Co-liquefaction of α-Cellulose and Phycocyanin: A Preliminary Study

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Hydrothermal liquefaction (HTL) is an efficient technology for converting biomass to platform compounds. It has great potential for reducing the dependence on fossil fuels. The HTL of waste biomass has been extensively studied in recent years due to both its environmental and economic benefits. However, most woody waste contains a large amount of cellulose, and it is difficult to be sufficiently decomposed to valuable chemicals. Phycocyanin, a key component of algae, is easily degraded under high-temperature liquefaction conditions. In this work, focusing on bio-oil generation properties, the co-liquefaction characteristics and synergistic mechanisms of α-cellulose and phycocyanin were explored. The findings revealed a maximum bio-oil yield of 33.1 wt% under the optimal conditions (300 °C for 40 min), with a notable positive synergistic effect of 13.5 wt%. Chemical composition analysis indicated distinct compositional differences between the bio-oils derived from individual and dual feedstock. The amounts of pyridine and pyrimidine compounds increased due to the enhanced co-liquefaction. The results also highlighted the influence of temperature on the degree of conversion and product distribution. Finally, preliminary chemical reaction pathway was elucidated, underscoring the potential of integrating microalgae and woody biomass for enhanced bio-oil production.

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Keywords: Phycocyanin; a-Cellulose; Liquefaction; Synergistic effect; Bio-oil

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INTRODUCTION

Due to climate change and energy security pressure, the world's demand for the research and development of environmentally friendly and renewable new energy sources is increasing. Biomass is attracting increasing attention as an abundant renewable resource. High energy density liquid biofuels can be obtained from system refinement of the biomass. Such liquid biofuels have many advantages, especially low sulfur content, sustainability, and carbon emissions that are 80 to 90% lower than those of fossil fuels (Shamoon *et al.* 2022). Various types of biomasses, including algae, agricultural wastes, and sludges, have been explored. Agricultural wastes are produced in large amounts every year; for example, there are an average of 740 million tons of agricultural straw in China, which is equivalent to 317 million tons of standard coal in calories (Cao *et al.* 2017). Currently, the most commonly applied solutions for addressing large-scale biomass waste are incineration, pyrolysis, and gasification. However, these methods have several problems, such as massive CO_2 and hazardous gas emissions during conversion and low efficiency due to the high moisture content of some biomass wastes.

Among the conversion technologies, hydrothermal liquefaction is an efficient way to transfer bio-organics to several useful products, such as bio-oil and hydro-char, in a water environment under high temperature and pressure (250 to 400 °C, 5 to 25 MPa) (Zhang *et al.* 2018; Yang *et al.* 2020). According to the biomass type, the generated biofuel has been developed from first-generation biofuel obtained from food crops to second-generation biofuel from woody biomass (Correa *et al.* 2017; Pereira *et al.* 2024). A typical woody biomass is primarily composed of cellulose, hemicellulose, and lignin (Asafu-Adjaye *et al.* 2022). Among these, cellulose is the largest component of woody plants, followed by hemicellulose and lignin (Zhang *et al.* 2019). Ching *et al.* (2017) conducted HTL of microcrystalline cellulose with an acid catalyst and reported 5-hydroxymethylfurfural and levulinic acid as the main compounds at 180 °C. With increasing reaction temperature, furan derivatives, carbonic acid, aldehydes, and acetic acid are produced (Ching *et al.* 2017). The HTL of woody biomass has a relatively low bio-oil yield at different temperatures, residence times, pressures, and particle sizes. Gao *et al.* (2012) performed liquefaction of cellulose under various conditions and reported a maximum oil yield of only 14.75 wt%. The stable structure of cellulose is the main reason for the lower conversion degree.

Microalgae, as aquatic plants, have the advantages of low feeding costs, high photosynthetic efficiency, a short growth cycle, and a relatively high value of the higher heating value (HHV) (Zou *et al.* 2010; Shin *et al.* 2016). High-fat microalgae are excellent feedstocks for biodiesel production, while low-fat microalgae are currently used as food for feeding animals. HTL is an effective option for improving biomass conversion to biofuel, especially for wet microalgae (Jena *et al.* 2011; Saber *et al.* 2016; Arun *et al.* 2018; Huang *et al.* 2018).

Microalgae have strong potential as sustainable biofuels (Hawrot-Paw *et al.* 2020). According to a previous study (Agbulut *et al.* 2023), the oil production percentage of microalgae can range from 32.2 wt% to 52.8 wt% and even up to 77.9 wt% under optimal conditions. However, the HTL process of low-fat microalgae suffers from several problems, including the high content of nitrogen compounds in the bio-oil (Vardon *et al.* 2011; Guo *et al.* 2015). According to previous investigations, hydrothermal co-liquefaction (HTCL) has emerged as a promising novel method for biofuel production to overcome the high-nitrogen problem (Gai *et al.* 2015; Leng *et al.* 2018; Tian *et al.* 2020; Wang *et al.* 2023).

