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Evaluation of antioxidant effect of gallic acid and its alkyl esters on freeze-dried scallop with different packaging by lipid stability, predicted shelf life, and thermodynamic study

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Abstract

Antioxidant effect of gallic acid alkyl esters in emulsions or complex food systems has a "cut-off effect". However, it is unclear whether this phenomenon can occur in food under low-oxygen environment. The present work compared the effects of gallic acid, octyl gallate, and hexadecyl gallate on lipid stability, predicted shelf life, and thermodynamic parameters of freeze-dried scallop with vacuum polyamide/polyethylene packaging (VPP) and non-vacuum polyamide/polyethylene packaging (NPP) during storage for 16 days. The results showed that regardless of the packaging type, the octyl gallate group showed the lowest peroxide value, thiobarbituric acid reactive substances value, and free radical levels, and loss of eicosapentaenoic acid and docosahexaenoic acid contents (46.5, 39.3, 35.7, and 22.6% lower than those of deionised water-treated sample (control group), respectively). Moreover, the octyl gallate group had the longest induction period and shelf life (6.9 and 1.3-fold longer than those in control group, respectively). These results indicated that the antioxidant capacity of gallic acid and its alkyl esters increased with the chain length (C0 to C8), and then decreased (C8 to C16). Thus, the "cut-off effect" existed in freeze-dried scallop with VPP or NPP. Thermodynamic analysis indicated that lipid oxidation of freeze-dried scallop during storage was an endothermic ($\Delta H^{++} > 0$) and non-spontaneous ($\Delta G^{++} > 0$) process, and the addition of octyl gallate in combination with VPP had the strongest antioxidant effect. The shelf life of freeze-dried scallop treated with octyl gallate and VPP was extended to about 120 days. The present work would be helpful for the reasonable selection of antioxidants and packaging to prolong shelf life of dried seafood during long-term storage.

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Introduction

Lipid oxidation is an unavoidable problem during food storage, since unsaturated fatty acids (UFAs) in foods are prone to oxidation. Lipid oxidation can lead to loss of some beneficial lipids, lowering the nutritional value of food. Moreover, lipid oxidation products can cause food quality and safety issues, resulting in a shorter shelf life of food (de Jorge Gouvêa *et al*., 2023). This phenomenon is more prominent in aquatic food products due to their high level of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Zhao *et al*., 2023). The bis-allylic carbons of PUFAs are susceptible to oxidation, resulting in a reduction in the sensory qualities (flavour, colour, texture, taste) and nutritional value of aquatic food products (Hammer *et al*., 2021).

Recently, natural phenolic antioxidants have been widely used to limit lipid oxidation in foods due to their safety and excellent antioxidant effect (Ahsan *et al*., 2024; Zhu *et al*., 2024). It is well known that lipid oxidation occurs at the oil-water interface. However, most of the phenolic antioxidants cannot be adequately distributed at the oxidation site due to their polarity, limiting their antioxidant effects (Zhao *et al*., 2023). Therefore, the polarity of phenolic antioxidants has been modified by linking alkyl chains to improve their antioxidant effects (McClements and Decker, 2018). Previous studies have found that the antioxidant effects of phenolic antioxidants such as rosmarinic acid (Panya *et al*., 2012), gallic acid (González *et al*., 2015), and caffeic

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acid (Sørensen *et al.*, 2017), with short or long alkyl chain length in emulsions, were worse than those with medium alkyl chain length. The antioxidant effect first increases and then decreases with the increase in alkyl chain length; this phenomenon is called as "cut-off effect". It occurs because the short-chain antioxidants are polar and mainly distributed in the water phase, while the long-chain antioxidants are non-polar and mainly distributed in the lipid phase. Only medium-chain antioxidants with the right polarity can be located at the oil-water interface, and play a stronger antioxidant role. However, the "cut-off effect" is mainly observed in emulsion systems, and its applicability to complex food systems still needs further research.

Scallop is an important marine cultured and captured bivalve, which has a global production volume of more than 2.8 million tonnes (Wu *et al*., 2022). Fresh scallop spoils within a few days; so, it is usually processed as dried scallop to extend its shelf life (Liu *et al*., 2023; Wang *et al*., 2024a). However, dried scallop is rich in PUFAs and easily oxidised during long-term storage, which reduces its nutritional value and shelf life (Zeng *et al.*, 2023). Gallic acid alkyl esters with different chain lengths are important polyphenolic antioxidants, which can be used to prevent lipid oxidation in seafood. Previous studies reported that the "cut-off effect" of gallic acid and its alkyl esters occurred during frying, drying, and ambient storage of dried oyster (Li *et al*., 2021; Zhao *et al.*, 2021; 2022; 2023). However, these dried oysters were stored unpackaged or within non-vacuum packaging. Oxygen is an important reactant of lipid oxidation, and its concentration affects lipid oxidation. The antioxidant effect of gallic acid alkyl esters on PUFAs-rich seafood, such as dried scallop, under low oxygen environment is still unclear. In addition, there are only a few studies on the effect of gallic acid alkyl esters on the shelf life of dried seafood.

Therefore, the present work aimed to explore the effects of gallic acid, octyl gallate, and hexadecyl gallate on the lipid stability and shelf life of freeze-dried scallop with vacuum polyamide/polyethylene packaging (VPP) and non-vacuum polyamide/polyethylene packaging (NPP). The primary oxidation products, secondary oxidation products, oxygen absorption, free radical generation, and oxidative substrate loss of sample during accelerated storage were determined to evaluate the lipid stability. Moreover, a shelf-life prediction model was created to predict the shelf life of freeze-dried scallop with different packaging conditions. Finally, a thermodynamic study was conducted to further verify the antioxidant effect of gallic acid and its alkyl esters.

Materials and methods

Materials

Fresh scallops (*Argopecten irradians*) with an average individual size of 5.7 ± 0.9 cm were purchased from Nanjing, Jiangsu, China. Polyamide/polyethylene bags (15 × 20 cm, 160 μm total thickness) were purchased from Jiangsu Ruifu Packaging Co., Ltd. (Zhenjiang, Jiangsu, China). Boron trifluoride (BF_3)-methanol solution (14%, w/w), gallic acid (95%), glyceryl triundecanoate (98%), hexadecyl gallate (95%), octyl gallate (98%), and 1,1,3,3-tetramethoxypropane (98%) were obtained from Aladdin (Shanghai, China). High-performance liquid chromatography (HPLC) grade reagents methanol and *n*-hexane were provided by Spectrum Chemical (Gardena, CA, USA).

