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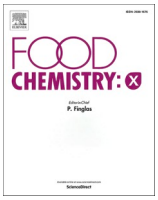

Arabic gum grafted with phenolic acid as a novel functional stabilizer for improving the oxidation stability of oil-in-water emulsion

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ABSTRACT

Three kinds of phenolic acids: ferulic acid (FA), caffeic acid (CA), and gallic acid (GA) with different chemical structures were individually grafted onto Arabic gum (AG) via a laccase mediated method, and their roles in stabilizing o/w emulsions were evaluated. The total phenolic content in modified AG increased from 2.7 ± 0.2 to 18.7 ± 0.2, 19.8 ± 0.6, 22.4 ± 0.8 mg/g after 4 h of laccase catalysis, respectively. FTIR spectra of modified AGs exhibited additional phenolic characteristics, revealing the successful grafting of phenolic acids to AG structure. Compared with natural AG, modified AGs showed remarkably enhanced thermal stability, as well as antioxidant capacity in an order of gallic acid > caffeic acid > ferulic acid. The incorporation of phenolic acids into AG dramatically improved its emulsification performance. Herein, gallic acid-modified AG evinced up to 17.6 % and 12.6 % increments in emulsifying activity and emulsion stability relative to natural AG, respectively. Moreover, the oxidative stability of AG emulsions was pronouncedly meliorated by the introduced phenolic acids, especially gallic acid, as manifested by the suppressed production of primary and secondary oxidation products.

1. Introduction

For emulsion-type products, it is a challenge to ensure their storage quality by solving the time-dependent oxidation of emulsion in the product system. In general, lipid peroxides in oil-in-water (o/w) emulsions accumulated at the interface and reacted with transition metal ions in the aqueous phase. The free radicals produced by the reaction would penetrate the interface layer and initiate a free radical chain reaction with polyunsaturated fatty acids (PUFA) in oil droplets, thereby accelerating oxidation (Wang et al., 2020). It has been demonstrated that the emulsifier existing at the interface or in the aqueous phase is closely related to the oxidation process of the emulsion (McClements and Decker, 2018). Therefore, enhancing the antioxidant activity of emulsifier would be conducive to solve the oxidation problem of o/w emulsions.

Arabic gum (AG) is defined by the FAO/WHO Joint Expert Committee for Food Additives (JECFA) as 'a dried exudate obtained from the stems of *A. senegal* (L.) Willdenow or closely related species of *Acacia* (family Leguminosae)' (Mortensen et al., 2017). It is a branched-chain, complex polysaccharide, either neutral or slightly acidic, consisting of high molecular polysaccharides, a small amount of protein, and its calcium, magnesium, and potassium salts (Araujo et al., 2020). AG, as one of the oldest and most well-known natural gums in the world, has been widely used in food industry as a preeminent emulsifier due to its amphiphilicity. The hydrophobic proteins can be adsorbed on the surface of oil droplets and hydrophilic carbohydrate components inhibit the flocculation and coalescence of molecules through electrostatic and spatial repulsion (Vuillemin, et al., 2020). However, the application of AG is largely limited by its poor antioxidant capacity due to the absence of phenolic functional groups in the chemical structure. In order to endow polysaccharides with preeminent antioxidant properties, more recent attention has been focused on grafting polyphenols onto polysaccharides (Aljawish et al., 2014). Several graft copolymerization approaches have been successfully developed to synthesize phenol-grafted polysaccharides, including carbodiimide based chemical coupling method and laccase mediated grafting reactions (Liu, Yong, Liu, & Bai, 2020; Zhao et al., 2022). Considering the potential toxicity of carbodiimide, the chemical coupling method are not suitable for food

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applications. In contrast, the laccase-mediated grafting method with high selectivity and substrate specificity is environmental-friendly and considered as a promising method for modifying AG. Recently, Vuillemin et al., 2020 has demonstrated that AG grafted with ferulic acid by laccase exhibited conspicuously improved antioxidant properties. From the perspective of food safety, the commonly used method for oxidation control in the food industry is to add chemically synthesized antioxidants. However, research reports about adverse effects of chemical synthesis additives on human health are not uncommon, and consumers tend to choose green processed products with natural ingredients (Lebelo, et al., 2021). Phenolic compounds are widely presented in nature, among which phenolic acid is one of the main dietary phenolic compounds (Delgado, Issaoui, & Chammem, 2019). Phenolic acids have high bioavailability and good water solubility, and are considered excellent antioxidants that can eliminate body damage and chronic diseases caused by excessive free radicals (Rahman et al., 2021). The antioxidant activity of phenolic acids largely depends on their competence to scavenge free radicals and provide hydrogen atoms, which are closely related to their number and position of phenolic hydroxyl groups (Spiegel et al., 2020). Ferulic acid (Cao, Fu, & He, 2007), caffeic acid (Kosaraju et al., 2010) and gallic acid (Yan, Li, Zhao, & Yi, 2011) are natural non-toxic and recognized as safe raw materials, which are allowed to be used in food. Their structures are different. Ferulic acid has one phenolic hydroxyl group on the benzene ring, caffeic acid has two phenolic hydroxyl groups, and gallic acid has three phenolic hydroxyl groups. Through laccase catalysis, these three phenolic acids can be oxidized to form covalent bonds with amino, sulfhydryl and other groups. However, there are scant information on how the chemical structure of phenolic acids affected the laccase-mediated grafting reactions with AG and the antioxidant capacity of the resulting phenolic acid-AG conjugates alone or in their stabilized emulsions. It is of great significance to explore the emulsifying properties of modified AG and the ability to inhibit oxidation reactions in emulsions to expand the application of AG.

