Preparation of preservative product for Xuong Com Vang longan based on chitosan and the oligochitosan-iodine complex

Du Duy Bui[1*], Tho Phuoc Tran[1], Kien Trung Chu[2], Tuan Nghiem Anh Le[1], Giang Ngoc Doan[1]

[1]*Institute of Advanced Technology, Vietnam Academy of Science and Technology, 1B TL29 Street, Quarter 1, An Phu Dong Ward, Ho Chi Minh City, Vietnam*
[2]*Institute of Agriculture Science for Southern Viet Nam, Vietnam Academy of Agriculture Science, 121 Nguyen Binh Khiem Street, Tan Dinh Ward, Ho Chi Minh City, Vietnam*

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Abstract:

Longan (*Dimocarpus longan* Lour.) is a tropical fruit characterised by a short shelf life, which necessitates the application of postharvest preservation methods to reduce losses during distribution and consumption. A preservation mixture based on chitosan (CS) and the oligochitosan-iodine (OC-I_2) complex was developed and evaluated for postharvest application in longan fruit storage. Ultraviolet-visible spectroscopy, X-ray diffraction, and Fourier-transform infrared spectroscopy confirmed that 2% H_2O_2 hydrolysis broke the β-1,4 glycosidic bond of CS without altering the OC structure. OC formed a complex with I_2 through -NH_2 and -OH groups. The chitosan-(oligochitosan--iodine) (CS-(OC-I_2)) mixture at 9.00-22.5 g/l extended the shelf life of Xuong Com Vang longan fruits from 5 days (control) to 15-20 days at room temperature. Treated fruits showed reduced respiration rates, microbial density, weight loss, total soluble solids, browning index, and disease index, while maintaining greater fruit hardness and stabilising total soluble solids. These results indicate that the CS-(OC-I_2) mixture is a promising preservative for longan and potentially other fruits, with the capacity to reduce postharvest losses during storage and distribution.

Keywords: **chitosan, iodine, oligochitosan, preservation, Xuong Com Vang longan.**

Classification numbers: **2.3, 3.1, 3.5**

1. Introduction

Longan (*Dimocarpus longan* Lour.) is a seeded fruit native to Asia, and belongs to the Sapindaceae family. The Xuong Com Vang (XCV) longan variety is a speciality, primarily grown in Ba Ria-Vung Tau province and the Mekong Delta in Vietnam [1]. One of the major drawbacks of longan is its very short postharvest shelf life, lasting only 3-4 days at ambient temperature, which limits commercialisation [2]. According to our observations, for XCV longan, once the fruit reaches harvest maturity, it typically begins to rot within 7 days if stored at ambient conditions, with a maximum shelf life of 10 days. Studies have shown that fruits deteriorate due to factors such as high humidity and temperature, which encourage microbial invasion [3]. It is estimated that approximately one-third of harvested fruits are lost annually to spoilage before reaching consumers [4]. As a result, new methods are continuously being developed to extend the postharvest shelf life of fruits. For longans, several preservation methods have been developed, including fungicide dipping, the use of plant growth regulators to delay ripening, wax or chitosan (CS) coating, bacterial antagonists such as *Bacillus subtilis*, sulphur fumigation, irradiation, and heat treatments (e.g., hot water for fungal control or cold storage) [2].

Currently, the development of non-toxic, edible coatings for fruit preservation represents an innovative approach, creating physical barriers on the fruit surface which decrease respiration rates and slow down oxidation-reduction reactions, thereby extending shelf life [5]. Edible coatings based on CS, the deacetylated product of chitin found in crustacean shells [6], have attracted considerable research attention due to their antimicrobial properties and

*Corresponding author: Email: vina9802@gmail.com

their ability to enhance surface texture without affecting the sensory or nutritional qualities of fruits [7]. When applied to fruits or plants, CS functions through three main mechanisms: stimulating the plant's defence system, exhibiting antimicrobial activity, and forming a protective film on the treated surface [8].

CS stimulates plants to produce defence enzymes such as chitinase and glucanase to combat microbial invasion, and induces the production of phytoalexins, protease inhibitors, callose, lignin biosynthesis and the activation of stress-responsive genes [9]. Low molecular weight (Mw) CS, particularly water-soluble oligochitosan (OC), has a stronger capacity to induce plant immune responses and defence-related genes compared with high Mw CS [10, 11], similar to the way vaccines stimulate immune responses in humans and animals [12]. Additionally, CS exhibits broad-spectrum antimicrobial properties, inhibiting the growth of harmful microorganisms [13], thus extending the shelf life of fruits. The antimicrobial activity of CS depends on its Mw, degree of deacetylation (DD), and pH [14]. The film-forming property of CS is advantageous for fruit preservation, as it prevents metabolic exchange with the environment [13]. Generally, high Mw CS forms denser films with greater hydrophobicity and higher mechanical strength, making it more effective for fruit preservation [15]. However, low Mw CS or OC exhibits stronger antifungal activity, significantly delaying colour changes, retaining moisture, increasing total phenolic content, reducing free radical production, lowering disease incidence, and maintaining fruit hardness [16]. Furthermore, CS possesses moisture-retention properties, preventing water loss due to the affinity of hydroxyl and amino groups for water molecules [17]. In terms of safety, CS is non-toxic, as it is degraded in the environment by various bacteria and fungi into harmless compounds [18]. Based on these properties, numerous studies have investigated the use of CS in postharvest longan preservation [19].

