.

The development of alternative green solvents has received a lot of attention to better protect human health and the environment from the risks associated with the use of toxic solvents. Deep eutectic solvents (DESs), which are Generally Recognized as Safe solvents (GRAS), are formed by the combination of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). Due to the hydrogen bonding interactions established between DES molecules and bioactive compounds, DESs are solvents that can dissolve bioactive compounds [5]. Many studies have shown the effectiveness of DESs in the extraction of bioactive compounds such as phenolic acids, proanthocyanidins, and saponins [6]-[8]. The types of DES used for extraction depend on the type of raw material and target compounds. DES solvent systems are often created by combining HBA (inorganic salts such as quaternary ammonium, tetralkylammonium, or phosphonium salts; amino acids such as alanine, proline, glycine, or betaine) with HBD (organic acids, alcohols, amides, or carbohydrates) [5], [9]. Organic acids are common, safe, and inexpensive substances, which makes them an attractive choice as hydrogen bond donors for DES.

In addition, DES systems produced by combining choline chloride with HBD from various organic acids have been reported to have high extraction efficiency on plant materials [5], [10].

Although DES systems have been used for extraction bioactive compounds from pomace of grape varieties which mainly used in wine grape varieties, the Red Cardinal grape variety (a table grape variety) has not been studied for the recovery of bioactive compounds. In addition, a research by Dwyer et al. (2014) [4] used DES based on combining choline chloride with alcohol and organic acids with high viscosity which is suitable for applying in oleogel and emulsions. In contrast, the DES based on combining choline chloride with organic acids has low viscosity and is suitable for convenient applications like spray drying. Thus, this study was conducted to deal with large amounts of by-products from the production of juice and wine from local producers, which mostly used as feeds for goats and sheep, in Ninh Thuan province (Vietnam). The application of DES (formed by choline chloride with organic acids) to extract bioactive compounds from Red Cardinal grape pomace can be a sustainable development in the future.

2. Materials and Methods

2.1. Materials

2.1.1. Grape pomace

Red Cardinal grape pomace (GP) was collected from Ba Moi Company (Ninh Thuan province, Vietnam). Grape pomace was pretreated following the method outlined by [6]. The GP (separated branches and seeds) was heat-pump dried at 40°C (moisture content ≤ 10%), ground into powder (≤ 0.5 mm). Grape pomace powder was stored at -18 °C for further experiments.

2.1.2. Standards and reagents

Choline chloride (Himedia, India), vanillin (Himedia, India), and Folin-Ciocalteau reagent (Himedia, India), citric acid (Jiangsu Guoxin Union Energy, China), lactic acid (Xiong, China), malic acid (Isegen, South Africa), sulfuric acid (Xilong, China), hydrochloric acid (Xilong, China), sodium carbonate (Xilong, China), sodium nitrite (Guangdong guanghua, China), aluminum chloride (Xilong, China), and sodium hydroxide (Xilong, China) were obtained from SBC Trading and Service Co., Ltd, Ho Chi Minh City, Vietnam. The standards were gallic acid (Himedia, India), aescin (Sigma-Aldrich, USA), and catechin (Thermo Fisher Scientific, UK).

2.2. Methods

2.2.1. Preparation of DESs

Choline chloride (HBA) was mixed with the appropriate amount of lactic, malic, or citric acids as the HBD at different ratios (choline chloride: lactic acid (ChCl–LA) = 1:2, choline chloride: malic acid (ChCl–MA) = 1:1, choline chloride: citric acid (ChCl–CA) = 2:1) [11], [12]. Then, water was added to DESs (30%). The mixtures were stirred with a magnetic stirrer at 80°C [13] at 260 rpm for 1.5 hours. The mixture formed a clear liquid and remained liquid after about 24 hours at ambient temperature.