It has been reported that woody residue HTCL with protein-rich feedstock, such as microalgae, exhibits intriguing disparities that could result in favorable outcomes (Zhang *et al.* 2020; Wang *et al.* 2023). Thus, in the present work, the HTCL of α-cellulose and phycocyanin (key components of microalgae) were investigated. It is crucial to comprehensively understand the coconversion mechanism to develop a better utilization strategy for solid waste and to produce high-quality bio-oil. The effects of temperature and feedstock composition on the hydrothermal liquefaction degree and the product properties were explored. Moreover, the bio-oil generation pathway of α-cellulose and phycocyanin was discussed based on the experimental results and product characterization. These findings should be valuable for understanding the performance of woody waste and protein-rich feedstock in hydrothermal co-liquefaction and provide helpful support for fuel conversion and renewable feedstock utilization.

EXPERIMENTAL

Materials

The α-cellulose powder was supplied by Macklin Biochemical Technology Ltd. The phycocyanin powder was purchased from Xi'an Zebang Biological Technology Ltd. All the feedstocks were first milled and passed through 40 mesh. Then, they were dried at 105 °C overnight. All the chemicals used in the experiments were of ACS reagent grade.

Liquefaction Process and Product Separation

The experimental setup for the HTL employed a high-temperature and high-pressure batch reactor featuring a 50 mL capacity and was equipped with a temperature-controlled heating plate. The reactor was constructed from 316 L stainless steel and was capable of withstanding pressures up to 40 MPa and temperatures of 400 °C. In a typical test, 3 g of feedstock was mixed with 30 mL of distilled water in the reactor to obtain a fixed solid loading of 10 wt%. The reactor was heated at approximately 10 °C/min in a heating furnace to the set temperature and kept stable during the designed reaction time. After the reaction, the reactor was rapidly cooled to room temperature using tap water, and the exhaust valve was opened to collect the gaseous products. According to a previous study (Duan *et al.* 2022), the effect of reaction temperature is the most significant parameter in the liquefaction reaction. Thus, temperatures ranging from 260 to 340 °C were selected for testing the temperature sensitivity, and the reaction time was maintained at 40 min.

The solid product was recovered using a Büchner funnel. It was washed three times using dichloromethane and dried at 105 °C overnight. The solid weight was obtained based on the weight difference between the spent and fresh filter paper. Then, a separating funnel was applied to recover the oil phase product with dichloromethane. After evaporation of the dichloromethane in a rotary evaporator at 40 °C for one hour, the bio-oil mass could be obtained. All experiments were conducted twice, and the yield reported was the average value. The product yields, energy recovery rates, and synergistic effects (SEs) were calculated using the following equations (Duan *et al.* 2022):

$$\text{Bio} - \text{oil yield}/(\text{wt.}\,\%) = m_{bio-oil}/m_{biomass} \times 100\% \tag{1}$$

$$\text{Solid residue yield}/(\text{wt.}\,\%) = m_{solidresiehe}/m_{biomass} \times 100\% \tag{2}$$

$$\text{Others yield }/(\text{wt.}\,\%) = 100\% - Y_{bio-oil} - Y_{\text{solid residue}} \tag{3}$$

$$\text{Conversion rate}/(\text{wt.}\,\%) = 100\% - Y_{solid\ residue} \tag{4}$$

$$\text{Energy recovery rate}/(\%) = HHV_{bio\text{-}oil} \times Y_{bio\text{-}oil}/HHV_{bionass} \times 100\% \tag{5}$$

$$\text{Synergistic effect} = Y_{mix} - \Sigma(Y_a \times \mathrm{m}_a) \tag{6}$$

In the foregoing equations, m_x represents the mass of the corresponding products or raw biomass and Y_x represents the yield of the related products. In the formula for synergistic enhancement, Y_{mix} represents the bio-oil yield obtained from the co-liquefaction experiments, m_a represents the mass fraction of *a* feedstock in the total biomass weight during hydrothermal co-liquefaction, and Y_a represents the bio-oil yield obtained when the corresponding feedstock is individually liquefied.

Product Characterization

The experimental products included bio-oil, aqueous phase, gas, and solid residue. The primary emphasis of the study is placed on the analysis and characterization of the composition and physicochemical properties of the bio-oil. An Agilent GC–MS 5977B was applied for compositional analysis, employing a complementary capillary column of HP-5MS with dimensions of 30 mm × 0.25 mm × 0.25 µm. Specific peaks in the data were compared with the National Institute of Standards and Technology (NIST) database, and compound identification results were obtained based on comparative analysis.

