### Development of Coconut Protein Concentrate-Xanthan Gum Conjugate by Wet-Dry Heating Method for Red Palm Oil Emulsification

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#### ABSTRACT

Protein-polysaccharide conjugation is commonly achieved by wet and dry heating methods. Therefore, this study aimed to produce red palm oil (RPO) emulsifiers by conjugating coconut protein concentrate (CPC) and xanthan gum (XG) through a combination of wet and dry heating method using a cabinet dryer. Several factors, including reaction time (3, 4, 5, 6, and 7 hours), pH (3, 5, 7, 9, and 11), and protein-polysaccharide ratio (1:3, 1:2, 1:1, 2:1, and 3:1) were evaluated for their effect on the Emulsion Activity Index (EAI) and Emulsion Stability Index (ESI). The ability of the obtaining conjugate to emulsify RPO was evaluated, and the results showed that CPC contained 67.40% protein. Reaction time, pH, and protein-XG ratio had a significant effect on EAI and ESI. Meanwhile, optimal conditions for the formation of the CPC-XG conjugate, based on EAI and ESI, were a reaction time of 5 hours, pH 9, and protein-polysaccharide ratio of 2:1. Fourier Transform Infrared (FTIR) analysis showed that the CPC-XG conjugate had a change in absorption at a wavelength number of around 1640 cm<sup>-1</sup>, indicating the presence of a Maillard reaction product. Furthermore, the CPC-XG conjugate used in RPO emulsion has a characteristic EAI value of 23.74 m<sup>2</sup>/g, ESI of 271.32 minutes, a droplet size of 790 nm, and a zeta potential of -36.9 mV. These results suggest that the CPC-XG conjugate produced by the wet-dry heating method has the potential for producing stable RPO emulsions.

Keywords: Coconut protein concentrate; conjugation; emulsification; xanthan gum

#### INTRODUCTION

The increasing demand for emulsifier material in red palm oil (RPO) emulsification process is a major factor contributing to high production costs. Several studies have explored the emulsification of RPO, including Lee et al. (2017), which focused on using 60% emulsifier material on 40% RPO. To address this issue and reduce production costs, an effective emulsifier is needed to stabilize RPO emulsion at low usage levels, facilitating a reduction in emulsifier material components. Improved stability can be achieved by using conjugate obtained through conjugation of protein and polysaccharide via glycation, an initial stage in the Maillard reaction. According to Oliveira et al. (2016), conjugation can be applied as an easy method to enhance protein functionality, such as solubility and thermal stability.

Conjugation process includes two heating methods, namely dry and wet heating. Conjugation through dry heating produces powder conjugate suitable for industrial applications, but the process requires a long reaction time. Additionally, it incorporates lyophilization using a freeze dryer with a generally small capacity, limiting its industrial development. In contrast, wet

DOI: http://doi.org/10.22146/agritech.76632 ISSN 0216-0455 (Print), ISSN 2527-3825 (Online) heating can proceed quickly with a larger production capacity, producing conjugate products that are less suitable for industrial use due to the more complex handling of liquid materials. This study aimed to combine the basic principles of dry and wet heating to produce powder conjugate through semi-dry heating using a cabinet dryer.

Factors such as reaction time, temperature, pH, and protein-polysaccharide ratio affect proteinpolysaccharide conjugation. Li et al. (2013) stated that the hydrophobicity of the Maillard reaction product surface decreases when the reaction time exceeds 5 minutes in the preparation of conjugate using wet heating at 100°C. Meanwhile, pH contributes to the conditioning of proteins and polysaccharides. O'Mahony et al. (2016) stated that at alkaline pH, deprotonation of amino acids occurs, enhancing the reaction in the early stage due to the formation of more reactive amine groups. Another factor affecting protein-polysaccharide interaction is protein-polysaccharide ratio. Phoebe et al. (2017) showed that the solubility of whey protein isolate increases by approximately 20% when conjugated with beet pectin at a lower beet pectin concentration, including a protein-pectin ratio of 2:1.

Coconut (*Cocos nucifera L.*) oil extraction using wet heating method produces a by-product called "blondo," which is protein sediment. According to Thaiphanit (2016), processed blondo concentrate contains 80.30% protein, presenting a valuable opportunity for use as an emulsifier. In this study, conjugation was carried out by reacting coconut protein concentrate (CPC) with xanthan gum (XG) polysaccharide due to several advantages, including rapid solubility in cold or hot water, good stability to acid and base, salts, as well as high temperatures (Leela et al., 2000). The CPC-XG conjugate produced from conjugation using optimal factors was used for RPO emulsification.