2.2.2. Effect of extraction conditions on bioactive compounds from grape pomace

DESs were prepared with different ratios of hydrogen bond acceptor to hydrogen bond donor (2:1, 1:1, 1:2, and 1:3). Then, an amount of GP powder was mixed with DESs according to the different solid-to-solvent ratios (1/15, 1/20, 1/25, and 1/30 (w/w)). The water additions in DESs were 10, 15, 20, 25, 30, and 35%. The mixture was stirred using a magnetic stirrer at 260 rpm for different extraction times (1.5, 2.0, 2.5, 3.0, 3.5, and 4 hours) at an extraction temperature of 30 ºC. Finally, the mixture was centrifuged in a refrigerated centrifuge at 6,000 rpm, at 4 °C for 10 min. The supernatant extract was collected and further analyzed.

2.2.3. Determination of TPC, TSC, and PAC

TPC was analyzed using the method outlined by Škulcova, et al. [14] with modifications. For tested samples, add 0.5 mL of each diluted extract to the test tube. Add 0.5 mL of 10% Folin-Ciocalteu's reagent and 2.5 mL of 20% Na_2CO_3 to each test tube and shake well. Leave the reaction tubes at ambient temperature for 1 hour, then measure the absorbance of the solution at 765 nm. TPC was expressed as gallic acid equivalent (GAE) per g of dry weight of GP (mg GAE/g DW).

TSC was determined according to the method of Tan, et al. [15]. The extract (0.3 mL) was mixed with 8% (w/v) vanillin solution (0.3 mL) and 72% (v/v) sulfuric acid (3 mL) and then incubated at 60°C for 15 min. The mixture was then cooled for 10 min and was measured the absorbance of the solution at 560 nm. TSC was expressed in mg aecsin equivalent per g of dry weight of GP (mg AE/g DW).

PAC was estimated according to the method of Li, et al. [16] with modifications. The extract (0.5 mL) was mixed with 0.3 mL of 4% (w/v) vanillin solution and 1.5 mL of 36% HCl. The mixture was then incubated at room temperature for 15 min. Measure the absorbance at 500 nm. PAC is expressed in mg catechin equivalents per g of dry weight of GP powder (mg CE/g DW).

The Total is computed by summing the individual quantities of TPC, TSC, and PAC.

2.2.4. Statistical analysis

Each single-factor experiment was repeated three times, and the experimental results were presented as the mean ± standard deviation. To analyze differences between the means, an analysis of variance (ANOVA) was performed, followed by the least significant difference (LSD) using JMP Pro 13.0 software with $p < 0.05$.

3. Results and Discussion

3.1. The effect of DESs type on the extraction of bioactive compounds

Each type of DES has physicochemical characteristics leading to different extraction efficiency. Therefore, for the extraction process of bioactive compounds, choosing the suitable DES type for the specific raw material and target compound is one of the most important factors. In this study, the effect of three DESs (choline chloride: lactic acid (ChCl–LA) = 1:2, choline chloride: malic acid (ChCl–MA) = 1:1, choline chloride: citric acid (ChCl–CA) = 2:1) on the extraction of bioactive compounds from grape pomace was investigated. The fixed factors included solid-to-solvent ratio (1/20, w/w), water addition (30%), extraction temperature (30 ºC), and extraction time (1.5 hours). The result is shown in Figure 1.