RESULTS AND DISCUSSION

Liquefaction of Single Phycocyanin and α-Cellulose

HTL was conducted by setting the reaction temperature range from 260 to 340 °C, with feedstock blending of 10 wt%. Under the same reaction conditions, liquefaction characteristics of phycocyanin and α-cellulose were compared to reveal the similarities and differences in the reactions of model compounds. Figure 1 shows the results of the HTL experiments of pure phycocyanin at different temperatures.

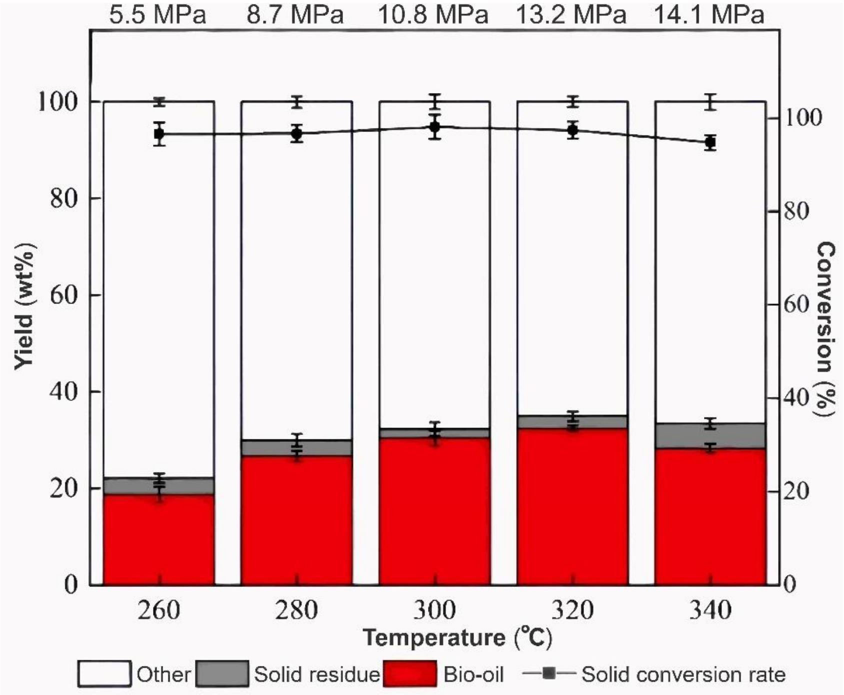

Fig. 1. Product distribution of phycocyanin HTL (reaction time: 40 min)

It can be seen that the conversion of phycocyanin is quite sensitive to temperature during the HTL process. During the liquefaction, the bio-oil yield increased as the temperature was elevated. The optimal reaction temperature was found to be approximately 320 °C. The maximum yield of phycocyanin was 31.7 wt%. Further increasing the temperature led to a decrease in yield, accompanied by a rise in solid residue yield. The experimental results indicate that there is an optimum temperature for the HTL of biomass feedstock, and above which might promote coking reactions. In addition, a higher temperature caused a higher pressure in the reaction system; over 14 MPa pressure of liquefaction was obtained at 340 °C. Greater gaseous fraction that generated at higher temperature could be one of the reasons that achieve the robust pressure. The result of research shows that phycocyanin could serve as a model compound, offering a representative basis for protein-rich microalgae and providing a feasible foundation for further exploration of reaction mechanisms in subsequent research endeavors.

Figure 2 displays the product distribution, final reaction pressure, and feedstock conversion rate of the HTL of α-cellulose. It can be seen that the bio-oil yield of the α-cellulose was quite low compared with that of the phycocyanin. Moreover, the conversion percentage of the feedstock was positively correlated with temperature, but the bio-oil yield showed different trends. As the temperature was increased to 280 °C, the bio-oil yield reached its maximum value of approximately 17 wt%, followed by a decreasing trend, indicating that the optimal reaction temperature is around 280 °C. The solid residue yield decreased first and then slightly increased, with the turning point of 300 °C reaction temperature. Since bio-oil was the main target product, the HTL temperature of α-cellulose was slightly lower than that of phycocyanin, ensuring the economical preparation of bio-oil. Final reaction pressure was increased when the temperature was raised from 280 to 300 °C, indicating the generation of massive amounts of gaseous products. In general, under a higher temperature the feedstock would decompose more thoroughly but also causes part of the oil phase to break down into smaller gaseous molecules. Thus, the balance between conversion rate and bio-oil yield should be carefully evaluated. Overall, approximately 280 °C is thought to be the optimal temperature for α-cellulose liquefaction, which is meaningful for woody biomass conversion.