Based on the above description, this study aimed to examine the effect of reaction time, pH, and protein-polysaccharide ratio on CPC-XG conjugation through the semi-dry heating method, and the process was confirmed using FTIR. Subsequently, CPC-XG conjugate was characterized in RPO emulsion to assess its performance based on the Emulsion Activity Index (EAI), Emulsion Stability Index (ESI), droplet size, and zeta potential.

### METHODS

#### Materials

Blondo was obtained from Heltico (Yogyakarta, Indonesia), while XG was obtained from Meihua (Shandong, China). Subsequently, commercial palm oil and RPO were purchased from the local market. NaOH, n-hexane, HCl, and SDS were obtained from Merck KGaA (Darmstadt, Germany).

### Production of CPC

The method for producing CPC was based on Permatasari et al. (2015) with modifications. Blondo in paste form was dispersed in n-hexane (1:2 w/v) and stirred for 1.5 hours at 2000 rpm using an overhead stirrer (IKA RW 20 digital). The stirring process continued with filter paper filtration to separate protein and oil trapped in the solvent. The filtrate obtained was dispersed in fresh n-hexane (1:2 w/v), followed by stirring and filtration. This oil removal process using n-hexane was conducted in three cycles. Subsequently, the filtrate was placed in a tray for 12 hours to eliminate any remaining n-hexane. All oil removal processes were carried out in the fume hood, and the filtrate was dried at 50 °C for 8 hours using a cabinet drver. The dried CPC was reduced in size using an electric blender (Philips) and sieved with a 40-mesh sieve.

#### Effect of Reaction Time on EAI and ESI of CPC-XG Conjugate

CPC and XG (2:1 w/w) were dissolved in 0.1 N pH 11 buffer solution. Subsequently, CPC was stirred for 30 minutes at 400 rpm, followed by stirring of XG for 30 minutes using an overhead stirrer at 2000 rpm (IKA RW 20 digital). CPC and XG were each stored at 4 °C overnight to complete the hydration process, and the pH of the CPC-XG mixture was adjusted to pH 11 with 1 N NaOH and 1 N HCI. The mixture was incubated for 3, 4, 5, 6, and 7 hours at 65°C using a cabinet dryer, and the conjugate obtained was placed in the freezer for further use.

#### Effect of pH on EAI and ESI of CPC-XG Conjugate

CPC and XG (2:1 w/w) were dissolved in 0.1 N pH 9 buffer solution. Subsequently, CPC was stirred for 30 minutes at 400 rpm, followed by stirring of XG for 30 minutes using an overhead stirrer at 2000 rpm (IKA RW 20 digital). CPC and XG were each stored at 4 °C overnight to complete the hydration process, while the pH of the CPC-XG mixture was adjusted to pH 3, 5, 7, 9, and 11 with 1 N NaOH and 1 N HCI. The mixture was incubated for 5 hours at 65 °C using a cabinet dryer, and the conjugate obtained was placed in the freezer for further use.

# Effect of Protein-Polysaccharide Ratio on EAI and ESI of CPC-XG Conjugate

CPC and XG (1:3, 1:2, 1:1, 2:1, and 3:1 w/w) were dissolved in 0.1 N pH 9 buffer solution. Subsequently,

CPC was stirred for 30 minutes at 400 rpm, followed by stirring of XG for 30 minutes using an overhead stirrer at 2000 rpm (IKA RW 20 digital). Both CPC and XG were stored at 4 °C overnight to complete the hydration process, while the pH of the CPC-XG mixture was adjusted to pH 9 with 1 N NaOH and 1 N HCl. The mixture was incubated for 5 hours at 65 °C using a cabinet dryer, and the conjugate obtained was placed in the freezer for further use.

# Production of RPO Emulsion using CPC-XG Conjugate

The method for producing CPC was based on Zhang et al. (2015) with modifications. Conjugate was dissolved in aquades to achieve a concentration of 2 mg/ml. RPO and conjugate solution (1:19 w/w) were homogenized using an Ultra-Turrax (IKA Digital T – 25) at 10,000 rpm for 3 minutes to produce a coarse emulsion. Subsequently, the coarse emulsion was homogenized using a High-Pressure Homogenizer at a pressure of 420 Mpa for one cycle.