In this current study, three kinds of phenolic acids (ferulic acid, caffeic acid, and gallic acid) with relevant chemical structures (mono-, di-, and triphenol) was grafted onto AG by laccase individually. The structural characteristics, thermal stability, and antioxidant activities of the obtained phenolic acids-grafted AG conjugates were investigated and compared with that of native AG (NAG). Specially, the effects of the incorporation of different phenolic acids on the AG structure on the emulsification performance (emulsifying activity, physical stability, and oxidation stability) of AG were further explored. This work would provide valuable information on the potential use of phenolic acid grafted AG conjugates as a novel functional stabilizer to produce high-quality emulsion-type products.

2. Materials and methods

2.1. Materials

AG (Pract Grade, molecular weights of 2.4–5.8 $\times$ 10^5 Da, initial density of 1.35 g/mL) was obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Ferulic acid (FA, purity 99 %), caffeic acid (CA, purity 99 %), gallic acid (GA, purity 99 %) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Laccase from *Rhus vernificera* (500 $\pm$ 21 LAMU/g) was acquired from Shanghai Yingxin Laboratory Equipment Co., Ltd. (Shanghai, China) in solid form. Soybean oil (hydrogen peroxide concentration less than 10 ppm) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All other chemicals and solvents of at least analytical grade were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, US) or Sangon Biotech Co., Ltd. (Shanghai, China).

2.2. Preparation of phenolic acid grafted AG

Phenolic acid was grafted into AG according to the method adapted from Vuillemin et al. (2020). In brief, 1 g of AG was dissolved in 45 mL of 0.2 mol/L phosphate buffer (pH 7.5). Phenol acid (50 mM, ferulic acid, caffeic acid, or gallic acid) dissolved in methanol solution (5 mL) and laccase (15 LAMU/mL) was added successively to the above AG solution, and the mixed solution was placed in a magnetic stirring reactor for 4 h under atmospheric conditions (the dissolved oxygen is not limited). An equal volume of ethanol (50 mL) was then added to the mixed solution to terminate the reaction. The obtained solution was centrifuged for 20 min at 6000 rpm to remove any insoluble substances. Afterwards, methanol was removed using a rotary evaporator (RE-52AA, Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China) at an operating temperature of 45 ℃. The concentrated solution was dialyzed in ultra-pure water with a biotechnology grade dialysis membrane (MWCO 10,000 Da from Labest Biotechnology Co., Ltd, Beijing, China) to remove excess salt. The dialysate was freeze-dried for 72 h. The obtained sample powder was stored in a sealed and dry environment until further use. The AG samples grafted by ferulic acid, caffeic acid, gallic acid were expressed as FA-AG, CA-AG, and GA-AG, respectively. Natural AG (NAG) without modification was used for comparison.

2.3. Evaluation of phenolic acid grafting content

The content of phenolic acid grafted to AG was evaluated by the Folin-Ciocalteu method (D. Chen et al., 2022). Briefly, phenolic acid grafted AG (1 mg/mL) was mixed with Folin-Ciocalteu reagent (0.25 mol/L) and allowed to react at room temperature in the dark for 3 min. Subsequently, sodium carbonate (150 g/L) solution was added. After incubation in the dark for 30 min, the absorbance of the mixture was read at 760 nm. The phenolic content in the modified AG samples was expressed as mg pyrogallol equivalents per g sample.

2.4. Fourier transform infrared spectroscopy (FTIR) analysis

A Cary 610/670 micro infrared spectrometer (Varian, USA) was used for FTIR analysis to reflect changes in the chemical structure of AG samples. The sample powder was placed on a universal diamond ATR top-plate, and then analyzed in the spectral range from 500 to 4000 cm^{-1}, with 32 scans and a resolution of 0.1 cm^{-1}.

2.5. Thermogravimetric analysis (TGA)

The thermal stability of both natural and modified AG samples was investigated by a Pyris 1 TGA (PerkinElmer Inc., USA) with reference to the method illustrated by Liu et al. (2016). The AG samples (3–5 mg) were heated from 30 °C to 600 °C at a heating rate of 20 °C/min in a nitrogen flow.

2.6. ABTS radical scavenging activity

The ABTS radical scavenging activity of AG samples was determined according to the protocol described by Yang, Chen, Hao, and Liu (2021). ABTS solution (7.4 mM) and potassium persulfate solution (2.6 mM) were mixed in a volume ratio of 1:1, and allowed to stand in the dark for 12–16 h. The mixture was then diluted with phosphate buffer (0.2 mol/L, pH = 7.4) to an absorbance of 0.7 $\pm$ 0.02 at 734 nm to obtain ABTS working solution. Thereafter, 1 mL of sample solution (1 mg/mL) was reacted with 1 mL ABTS working solution for 10 min. The absorbance was measured at 734 nm. The ABTS radical scavenging activity of samples was calculated as follows:

$$\text{ABTS radical scavenging activity}(\%) = \left(1 - \frac{A_s}{A_c}\right) \times 100 \quad (1)$$

Here, A_c represents the original absorbance of ABTS working solution; A_s indicates the absorbance of samples.

2.7. Ferric reducing antioxidant power

The ferric reducing antioxidant power was determined with reference to Bagchi and Kumar (2016) with slight modifications. Briefly, 2 mL of sample solution (1 mg/mL) was incubated with 2.5 mL of phosphate buffer (0.2 M) and 2.5 mL of 1 % (w/v) potassium ferricyanide solution at 50 ℃ for 20 min. The reaction was then terminated by 2.5 mL trichloroacetic acid (10 %, w/v). Subsequently, the mixture was centrifuged at 2000 rpm for 10 min. The obtained supernatant (2 mL) was mixed with 2.5 mL ultrapure water and 0.5 mL of 0.1 % (w/v) $FeCl_3$ for 10 min. Finally, the absorbance of the mixture was read at 700 nm.