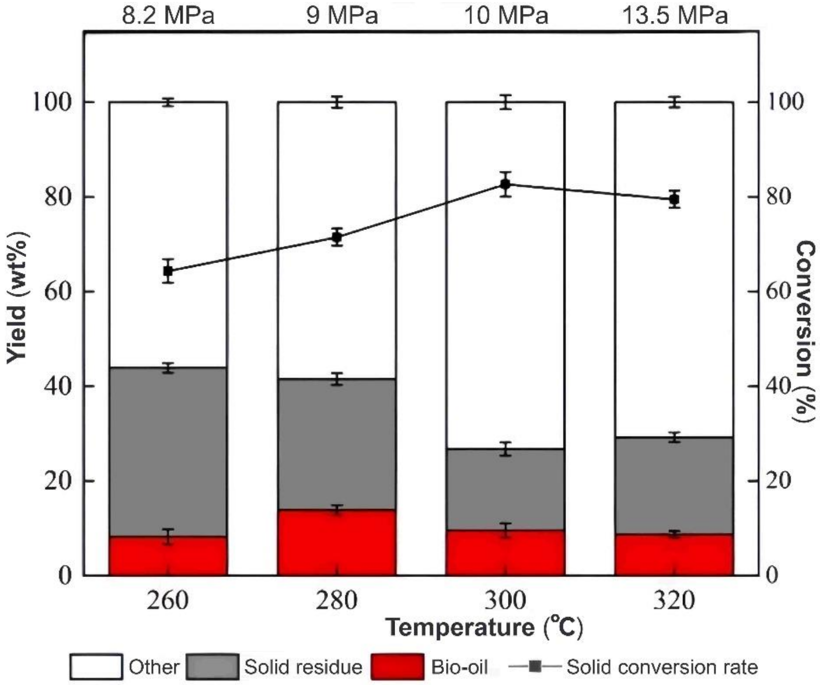

Fig. 2. Product distribution of α-cellulose HTL (reaction time: 40 min)

Physical properties of the feedstock, such as solubility in water, may lead to different performance in the liquefaction process. For instance, phycocyanin exhibits a strong affinity for water and can be premixed to form a uniform feedstock slurry before the reaction. In contrast, the cellulose sample demonstrates poor hydrophilicity but exhibits improved solubility in alcohol or alcohol-water cosolvents (Yuan *et al.* 2011; Xu and Savage 2014; Ji *et al.* 2017; Watson *et al.* 2019). Therefore, during the HTL process in pure water, α-cellulose is prone to form solid residues at the bottom and wall of the reactor. This characteristic might be a factor affecting the bio-oil yield, conversion rate, and final reaction pressure between the two model compounds under the same conditions that cannot be ignored.

Synergistic Liquefaction Effect between Phycocyanin and α-Cellulose

The synergistic effect is defined as mentioned in Eq. (7). Its principle involves calculating the variance between the experimental yield and the theoretical yield of HTCL. Figure 3 presents the experimental results related to the bio-oil yield, product distribution, and final reaction pressure obtained from the co-liquefaction using phycocyanin and α-cellulose dual feedstocks from 260 to 320 °C, with a reaction time of 40 min and a 1:1 blending ratio. It provides the basis for the evaluation of quantitative indexes and the study of the influence of co-liquefaction strategy on bio-oil production.

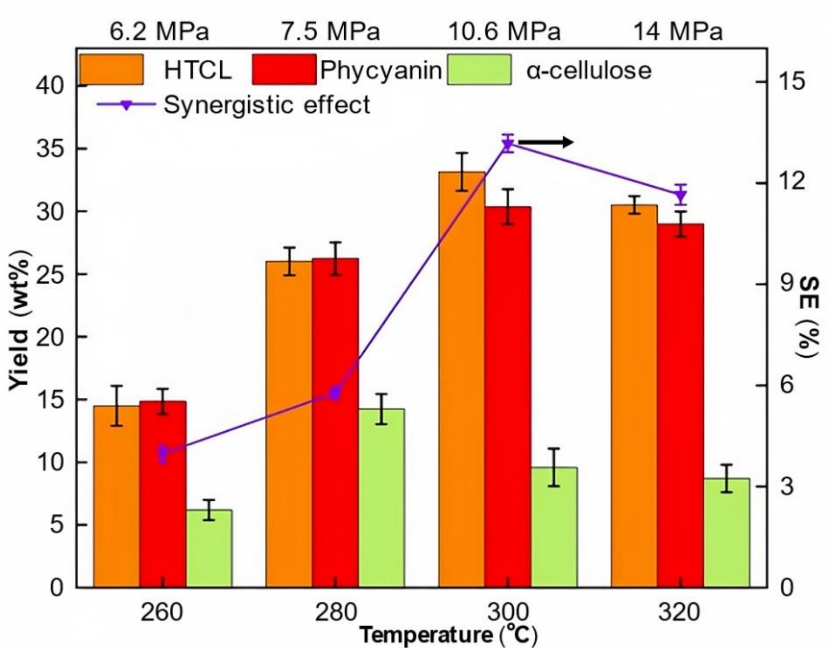

Fig. 3. Synergistic effect of phycocyanin and α-cellulose HTCL co-liquefaction regarding bio-oil