To enhance the antimicrobial activity of CS through synergistic interactions, complexes formed between CS and iodine (I_2) have been studied and applied in recent years. The reaction between CS and I_2 produces an iodophor complex (containing molecular I_2 and dissolved iodine ions) [20]. I_2 is well known for its broad-spectrum antimicrobial activity against bacteria, fungi, and viruses. Molecular I_2 can be toxic at high doses, with a median lethal dose (LD_{50}) ranging from 2 to 4 g/kg body weight [21]. However, iodophors such as povidone-iodine (PVP-I_2), commonly used as surface disinfectants, exhibit low toxicity due to their controlled-release mechanism. This slow release prevents I_2 from diffusing through the stratum corneum of human skin [19, 22]. Similarly, CS-based iodophor complexes release I_2 slowly, as it is retained by -NH_2 and -OH functional groups through exciton bonding. In addition, the CS-I_2 complex does not exhibit cytotoxicity under acidic gastric conditions [19].

Thus, the application of the CS-(OC-I_2) mixture is considered a safe and effective method for preserving longan after harvest. The CS-(OC-I_2) mixture has already been investigated for applications such as edible I_2 coatings for fruit preservation [23] and I_2 biofortification in plants [24]. However, studies on the use of the CS-(OC-I_2) mixture for postharvest fruit preservation are still limited. Based on the antimicrobial and biostimulant properties of CS-(OC-I_2) mixture, in this study, we have synthesised a mixture of CS-(OC-I_2) including CS with a Mw of 120.12 kDa and OC with a Mw of 5.31 kDa to preserve XCV longan fruits with the aim of extending their shelf life.

2. Materials and methods

2.1. Materials

Chemicals used in the experiment were of analytical grade, including CS powder (Suntze Chemical, Vietnam), H_2O_2 30%, I_2, lactic acid, acetic acid, C_2H_5OH 99.7° (Xilong, China); Potato Dextrose Agar - PDA (Himedia, India); pullulan with Mw of 1.3, 6, 12, 22, 50, and 130 kDa (Sigma Aldrich, Germany). Deionised water was used throughout the experiment.

2.2. Methods

Preparation of OC: OC was prepared following the method of T.H.P. Nguyen, et al. (2023) [25] with some modifications. Briefly, ten grams of CS powder was hydrolysed in 2% H_2O_2 solution over 10 days. The reaction

mixture was adjusted to pH 7.5 using 5% NH_4OH solution, and OC was precipitated by ethanol at a 6:1 (v/v) ethanol-to-sample ratio. The precipitate was washed three times with ethanol, filtered, dried at 60°C and ground to obtain OC powder.

Preparation of OC-I_2 complex: The OC-I_2 complex was prepared following the method of B.D. Du, et al. (2023) [26] with slight modifications. Four grams of OC powder was dissolved in 80 ml of H_2O in a glass beaker, then 20 ml of 5% (w/v) I_2 solution was added to the OC solution to form an OC-I_2 complex containing 4% OC and 1% I_2. The mixture was stirred until the I_2 colour disappeared, at which point the complexation reaction was considered complete.

Preparation of the CS-(OC-I_2) complex: Four grams of CS was dissolved in 100 ml of 4% lactic acid solution to obtain 100 ml of 4% CS (w/v). The CS-(OC-I_2) complex was prepared by mixing 100 ml of CS solution with 100 ml of OC-I_2 complex, followed by stirring, resulting in 100 ml of complex solution containing 2% CS, 2% OC, and 0.5% I_2 (w/v).

Characterisation of materials: The Mw was determined by gel permeation chromatography (GPC) using a GPC LC-20AD system (Shimadzu, Japan) with a RID 20A detector, Shodex SB803 HQ column and pullulan standards with Mw ranging from 0.78 to 130 kDa. Optical properties were analysed using UV-visible spectroscopy (UV-Vis) on a V630 spectrophotometer (JASCO, Japan); samples were diluted in a 0.2% acetic acid solution to achieve a CS concentration of 0.1% (w/v). The crystal structure was determined by X-ray diffraction (XRD) on a D8 Advance diffractometer (Bruker, Germany), using Cu Kα radiation (λ=1.5405 Å) with a constant voltage of 40 kV and a diffraction angle (2θ) scan from 10° to 80°. Functional group characterisation, bond identification and degree of deacetylation (DD) were performed using FT-IR spectroscopy on an FT-IR 8400S spectrometer (Shimadzu, Japan) over the wavenumber range of 4000-400 cm^{-1}. DD was calculated using formula (1) [27]:

$$DD\,(\%) = 100 - \left(\frac{A_{1655}}{A_{3450}}\right) \times 115 \quad (1)$$

where A_{1655} and A_{3450} are the absorbance intensities at 1655 cm^{-1} (related to the stretching vibration of the -C=O bond) and 3450 cm^{-1} (related to the stretching vibration of the O-H or N-H bonds), respectively.