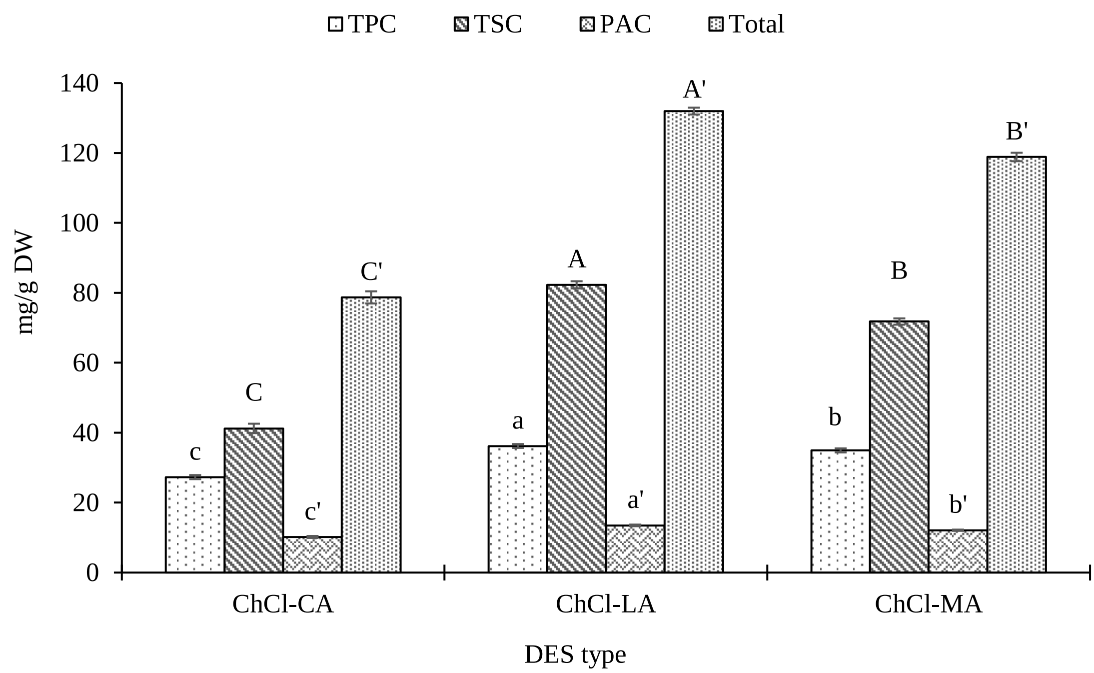

Figure 1. *The effect of DES type on the extraction of bioactive compounds from grape pomace*

Distinct letters within the same parameters indicated statistically significant differences between extracts obtained by different DES types ($p \leq 0.05$).

Statistical results showed that there was a significant difference in the ability of the extraction solvents to extract bioactive compounds from grape pomace ($p < 0.05$). Among the DESs, the one based on choline chloride and lactic acid (ChCl–LA) gave the highest extraction yield. This is evident in the total extraction yield, which amounted to 131.99 ± 0.97 mg/g DW, including TPC, TSC, and PAC of the extract of 36.19 ± 0.55 mg GAE/g DW, 82.30 ± 1.03 mg AE/g DW, and 13.49 ± 0.24 mg CE/g DW, respectively. These values were higher than those obtained with the other two solvents and had statistical significance ($p < 0.05$). In general, the highest total extraction yield was monocarboxylic acid (lactic acid), followed by dicarboxylic acid (malic acid) and tricarboxylic acid (citric acid). This result is similar to previous studies in that the extraction efficiency of bioactive compounds using ChCl–LA was higher than that of ChCl–MA and ChCl–CA [10], [17]. Many studies have also shown that when the molecular structure of HBD has more hydroxyl (OH) and/or carboxyl (COOH) groups, the recovery of bioactive compounds is reduced [8]. In solid-liquid extraction, the viscosity of the solvent is important for practical application. ChCl–LA showed higher extraction efficiency than ChCl–MA and ChCl–CA possibly due to the difference in their viscosities. The DES with more hydroxyl group (OH), carboxyl group (COOH) in the molecular structure has a higher viscosity. According to Crespo, et al. [18], viscosity decreases as the number of functional groups decreases: citric acid has a higher viscosity, followed by malic acid, then lactic acid. The high viscosity of ChCl–MA and ChCl–CA will restrict the mobility of target compounds inside DES thereby reducing extraction efficiency. Therefore, the DES generated by choline chloride and lactic acid, has the lowest viscosity, and was more suitable for the bioactive compound extraction from grape pomace than ChCl–MA and ChCl–CA.

3.2. The effect of HBA: HBD ratio on the extraction of bioactive compounds

Determining the appropriate HBA: HBD ratio is important to improve the extraction rate of total bioactive compounds. To investigate the effect of the HBA: HBD ratio of ChCl–LA on the extraction of bioactive compounds from grape pomace, the extraction processes were performed with DESs of different choline chloride: lactic acid ratios from 2:1 to 1:3. The fixed factors included solid-to-solvent ratio (1/20, w/w), water addition (30%), extraction temperature (30 ºC), and extraction time (1.5 hours). The result is shown in Figure 2.