Experimental design

Fresh scallop adductor muscle was separated from the shell, and randomly divided into four groups (control, GA-0, GA-8, and GA-16 groups). Then, each group was randomly divided into six batches for the following treatment. Control group: each batch was boiled in deionised water for 15 min (material to liquid ratio, 1:2, w/v), cooled down to room temperature, and freeze-dried by a Scientz-10N vacuum freeze-dryer (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, Zhejiang, China) with a cold trap temperature of -50°C, and a chamber pressure of 100 Pa for 72 h, respectively (Zhao *et al*., 2021). For the treatment groups, the antioxidant was added in equimolar concentration, following the maximum amount of propyl gallate as specified in the Chinese National Standard GB 2760-2014. The GA-0, GA-8, and GA-16 groups were treated in the same way as the control group, except that they were boiled in 5 mM of gallic acid solution, octyl gallate solution, and hexadecyl gallate solution, for 15 min, respectively. There was no significant difference in the total molar content of gallic acid alkyl esters (the sum in scallop tissue and water solution) before and after boiling process. This may be because the boiling temperature and short boiling time were not enough to degrade the gallic acid alkyl esters. Moreover, the boiling process

was an aqueous solution deoxygenation process, which reduced the oxidation of the scallop lipids and the loss of gallic acid alkyl esters.

Each batch of dried scallop adductor muscle in each group was randomly placed into 60 polyamide/polyethylene bags (160 μm total thickness; Jiangsu Ruifu Packaging Co., Ltd., Zhenjiang, Jiangsu, China). Then, 30 bags were sealed under VPP, and the other 30 bags were sealed under NPP using a TC-5000 vacuum packaging machine (Shanghai Starpack Machine Co., Ltd., Shanghai, China). The instrument conditions were as follows: VPP (vacuum time, 15 s; vacuum degree, -0.1 MPa; heat sealing time, 3 s), and NPP (sealing time, 3 s).

The packed samples were stored in four DHG-9423A ovens in the dark (Shanghai Jing Hong Laboratory Instrument Co., Ltd., Shanghai, China). Briefly, 15 bags of each batch from the control, GA-0, GA-8, and GA-16 groups with NPP or VPP were stored at 25°C. Samples for analysis were collected every 5 d from day 20 to 90. Five bags of each batch from the control, GA-0, GA-8, and GA-16 groups with NPP or VPP were stored at 45, 55, and 65°C, respectively. Samples for analysis were collected at 0, 4, 8, 12, and 16 d. All collected samples were stored at -80°C (DW-86L338J, Haier, Qingdao, China) until further use. Pictures of fresh scallop, fresh scallop adductor muscle, polyamide/polyethylene packaging bag, and scallop adductor muscle treated with deionised water or gallic acid alkyl esters under different packaging conditions are shown in Figure 1.

Figure 1. (A) fresh scallop. **(B)** fresh scallop adductor muscle. **(C)** polyamide/polyethylene packaging bag. **(D)**, **(E)**, **(F)**, **(G)** are the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in non-vacuum polyamide/polyethylene packaging (NPP), respectively. **(H)**, **(I)**, **(J)**, **(K)** are the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in vacuum polyamide/polyethylene packaging (VPP), respectively.

Analysis of gallic acid, octyl gallate, and hexadecyl gallate contents

The contents of gallic acid and its alkyl esters in freeze-dried scallop were analysed following a previously described method (Zhao *et al*., 2022). First, 0.5 g of sample powder and 2.5 mL of ethanol were combined and vortexed for 1 min. After centrifugation at 7,800 *g* for 10 min, the supernatant was filtered through a 0.22-μm filter. Gallic acid and its alkyl esters were detected by an Agilent 1260 Infinity HPLC (Santa Clara, CA, USA) with the following conditions: column, ZORBAX SB-C18 (4.6 × 250 mm, 5 μm); injection volume, 5 μL; absorption wavelength, 272 nm; flow rate, 0.7 mL/min; mobile phase, 10% methanol aqueous solution (for gallic acid) and 100% methanol (for octyl gallate and hexadecyl gallate). The contents of gallic acid, octyl gallate, and hexadecyl gallate were calculated based on standard external curves, and the results were given as μmol/g dry basis.

Analysis of total phenolic content

The total phenolic content of freeze-dried scallop was analysed following a previously described method (Hussain *et al*., 2021). First, 0.5 g of sample powder and 3 mL of distilled water were combined and homogenised for 20 s at 3,000 rpm. Then, 4.5 mL of chloroform was added to the mixture. Subsequently, the aqueous supernatant (0.5 mL), Folin-Ciocalteu reagent (0.5 mL), and 10% Na_2CO_3 (1 mL) were mixed and incubated for 2 h at room temperature in the dark. After that, the mixture was analysed at a wavelength of 700 nm using a microplate reader (SpectraMax 190, Molecular Devices, Sunnyvale, CA, USA). Gallic acid was used to create a standard curve ($y = 0.0112x – 0.0336$, $R^2 = 0.9999$) to calculate the total phenolic content. The results were represented as μg/g of dry basis, and the total phenolic content found in the control group was used as the blank.

Lipid extraction

Lipids were extracted using the Folch method with some modifications (Xie *et al*., 2019). Briefly, 15 g of sample powder was mixed with 25 mL of deionised water. Then, 50 mL of chloroform/methanol (2:1, v/v) was added. After thorough mixing, 20 mL of chloroform was added to the mixture, and stirred for 60 min at 25°C. After that, the mixture was centrifuged at 8,000 *g* for 10 min. The organic phase was collected and dried using a vacuum evaporator (RE-2000, Yarong, Shanghai, China). The extracted lipids were stored at -80°C (DW-86L338J, Haier, Qingdao, China) until further analysis.

Peroxide value (PV)

The PV was analysed following the method described in Chinese National Standard GB 5009.227-2016. The sodium thiosulfate concentration was 1 mM, and the mass of lipid was 100 mg. The result in g/100 g was transformed to meq/kg by the conversion coefficient of 78.8.

Thiobarbituric acid reactive substances (TBARS)

The TBARS was determined following a previously described method (Khan *et al*., 2006) with some modifications. First, 100 mg of sample powder, 0.4 mL of 10% (w/v) trichloroacetic acid, and 0.4 mL of deionised water were combined and vortexed for 2 min. After centrifugation at 8,000 *g* for 5 min, the supernatant (1 mL) was combined with 10 mmol/L of 2-thiobarbituric acid solution (1 mL). Then, the mixture was placed in a boiling water bath for 25 min, and its absorbance at 532 nm was measured using SpectraMax 190 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). The TBARS was calculated using a 1,1,3,3-tetramethoxypropane standard curve ($y = 0.0997x – 0.0024$, $R^2 = 0.9999$). The results were expressed as malondialdehyde (MDA)/kg dry basis.