Preservation of XCV longan fruits: Ten freshly harvested XCV longan fruits (within 2 h of harvest) were washed and fully soaked in CS-(OC-I_2) mixture solutions at concentrations of 9.00 (T1), 11.25 (T2), 15.00 (T3), and 22.50 (T4) g/l. The control treatment consisted of fruits without preservative application. After treatment, the fruits were drained and placed in sealed 3 L containers with lids fitted with a valve connected to a gas analyser. The experiment was repeated three times, with each replication conducted in a separate container. The fruits were sampled and evaluated at 0, 5, 10, 15, 20, 25, and 30 days of storage.

Evaluation parameters for XCV longan fruits: The hardness of the longan pericarp was determined using an FHT-15 Fruit Hardness Tester Meter (Total Meter, China). The disease index of the fruits was assessed by measuring the diseased area on the fruit surface according to the following scale:

0-No disease

1-Disease area <1/4

2-Disease area ≥1/4 and <1/2

3-Disease area ≥1/2 and <3/4

4-Disease area ≥3/4.

The disease index was calculated using formula (2) [28]:

$$\text{Disease index (\%)} = \sum\left(\frac{\text{Disease scale}}{\text{Highest scale}}\right) \times \text{Proportion of corresponding fruit within each class} \quad (2)$$

Weight loss was calculated using the formula (3) [29]:

$$\text{Weight loss}\,(\%) = \frac{\text{Initial weight} - \text{Weight at inspect date}}{\text{Initial weight}} \times 100 \quad (3)$$

Browning index is assessed by measuring the browned area of the fruit pericarp according to the following scale:

1-No browning

2-Slight browning

3-Browning area <1/4

4-Browning area ≥1/4 and <1/2

5-Browning area ≥1/2

The browning index was calculated using the following formula (4) [28]:

Browning index=Browning scale×Proportion of corresponding fruit within each class (4)

Total soluble solids (TSS) were determined using an Atago PAL-1 refractometer (Atago, Japan) [29]. The microbial density on the fruit pericarp was determined as follows: three longan fruits were stirred and washed in sterile saline; the resulting solution was serially diluted and plated onto Petri dishes containing PDA medium, then incubated for 72 h at room temperature. Microbial density was expressed as log cfu/g [30]. Respiration rate was determined according to the method of A.A. Saquet, et al. (2001) [31] with several modifications. 30 uniform longan fruits, free from spoilage, were individually weighed and enclosed in a 5 L airtight plastic container. After 60 min of incubation, gas samples were withdrawn using a 5 ml gas-tight syringe and analysed using a SCION 456-GC gas chromatograph (SCION, the Netherlands) to determine CO_2 (ml/kg/h) and C_2H_4 (µl/kg/h) concentrations. After 15 days of storage, longan pedicels from the samples were inoculated onto PDA medium, and fungal growth was observed after 5 days of incubation.

Statistical analysis: All data were statistically analysed using IRRISTAT 5.0 and Microsoft Excel 2016 software, and results were presented as mean ± standard error. Mean values were compared using the least significant difference (LSD) test at the 5% probability level ($p<0.05$).

3. Results and discussion

3.1. Preparation of chitosan-(oligochitosan-iodine) mixture

The GPC chromatograms of CS and OC are shown in Fig. 1. The Mw of the initial CS and OC, calculated from the retention time in Fig. 1, were 120.12 and 5.31 kDa, respectively. The oxidative hydrolysis method using H_2O_2 was effective in breaking the β-1,4 glycosidic bonds of CS in powder form without the need for organic acids for dissolution, thereby producing water-soluble OC at high concentrations of up to 10% (w/v). This method was considered efficient, environmentally friendly, and energy saving [32].

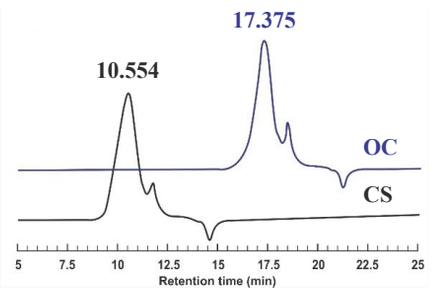

Fig. 1. Gel permeation chromatography (GPC) chromatograms of chitosan and oligochitosan.

The photographs of the CS, OC, and CS-(OC-I_2) mixture solutions are presented in Fig. 2. The CS solution exhibited a characteristic pale yellow colour, while the OC solution appeared lighter in comparison. In contrast, the CS-(OC-I_2) complex solution was noticeably darker. In our previous study, water-soluble OC was shown to form a complex almost instantaneously with the I_2 solution, with the resulting complex solution nearly losing the initial brown colour of I_2 [26].

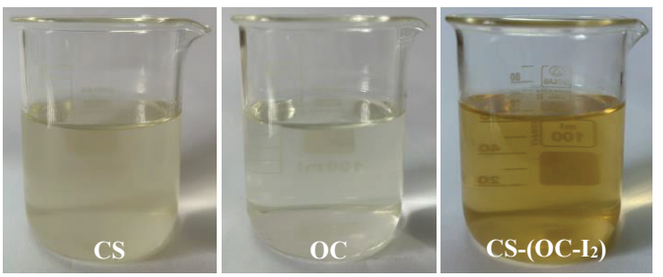

Fig. 2. Photographs of the chitosan, oligochitosan, and chitosan-(oligochitosan-iodine) mixture solutions.