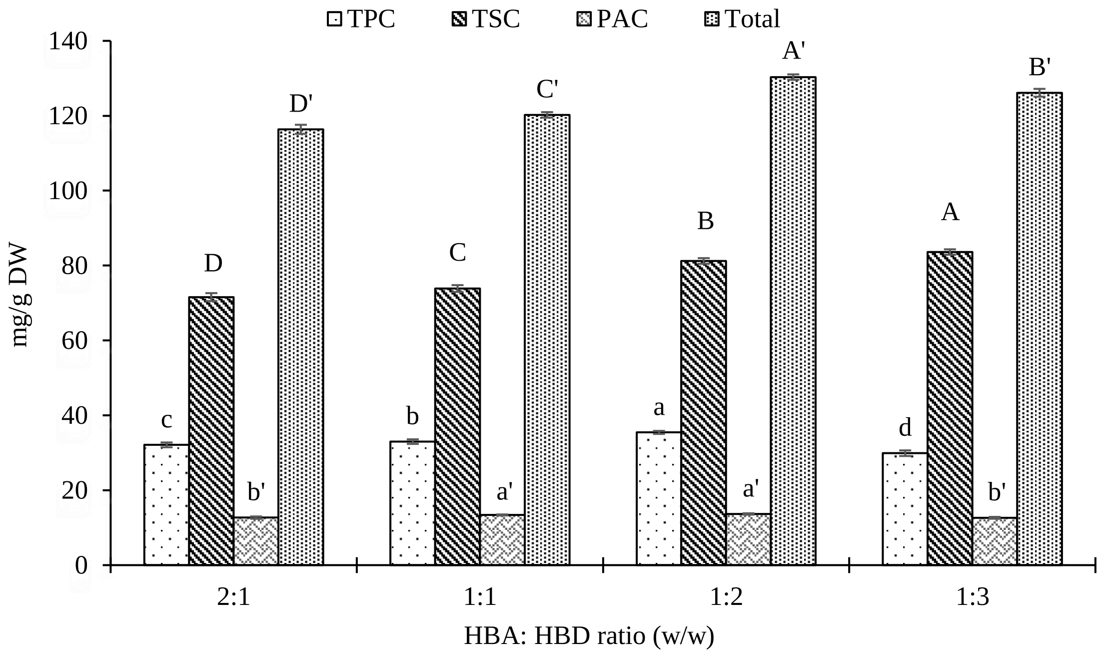

Figure 2. *The effect of HBA: HBD ratio on the extraction of bioactive compounds from grape pomace*

Distinct letters within the same parameters indicated statistically significant differences between extracts obtained by DES with different HBA: HBD ratios ($p \leq 0.05$).

Statistical results showed that the difference in the ratio of choline chloride and lactic acid significantly affected the ability to extract bioactive compounds in grape pomace ($p < 0.05$). As can be seen in Figure 2**,** ChCl–LA with a ratio of 1:2 gave the highest extraction yield. The TPC, TSC, and PAC of the extract were 35.44 ± 0.40 mg GAE/g DW, 81.22 ± 0.76 mg AE/g DW, and 13.66 ± 0.18 mg CE/g DW, respectively. The total extraction yield increased with a ratio range of 2:1 to 1:2 (w/w). That corresponded to the decrease in choline chloride and increased lactic acid in the DES system. The total extraction yield decreased when the choline chloride: lactic acid ratio changed from 1:2 to 1:3 (w/w). This phenomenon is similar to previously reported results [13], [19]. The ability to extract some bioactive compounds with DES is attributed to hydrogen bonding interactions between molecules of DES and bioactive compounds. The decrease in extraction yield may be due to a decrease in the amount of choline chloride (HBA in the DES system), leading to a decrease in hydrogen bond strength because the chloride anion is a proportion of hydrogen bond receptors [13]. Furthermore, lactic acid is acidic. Because of the advantageous partially acidic environment for phenolic compound extraction, extraction efficiency rises when the HBA-HBD ratio decreases. However, high acid causes DES's physicochemical characteristics, such as pH and polarity, to alter significantly, which may lower extraction efficiency [20]. Therefore, the HBA: HBD ratio of 1:2 was selected for the further experiments.