### EAI and ESI

The measurement method for EAI and ESI was based on Li et al. (2014) with slight modifications. Conjugate was dissolved in aguades to obtain a final concentration of 0.8 mg/mL, while the effect of reaction time, pH, and CPC-XG ratio was evaluated using RPO (1:9 w/w) on conjugate. Furthermore, RPO (1:9 w/w) was used to evaluate the performance of conjugate in stabilizing the emulsion. The mixture was homogenized at 10,000 rpm for 3 minutes with an Ultra-Turrax (IKA Digital T – 25). After homogenization, 50  $\mu$ l of the emulsion was immediately taken from the bottom of the container at 0 minute and diluted in a 0.1% SDS solution (1:200 v/v) after 10 minutes. The absorbance of the diluted emulsion was recorded at 500 nm. Subsequently, EAI  $(m^2/g)$  and ESI (minutes) were calculated using the Equation 1.

where DF is the dilution factor (200), C is protein concentration (g/mL),  $\phi$  is the optical path (1 cm),  $\theta$  is the oil volume fraction (0.1), A0 and A10 are the absorbances of the emulsion at 0 and 10 minutes, respectively.

#### FTIR Analysis of CPC-XG Conjugate

Fourier Transform Infrared (FTIR) analysis on the samples was conducted by placing the sample into the

Agilent Cary 630 FTIR device. The sample was ground using a diamond inside the device before infrared light was targeted at the sample.

# Measurement of Zeta Potential and Oil Droplet Diameter

Zeta potential and oil droplet diameter were measured using the dynamic light scattering method (Zetasizer NanoZS, model ZEN3600 Malvern Instruments, Malvern, UK) with version 3.1 of the automatic measurement software (Malvern Instruments). Before measurement, the samples were diluted 250 times in aquades to avoid double scattering effects.

#### **Statistical Analysis**

One-way ANOVA was used to determine the statistical significance of EAI and obtained using SPSS 25 (IBM) at a significance level of 95%.

#### **RESULTS AND DISCUSSION**

#### **Characteristics of CPC**

The defatting process was carried out to obtain a product with a high protein fraction from crude CPC. As presented in Table 1, the characteristics of defatted CPC have a protein content of 67.40%. Previous study conducted by Minj and Anand (2020) showed that whey protein concentrate has a content of 34-89%. Based on the comparison with whey protein concentrate, the defatted blondo in this study is categorized into protein concentrate group.

# Effect of Reaction Time on EAI and ESI of CPC-XG Conjugate

Figure 1 shows a significant improvement in EAI when the reaction time increases from 3 to 4 hours. However, a further increase from 4 to 7 hours did not show a significant increase in EAI, indicating the protein's ability to reduce the surface tension at the oil-water interface (Naik et al., 2012). The increase in EAI

Table 1. Characteristics of CPC

Parameter	Content (%)
Fat Content	8.57 ± 0.08
Protein Content	$67.40 \pm 0.93$
Water Content	$5.15 \pm 0.01$
Ash Content	$3.27 \pm 0.00$
Carbohydrate (by difference)	15.61

at a 4-hour reaction time was attributed to the heating process, which opened the protein structure, exposing hydrophobic protein areas to the surface and interacting with the oi.

The ESI value was relatively low and not significantly different at 3 and 4 hours, namely 31.99 and 39.73 minutes. However, a significant increase was obtained with a further extension of reaction time from 4 to 5 hours. The ESI values for 5 and 6 hours were not significantly different, at 106.64 and 101.81, but decreased after 7 hours, at 91.60 minutes. This phenomenon occurred because more prolonged incubation or heating could decrease the solubility and hydrophilicity of the conjugate surface (Chen et al., 2019).

effectiveness The of protein-polysaccharide conjugate in improving emulsification properties is determined by reaction time. There needs to be more reaction time to ensure the optimal progression of the glycation process, as CPC and XG lack the opportunity to react adequately. This phenomenon results in poor conjugate performance in forming emulsions with high EAI and ESI values, as observed at a 3-hour reaction time. However, excessive reaction time increases the risk of decreasing the conjugate's performance in producing good emulsification properties as the Maillard reaction continues to the advanced stage. Conjugate formed at a 7-hour reaction time did not experience a reduction in EAI but in ESI due to the Maillard reaction entering the advanced stage. Meanwhile, unchanged EAI is possible because some proteins do not react with XG, contributing to the consistently high EAI value.