2.8. Preparation of emulsions

Both NAG and modified AGs (FA-AG, CA-AG, GA-AG) were dissolved in ultrapure water and mixed overnight to ensure full hydration. Emulsions were generated by mixing the AG solution (5 %, w/v) with soybean oil (10 %, w/v) using an Ultra-Turrax homogenizer (IKA T18 Basic; IKA, Staufen, Germany) at 10,000 rpm for 3 min.

2.9. Emulsifying activity and emulsion stability

The emulsifying activity index (EAI) and emulsion stability index (ESI) of AG samples were analyzed as previously reported by Liu et al. (2016). Aliquots of samples (20 μL) were pipetted from the bottom of the resulting emulsions at 0 min and 10 min, respectively, and diluted with 5 mL of 0.1 % (w/v) SDS (251 dilution factor). The absorbance was measured at 500 nm. EAI and ESI were calculated using the following equations, respectively.

$$\mathrm{EAI} = \frac{2 \times 2.303 \times A_0 \times N}{C \times (1 - \Phi) \times 10^4} \quad (2)$$

$$\mathrm{ESI}(\backslash\%)) = \frac{A_{10}}{A_0} \times 100 \quad (3)$$

where N is the dilution factor (251); C represents the protein concentration (g/mL) before emulsification; Φ represents the oil volume fraction; A_0 and A_{10} are the absorbance of the emulsions at 0 min and 10 min, respectively.

2.10. Microstructure of emulsions

The microstructure of emulsions stabilized by NAG and modified AGs were visualized by a LSM 800 confocal laser scanning microscopy (CLSM, Carl Zeiss Microimaging Inc., NY, USA). The oil droplets in the emulsions were stained with 1 mg/mL Nile Red, which was excited with a 488 nm argon laser line.

2.11. Oxidation stability of emulsions

Emulsions were transferred into covered test tubes, and stored in a ventilated constant temperature incubator at 50 ℃ (to accelerate the oxidation reaction) for 7 days. The oxidation stability was evaluated by monitoring the formation of both primary (peroxide value and conjugated dienes) and secondary (thiobarbituric acid reactive substances and conjugated trienes) oxidation products.

2.11.1. Peroxide value (POV)

POV was determined according to the method illustrated by Noon, Mills, and Norton (2020). The emulsion samples (1 mL) were thoroughly mixed with isooctane/isopropanol solution (5 mL, 2:1, v/v) by a vortex for 30 s. After centrifugation at 3000 rpm for 2 min, 2 mL of the supernatant was collected and added with 20 μL of potassium thiocyanate solution and 20 μL of ferrous chloride solution. Thereafter, methanol/*n*-butanol solution (2:1, v/v) were mixed with the above solution to fix the volume to 5 mL. After reaction at room temperature in the dark for 20 min, the absorbance was recorded at 510 nm. The hydroperoxide concentration was calculated based on a cumene hydroperoxide standard curve.

2.11.2. Thiobarbituric acid reactive substances (TBARS)

For TBARS measurement, AG emulsions were mixed with the assay solution containing 15 % (w/v) thiobarbituric acid and 0.375 % (w/v) trichloroacetic acid followed by heating in a boiling water bath for 30 min. Afterwards, the mixtures were cooled rapidly and then centrifuged at 4500 rpm for 10 min. The absorbance of the supernatant was read at 532 nm. TBARS content were determined according to a standard curve prepared by 1,1,3,3-tetraethoxypropane (Yong et al., 2019).

2.11.3. Conjugated diene (CD) and conjugated triene (CT) hydroperoxides

CD and CT hydroperoxides in AG emulsions were quantified as detailed by Bastürk, Ceylan, Cavus, Boran, and Javidipour (2018). Emulsion samples (20 μL) were vortexed with 10 mL of isooctane solution for 1 min. Subsequently, the absorbance was measured at 234 nm and 268 nm, respectively. The contents of CD and CT were calculated using the molar extinction coefficients of 26,000 and 36300 M^{-1} cm^{-1}, respectively. The results were expressed by the relative changes of CD or CT, that is, the difference between the measured values of CD or CT on day n and day 0.

2.12. Statistical analysis

All experiments were repeated three times, and triplicate samples were prepared for each repeated trial. Data were analyzed by SPSS 22 (SPSS Statistical Software, Inc., Chicago, IL, USA). Significant differences ($P < 0.05$) between means were identified using Duncan's multiple range tests.

3. Results and discussion

3.1. Evaluation of phenolic acid grafting content

The grafting ratio of phenolic acids onto AG was evaluated using the Folin-Ciocalteu method. As presented in Table 1, NAG had a very low phenol content (2.7 ± 0.2 mg equivalent pyrogallol/g sample). After laccase catalysis for 4 h, the total phenol contents in all three kinds of modified-AG were significantly increased ($P < 0.05$), with an order of GA-AG > CA-AG > FA-AG, indicating that GA was of greater affinity for AG than CA and FA. The grafting of phenolic acid onto AG catalyzed by laccase follows a two-step reaction. The reaction is initiated by the oxidation of phenolic acid by laccase to produce *o*-quinone reactive intermediates, which are subsequently crosslinked with the nucleophilic groups (e.g., amines or carboxylic acids) of AG via Schiff-base and/or Michael adduct reactions (Adam et al., 2022). The reaction of phenolic

Table 1
Quantitative determination of total phenols in natural Arabic gum and three kinds of modified Arabic gum.