Figure 3A presents the UV-Vis spectra of CS, OC, the OC-I_2 complex, and the CS-(OC-I_2) mixture. For the CS sample, characteristic peaks were observed at 205 nm, attributed to n→σ* electron transitions due to the presence of amino groups (-NH2) in the CS structure [32]. In addition, the peak at 256 nm could be assigned to the carbonyl group [33]. This peak was shifted to 290 nm in the UV-Vis spectrum of OC, which resulted from the breakdown

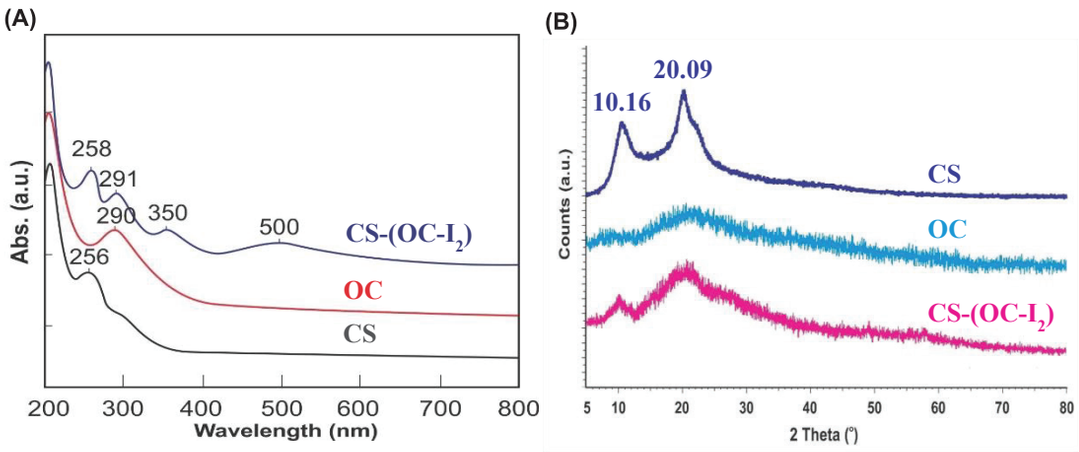

Fig. 3. UV-Vis spectra (A) and XRD patterns (B) of chitosan, oligochitosan, and the chitosan-(oligochitosan-iodine) mixture.

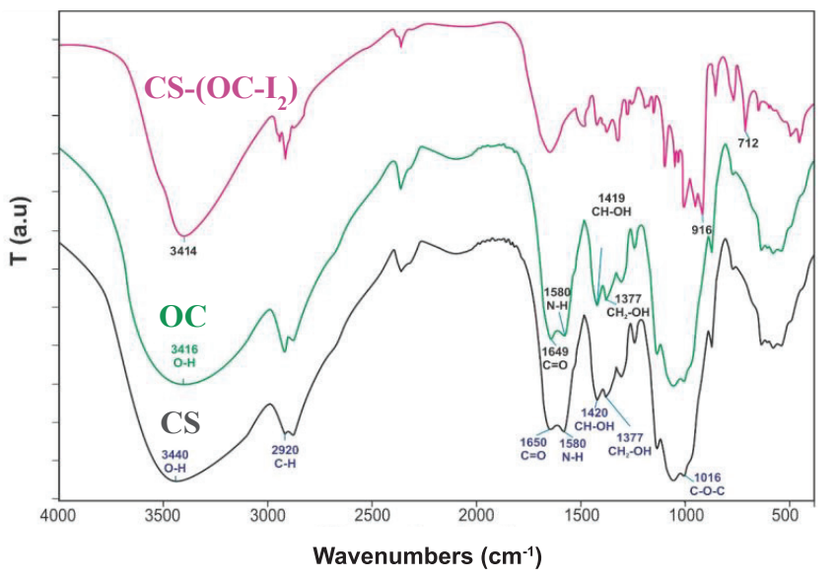

Fig. 4. FT-IR spectra of chitosan, oligochitosan, and the chitosan-(oligochitosan-iodine) mixture.

of glycosidic bonds at positions C1 and C4 during the oxidative hydrolysis of CS [34]. The UV-Vis spectrum of OC also displayed a peak at 205 nm. The UV-Vis spectrum of the CS-(OC-I_2) mixture exhibited not only the two characteristic peaks of CS and OC but also additional signal peaks at 291 and 350 nm, assigned to the absorption of I_3^- and IO_3^-, respectively [20], as well as a weak signal at 500 nm corresponding to polyiodide structures, which are characteristic of the complexation between OC and I_2 [35]. Therefore, the peak at 290 nm of OC and the I_3^- absorption in the CS-(OC-I_2) mixture may have overlapped to form the observed peak at 291 nm.

Table 1. The relationship between the hardness of XCV longan pericarp and the concentration of the chitosan-(oligochitosan-iodine) mixture during storage.