3.3. The effect of water addition on the extraction of bioactive compounds

Despite their potential, one of the limitations of DES is their high viscosity. Therefore, an appropriate amount of water is often added with a deep eutectic solvent to improve this drawback. Accordingly, this experiment focused on the influence of different water additions on the extraction performance of ChCl–LA (HBA: HBD ratio of 1:2). The extraction processes were performed with ChCl–LA of different water additions (10–35%). The fixed factors included the solid-to-solvent ratio (1/20, w/w), extraction temperature (30 ºC), and extraction time (1.5 hours). The effect of water addition on the extraction of bioactive compounds is shown in Figure 3.

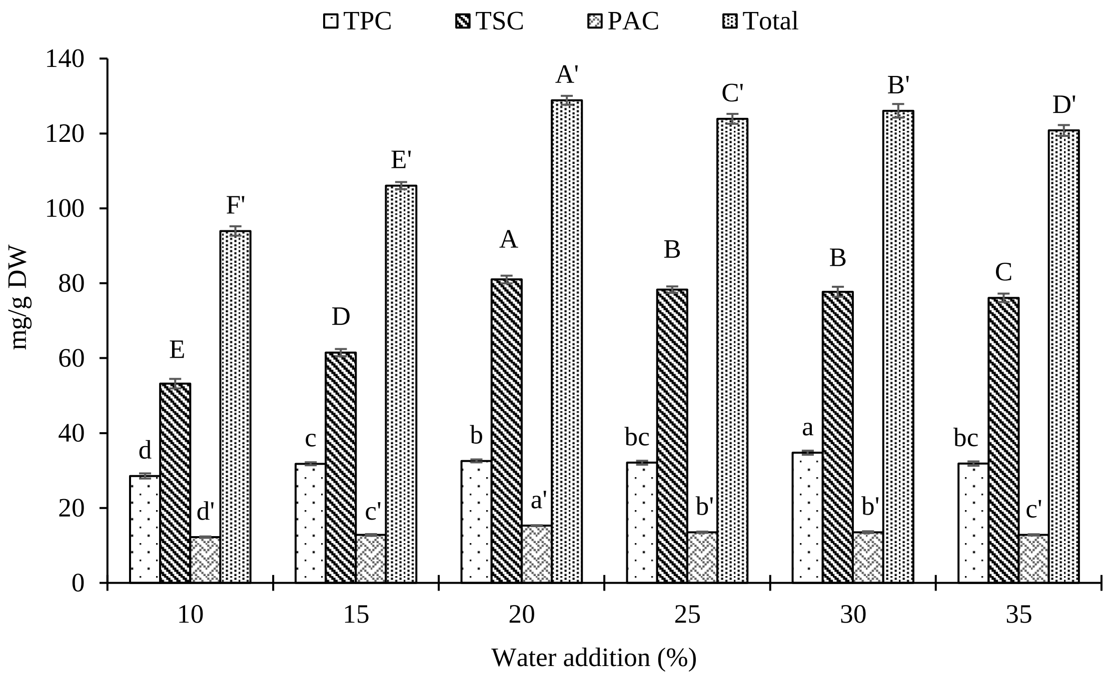

Figure 3. *The effect of water addition on the extraction of bioactive compounds from grape pomace*

Distinct letters within the same parameters indicated statistically significant differences between extracts obtained by DES with different water addition ($p \leq 0.05$).