	Amount of total phenol (mg equivalent pyrogallol/g sample)
NAG	2.7 ± 0.2^{d}
FA-AG	18.7 ± 0.2^{c}
CA-AG	19.8 ± 0.6^{b}
GA-AG	22.4 ± 0.8^{a}

Notes: Values are means ± the standard deviation (SD). Different lowercase letters indicate significant differences ($P < 0.05$). NGA: natural Arabic gum; FA-AG: ferulic acid grafted Arabic gum; CA-AG: caffeic acid grafted Arabic gum; GA-AG: gallic acid grafted Arabic gum. The phenolic content was expressed in mg equivalent pyrogallol/g sample.

acids with AG was affected by the number and position of phenolic hydroxyl groups. FA and CA have similar chemical structures, both of which are derivatives of hydroxycinnamic acid. The difference is that the benzene ring of CA has two adjacent phenolic hydroxyl groups, while the benzene ring structure of FA has only one phenolic hydroxyl group. The benzene ring of GA contains three adjacent phenolic hydroxyl groups with methoxy groups in its structure. Consequently, the superior binding capacity of AG with GA in comparison with FA and CA could be related to the presence of more active hydroxyl groups (three) in GA. Similar findings have been reported in the case of lotus root polysaccharide grafted with different phenols (Yi et al., 2022).

3.2. FTIR spectra

FTIR analyses were exploited to identify changes in the chemical structure of AG induced by the modification process. NAG exhibited a broad peak between 3000 and 3600 cm^{-1}, corresponding to the O—H stretching vibration of hydroxyl groups. The characteristic absorption peaks at 2925 cm^{-1}, 1600 cm^{-1}, and 1053 cm^{-1} denoted the stretching vibrations of C—H, C═O, and C—O, respectively (Preparation and antimicrobial activity of thyme essential oil microcapsules prepared with gum arabic). As shown in Fig. 1A and 1B, the fingerprint (between 900 cm^{-1} and 1200 cm^{-1}) of NAG was maintained in the modified AGs, demonstrating that the laccase-mediated reaction had little effect on the AG backbone. However, we also observed a significant red shift in the peak of modified AG, which was due to a decrease in the separation distance between particles (Vasicek, Jenkins, Vaz, Chen, & Stenken, 2017). It also indicates that phenolic acid has been successfully grafted onto AG, resulting in the molecular spacing of modified AG. Compared with the spectra of NAG, a pronounced increment in the intensity of the absorption peak at around 1125 cm^{-1} assigned to C—O stretching was detected in the modified AG samples (GA-AG, FA-AG, and CA-AG), which could derive from the elongation of ester bonds between the carboxyl groups of AG and phenolic acid (Vuillemin et al., 2020). The absorption band at 1645 cm^{-1} could be ascribed to the C═N stretching mode of the imines. The intensity of this band was notably strengthened when AG was treated with phenolic acids, suggesting the occurrence of a Schiff-base reaction between AG and phenolic acid (Gnanasambandam & Proctor, 2000). Additionally, the absorption enhancement at approximately 870 cm^{-1} could be linked to the *para*-substituted aromatic rings of phenolic acid-oxidation products induced by laccase (Karaki, et al., 2017). These phenomena indicated that phenolic acids had been successfully grafted onto AG.

3.3. Thermogravimetric analysis (TGA)

The decomposition pattern and thermal stability of both NAG and modified AGs were assessed by the thermogravimetric analysis. As it can be seen in Fig. 2, all AG samples presented an emblematic thermogram characterized by two principal stages of weight loss, which could be ascribed to the loss of absorbed and bound water in the AG samples (30 ℃–200 °C) and the decomposition/depolymerization of the grafted phenolic acids and AG backones (200 ℃–800 ℃), respectively (Guo et al., 2022). In the first stage, the weight loss of NAG (10.48 %) was remarkably higher than that of the three modified AGs, in an order of FA-GA (10.29 %) > CA-GA (9.49 %) > GA-AG (7.80 %). The results could be attributable to the introduced phenolic acid groups in the AG structure improved its hydrophilicity, consequently leading to an increment in the amount of water retained by the modified AGs. Similar phenomenon has been observed in the curcumin-modified AG (Adam et al., 2022). In the second stage (200 ℃–800 ℃), a severe weight loss was noted in all samples. At the end of heating, the modified GAs (64.73 %–73.82 %) with greater weight loss appeared to be more susceptible to decomposition than NAG (61.64 %). This is due to the oxygen-