Treatments	Hardness (N)						
	0	*5*	*10*	*15*	*20*	*25*	*30*
Control	17.5^{ns}±0.2	14.5^{ns}±0.5	6.4^{a}±0.4	-	-	-	-
T1	17.4^{ns}±0.3	14.5^{ns}±0.3	13.9^{b}±0.4	13.6^{ns}±0.5	13.3^{ns}±0.3	13.1^{a}±0.2	13.0^{ns}±0.5
T2	17.6^{ns}±0.2	14.7^{ns}±0.5	14.1^{b}±0.3	13.8^{ns}±0.4	13.5^{ns}±0.4	13.3^{a}±0.4	13.2^{ns}±0.1
T3	17.8^{ns}±0.2	14.9^{ns}±0.4	14.4^{b}±0.4	14.1^{ns}±0.5	13.8^{ns}±0.5	13.7^{a}±0.4	13.5^{ns}±0.5
T4	17.8^{ns}±0.2	15.3^{ns}±0.3	14.9^{c}±0.3	14.5^{ns}±0.2	14.3^{ns}±0.4	14.1^{b}±0.2	13.9^{ns}±0.2

T1, T2, T3, and T4 were CS-(OC-I_2) mixtures at concentrations of 9.00, 11.25, 15.00, and 22.50 g/l, respectively. The mean values in a column with the same letter are not significantly different at $p<0.05$.

The XRD patterns of CS, OC, and the CS-(OC-I_2) mixture are shown in Fig. 3B. The XRD pattern of CS displayed two characteristic peaks at 2θ≈10.16° and 20.09°, consistent with the results of H.N. Nguyen, et al. (2024) [36]. In the OC sample, the peak at 2θ≈10.16° disappeared, while the peak at 2θ≈20.09° decreased in intensity, which may be attributed to oxidative hydrolysis with H_2O_2 reducing the crystallinity of CS [25]. The XRD pattern of the CS-(OC-I_2) mixture retained both characteristic peaks of CS but with reduced intensity, likely due to complex formation between I_2 and the -OH and -NH_2 functional groups of CS through electron donor-acceptor interactions [26, 37].

The FT-IR spectra of CS, OC, and the CS-(OC-I_2) mixture are presented in Fig. 4. The FT-IR spectrum of OC displayed the characteristic peaks of CS, including those at 3457 cm^{-1}, 2876 and 2924 cm^{-1}, 1650 cm^{-1}, 1567 cm^{-1}, and 1425 cm^{-1}, which were assigned to the N-H, O-H, -CH, C=O, N-H, and CH_2 functional groups, respectively [38]. For the CS-(OC-I_2) mixture, the peak at 1580 cm^{-1} disappeared, indicating that the N-H group of OC was complexed with I_2 [37]. However, the FT-IR spectra in this study showed that the characteristic hydroxyl peak of the CS-(OC-I_2) mixture at 3414 cm^{-1} did not differ significantly from that of OC. In our opinion, this may be because OC had a low Mw, so the complexation process primarily occurred at the N-H group. In addition, two new peaks appeared in the FT-IR spectrum of the CS-(OC-I_2) mixture at 916 and 712 cm^{-1}, though their origin remains unknown. The DD of CS and OC, determined according to formula (1), were 92.6% and 91.7%, respectively. These results confirm that the heterogeneous hydrolysis method using H_2O_2 to produce water-soluble OC did not alter the chemical structure compared to the original CS, but only cleaved the β-1,4 glycosidic bonds.

3.2. Preservation of XCV longan by the chitosan-(oligochitosan-iodine) mixture

The hardness results of XCV longan pericarp treated with the CS-(OC-I_2) mixture are presented in Table 1. In the control sample, the pericarp hardness decreased rapidly from 17.5 N to 6.4 N after 10 days of storage, and by day 15, the fruits had spoiled. In contrast, longan fruits treated with the CS-(OC-I_2) mixture exhibited a significantly slower decline in hardness over time compared with the control. This effect may be attributed to the formation of a biopolymer coating, which helps delay the ripening process by reducing respiration rates and inhibiting microbial growth on the fruit surface as well as from environmental contamination [39, 40]. Notably, after 10 days of storage, the hardness of fruits in the T4 treatment was significantly higher than that observed in the T1, T2, and T3 treatments Table 1.

The disease index results of longan fruits during storage are presented in Table 2. In the control treatment, the disease index was 0.60% on day 10 and increased to 1.80% by day 30. In contrast, all samples treated with the CS-(OC-I_2) mixture showed a significantly reduced disease index compared with the control. Specifically, fruits in the T1 and T2 treatments were protected from microbial invasion for up to 20 days. At higher concentrations, the T3 and T4 treatments completely inhibited disease development after 30 days. According to N. Limchoowong, et al. (2016) [23], the CS-I_2 complex exhibited greater antimicrobial activity than CS when used as a coating to preserve tomatoes.

The results of weight loss in XCV longan fruits during 30 days of storage are presented in Table 3. In the control treatment, fruit weight decreased by 3.37% after 10 days, and by day 15 the fruits had begun to rot and spoil. In contrast, longan fruits treated with the CS-(OC-I_2) mixture showed a significant reduction in weight loss compared with the control, with weight loss decreasing in direct proportion to the concentration of the complex. The lowest weight loss was observed in the T4 treatment, with only 1.21% after 30 days. This finding is consistent with the results of Y. Lin, et al. (2020) [40] and M.G. Lin, et al. (2018) [41], who reported the effectiveness of CS in preserving longan.

Table 2. The relationship between the disease index of XCV longan fruits and the concentration of the chitosan-(oligochitosan-iodine) mixture during storage.