Statistical results showed that the difference in water addition in ChCl–LA affected the ability to extract bioactive compounds in grape pomace ($p < 0.05$). As shown in Figure 3, the total extraction yield increased with the increase in water content added to ChCl–LA from 10% to 20%. In particular, the ChCl–LA with 20% water addition gave the highest extraction yield. The TPC, TSC, and PAC of the extract were 32.57 ± 0.39 mg GAE/g DW, 81.00 ± 1.03 mg AE/g DW, and 15.28 ± 0.12 mg CE/g DW, respectively. The main drawback of DES in comparison to conventional solvents is its high viscosity, which makes it difficult to transfer mass from the plant matrix to the extraction solution. Water can be added to a DES to decrease its viscosity, which is beneficial for increasing the dissolution rate of the compounds, extracting the target compounds, and reducing the time of preparation [5]. However, the total extraction yields continuously decreased when the water content in the ChCl–LA continued to increase by over 20%. The viscosity of the DES system can be significantly decreased by adding water, but too much water would interfere with the ability of HBD and HBA to establish hydrogen bonds and destroy the DES structure [21]. That will cause the interaction between the DES and the target components to be weakened, which can reduce the extraction yields. There have been many reports that adding water to DES does not always increase extraction yield [6], [8], [19]. Therefore, a 20% water addition in ChCl–LA was selected for the subsequent experiments.

3.4. The effect of solid-to-solvent ratio on the extraction of bioactive compounds

The ratio of grape pomace to DES is an important factor affecting extraction efficiency. To investigate the effect of the solid-to-solvent ratio on the extraction of bioactive compounds, the extraction processes were performed with different solid-to-solvent ratios (1/15, 1/20, 1/25, and 1/30 (w/w)) (see Figure 4). The fixed factors included ChCl–LA, HBA: HBD ratio of 1:2, water addition (20%), extraction temperature (30 ºC), and extraction time (1.5 hours).

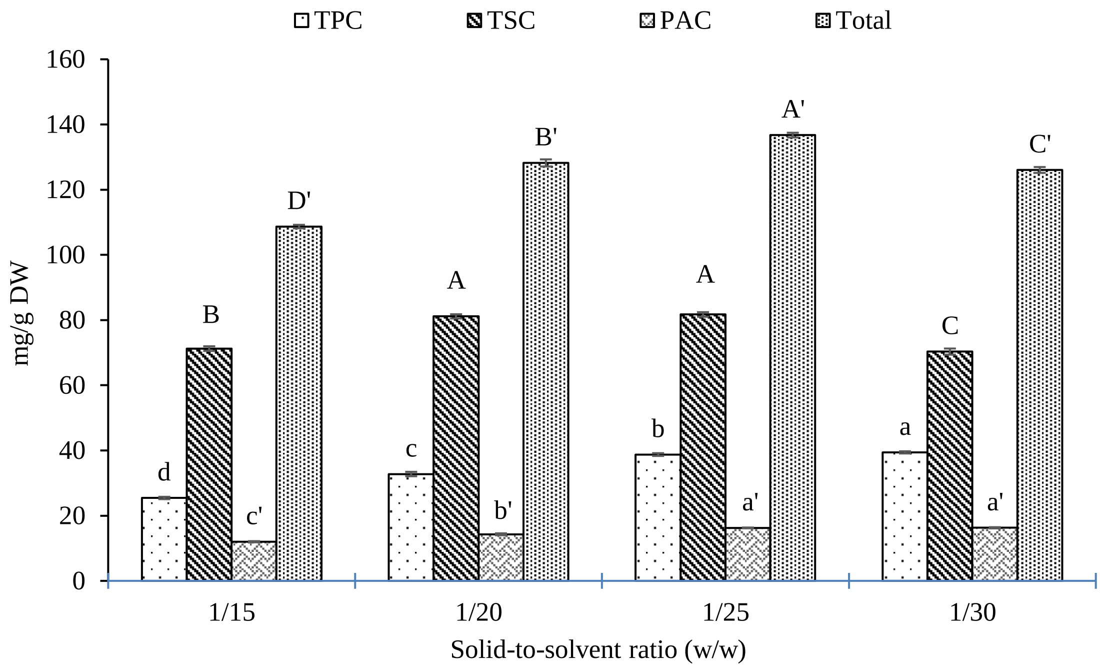

Figure 4. *The effect of solvent-to-material ratio on the extraction of bioactive compounds from grape pomace*

Distinct letters within the same parameters indicated statistically significant differences between extracts obtained with different solid-to-solvent ratios ($p \leq 0.05$).