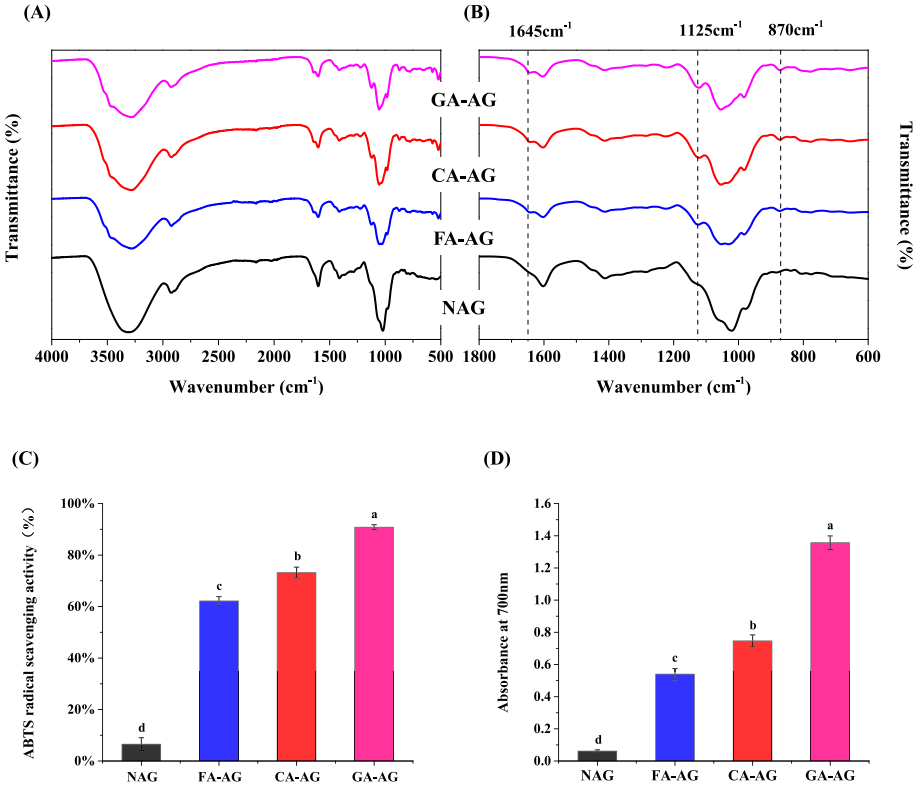

Fig. 1. Fourier-transform infrared spectroscopy (FTIR) of natural Arabic gum (NAG), ferulic acid grafted Arabic gum (FA-AG), caffeic acid grafted Arabic gum (CA-AG), and gallic acid grafted Arabic gum (GA-AG). A: whole wavenumber ranging from 500 cm^{-1} to 4000 cm^{-1}, B: focused between 600 cm^{-1} and 1800 cm^{-1}. ABTS radical scavenging activity (C) and ferric reducing power (D) of NAG and three kinds of modified AG (FA-AG, CA-AG, and GA-AG). Means without common lowercase letters differ significantly ($P < 0.05$).

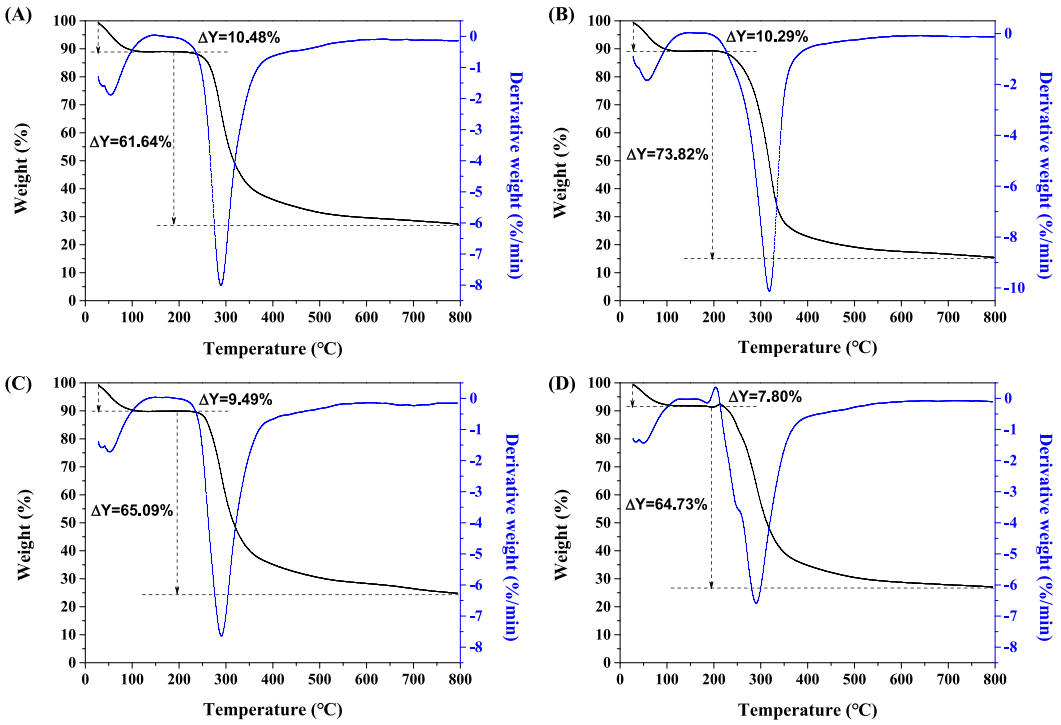

Fig. 2. Primary thermograms (black line) and derivative thermograms (blue line) of NAG (A), FA-AG (B), CA-AG, (C), and GA-AG (D). ΔY indicates the percentage of weight loss (%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

containing groups on the chemical structure of modified AGs, especially the highly active phenolic hydroxyl groups, involved in the pyrolysis reaction first and turned into small oxygen-containing molecules which would take part in the full combustion reaction (Zhong, Jing, Wang, & Jia, 2016).

Furthermore, the derivative thermogravimetric curves were obtained to provide additional information on the relative decomposition rate, where the peak maxima represented the maximum weight loss rate of polymers. As shown in Fig. 2 (blue line), the rapidest weight loss of NAG appeared at 289.5 ℃. After modification by phenolic acids, the temperature for the rapidest weight loss was shifted towards a higher value (290.4 ℃–318.3 ℃), especially for FA-AG, insinuating an improvement in the thermal stability of AG after graft copolymerization. This could be associated with the enhanced intra- and intermolecular interactions caused by the laccase-catalyzed grafting reaction (Ren, Guo, Zhao, & Qiang, 2019).