Starting at 260 °C, the bio-oil yield of co-liquefaction increased, and the yield under the same conditions was significantly greater than that for phycocyanin and α-cellulose individually liquefied. The yield increment of the HTCL decreased when temperature approaching 280 °C, while at 320 °C, the bio-oil yield obviously decreased. The results showed that the maximum bio-oil yield located at 300 °C and 40 min, reaching approximately 33.1 wt%, which is the highest yield throughout this study.

The maximum liquefaction bio-oil yield from the α-cellulose and phycocyanin dual feedstocks was close to that yield reported for the mixture of spirulina and oil tea residue (Duan *et al.* 2022) but at a lower temperature. In general, high pressure is favorable for bio-oil production.

An increase in pressure up to supercritical condition is beneficial to liquefaction by keeping the water in the liquid state while increasing its extraction and penetration ability.

An increase in pressure prevents fragmentation of the C-C bond by increasing the local solvent density (Shahbeik *et al.* 2024). Since liquefaction pressure and temperature are positively correlated, it is difficult to study the role of pressure alone in the liquefaction process. Figure 3 shows that when the temperature was increased to 300 °C, both the pressure (over 10 MPa) and the oil yield were increased. However, after further increasing to 320 °C, the final reaction pressure reached 14 MPa, and the oil yield decreased. This phenomenon is similar to the co-liquefaction of real biomass (Anamul Haque *et al.* 2023), indicating that using the model compound would be representative in terms of bio-oil yield and reaction to some extent.

When the temperature was increased from 260 to 280 °C, the SE value increased significantly, reaching 7.27 wt%. Subsequently, as the temperature was further increased to 300 °C, the highest SE value was approached, reaching approximately 13.5 wt. %. When the temperature was raised to 320 °C, the value decreased to approximately 11.6 wt. %. The experimental findings revealed that the synergistic effect consistently remained positive in the HTCL reaction. This indicates that co-liquefaction always enhanced the bio-oil yield across the entire temperature range.

Figure 3 shows that within the temperature range studied, co-liquefaction of phycocyanin and α-cellulose have the potential to achieve a considerable bio-oil yield between 280 and 300 °C. Since the synergies were always positive, this finding also supports the good benefit for co-liquefaction of protein-rich algae and the woody residue for bio-oil production.

GC-MS Analysis of Bio-oil from the Single Feedstock and their Mixture

During hydrothermal co-liquefaction, the extent of the reaction of the feedstock is influenced by varying parameters. To gain a deeper understanding of the hydrothermal reaction mechanism and bio-oil generation pathways of specific biomasses, a comprehensive analysis of the bio-oil composition resulting from the hydrothermal liquefaction (HTL) of phycocyanin and α-cellulose was conducted. The bio-oils from HTCL cases obtained at 260 and 320 °C were analyzed by GC-MS. The GC-MS spectra of the bio-oil samples are shown in Fig. 4, and the distributions of organic matter classified according to functional groups are shown in Fig. 5.

Composition analysis of the phycocyanin bio-oil

GC-MS analysis of the phycocyanin bio-oil obtained at 320 °C is displayed in Fig. 4A. It can be seen that the bio-oil contained several main components such as organic acids, including oleic acid and n-hexadecanoic acid. Additionally, compounds such as p-toluene and phenol were identified.

The peaks for organic acids and amides appeared in the residence time range of 30 to 35 min. Their abundances were high, indicating that the bio-oil contained more heavy components. The significant peak in 5 to 15 min was less, indicating that the content of light components in bio-oil was low. Figure 5 confirms that bio-oil from the protein sample had a large amount of heterocycles and organic acids, resulting from the abundance of amino acids in the protein sample.