EAI at a 4-hour reaction time shows a significant increase, while ESI experienced insignificant improvement. This is because a 4-hour reaction time has not allowed CPC and XG to react optimally. Consequently, the stabilization effect by the polysaccharide group through steric hindrance has not been manifested. Based







Figure 2. Effect of pH on emulsion activity and stability. The reaction was carried out at 65 °C for 5 hours, with a CPC-XG ratio of 2:1. Samples with the same alphabet show no statistical difference in the samples.

on the results, a 5-hour reaction time was selected as the optimal value in the CPC-XG conjugation process.

#### Effect of pH on EAI and ESI of CPC-XG Conjugate

Based on Figure 2, EAI shows a significant fluctuation when the reaction was conditioned at pH 7, reaching its peak at pH 9. However, a significant decrease was observed with a further increase to pH 11. An increase in ESI and pH value was also observed from acidic to basic, which decreased significantly at pH 11. The highest ESI occurred when the conjugate was conditioned at pH 9. This significant increase in ESI values from pH 7 to pH 9 was also obtained in EAI values.

According to Evans et al. (2013), at pH between 4.8 and 3.0, depending on the mixing ratio, Gum Arabic (GA) and Bovine Serum Albumin (BSA) have opposite charges, forming insoluble complexes. This observation was relevant to the results obtained at pH 3, where the conjugate preparation process encountered difficulties as CPC and XG interacted to form insoluble complexes, resulting in uneven mixing of CPC-XG. Furthermore, CPC is positively charged at acidic pH (below the isoelectric pH), while XG shows a negative charge, limiting the chance of repulsion force to create stable emulsion conditions.

The results obtained in this study were similar to A'yun et al. (2020), where at higher pH values, amino groups in protein became less protonated and were available for conjugation with reducing sugars. Conditioning at pH 8 accelerated the Maillard reaction during conjugation using the dry heating method for whey protein concentrate samples. This condition reduced the incubation time needed to produce stable whey protein concentrate conjugate against heat. Meanwhile, increasing the pH to 10 potentially accelerated the rate of the Maillard reaction, leading to the formation of insoluble complexes after 1 hour



Figure 3. Effect of protein-polysaccharide ratio on activity and stability of the emulsion. The reaction was carried out at 65 °C for 5 hours, pH 9. Samples with the same alphabet show no statistical difference in the samples.

of incubation at 80°C, a product produced from the Advanced stage. Based on this result, pH 9 was selected as the optimal pH in the CPC-XG conjugation process.

# Effect of Protein-Polysaccharide Ratio on EAI and ESI of CPC-XG Conjugate

Figure 3 shows that higher CPC content resulted in greater EAI due to protein serving as emulsifiers capable of interacting with the hydrophilic groups of water and the hydrophobic groups of oil. At a 2:1 ratio, it was observed that the obtained EAI was significantly high, while the ESI was at the highest position compared to other ratios. The composition at a 2:1 ratio consisted of 66.66% protein, while XG was 33.33%. Similarly, Phoebe et al. (2017) stated that the solubility of WPI increased by approximately 20% when reacted with conjugation and beet pectin at a lower concentration of 2:1 protein-pectin ratio.

A higher protein ratio of 3:1 provided low emulsion stability with greater conjugate values. This phenomenon was associated with droplet aggregation induced by a higher protein ratio, causing a reduction in the steric hindrance effect from thinning at the interface due to a lower polysaccharide ratio. The denser and thicker layer at the interface resulted in stronger steric hindrance (McClements and Gumus, 2016). Generally, emulsifiers differ significantly in composition and molecular arrangement at the oil-water interface, affecting their ability to produce steric hindrance between droplets. Polysaccharides that form a thick layer at the interface, such as Gum Arabic, effectively inhibit droplet aggregation through steric interaction. However, globular proteins such as whey protein forming a thin interface layer are ineffective in preventing

droplet aggregation through steric hindrance. Based on the results obtained, a 2:1 ratio was selected as the best ratio in the CPC-XG conjugation process.