3.4. Evaluation of antioxidant activity

3.4.1. $ABTS^{\cdot+}$ radical scavenging activity

The radical scavenging activities of NAG and modified AGs were evaluated by the ABTS assay. As depicted in the Fig. 1C, NAG had a weak $ABTS^{\cdot+}$ radical scavenging activity (6.5 %). With the grafting of phenolic acids onto AG structure, the $ABTS^{\cdot+}$ radical scavenging activity of the modified AGs was dramatically boosted, evidently originating from the competence of phenolic acids. Likewise, an increase in the $ABTS^{\cdot+}$ radical scavenging activity was observed in other polysaccharides such as chitosan and pectin grafted with phenolic acids mediated by laccase (Wang et al., 2020; Yu, Liu, Pei, & Wang, 2020). Among three kinds of modified AGs, GA-AG (90.8 %) owned the greatest $ABTS^{\cdot+}$ radical scavenging activity, followed by CA-AG (73.2 %), while FA-AG (62.2 %) exhibited the lowest scavenging activity. Previous studies have revealed that the antioxidant activity of phenolic acids was largely dependent on the number and position of hydroxyl groups, as well as the subcategory of phenolic acids (Sroka & Cisowski, 2003). The presence of more hydroxyl groups in the ortho or para position was conducive to the antioxidant activity, owing to additional resonance stabilization (Spiegel et al., 2020). Consequently, GA with three hydroxyl groups (para and ortho substituted on the aromatic ring) owned stronger $ABTS^{\cdot+}$ radical scavenging activity relative to FA with one hydroxyl group (para substituted on the aromatic ring) and CA with two hydroxyl groups (para and ortho substituted on the aromatic ring). Additionally, the stronger antioxidant capacity of GA-AG might also be explained by its higher phenol content, as more GA were grafted onto AG than FA and CA (Table 1).

3.4.2. Ferric reducing antioxidant power (FRAP)

FRAP assay based on the single electron transfer reaction is usually adopted to evaluate the redox potential of compounds (Munteanu & Apetrei, 2021). As shown in Fig. 1D, the modified AGs displayed tremendously stronger FRAP than NAG (0.062), which was in good corroboration with the ABTS results, vindicating that the antioxidant activities of AG were extensively reinforced by graft copolymerization. Analogous findings have been reported on protocatechuic acid grafted carboxymethyl chitosan (Xu et al., 2021). For the three modified AGs, the FRAP increased in the following order: FA-AG (0.54) < CA-AG (0.75) < GA-AG (1.36).

3.5. Emulsifying properties

The emulsifying properties of modified AG conjugates were evaluated on the basis of emulsifying activity (EAI) and emulsion stability (ESI) and compared with NAG. As enumerated in Table 2, the EAI of NAG was 35.23 m^2/g. After grafting with phenolic acids, the EAI of modified AGs was markedly risen to 38.27 m^2/g for FA-AG, 38.09 m^2/g for CA-AG, and 42.59 m^2/g for GA-AG. The higher EAI could be due to the fact the incorporation of phenolic groups into the AG structure caused an increase in the hydrophobicity of AG, thus favoring the rapid absorption at the oil–water interface. Analogous phenomenon has been observed in pectin modified with p-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, and gallic acid (Jingna Liu et al., 2021).

In terms of ESI, it seemed that the modified AG conjugates were more

Table 2
Emulsifying properties of natural Arabic gum and three kinds of modified Arabic gum.

Samples	EAI (m^2/g)	ESI (%)
NAG	35.23 ± 0.13^{c}	62.63 ± 0.39^{c}
FA-AG	38.27 ± 0.78^{b}	64.76 ± 0.50^{b}
CA-AG	38.09 ± 0.53^{b}	63.83 ± 0.56^{b}
GA-AG	42.59 ± 0.67^{a}	70.96 ± 0.81^{a}

Notes: Values are means ± the standard deviation (SD). Different lowercase letters indicate significant differences ($P < 0.05$). NGA: natural Arabic gum; FA-AG: ferulic acid grafted Arabic gum; CA-AG: caffeic acid grafted Arabic gum; GA-AG: gallic acid grafted Arabic gum. EAI: emulsifying activity index; ESI: emulsion stability index.

adept at stabilizing the emulsion droplets when compared with NAG (Table 2). The phenolic acids introduced on the AG could be well anchored at the o/w interface due to their high affinity for the oil phase, impelling the formation of a dense interfacial layer around the oil droplets, which favored the stabilization of emulsions. Among the three modified AGs, the ESI of GA-AG (70.96 %) was notably higher than that of FA-AG (64.76 %) and CA-AG (63.83 %), potentially resulting from the larger grafting ratio and stronger hydrophobicity of GA over FA and CA.

3.6. Microstructure of AG emulsions

The microstructure of emulsions stabilized by NAG and modified AGs was visualized by CLSM (Fig. 3). It could be seen that the incorporation of phenolic acids into AG resulted in a drastic diminution in the droplet size, irrespective of the type of phenolic acids, which was instrumental in the emulsion stability as depicted in Table 2. Similar droplet size reduction effects have been monitored in phenolic acids grafted pectin (Ilyasoglu & Guo, 2019; Zhang, Huang, Zheng, Chen, & Fei, 2021). The observations could be because the modified AGs could more promptly absorb at the o/w interface and ensure a sufficient interfacial coverage when lowering the interfacial tension, conducing to the fabrication of smaller oil droplets. Indeed, Vuilemin and coworkers (2020) have testified that AG modified with ferulic acid acted more vigorously in reducing the interfacial tension than NAG (Vuillemin et al., 2020). GA-AG evinced a smaller droplet size with respective to FA-AG and CA-AG, which was in tandem with the findings in the emulsifying properties (Table 2).

3.7. Oxidation stability of AG emulsions

Lipid oxidation leads to the oxidative degradation of bioactive components in the oil phase and affects the shelf life of emulsion products (Zhao, Fan, Liu, & Li, 2022). The effect of the grafting of phenolic acids onto AG on the oxidation stability of oil droplets was explored by monitoring the production of the primary and secondary oxidation products during storage.