Statistical results showed that the difference in the solid-to-solvent ratio affected the ability to extract bioactive compounds in grape pomace ($p < 0.05$). The solid-to-solvent ratio of 1/25 gave the highest extraction yield, which was demonstrated in the highest total extraction yield. The TPC, TSC, and PAC of the extract were 38.75 ± 0.43 mg GAE/g DW, 81.73 ± 0.70 mg AE/g DW, and 16.26 ± 0.11 mg CE/g DW, respectively. The total extraction yield of the bioactive compounds obviously increased with increasing solvent volume, specifically when the solid-to-solvent ratio increased from 1/15 to 1/25. As the solvent volume continues to increase to a solid-to-solvent ratio of 1/30, the total extraction yield of the bioactive compounds decreased. The solid-to-solvent ratio plays a significant role in the extraction. Commonly, as the solid-to-solvent ratio rises, the yield of phenolic compounds increases and the extraction time decreases. To a certain extent, increasing the solid-to-solvent ratio can cause the sample

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to be completely immersed and increase the contact area, resulting in a higher extraction yield. In other studies, Zhang, et al. [20] and Nguyen, et al. [6] also observed that increasing the solid-to-solvent ratio leads to a decrease in bioactive compound yield. This is explained by the extract concentration gradient between the solid and liquid phases will be higher, corresponding to a higher solid-solvent ratio. Increased concentration gradients facilitate the mass transfer of bioactive compounds [9]. A high solvent-to-solid ratio typically supported target compound transfer and thereby enhanced extraction efficiency. However, high amounts of solvent also increase costs. Therefore, from this investigation, the solid-to-solvent ratio of 1/25 (w/w) was the most suitable solid-to-solvent ratio for the extraction of bioactive compounds from grape pomace using ChCl-LA.

3.5. The effect of extraction time on the extraction of bioactive compounds

The extraction time was considered one of the important factors that could affect the bioactive compounds in the extract from grape pomace. In this study, the effect of time on the extraction of bioactive compounds was investigated. The extraction processes were performed for different periods (1.5 to 4.0 hours) (see Figure 5). The fixed factors included ChCl–LA, HBA: HBD ratio of 1:2, the solid-to-solvent ratio of 1/25 (w/w), water addition (20%), and extraction temperature (30 ºC).