#### FTIR Analysis of CPC-XG Conjugate

In Figure 4, there are four peak zones in the spectrum from the functional group analysis of the samples. In the first zone, the optimum conjugate and native CPC have a maximum peak at approximately 3300 cm-1, associated with the stretching vibration of NH groups found in protein. Similarly, Farshi et al. (2019) stated that the maximum stretching vibration of WPI was approximately 3325 cm<sup>-1</sup>, while the stretching at 1646 and 1535 cm<sup>-1</sup> corresponded to amides I and II. Moreover, Amide I was observed at a wavenumber of 1640 cm<sup>-1</sup>, specifically for the Amide I C=O functional group. At 1530 cm<sup>-1</sup>, it represented the functional group for C-N of Amide II, and at approximately 1025 cm<sup>-1</sup>, there was a specific peak from the C-O band of XG in conjugate. A new band was detected at around 1640 cm<sup>-1</sup> related to specific Maillard reaction products, such as Schiff bases, including amino groups, enaminol, or Amadori products, namely ketoamines.

# EAI and ESI of CPC-XG Conjugate on Palm Oil and RPO

Table 2 indicates that the use of native CPC at concentrations of 0.8% and 2% shows no emulsion formation. This phenomenon is attributed to the dominance of polar groups in the surface molecules of native CPC, leading to the ability to reduce interfacial tension. According to McClements and Jafari (2018), protein reside at the interface between the oil and water phases, facilitating emulsion droplet formation and stabilization by reducing interfacial tension. The ability of proteins as emulsifiers depends on the balance of polar and non-polar groups constituting the surface



Figure 4. FTIR of CPC-XG conjugate and native CPC

Formulation		$E\Lambda I(m^2/a)$	FSI (minutos)
Surfactant	Oil		LSI (IIIIIules)
CPC native 0.8%	Palm oil	Not detected	Not detected
CPC native 0.8%	Red palm oil	Not detected	Not detected
CPC native 2%	Palm oil	Not detected	Not detected
CPC native 2%	Red palm oil	Not detected	Not detected
CPC-XG 0.8% Conjugate	Palm oil	$75.14 \pm 1.22^{\circ}$	$326.25 \pm 5.30^{\circ}$
CPC-XG 0.8% Conjugate	Red palm oil	Not detected	Not detected
CPC-XG 2% Conjugate	Palm oil	$36.7 \pm 1.00^{b}$	249.32 ± 11.53°
CPC-XG 2% Conjugate	Red palm oil	23.74 ± 0.06 <sup>a</sup>	$271.32 \pm 11.46^{\circ}$

Table 2. Characterization of conjugate based on EAI and ESI

Samples with the same alphabet show no statistical difference among the samples

molecules. When non-polar groups dominate the surface molecules of protein, it is not easily dissolved in water. However, when the polar group dominates, it will not function as a surfactant.

The CPC-XG conjugate used in palm oil produced EAI and ESI values of 75.14 m<sup>2</sup>/g and 326.25 minutes, respectively, at a concentration of 0.8%. However, when the CPC-XG conjugate was applied to RPO at a 0.8% conjugate concentration, no emulsion could be formed due to the occurrence of rapid creaming immediately after homogenization using Ultra-turrax was stopped. Samples experiencing rapid creaming, which occurred due to differences in composition between RPO and palm oil, were not representative for further analysis. Consequently, conjugate cannot form a stable emulsion at the same concentration. According to Mba et al. (2015), the micro-nutrients contained in RPO consist of carotenoids with a concentration of 500-700 ppm, vitamin E in the form of tocopherols and tocotrienols at 500-1000 ppm, sterols, and ubiquinone. Chandi and Gill (2011) reported that carotenoids were long-chain polyene with 35-40 carbon atoms affecting the performance of conjugate in RPO emulsification. Ariviani et al. (2018) stated that nanoemulsions of  $\beta$ -carotene with a long-chain triglyceride (LCT) oil phase had an average droplet diameter greater than the medium-chain triglyceride (MCT) oil phase. The results were in line with Yang and McClements (2013), where emulsions with an MCT oil phase had an average particle diameter, which was significantly smaller than the LCT oil phase. The size of the droplet diameter in the emulsion contributes to an increase in Stokes velocity (Vstokes), potentially leading to instability. Specifically, droplets tend to move in the same direction as the Stokes force, ascending from the liquid surface. This phenomenon leads to a creaming tendency in the emulsion.