3.7.1. Primary oxidation products

POV, a monumental indicator of lipid oxidation, mirrors the content of primary oxidation products (hydroperoxides). As presented in Fig. 4A, with the prolong of storage time, the POV of NAG-stabilized emulsion expeditiously increased from 20.17 meq/kg to 45.12 meq/kg. Contrastively, the emulsions prepared with modified AGs emanated a slower POV evolution over 7 days of storage, suggesting a better oxidation stability. The findings could be interpreted as the fact that phenolic acids bound to AG could effectively eliminate free radicals and/or inactivate

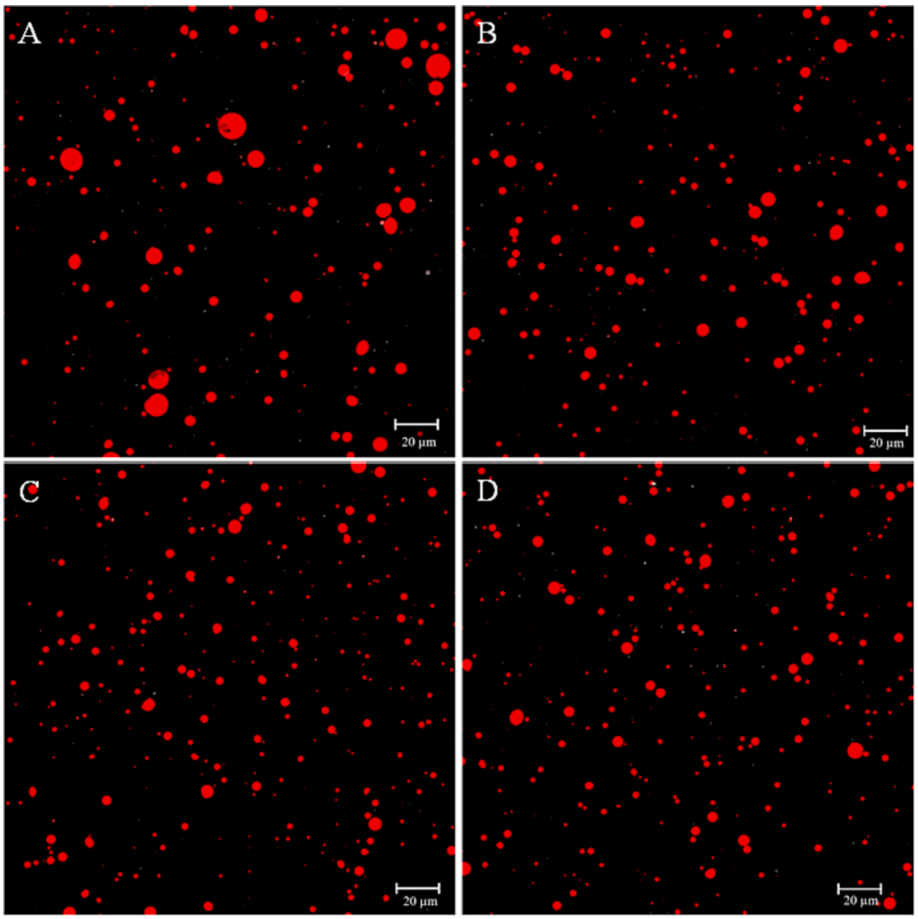

Fig. 3. Microstructure of emulsions stabilized with NAG (A) and three kinds of modified AG (B: FA-AG; C: CA-AG; D: GA-AG).

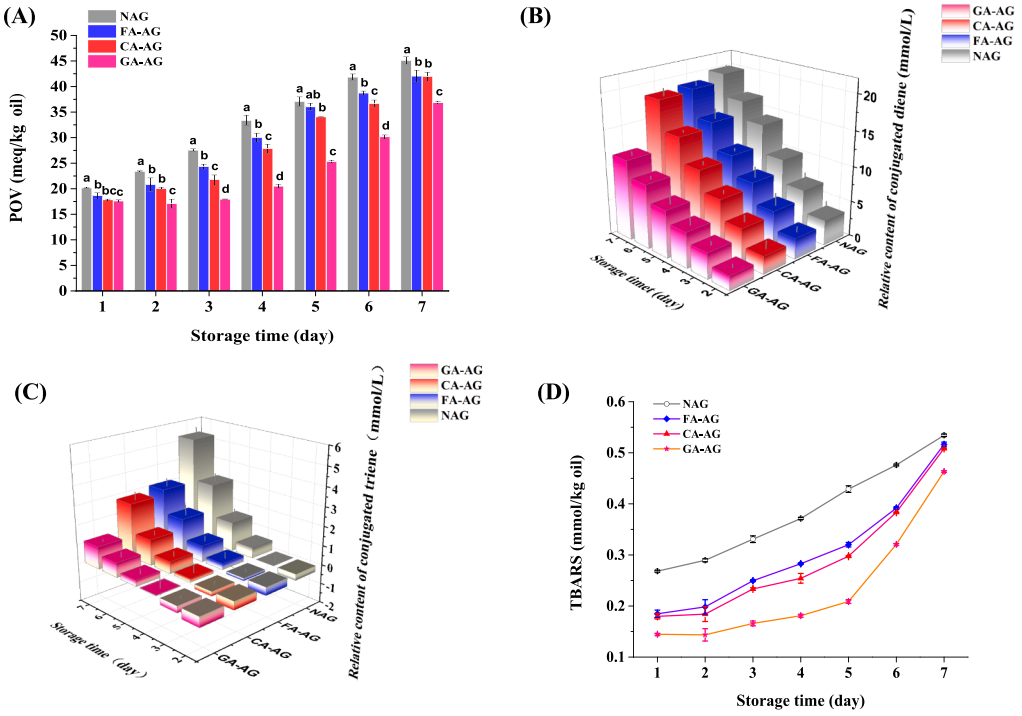

Fig. 4. Peroxide values (A), conjugated diene hydroperoxides (B), conjugated triene hydroperoxides (C) and Thiobarbituric acid reactive substances (TBARS) (D) of emulsions stabilized with NAG and three kinds of modified AG (FA-AG, CA-AG, and GA-AG) within a 7-day storage period. Means within the same day without a common lowercase letter differ significantly ($P < 0.05$).