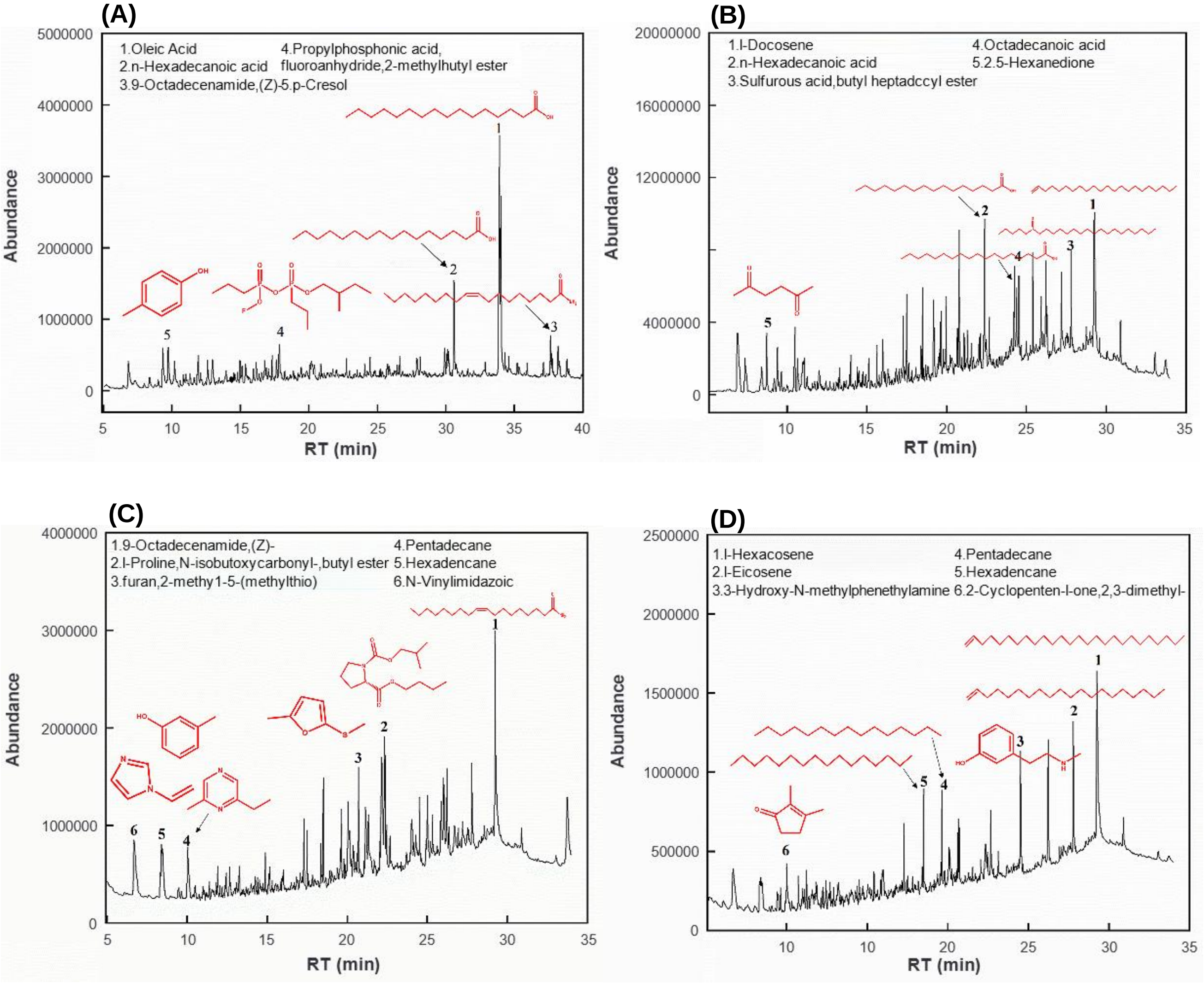

Fig. 4. GC-MS spectra of HTL bio-oils from (A) phycocyanin at 320 °C, (B) α-cellulose at 320 °C, (C) phycocyanin+α-cellulose at 260 °C and (D) phycocyanin+α-cellulose at 320 °C

Composition of the α-cellulose bio-oil

Figure 4B shows the GC-MS characteristic peak chromatogram of the α-cellulose bio-oil obtained at 320 °C, where the characteristic peaks were primarily distributed within the retention time ranges of 5 to 10 min and 20 to 28 min. Unlike the bio-oil from phycocyanin, the α-cellulose bio-oil did not contain large amounts of organic acids within the retention time range of 28 to 32 min.

The distribution in Fig. 5 reveals relatively high abundances of hydrocarbons due to the presence of rich components such as docosahexene, 5-hydroxymethylfurfural, and hexacosene in the bio-oil. Moreover, HTL oil from α-cellulose comprised a larger proportion of esters, ketones, and less heavy components than phycocyanin bio-oil. For these reasons, the α-cellulose bio-oil displayed a lighter color and better fluidity.