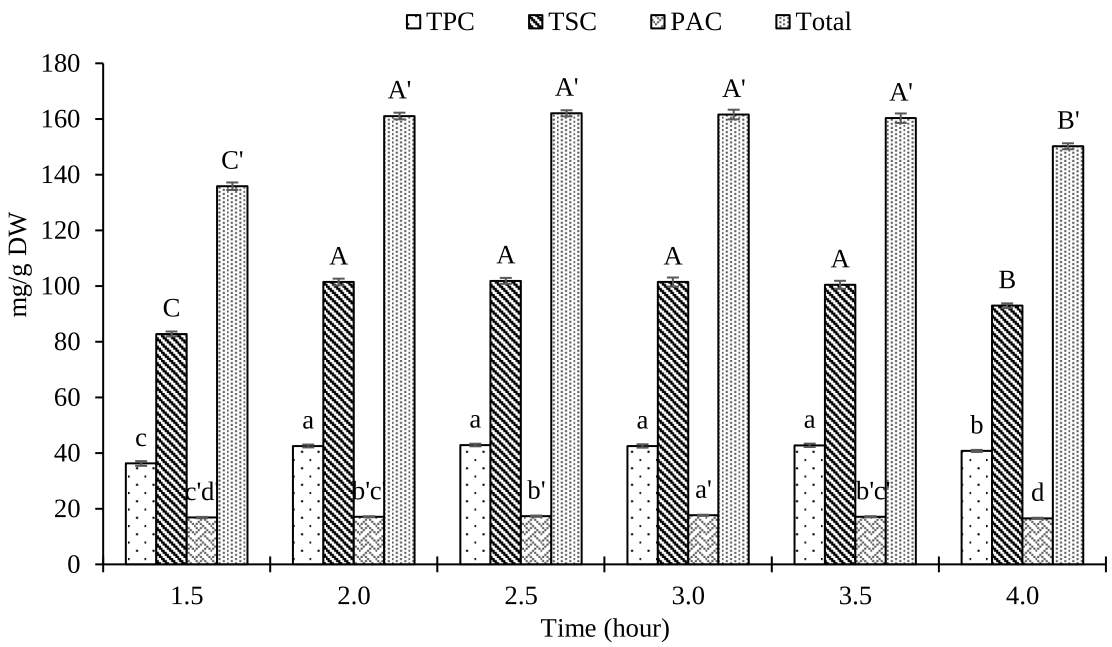

Figure 5. *The effect of extraction time on the extraction of bioactive compounds from grape pomace*

Distinct letters within the same parameters indicated statistically significant differences between extracts obtained with different extraction time ($p \leq 0.05$).

Statistical results showed that the difference in extraction time affected the ability to extract bioactive compounds in grape pomace ($p < 0.05$). As can be seen in Figure 5, when increasing the extraction time from 1.5 to 2.0 hours, the total extraction efficiency increased. However, the total extraction yield did not increase in the periods of 2.5 to 3.5 hours. After 4.0 hours, PAC decreased slightly. After 2.0 hours of extraction, the total extraction yield reached 161.07 ± 1.20 mg/g DW. The TPC, TSC, and PAC of the extract were 42.53 ± 0.52 mg GAE/g DW, 101.43 ± 1.22 mg AE/g DW, and 17.11 ± 0.18 mg CE/g DW, respectively, and were not significantly different ($p > 0.05$) compared to 2.5, 3.0, and 3.5 hours. In general, extraction time affects the extraction yield of bioactive compounds using DES. According to studies, the bioactive compounds dissolve more quickly with time, but once the osmotic pressure of the solution system achieves equilibrium, the rate of dissolution will not change substantially [9]. There was no difference in the ability to extract bioactive compounds after an extraction time of 2.0 hours. However, after the extraction time reached 4 hours, the extractability decreased slightly, possibly because some bioactive compounds became less stable over time. Furthermore, greater extraction times increase the cost, which makes the process inefficient. On the other hand, the ideal extraction time also depends on the characteristics of the material and the DES used. From this investigation, the extraction time of 2.0 hours was selected as the optimal extraction time for the extraction of bioactive compounds from grape pomace.

4. Conclusions

The results of the investigation demonstrated that DESs based on organic acids exhibited efficiency in extracting bioactive compounds from grape pomace. This research also showed that DES type, HBA: HBD ratio, water addition, solid-to-solvent ratio, and extraction time have a significant influence on the extraction of bioactive compounds from grape pomace. Among DES solvents based on organic acids including citric acid, malic acid, and lactic acid, the one based on choline chloride and lactic acid with a ratio of choline chloride to lactic acid of 1:2 and a 20% water content has the highest extraction yield. The suitable conditions for the extraction of bioactive compounds from grape pomace using choline chloride and lactic acid-based DES were a solid-to-solvent ratio of 1/25 (w/w) and an extraction time of 2.0 hours at ambient temperature. The extract obtained under these most suitable conditions exhibited high levels of TPC, TSC, and PAC. Specifically, the TPC, TSC, and PAC contents of the extract were measured as 42.53 ± 0.52 mg GAE/g DW, 101.43 ± 1.22 mg AE/g DW, and 17.11 ± 0.18 mg CE/g DW, respectively. Future research on optimizing the extraction of bioactive compounds extracted from grape pomace should be carried out to provide the most optimal extraction efficiency.

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Conflict of Interest

The authors declare no conflict of interest.

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