Induction period (IP)

The IP was determined using an OXITEST device (Velp Scientifica, Usmate, MB, Italy) according to Xie *et al*. (2019). The instrument parameters were defined as: sample powder, 3.0 g; initial oxygen pressure, 6 bar; and temperature, 90°C. The result was calculated automatically by OXISoft software installed in the OXITEST equipment.

Free radicals

The free radical levels were measured using an electron spin resonance spectrometer (A300, Bruker, Karisruhe, Baden-Württemberg, Germany) according to Xie *et al*. (2019). The instrument parameters were defined as: sample powder, 100 mg; microwave power, 5.32 mW; centre field, 3453.00 G; sweep width, 100.00 G; modulation frequency, 100.00 kHz; amplitude,1.00 G; conversion time, 480 ms; and time constant, 5242.88 ms. The intensity of the first peak's signal was defined as the free radical level.

EPA and DHA contents

Lipids containing EPA and DHA were methylated and analysed using an Agilent 6890N gas chromatography system with flame ionisation detector (GC-FID) (Santa Clara, CA, USA) according to Xie *et al*. (2020). Briefly, 5 mg of lipid, 0.2 mg of internal standard (glyceryl triundecanoate), and 2 mL of 0.5 M sodium hydroxide-methanol solution were added to a 10 mL round-bottom flask, and refluxed at 80°C for 5 min. Then, 2 mL of BF_3-methanol solution (14%, w/w) was added and refluxed at 80°C for 2 min. After cooling to room temperature, the mixture was extracted with 1.5 mL of hexane. Trace water was removed from the hexane containing fatty acid methyl esters (FAMEs) by anhydrous Na_2SO_4 and then it was passed through a 0.22-μm filter. The FAMEs were separated by a Supelco SP 2560 capillary column (100 m × 0.25 mm i.d., 0.2 μm; Supelco, Bellefonte, PA, USA). The GC-FID instrument parameters were set as: injector

temperature, 270°C; injection volume,1 μL; split ratio, 5:1; carrier gas, N_2; flow rate, 1 mL/min; and detector temperature, 270°C. The oven temperature was 100°C and held for 13 min; increased to 180°C at 10°C min^{-1} and held for 6 min; increased to 215°C at 1°C/min; and increased to 230°C at 5°C/min and held for 12 min. The EPA and DHA contents were calculated using Eq. 1:

$$\text{EPA or DHA content} = \text{RF} \times \frac{A_{\text{EPA or DHA}}}{A_{C11}} \times \frac{\text{IS} \times 1.0067}{\text{m}} \times F_{\text{EPA or DHA}} \times \text{c} \quad \text{(Eq. 1)}$$

where, RF = response factor; $A_{EPA\ or\ DHA}$ = peak area of EPA or DHA methyl esters; A_{C11} = peak area of undecanoic acid methyl ester; IS = mass of internal standard (mg); 1.0067 = conversion coefficient of glyceryl triundecanoate to undecanoic acid methyl ester; m = mass of lipid (mg); $F_{EPA\ or\ DHA}$ = conversion coefficient of EPA methyl ester to EPA (0.9557) or DHA methyl ester to DHA (0.9590); and c = lipid content of the sample (mg/g dry basis). The results were presented as mg/g dry basis.

Shelf-life prediction

Following a previous study, the shelf-life prediction model for freeze-dried scallop stored at 25°C was created (Tavakolipour *et al*., 2017; Xie *et al*., 2019). First, the PV data of freeze-dried scallop during accelerated storage at 45, 55, and 65°C were fitted by zero-order Eq. 2 and first-order Eq. 3. Then, the reaction rate constant k_1 obtained from the first-order equation was used to develop the Arrhenius Eq. 4. Subsequently, the k_A and E_A obtained from the Arrhenius equation were used to create the shelf-life prediction model (Eq. 5). Moreover, the PV of dried scallop stored at 25°C was also measured to confirm the accuracy of the model findings.

$$\text{PV} = k_0 \text{t} + \text{PV}_0 \quad \text{(Eq. 2)}$$

$$\ln(\text{PV}) = k_1 \text{t} + \ln(\text{PV}_1) \quad \text{(Eq. 3)}$$

$$\ln(k_1) = -\frac{E_A}{RT} + \ln k_A \quad \text{(Eq. 4)}$$

$$\text{shelf life} = \frac{\ln(\text{PV}_{\text{limit}}) - \ln(\text{PV}_{\text{initial}})}{k_A \times e^{-\frac{E_A}{R}}} \quad \text{(Eq. 5)}$$

where, k_0 and k_1 = reaction rate constants of zero-order and first-order equations, respectively; PV_0 (meq/kg lipid) and PV_1 (meq/kg lipid) = initial PV obtained from the zero-order and first-order equations, respectively; t = storage time (d); E_A = activation energy (KJ/mol); R = molar gas constant (8.3144 J/(mol·K)); T = absolute temperature (K); k_A = pre-exponential factor; PV_{limit} = highest PV allowed by the Chinese national standard GB 10136-2015 (47.28 meq/kg lipid) for freeze-dried scallop; and $PV_{initial}$ = initial PV of the freeze-dried scallop.

Thermodynamic study

Enthalpy (ΔH^{++}, kJ/mol) and entropy (ΔS^{++}, kJ/mol/K) were obtained from Eyring-polni Eq. 6, and the Gibbs free energy (ΔG^{++}, kJ/mol) was obtained from the basic equation of thermodynamics (Eq. 7) (Tavakolipour *et al.*, 2017; Kaseke *et al.*, 2021):

$$Ln\frac{k}{T} = -\frac{\Delta H^{++}}{R} \times \frac{1}{T} + \left[\text{Ln}\frac{k_B}{h} + \frac{\Delta S^{++}}{R}\right] \quad \text{(Eq. 6)}$$

$$\Delta G^{++} = \Delta H^{++} - T\Delta S^{++} \quad \text{(Eq. 7)}$$

where, k = reaction rate constant; T = absolute temperature (K); k_B = Boltzmann constant ($1.3806488 \times 10^{-23}$ J/K); h = Planck constant (6.6261×10^{-34} J/s); and R = universal gas constant (8.314 J/mol/K).