The optimum conjugate at a concentration of 2% can emulsify RPO with EAI and ESI values of 23.74  $m^2/g$  and 271.32 minutes, respectively. The EAI value produced was lower than the emulsion formed on palm oil (palm olein) at the same conjugate concentration, which was 36.7  $m^2/g$ . However, the ESI value obtained was higher than the RPO emulsion, while EAI results in the application of palm oil and RPO were lower compared to Li et al. (2015) on soy protein-XG conjugation (191.6  $m^2/g$ ). The ESI from this study was higher, showing that CPC-XG conjugate can be applied to emulsion-based products due to its ability to produce high emulsion stability.

#### Zeta Potential and Oil Droplet Size

Based on Table 3, the particle size produced in the emulsion formed by CPC-XG conjugate is in the nanoemulsion range. Shah et al. (2010) stated that nanoemulsion is an oil-in-water emulsion system with droplet sizes ranging from 50-1000 nm. In this study, the droplet diameter formed by the CPC-XG conjugate was smaller than the native CPC, according to Candraningrum et al. (2022). This phenomenon showed that conjugation could enhance the functional properties of CPC. Protein and polysaccharide conjugation is a method used to enhance emulsion-forming ability, which depends on the balance of polar and non-polar groups in surface molecules. When non-polar groups dominate the surface of protein molecules, it is not easily dissolved in water. However, when polar groups dominate, it will not function as an active surface (McClements and Jafari, 2018). Conjugation of CPC-XG through the Maillard reaction causes the opening of protein structure, increasing the hydrophobicity of CPC and enhancing its adsorption ability on the surface of oil droplets during emulsification. Conjugation also contributes to improve emulsion stability due to additional steric forces from

Table 3. Zeta potential and particle size of CPC-XG conjugate

Surfactant	Zeta potential (mV)	Particle size (nm)
CPC native	$-30.65 \pm 0,63$	4730 ± 1281.27
CPC-XG conjugate	$-36.90 \pm 0,28$	790 ± 84.85

XG. Similarly, Setyowati et al. (2017) reported increased emulsion activity in emulsions formed by Whey Protein Isolate (WPI)-Low Methoxyl Pectin (LMP) conjugate due to the additional steric forces provided by LMP conjugated to the oil droplet surface. This resulted in better adsorption ability of conjugate to the oil droplet surface during emulsification due to increased hydrophobicity of WPI caused by the exposed protein structure during glycosylation.

According to Hakansson et al. (2013) and Maindarkar et al. (2015), emulsifiers can accelerate the production of small droplets by rapidly absorbing at the droplet interface and reducing interfacial tension. The efficacy of emulsifiers in reducing interfacial tension directly influences the size of droplets produced under the same homogenizer operating conditions, such as pressure and number of cycles. During this process, the adsorption rate must be higher than the droplet breakup, ensuring that new droplets formed in the homogenization are completely coated by emulsifiers (Lee et al., 2013; Hakansson and Hounslow, 2013; Hakansson et al., 2012).

A significant increase is observed in the interfacial area as large droplets are broken down in the homogenizer. At this point, there is a possibility that droplets that are not immediately coated by emulsifiers will coalesce. The droplets are stable when the available emulsifiers can cover the entire droplet formed and have a sufficiently strong repulsive force. However, when emulsifiers only cover part of the droplet, coalescence will occur during the collision in the homogenization process (McClements and Gumus, 2016). Generally, emulsifiers that tend to be quickly absorbed on the surface of oil droplets are more effective in inhibiting coalescence during homogenization.

#### CONCLUSION

In conclusion, this study showed that CPC contains 67.40% protein. Reaction time, pH, and protein-XG ratio significantly affect (p < 0.05) EAI and ESI. Based on the results, the best conditions for forming CPC-XG conjugate were a reaction time of 5 hours, pH 9, and protein-polysaccharide ratio of 2:1. Based on FTIR analysis, CPC-XG conjugate showed changes

in absorption at a wavenumber around 1640 cm-1, which was a component of specific Maillard reaction products. Furthermore, the CPC-XG conjugate used in RPO emulsion had characteristics of EAI of 23.74 m<sup>2</sup>/g, ESI of 271.32 minutes, droplet size of 790 nm, and zeta potential of -36.9 mV. These results indicated that CPC-XG conjugate obtained through a wet-dry heating combination showed excellent potential as an alternative for developing emulsion systems, including for RPO encapsulation.

#### **CONFLICT OF INTEREST**

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