Treatments	Disease index (%)						
	0	*5*	*10*	*15*	*20*	*25*	*30*
Control	$0.0^{ns}\pm0.00$	$0.0^{ns}\pm0.00$	$0.6^{a}\pm0.24$	$0.8^{a}\pm0.20$	$1.0^{ns}\pm0.00$	$1.2^{a}\pm0.20$	$1.8^{a}\pm0.20$
T1	$0.0^{ns}\pm0.00$	$0.0^{ns}\pm0.00$	$0.0^{b}\pm0.00$	$0.0^{b}\pm0.00$	$0.0^{ns}\pm0.00$	$0.2^{b}\pm0.20$	$0.4^{b}\pm0.24$
T2	$0.0^{ns}\pm0.00$	$0.0^{ns}\pm0.00$	$0.0^{b}\pm0.00$	$0.0^{b}\pm0.00$	$0.0^{ns}\pm0.00$	$0.2^{b}\pm0.20$	$0.2^{b}\pm0.20$
T3	$0.0^{ns}\pm0.00$	$0.0^{ns}\pm0.00$	$0.0^{b}\pm0.00$	$0.0^{b}\pm0.00$	$0.0^{ns}\pm0.00$	$0.0^{b}\pm0.00$	$0.0^{b}\pm0.00$
T4	$0.0^{ns}\pm0.00$	$0.0^{ns}\pm0.00$	$0.0^{b}\pm0.00$	$0.0^{b}\pm0.00$	$0.0^{ns}\pm0.00$	$0.0^{b}\pm0.00$	$0.0^{b}\pm0.00$

T1, T2, T3, and T4 were CS-(OC-I_2) mixtures at concentrations of 9.00, 11.25, 15.00, and 22.50 g/l, respectively. The mean values in a column with the same letter are not significantly different at $p<0.05$.

Table 3. The relationship between the weight loss of XCV longan fruits and the concentration of the chitosan-(oligochitosan-iodine) mixture during storage.

Treatments	Weight loss (%)						
	0	*5*	*10*	*15*	*20*	*25*	*30*
Control	$0.00^{ns}\pm0.00$	$1.66^{a}\pm0.11$	$3.37^{a}\pm0.13$	-	-	-	-
T1	$0.00^{ns}\pm0.00$	$0.20^{b}\pm0.07$	$0.28^{b}\pm0.11$	$0.42^{ns}\pm0.08$	$2.84^{ns}\pm0.13$	$1.51^{a}\pm0.09$	$1.58^{a}\pm0.12$
T2	$0.00^{ns}\pm0.00$	$0.18^{b}\pm0.05$	$0.26^{b}\pm0.06$	$0.38^{ns}\pm0.07$	$2.79^{ns}\pm0.14$	$1.38^{a}\pm0.09$	$1.42^{a}\pm0.11$
T3	$0.00^{ns}\pm0.00$	$0.15^{b}\pm0.07$	$0.23^{b}\pm0.07$	$0.37^{ns}\pm0.05$	$2.71^{ns}\pm0.15$	$1.27^{a}\pm0.07$	$1.28^{a}\pm0.11$
T4	$0.00^{ns}\pm0.00$	$0.11^{b}\pm0.05$	$0.19^{b}\pm0.04$	$0.32^{ns}\pm0.04$	$2.61^{ns}\pm0.05$	$1.25^{b}\pm0.05$	$1.21^{b}\pm0.03$

T1, T2, T3, and T4 were CS-(OC-I_2) mixtures at concentrations of 9.00, 11.25, 15.00, and 22.50 g/l, respectively. The mean values in a column with the same letter are not significantly different at $p<0.05$.

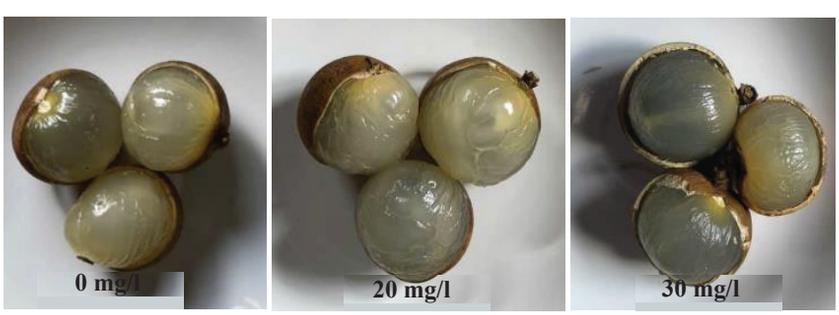

Fig. 5. Photographs of peeled XCV longan fruits in T3 treatment after 0, 20, and 30 days of storage at room temperature.

Throughout the monitoring period, no disease symptoms were observed on the longan fruits in the T3 and T4 treatments (Table 2). However, due to moisture loss, the shell and pulp of the longan began to separate, and the recorded weight loss reached 2.6-2.7% after 20 days of storage. Photographs of the peeled longan fruits are shown in Fig. 5. In particular, the pulp of XCV longan fruits in the T3 treatment showed visible shrinkage after 20 and 30 days of storage compared with the initial state (day 0).