Statistical analysis

All tests were repeated three times for different batches of samples in different groups. Data were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Data were analysed using One-way analysis of variance (ANOVA) followed by Duncan's test; $p < 0.05$ was considered to be statistically significant.

Results

Contents of gallic acid, gallic acid alkyl esters, and total phenolics in freeze-dried scallop

Before accelerated storage, the GA-0, GA-8, and GA-16 groups contained 8.55 ± 0.37 μmol/g of gallic acid, 24.64 ± 0.63 μmol/g of octyl gallate, and 11.12 ± 0.55 μmol/g of hexadecyl gallate, respectively (Figure 2). The total phenolic contents of the GA-0, GA-8, and GA-16 groups before accelerated storage were 278.33 ± 14.55, 689.56 ± 26.87, and 314.51 ± 11.16 μg/g, respectively. In the scallop muscle, the GA-8 group showed the highest levels of octyl gallate and total phenolic content.

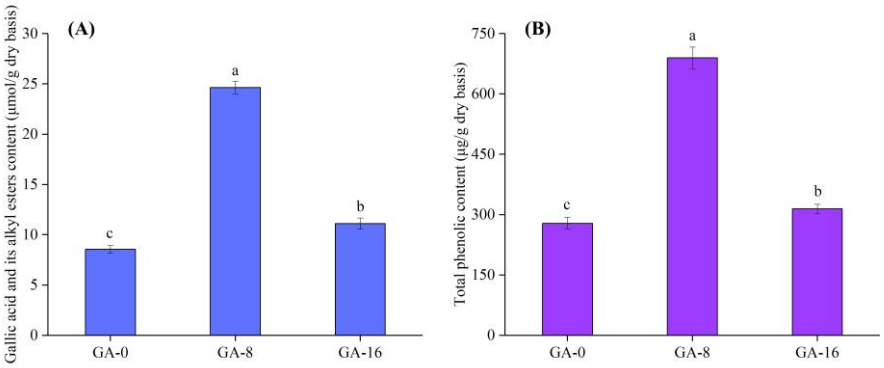

Figure 2. Contents of gallic acid and its alkyl esters **(A)** and total phenolic content **(B)** of freeze-dried scallop. GA-0, GA-8, and GA-16 represent the scallop adductor muscle boiled in 5 mM of gallic acid solution, octyl gallate solution, and hexadecyl gallate solution for 15 min and freeze-dried for 72 h, respectively. Different lowercase letters indicate significant differences between samples ($p < 0.05$).

Changes in PV and TBARS

The PV and TBARS of all samples significantly increased during accelerated storage (Figure 3). Regardless of NPP or VPP, the PV and TBARS of GA-0, GA-8, and GA-16 groups were lower than those of control group, and the GA-8 group had the lowest PV and TBARS following 4-d storage.

Changes in IP value

The initial IP values of the control, GA-0, GA-8, and GA-16 groups were 76.5 ± 5.9, 191.5 ± 7.0, 222.6 ± 12.1, and 163.6 ± 9.0 h, respectively (Figure 4). These values decreased to 0, 0, 15.6 ± 2.9, and 0 h following 16-d storage with NPP, and decreased to 8.6 ± 1.3, 37.9 ± 5.7, 59.6 ± 6.7, and 27.5 ± 4.7 h following 16-d storage with VPP.

Changes in free radical levels

The control, GA-0, GA-8, and GA-16 groups with NPP or VPP showed similar free radical levels before accelerated storage (Figure 5). Following 16-d storage, the free radical levels increased by 9.5, 5.1, 3.7, and 6.8-fold in the abovementioned four groups with NPP, respectively. The corresponding free radical levels with VPP increased by 6.0, 2.9, 1.7, and 4.1-fold following 16-d storage.

Changes in EPA and DHA contents

Similar initial EPA+DHA contents were observed in the control, GA-0, GA-8, and GA-16 groups, which were 9.22 ± 0.23, 8.98 ± 0.21, 9.19 ± 0.18, and 9.13 ± 0.09 mg/g dry basis, respectively (Figure 6). Following 16-d storage, the EPA+DHA contents of the four groups with NPP decreased by 32.5, 17.0, 10.6, and 23.2% to 6.22 ± 0.12, 7.45 ± 0.13, 8.22 ± 0.22, and 7.01 ± 0.11 mg/g dry basis, respectively. The EPA+DHA contents of the four groups with VPP decreased by 20.3, 4.1, 3.3, and 12.2% to 7.35 ± 0.28, 8.61 ± 0.22, 8.89 ± 0.22, and 8.02 ± 0.18 mg/g dry basis, respectively.

Changes in shelf life

The PV of the control, GA-0, GA-8, and GA-16 groups with NPP or VPP significantly increased during accelerated storage at 45, 55, and 65°C (Figure 7). As listed in Table 1, the R^2 values derived from the first-order equation of these four groups were higher than those obtained from the zero-order equation of the corresponding group, indicating that the PV changes were in agreement with the first-order equation. Consequently, k_1 values were used to establish the Arrhenius equation. The E_A values of the control, GA-0, GA-8, and GA-16 groups with NPP were found to be 19.4, 30.9, 33.0, and 30.6 KJ/mol, respectively, and the corresponding values with VPP were 25.4, 32.0, 34.0, and 31.3 KJ/mol. Additionally, the E_A and k_A values obtained from the Arrhenius equation were used to establish the shelf-life prediction model.

As seen in Table 1, the predicted shelf-life values of the GA-0, GA-8, and GA-16 groups with NPP were 1.08, 1.45, and 0.72-fold higher than the control group, respectively. The corresponding values with VPP were 0.60, 1.29, and 0.39-fold higher than the control group, respectively. Furthermore, the predicted shelf-life values of the control, GA-0, GA-8, and GA-16 groups with VPP were 0.79, 0.38, 0.67, and 0.44-fold higher than those with NPP, respectively.