the prooxidants (e.g., transition metals) at the o/w interface (Feng, Cai, Wang, Li, & Liu, 2018). Besides, the phenolic acid-AG conjugates were more liable to concentrate at the o/w interface, generating a more compact and impeccable interfacial layer (physical barrier) around the oil droplets to obstruct the penetration and diffusion of prooxidants. The POV of oil droplets stabilized by FA-AG, CA-AG, and GA-AG reached to 42.05 meq/kg, 41.97 meq/kg, and 36.84 meq/kg, respectively, after 7 days of storage. It appeared that the stronger oxidation stability of GA-AG-stabilized emulsion was largely associated with the superior antioxidant capacity of GA as revealed in Fig. 1C and 1D.

The allyl structure of polyunsaturated fatty acids is vulnerable to the attack of oxygen to form free radicals, which then undergo double bond rearrangement to form conjugated diene hydroperoxides. The accumulation of conjugated dienes and conjugated trienes in the different emulsions was further monitored to reflect the production of hydroperoxides. Fig. 4B revealed that the variations of conjugated dienes were similar to POV. The conjugate dienes in NAG-stabilized emulsion increased by 19.90 mmol/L after 7 days of storage, which were significantly higher than those in modified AG-stabilized emulsions ($P < 0.05$). Of the three modified AGs, GA-AG exhibited the best inhibitory effect on the formation of conjugated dienes over the whole storage period. Comparatively, the inhibitory effect of FA-GA and CA-AG on conjugated dienes seemed to be ineffective in the later stage of storage (Day 7).

On the other sides, it is noteworthy that the relative content of conjugated trienes in all emulsions encountered an initial declination but a subsequent increment with the extension of storage time (Fig. 4C). A possible explanation for this phenomenon was that the conversion rate of conjugated dienes into conjugated trienes was slower than the degradation rate of conjugated trienes in the early stage of oxidation, but faster in the later stage of oxidation. Similar to the conjugated dienes results, modified AG-stabilized emulsions exhibited a stronger inhibitory effect on the formation of conjugated trienes than NAG emulsion, of which GA-AG manifested the strongest. Collectively, the three modified AGs by phenolic acids with distinct chemical structures as emulsifiers behaved differentially in suppress the generation of primary oxidation products.

3.7.2. *Secondary oxidation products*

The primary oxidation products (e.g., hydroperoxides) are extremely unstable and will further decompose to form various secondary oxidation products such as alkanes, ketones, and aldehydes (Khanum & Thevanayagam, 2017). Malondialdehyde is one of the predominant aldehydes emerged in the secondary oxidation of lipid. To gain more information about the influence of the incorporation of phenolic acids into AG structure on the production of secondary oxidation products, the TBARS (for malondialdehyde) in the emulsions were further determined. From Fig. 4D, it can be seen that the TBARS values of NAG-stabilized emulsions aggrandized tremendously with the increased storage time, ascending to 0.53 mmol/kg at day 7. When compared with NAG-stabilized emulsions, modified AG-stabilized emulsions had a remarkable slower increment in TBARS, manifesting the splendid capacity of phenolic acids in regarding the generation of secondary products from lipid oxidation. A similar phenomenon was observed by İlyasoğlu, Nadzieja, and Guo (2019) for an emulsion prepared with caffeic acid grafted chitosan. The TBARS values in modified-AG-stabilized emulsions during storage declined in the order of GA-AG > CA-AG > FA-AG, which was consistent with the variation tendency of primary oxidation products (Fig. 4A, 4B and 4C), suggesting a good correlation between the suppression of lipid oxidation and the antioxidant capacity of emulsifiers (Fig. 1C and1D).

4. Conclusions

In summary, three kinds of phenolic acids (ferulic acid, caffeic acid, and gallic acid) with different chemical structures were successfully grafted onto AG by laccase in this study. The incorporation of phenolic acids into AG structure barely affected the main skeleton of natural AG. Among the three phenolic acids, gallic acid with more hydroxyl groups was prone to conjugate with AG. The thermal stability and antioxidant

activity of AG was significantly improved by the grafting of phenolic acids, notably gallic acid. Moreover, the introduced phenolic acids could improve the amphiphilicity of AG, which was conducive to the rapid absorption of AG at the o/w interface, consequently contributing to the markedly enhanced emulsification performance of AG. Yet, emulsions stabilized by modified AGs had better oxidation stability than that prepared with natural AG, which was closely related to the antioxidant capacity of the introduced phenolic acids. Applying modified AGs as an emulsifier in oil-in-water emulsions can couple with proteins, through the amphiphilic nature of protein, the modified AGs could exert anti-oxidant activity at the interface of lotion. Further research is needed to demonstrate the application of modified AGs coupled with proteins or other substances with interfacial activity in emulsions. The findings presented here would provide valuable information for broadening the application of phenolic acid-modified AG as a functional stabilizer in emulsified food products.

CRediT authorship contribution statement

Yi Luan: Methodology, Writing – original draft, Software. **Qingling Wang:** Formal analysis, Funding acquisition, Writing – original draft. **Songnan Li:** Methodology, Investigation, Formal analysis. **Chen Gu:** Formal analysis. **Rui Liu:** Methodology, Validation. **Qingfeng Ge:** Validation. **Hai Yu:** Validation. **Mangang Wu:** Supervision, Funding acquisition, Writing – review & editing, Project administration.

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.

Data availability

Data will be made available on request.

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