The results of the browning index of XCV longan pericarps treated with the CS-(OC-I_2) mixture during storage are presented in Table 4. Longan fruits in the control group exhibited a rapid increase in browning, with the index rising from 1.4 on day 10 to 4.6 on day 30, indicating spoilage that accelerates oxidation and pericarp browning. In contrast, fruits treated with the CS-(OC-I_2) mixture showed a slower increase in the browning index, with statistically significant differences compared to the control. These findings demonstrate that the CS-(OC-I_2) mixture is more effective in controlling longan browning than the method reported by Y. Lin, et al. (2020) [40], who used CS alone as a preservation coating. In addition to the synergistic antimicrobial effects of CS and OC complexed with I_2, the slower progression of browning could also be attributed to the presence of low-Mw OC (5.31 kDa), which possesses antioxidant activity in fruits [16, 42].

The results of the TSS content analysis in longan fruits after storage are presented in Table 5. In the control group, the TSS content decreased from 20.44 mg/l to 18.20 mg/l

Table 4. The relationship between the browning index of XCV longan pericarp and the concentration of the chitosan-(oligochitosan-iodine) mixture during storage.

Treatments	Browning index						
	0	*5*	*10*	*15*	*20*	*25*	*30*
Control	1.0^{ns}±0.0	1.0^{ns}±0.0	1.4^{a}±0.2	2.6^{a}±0.2	3.8^{a}±0.2	4.2^{a}±0.2	4.6^{a}±0.2
T1	1.0^{ns}±0.0	1.0^{ns}±0.0	1.0^{b}±0.0	1.0^{b}±0.0	1.2^{b}±0.2	1.6^{b}±0.2	1.8^{b}±0.2
T2	1.0^{ns}±0.0	1.0^{ns}±0.0	1.0^{b}±0.0	1.0^{b}±0.0	1.0^{b}±0.0	1.2^{b}±0.2	1.6^{b}±0.2
T3	1.0^{ns}±0.0	1.0^{ns}±0.0	1.0^{b}±0.0	1.0^{b}±0.0	1.0^{b}±0.0	1.2^{b}±0.2	1.4^{b}±0.2
T4	1.0^{ns}±0.0	1.0^{ns}±0.0	1.0^{b}±0.0	1.0^{b}±0.0	1.0^{b}±0.0	1.2^{b}±0.2	1.2^{b}±0.2

T1, T2, T3, and T4 were CS-(OC-I_2) mixtures at concentrations of 9.00, 11.25, 15.00, and 22.50 g/l, respectively. The mean values in a column with the same letter are not significantly different at $p<0.05$.

Table 5. The relationship between the TSS content of XCV longan fruits and the concentration of the chitosan-(oligochitosan-iodine) mixture during storage.

Treatments	TSS (%)						
	0	*5*	*10*	*15*	*20*	*25*	*30*
Control	20.44^{ns}±0.18	20.11^{a}±0.13	19.80^{a}±0.15	18.20^{a}±0.00	-	-	-
T1	20.60^{ns}±0.13	20.16^{a}±0.07	20.02^{a}±0.17	19.59^{b}±0.09	18.34^{a}±0.16	17.57^{a}±0.09	16.81^{a}±0.16
T2	20.63^{ns}±0.19	20.40^{a}±0.12	20.30^{b}±0.14	19.78^{b}±0.15	18.54^{a}±0.12	17.80^{b}±0.11	17.13^{a}±0.07
T3	20.47^{ns}±0.14	20.19^{a}±0.11	20.06^{a}±0.12	19.66^{b}±0.10	19.16^{b}±0.15	18.68^{c}±0.02	18.12^{b}±0.15
T4	20.63^{ns}±0.11	20.54^{b}±0.05	20.52^{b}±0.12	20.11^{c}±0.09	19.75^{c}±0.11	19.51^{d}±0.08	18.96^{c}±0.06

T1, T2, T3, and T4 were CS-(OC-I_2) mixtures at concentrations of 9.00, 11.25, 15.00, and 22.50 g/l, respectively. The mean values in a column with the same letter are not significantly different at $p<0.05$.

after 15 days. In contrast, the decline in TSS content was significantly slower in fruits coated with the CS-(OC-I_2) mixture. Notably, the TSS content of longan fruits in the T4 treatment was significantly higher than that of T1, T2, and T3 treatments at day 10. After 15 days, the TSS content in T1 and T2 treatments decreased by approximately 0.85-1.01%. In T3 and T4 treatments, the reduction was around 0.88-1.31% after 20 days, which remained within the acceptable range for maintaining fruit sweetness [43]. The TSS content of fruits depends on the concentration of soluble sugars, primarily glucose and fructose, hydrolysed from sucrose [44]. Following harvest, fruit respiration consumes carbohydrates and reducing sugars [45], leading to a decline in TSS content during storage.

The results of microbial growth on longan pericarp during storage with the CS-(OC-I_2) mixture are presented in Table 6. In the control treatment, microbial density increased rapidly, with log cfu/g rising from 4.23 to 4.36 after 5 days, and continuing to 5.12 by day 10. By day 15, the fruits spoiled due to extensive microbial growth. In treatments T1 and T2, microbial density increased from approximately 3.18 on day 5 to about 4.32 on day 30. Meanwhile, fruits treated with higher concentrations of the CS-(OC-I_2) mixture (T3 and T4) showed a slower increase in microbial density, from around 2.71 on day 5 to 3.41 on day 30. Overall, all fruits treated with the CS-(OC-I_2) mixture exhibited a lower increase in microbial density than the control during the storage period.