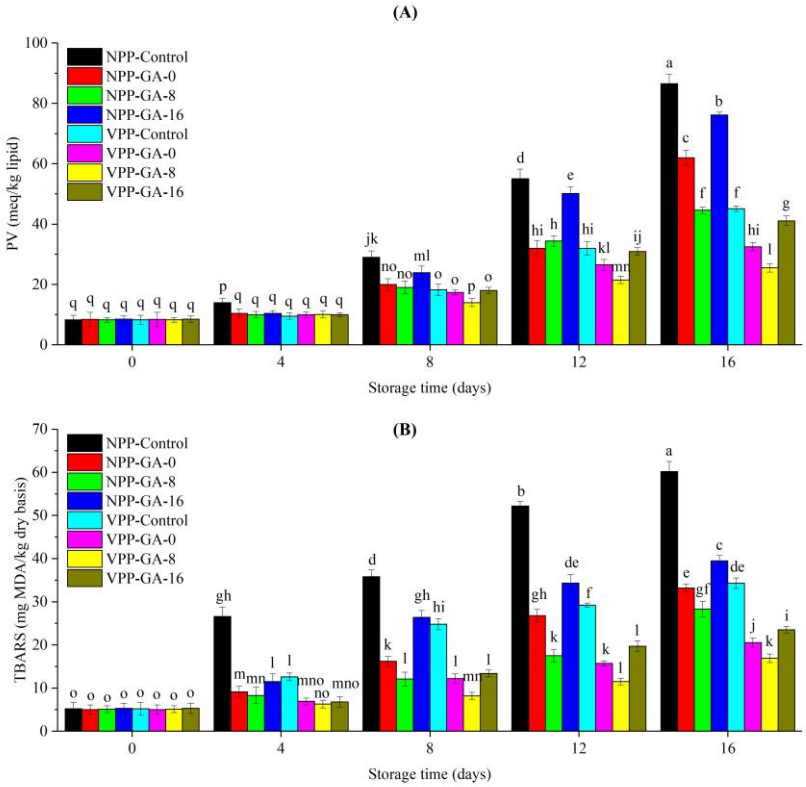

Figure 3. Changes in peroxide value (PV) **(A)** and thiobarbituric acid reactive substances (TBARS) **(B)** of freeze-dried scallop during accelerated storage at 65°C. NPP-Control, NPP-GA-0, NPP-GA-8, and NPP-GA-16 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in non-vacuum polyamide/polyethylene packaging (NPP), respectively. VPP-Control, VPP-GA-0, VPP-GA-8, and VPP-GA-16 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in vacuum polyamide/polyethylene packaging (VPP), respectively. Different lowercase letters indicate significant differences between samples ($p < 0.05$).

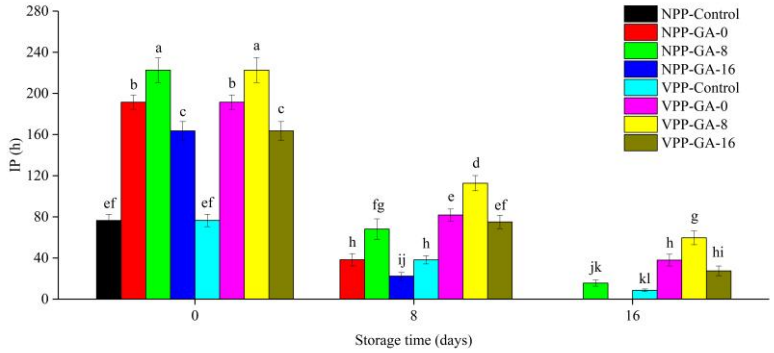

Figure 4. Changes in induction period (IP) values of freeze-dried scallop during accelerated storage at 65°C. NPP-Control, NPP-GA-0, NPP-GA-8, and NPP-GA-16 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in non-vacuum polyamide/polyethylene packaging (NPP), respectively. VPP-Control, VPP-GA-0, VPP-GA-8, and VPP-GA-16 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in vacuum polyamide/polyethylene packaging (VPP), respectively. Different lowercase letters indicate significant differences between samples ($p < 0.05$).

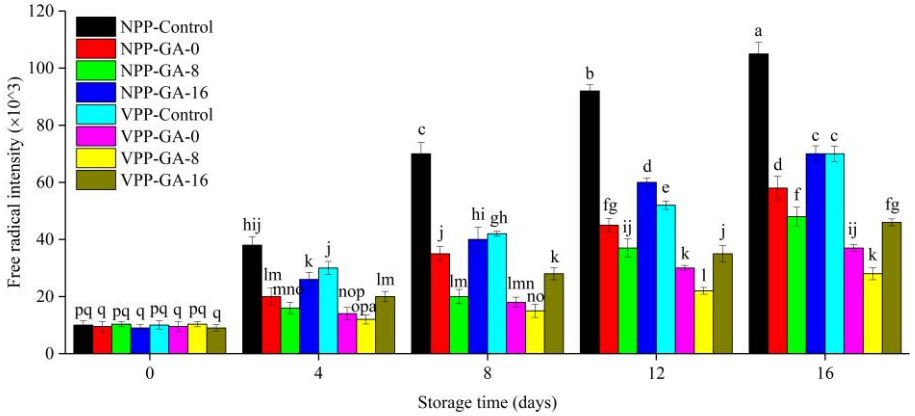

Figure 5. Changes in free radical levels of freeze-dried scallop during accelerated storage at 65°C. NPP-Control, NPP-GA-0, NPP-GA-8, and NPP-GA-16 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in non-vacuum polyamide/polyethylene packaging (NPP), respectively. VPP-Control, VPP-GA-0, VPP-GA-8, and VPP-GA-16 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in vacuum polyamide/polyethylene packaging (VPP), respectively. Different lowercase letters indicate significant differences between samples ($p < 0.05$).

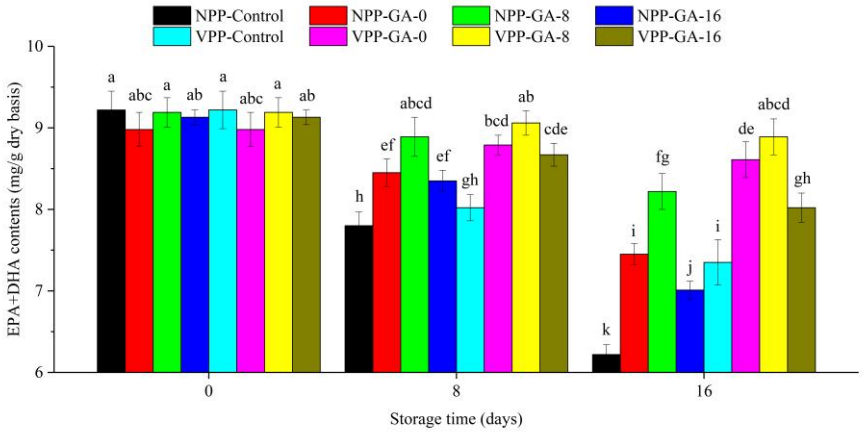

Figure 6. Changes in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents of freeze-dried scallop during accelerated storage at 65°C. NPP-Control, NPP-GA-0, NPP-GA-8, and NPP-GA-16 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in non-vacuum polyamide/polyethylene packaging (NPP), respectively. VPP-Control, VPP-GA-0, VPP-GA-8, and VPP-GA-16 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in vacuum polyamide/polyethylene packaging (VPP), respectively. Different lowercase letters indicate significant differences between samples ($p < 0.05$).