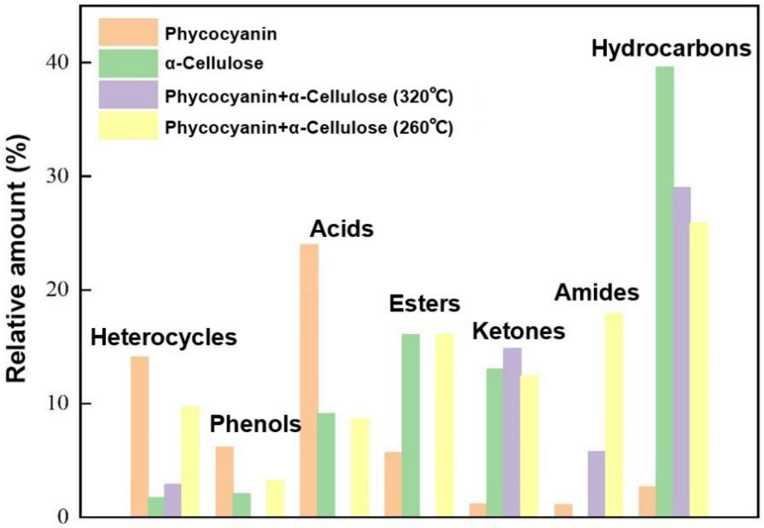

Fig. 5. Distribution of the identified chemicals according to functional group

Composition of the bio-oil from the dual feedstock

Figure 4C shows the characteristic peak curve of the bio-oil obtained from the HTCL of phycocyanin and α-cellulose at 260 °C. The chromatogram revealed that the characteristic peak distribution of the hydrothermal co-liquefied bio-oil differed from that of the phycocyanin and α-cellulose bio-oils. These differences were mainly located within the retention time intervals of 5 to 10 min and 20 to 30 min. Compared to the abundant heavier molecules at 30 to 35 min in the phycocyanin bio-oil, the compound distribution in the co-liquefied bio-oil was closer to the shorter retention time side, indicating that fewer heavy components were present in the bio-oil from HTCL, and the viscosity tended to decrease.

To understand the influence of temperature on the compound distribution of co-liquefied bio-oil, compositional analysis was conducted on the bio-oil generated at a high temperature of 320 °C, and the results are shown in Fig. 4D. A significant difference in components was observed compared to bio-oil obtained under low-temperature conditions. Bio-oils obtained under low-temperature conditions mainly contain pyrazine and furan compounds, while obvious pyridine and pyrimidine compounds are found under high-temperature conditions. Moreover, the high-temperature co-liquefied bio-oil mainly contained methylpyrazine and dimethyl pyrimidine, possibly because of the pyrazine-type reaction. In addition, components such as long-chain hydrocarbons, amides, and ketones were significantly present in the high-temperature bio-oil. Almost no furan or pyrazine compounds were identified. This suggests that as the temperature increases, these substances participate in the reaction and are further consumed.

Figure 5 shows that the co-liquefied bio-oil was more similar to the phycocyanin bio-oil in terms of compound distribution. Moreover, typical compounds such as 9-octadecenamide and furan were also found in bio-oils derived from hydrothermal co-liquefaction of spirulina and oil tea residue (Duan *et al.* 2022). However, they were not present in the bio-oils resulting from the hydrothermal liquefaction of a single phycocyanin or α-cellulose. Therefore, it could be deduced that these compounds came from the deep mutual interactions of phycocyanin and α-cellulose in the HTCL environment.

Discussion of the Co-liquefaction Reaction Pathway

By comparing the differences in the composition of the bio-oils derived from algae phycocyanin, α-cellulose, and their mixture, the chemical reaction pathways involved in the HTCL process were analyzed (Mathanker *et al.* 2021). The characterization results of the HTCL bio-oil indicate that during the HTCL process, stronger polymerization, condensation, and cyclization reactions occurred compared to the liquefaction of individual feedstocks, resulting in a significantly increased macroscopic bio-oil yield. Based on the characterization of samples from different reaction conditions, Fig. 6 shows some of the possible bio-oil generation pathways involved in the HTCL process.