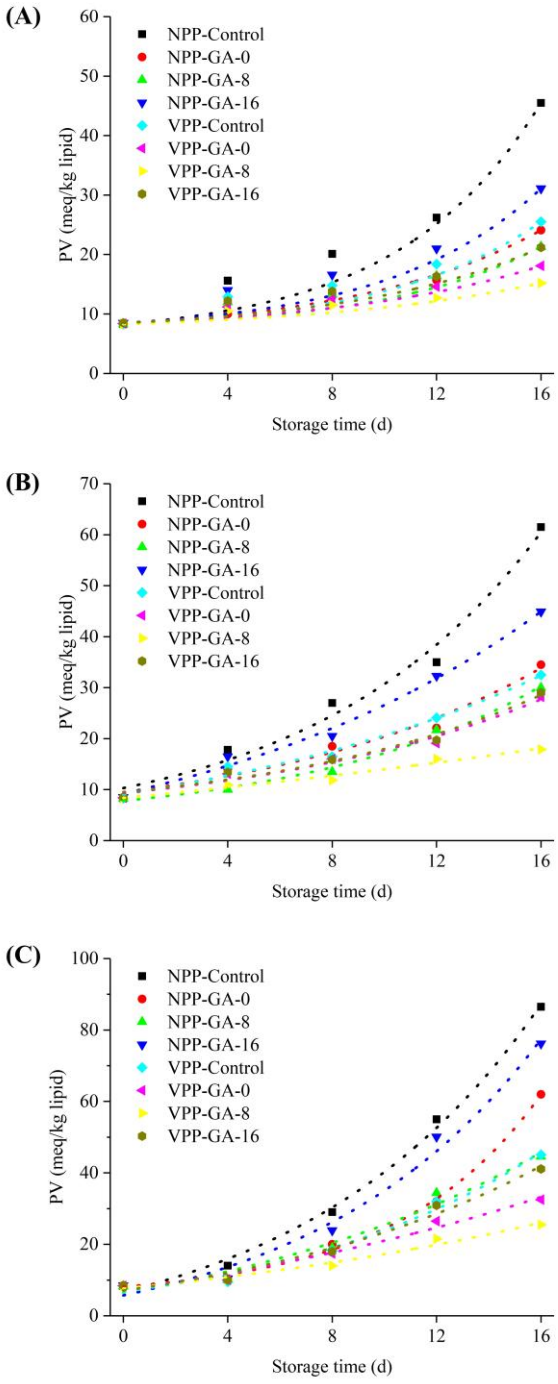

Figure 7. Changes in peroxide value (PV) of freeze-dried scallop during accelerated storage at 45°C **(A)**, 55°C **(B)**, and 65°C **(C)**. NPP-Control, NPP-GA-0, NPP-GA-8, and NPP-GA-16 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in non-vacuum polyamide/polyethylene packaging (NPP), respectively. VPP-Control, VPP-GA-0, VPP-GA-8, and VPP-GA-16 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in vacuum polyamide/polyethylene packaging (VPP), respectively.

Table 1. Kinetic models of peroxide value (PV), shelf-life prediction model, and predicted shelf life of freeze-dried scallop.

		Kinetic model			Shelf-life prediction model	Predicted shelf life (days)
		Zero-order model, R^2	First-order model, R^2	Arrhenius model, R^2		
1	45°C	y = 2.1250x+6.14, 0.9081	$y = 9.0726e^{0.098x}$, 0.9710	y = 5.0000-2334.6x, 0.9847	$\frac{Ln(PV_{limit}) - Ln(PV_{initial})}{e^{-2334.6/T+5.0000}}$	29.49 ± 0.50[f]
	55°C	y = 3.0900x+5.20, 0.9282	$y = 9.5524e^{0.117x}$, 0.9710			
	65°C	y = 4.9350x−0.92, 0.9325	$y = 8.2219e^{0.1514x}$, 0.9947			
2	45°C	y = 0.9275x+6.82, 0.8998	$y = 7.9607e^{0.064x}$, 0.9718	y = 8.8977-3716.6x, 0.9677	$\frac{Ln(PV_{limit}) - Ln(PV_{initial})}{e^{-3716.6/T+8.8977}}$	61.23 ± 3.55[d]
	55°C	y = 1.3775x+5.68, 0.9268	$y = 7.6224e^{0.0836x}$, 0.9794			
	65°C	y = 3.2200x+0.80, 0.8642	$y = 7.2964e^{0.128x}$, 0.9789			
3	45°C	y = 0.7625x+8.17, 0.9650	$y = 8.7500e^{0.055x}$, 0.9728	y = 9.5963-3971.9x, 0.9964	$\frac{Ln(PV_{limit}) - Ln(PV_{initial})}{e^{-3971.9T+9.5963}}$	72.19 ± 7.07[c]
	55°C	y = 1.5075x+7.44, 0.9415	$y = 9.0968e^{0.082x}$, 0.9732			
	65°C	y = 2.4250x+3.86, 0.9435	$y = 7.5393e^{0.115x}$, 0.9708			
4	45°C	y = 1.3050x+7.80, 0.9444	$y = 9.1532e^{0.075x}$, 0.9714	y = 8.9720-3685.3x, 0.9878	$\frac{Ln(PV_{limit}) - Ln(PV_{initial})}{e^{-3685.3/T+8.972}}$	50.83 ± 3.86[e]
	55°C	y = 2.2150x+6.82, 0.9656	$y = 9.4743e^{0.100x}$, 0.9761			
	65°C	y = 4.3775x-1.20, 0.9155	$y = 7.3072e^{0.149x}$, 0.9720			
5	45°C	y = 0.9975x+8.00, 0.9570	$y = 8.8803e^{0.065x}$, 0.9800	y = 6.8639-3061.0x, 0.9780	$\frac{Ln(PV_{limit}) - Ln(PV_{initial})}{e^{-3061.0/T+6.8639}}$	52.67 ± 2.21[de]
	55°C	y = 1.4500x+7.58, 0.9637	$y = 9.0597e^{0.081x}$, 0.9852			
	65°C	y = 2.4025x+3.40, 0.9328	$y = 7.3066e^{0.115x}$, 0.9852			
6	45°C	y = 0.5700x+8.40, 0.9769	$y = 8.7519e^{0.045x}$, 0.9844	y = -3854.8x+9.0388, 0.9862	$\frac{Ln(PV_{limit}) - Ln(PV_{initial})}{e^{-3854.8/T+9.0388}}$	84.53 ± 4.82[b]
	55°C	y = 1.1400x+7.84, 0.9451	$y = 8.9588e^{0.070x}$, 0.9762			
	65°C	y = 1.6175x+6.02, 0.9636	$y = 7.9488e^{0.092x}$, 0.9692			
7	45°C	y = 0.4000x+8.44, 0.9744	$y = 8.6237e^{0.035x}$, 0.9758	y = 9.4823-4091.3x, 0.9866	$\frac{Ln(PV_{limit}) - Ln(PV_{initial})}{e^{-4091.3/T+9.4823}}$	120.76 ± 8.57[a]
	55°C	y = 0.6075x+8.13, 0.9707	$y = 8.5230e^{0.048x}$, 0.9716			
	65°C	y = 1.1450x+6.72, 0.9599	$y = 7.9633e^{0.075x}$, 0.9756			
8	45°C	y = 0.7385x+8.50, 0.9694	$y = 9.0137e^{0.053x}$, 0.9794	y = 8.8943-3771.4x, 0.9870	$\frac{Ln(PV_{limit}) - Ln(PV_{initial})}{e^{-3771.4/T+8.8943}}$	73.34 ± 6.09[c]
	55°C	y = 1.1825x+7.84, 0.9430	$y = 9.0426e^{0.071x}$, 0.9782			
	65°C	y = 2.1525x+4.48, 0.9434	$y = 7.6902e^{0.107x}$, 0.9798			

1-4 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in non-vacuum polyethylene packaging (NPP), respectively. 5-8 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in vacuum polyethylene packaging (VPP), respectively.

Comparison of thermodynamic parameters

As seen in Table 2, for both VPP and NPP, GA-8 group showed the highest ΔH^{++} value followed by GA-0, GA-16, and control group. Moreover, the ΔH^{++} values of control, GA-0, GA-8, and GA-16 group with VPP were higher than those of corresponding groups with NPP. Regardless of the packaging used, GA-8 group had the lowest absolute value of ΔS^{++} while the control group had the highest absolute value of ΔS^{++}. In addition, the ΔS^{++} values of control, GA-0, GA-8, and GA-16 groups with VPP were lower than the corresponding values with NPP. Furthermore, samples from all groups with NPP or VPP showed positive ΔG^{++} values.

Table 2. Thermodynamic parameters for lipid oxidation occurring in freeze-dried scallop.

		ΔH^{++} (KJ/mol)	ΔS^{++} (KJ/mol/K)	ΔG^{++} (KJ/mol)
1	45°C	16.7	-0.21247	84.2
	55°C			86.4
	65°C			88.5
2	45°C	28.2	-0.17997	85.4
	55°C			87.2
	65°C			89.0
3	45°C	30.3	-0.17638	86.3
	55°C			88.1
	65°C			89.9
4	45°C	27.9	-0.17946	85.0
	55°C			86.8
	65°C			88.6
5	45°C	22.7	-0.19698	85.3
	55°C			87.3
	65°C			89.3
6	45°C	29.3	-0.17691	85.6
	55°C			87.3
	65°C			89.1
7	45°C	31.3	-0.17523	87.0
	55°C			88.7
	65°C			90.5
8	45°C	28.6	-0.17811	85.2
	55°C			87.0
	65°C			88.8

1-4 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in non-vacuum polyethylene packaging (NPP), respectively. 5-8 represent the scallop adductor muscle boiled in distilled water, gallic acid solution (5 mM), octyl gallate solution (5 mM), and hexadecyl gallate solution (5 mM) for 15 min, freeze-dried for 72 h, and packed in vacuum polyethylene packaging (VPP), respectively.

Discussion

Lipid oxidation is usually evaluated in terms of primary oxidation products, secondary oxidation products, oxygen absorption, concentration of free radicals, and loss of oxidative substrate (Shahidi and Zhong, 2010; Shabani *et al*., 2021). The present work showed that the PV, TBARS, and free radical levels significantly increased, while the IP value and EPA+DHA content of the control group significantly decreased during accelerated storage. These findings were consistent with a previous study which showed that PV and TBARS significantly increased while UFAs significantly decreased during accelerated storage of dried shrimps (Wang *et al*., 2024b). These results can be explained by the lipid autoxidation occurring in freeze-dried scallop during accelerated storage since lipoxygenase was inactivated in the boiling process, and light was avoided during storage. Autoxidation is a free radical chain process composed of chain initiation, propagation, and termination steps (Ahonen *et al*., 2022). In the initiation stage, the CH_2 group near the C=C bond in unsaturated lipid molecules, especially EPA and DHA, loses hydrogen atom to form alkyl radicals (R·). In the propagation stage, R· reacts with O_2 to form peroxy radical (ROO·), which can attack new unsaturated lipid molecules and produce hydroperoxide (ROOH), leading to an increase in the PV. ROOH is unstable and partially decomposes into free radicals (such as alkoxy and hydroxyl radical) and various secondary oxidation products (such as aldehydes). Thus, the levels of free radicals and TBARS increase, but the EPA+DHA contents decrease (Amaral *et al*., 2018; Domínguez *et al*., 2019). All these changes can culminate in reduced oxidation stability of freeze-dried scallop, corresponding to a decrease in the IP value (Shahidi, 2005).

The PV, TBARS, free radical levels, and EPA+DHA loss of the GA-0, GA-8, and GA-16 groups were lower than those of the control group during accelerated storage, while higher IP values and longer shelf lives were found for the GA-0, GA-8, and GA-16 groups compared to the control group. These results indicated that gallic acid and its alkyl esters inhibited the production of primary oxidation products, secondary oxidation products, and free

radicals. They also limited the degradation of oxidative substrate, and improved the oxidation stability of freeze-dried scallop. This is because the phenolic OH groups present in gallic acid and its alkyl esters donate hydrogen atoms to quench the free radicals (such as R·, RO·, and ROO·) (Bensid *et al*., 2022). A previous study also found that gallic acid and its alkyl esters including butyl gallate, octyl gallate, lauryl gallate, and hexadecyl gallate slowed down the increase in levels of PV and TBARS, as well as loss of EPA and DHA in dried oyster during storage (Zhao *et al*., 2022).