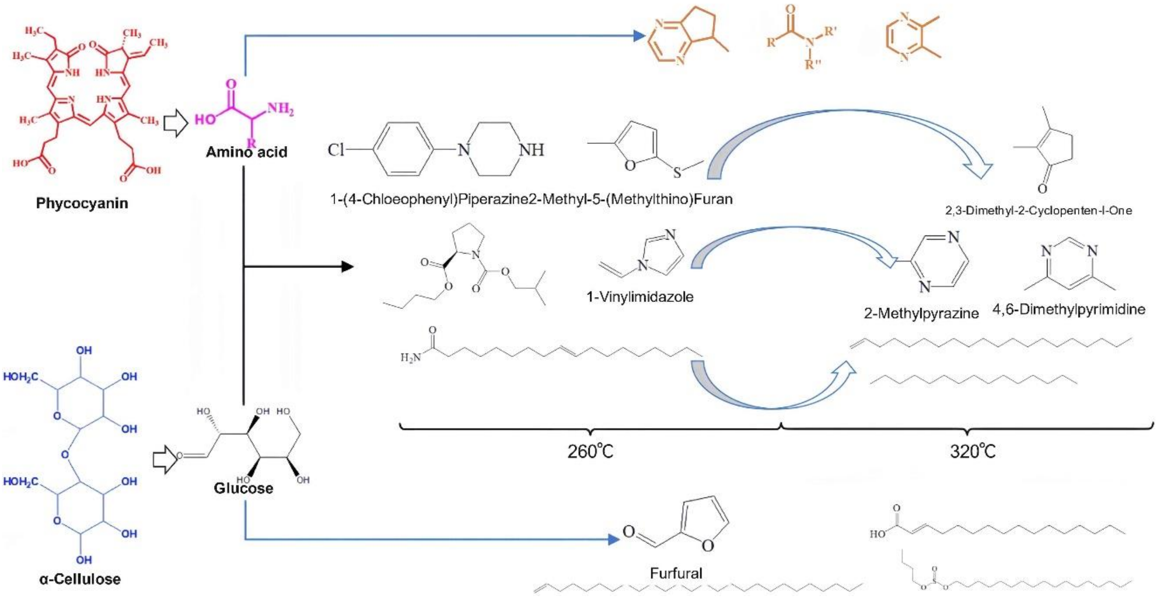

Fig. 6. Co-liquefaction mechanism of phycocyanin and α-cellulose

Generally, as the temperature increases, proteins and α-cellulose undergo hydrolysis, yielding fatty acids, amino acids, and monosaccharides. With further increases in temperature, organic acids undergo decarboxylation reactions to form long-chain hydrocarbons, while pyrazine compounds may be formed through condensation polymerization of amino acids. The hydrolysis of α-cellulose produces disaccharides, which are hydrolyzed to form monosaccharides, and through a series of reactions, aldehydes, ketones, and 2-hydroxymethylfurfural are generated, ultimately forming long-chain hydrocarbons *via* aldol condensation reactions. At the same time, α-cellulose hydrolysis may also produce complex pentatomic nitrogen-containing cyclic compounds. However, the dehydration reaction of pyrazines generated from the hydrothermal liquefaction of algae phycocyanin is enhanced, leading to the appearance of more pyridine compounds in the bio-oil.

Upon further increasing the reaction temperature, decarboxylation and dehydration reactions proceed, leading to an increase in the content of pyridine and pyrimidine compounds. Moreover, the contents of furan and pyrazine compounds decreased sharply, and coupling reactions occurred due to thermal cracking to generate other compounds. In the mid-to-high temperature range, the yield of HTCL bio-oil significantly increased compared to the theoretical values. This may be due to the intensive polymerization, condensation, cyclization, and Maillard reactions (Chacón-Parra *et al.* 2022) of hydrolyzed amino acids and polysaccharides in the hydrothermal liquefaction environment. The hydrolysis products of α-cellulose dissolve within the aqueous phase, reducing the bio-oil yield from the liquefaction. According to the GC-MS characterization results, hydrothermal co-liquefaction led to an increase in bio-oil components through coupling reactions between small molecular intermediates, including amide and furan compounds, which were absent in bio-oils from individual feedstocks. As the temperature further increased, the polymerization reactions of furan and nitrogen-containing compounds may generate additional bio-oil components. Nevertheless, they may also lead to a decline in bio-oil quality. Overall, the increase in bio-oil yield from HTCL may be attributed to the intensive polymerization, condensation, cyclization, and Maillard reactions of amino acids and polysaccharides in the hydrothermal liquefaction environment.

CONCLUSIONS

This study presented a preliminary investigation into the co-liquefaction of phycocyanin and α-cellulose to produce bio-oil. The maximum bio-oil yield obtained from the co-liquefaction of algae phycocyanin and α-cellulose was 33.1 wt% at 300 °C for 40 min, demonstrating a synergistic effect of 13.5 wt%. GC-MS analysis indicated that bio-oil derived from algae phycocyanin hydrothermal liquefaction predominantly consisted of fatty acids and phenols. The bio-oil produced from α-cellulose hydrothermal liquefaction was characterized by its content of long-chain hydrocarbons and 5-hydroxymethylfurfural. At lower temperatures, the main components identified in bio-oil from co-liquefaction of phycocyanin and α-cellulose were amides, furans, and the derivatives, whereas at higher temperatures, the composition shifted toward primarily pyridine and pyrimidine compounds. The preliminary chemical reaction pathway involved in the co-liquefaction process was also discussed in this work.

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