The powerful antioxidant effect is apparent only when the antioxidant is concentrated at the site of oxidation (McClements and Decker, 2018). The distribution of antioxidant is affected by its hydrophobicity, which in turn is determined by the chain length of gallic acid alkyl ester (Laguerre *et al*., 2017). Octyl gallate exhibited the best capacity for improving lipid oxidation and extending shelf life of freeze-dried scallop during accelerated storage. Moreover, the findings showed that the content of octyl gallate in freeze-dried scallop was significantly higher than that of gallic acid and hexadecyl gallate. Similarly, Zhao *et al*. (2021) reported that the content of gallic acid and its alkyl esters in oyster tissues increased first and then decreased with the increase in chain length, and the content of octyl gallate was the highest in oyster tissues. The lower content of gallic acid was because of its tendency to remain in the water rather than in the scallop adductor muscle, while the lower content of hexadecyl gallate was due to its lower solubility in water, so only a portion of hexadecyl gallate was able to enter muscle tissue. This result suggested that the distribution and accumulation of gallic acid and its alkyl esters in muscle tissue modulated the antioxidant activity for freeze-dried scallop. Specifically, the antioxidant properties of gallic acid alkyl esters improved as the alkyl chain length increased until octyl gallate, and then decreased, which was in accordance with the "cut-off effect". This "cut-off effect" of gallic acid and its alkyl esters was also observed during frying (Li *et al*., 2021), drying (Zhao *et al*., 2021), and storage of oyster (Zhao *et al*., 2022; 2023; Luo *et al*., 2023). This result can be explained by the fact that polar gallic acid is mainly distributed in the aqueous phase, medium polar octyl gallate is mainly distributed on the oil-water interface (*i.e*., the lipid oxidation site), and non-polar hexadecyl gallate crosses the oil-water interface into the lipid phase during the soaking and boiling process of scallop adductor muscle (McClements and Decker, 2000; 2018; Laguerre *et al*., 2017; Zhao *et al*., 2021). Thus, gallic acid and hexadecyl gallate showed lower antioxidant capacity for freeze-dried scallop during accelerated storage than octyl gallate. It should be noted that this antioxidant effect still showed a "cut-off effect" in vacuum packed freeze-dried scallops.

Moreover, the results showed that the control, GA-0, GA-8, and GA-16 groups with VPP had lower PV, TBARS, free radical levels, and EPA+DHA loss but longer IP value and shelf life than the four groups with NPP, suggesting that VPP significantly prevented the lipid oxidation of freeze-dried scallop. It has been reported that oxygen atom is the main reactant in lipid oxidation (Johnson and Decker, 2015). Based on the earlier discussion, the lipid oxidation rate can be modulated by reducing the oxygen concentration, thus improving the lipid stability (Johnson and Decker, 2015). Therefore, VPP improved the oxidation stability and prolonged the shelf life of freeze-dried scallop. Debbarma and Saha (2019) reported that VPP inhibited the TBARS value of dried Chandana (*Hilsa toli*) during storage, and extended its shelf life to 210 days. Teeraporn *et al*. (2014) found that VPP effectively controlled the lipid oxidation of salted dried snakehead fish during refrigerated storage based on the changes in PV, TBARS, and fatty acid composition.

Freeze-drying can provide higher quality dried aquatic food products because it can remove the moisture at low temperature and low oxygen concentration, which inhibits the lipid oxidation (Cheng *et al*., 2021). Thus, the freeze-dried scallop with a low initial oxidation degree had a longer shelf life. Moreover, freeze-drying inhibited oxidative loss of gallic acid, octyl gallate, and hexadecyl gallate added to the three groups of freeze-dried scallops, ensuring that they can improve the oxidative stability, and extend shelf life of freeze-dried scallops during storage.

Thermodynamic study is essential to determine whether the lipid oxidation process is endothermic or exothermic, exergonic or endergonic, and spontaneous or non-spontaneous (Kaseke *et al*., 2021). Greater ΔH^{++} value indicates that more energy is required for the material to reach activated state (Mihaylova *et al*., 2020). Smaller absolute value of ΔS^{++} implies less abundance of substances in the activated state, and consequently, a lower conversion rate of UFAs to ROOH (Tavakolipour *et al*., 2017;

Farhoosh, 2021). Therefore, positive values of ΔH^{++} of all groups with NPP or VPP indicated that the UFAs oxidation and ROOH formation in freeze-dried scallops during storage were endothermic reactions. Moreover, the highest ΔH^{++} value of GA-8 group and samples with VPP, as well as the lowest ΔS^{++} value (absolute value) of GA-8 group and samples with VPP, were observed. This suggested that octyl gallate had a better antioxidant effect than gallic acid and hexadecyl gallate, and VPP had a better antioxidant effect than NPP during storage of freeze-dried scallops. Positive values of ΔG^{++} indicated that the UFAs oxidation and ROOH formation of freeze-dried scallops during storage are non-spontaneous processes (Tavakolipour *et al.*, 2017; Ciğeroğlu *et al.*, 2022). Hence, the lipid oxidation stability of freeze-dried scallops during storage can be regulated by selecting appropriate antioxidants and packaging. The thermodynamic study showed that addition of octyl gallate in combination with VPP can slow down the increase in PV, and minimise lipid oxidation sensitivity of freeze-dried scallops during storage.

Conclusion

The present work found that gallic acid and its alkyl esters extended the shelf life of freeze-dried scallop by inhibiting lipid oxidation. A "cut-off effect" was observed, wherein the antioxidant effects of gallic acid and its alkyl esters first increased with the alkyl chain length, and then decreased, and octyl gallate showed the best antioxidant capacity. Moreover, the concentration of octyl gallate in freeze-dried scallop was the highest among gallic acid and its alkyl esters. Therefore, it can be inferred that the hydrophobicity of gallic acid and its alkyl esters is crucial for their effective distribution on the oil-water interface (the oxidation site) and their antioxidant effect. Additionally, VPP can enhance the oxidation stability of freeze-dried scallop, and prolong its shelf life during storage. These results were further supported by the thermodynamic analysis: samples treated with octyl gallate and VPP had the highest ΔH^{++} value and the lowest absolute value of ΔS^{++}. The present work provided useful information for selecting antioxidants to improve the oxidation stability, and prolong the shelf life of dried marine shellfish during long-term storage, as well as presented an efficient tool for the food industry to control lipid oxidation in dried seafood.

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