The antimicrobial activity of CS-I_2 and OC-I_2 complexes, even at lower concentrations compared with CS and OC alone, has been reported by several authors. A.M. Sklyar, et al. (2023) [19] concluded that the minimum inhibitory concentration of the CS-I_2 complex against *Escherichia coli* and *Staphylococcus aureus* was 2.5 mg, while for *Candida* spp. it was 0.62 mg. The OC-I_2 complex (Mw of OC was 3.32 kDa) was effective in inhibiting *Pantoea stewartii* at concentrations of 425-500 mg/l, while OC alone showed no activity [46].

Table 6. The relationship between the microbial density of XCV longan fruits and the concentration of the chitosan-(oligochitosan-iodine) mixture during storage.

Treatments	Microbial density (log cfu/g)						
	0	*5*	*10*	*15*	*20*	*25*	*30*
Control	$4.23^{a}\pm0.19$	$4.36^{a}\pm0.15$	$5.12^{a}\pm0.14$	-	-	-	-
T1	$0.00^{b}\pm0.00$	$3.23^{b}\pm0.16$	$3.75^{b}\pm0.08$	$3.98^{a}\pm0.13$	$4.14^{a}\pm0.10$	$4.25^{a}\pm0.14$	$4.34^{a}\pm0.14$
T2	$0.00^{b}\pm0.00$	$3.13^{b}\pm0.14$	$3.71^{b}\pm0.16$	$3.95^{a}\pm0.16$	$4.10^{a}\pm0.10$	$4.22^{a}\pm0.15$	$4.31^{a}\pm0.14$
T3	$0.00^{b}\pm0.00$	$2.84^{b}\pm0.10$	$2.93^{c}\pm0.15$	$3.04^{b}\pm0.13$	$3.16^{b}\pm0.15$	$3.38^{b}\pm0.12$	$3.57^{b}\pm0.14$
T4	$0.00^{b}\pm0.00$	$2.57^{c}\pm0.09$	$2.58^{d}\pm0.07$	$2.87^{b}\pm0.08$	$3.04^{b}\pm0.10$	$3.16^{b}\pm0.09$	$3.25^{b}\pm0.09$

T1, T2, T3, and T4 were CS-(OC-I_2) mixtures at concentrations of 9.00, 11.25, 15.00, and 22.50 g/l, respectively. The mean values in a column with the same letter are not significantly different at $p<0.05$.

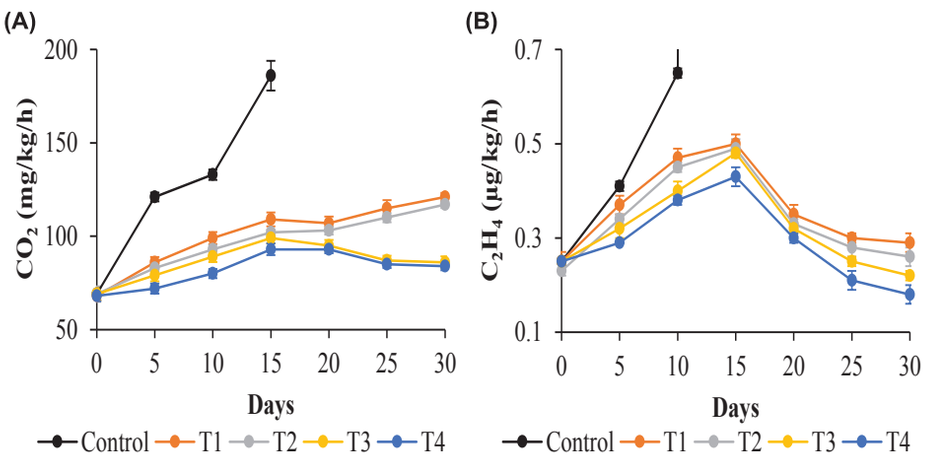

Fig. 6. Relationship between CO_2 (A) and C_2H_4 (B) contents of XCV longan fruits and the concentration of the chitosan-(oligochitosan-iodine) mixture during storage.

The results of the respiration rate of XCV longan fruits during storage are shown in Figs. 7 and 8. In the control sample, the CO_2 level (Fig. 6A) increased from 68.50 to 132.72 mg/kg/h after 10 days, then rose sharply to 185.58 mg/kg/h by day 15, coinciding with fruit spoilage. Similarly, the initial C_2H_4 level (Fig. 6B) was 0.25 µg/kg/h, which increased to 0.65 µg/kg/h on day 10 and 24.10 µg/kg/h on day 15.

In the treatments with the CS-(OC-I_2) mixture, the production rates of CO_2 and C_2H_4 were consistently lower than in the control. After 15 days, CO_2 levels in treated samples remained ≤108.86 mg/kg/h, while C_2H_4 levels were ≤0.50 µg/kg/h. By day 30, treatments T3 and T4 showed further decreases in both CO_2 and C_2H_4 release rates. These findings confirm that the CS-(OC-I_2) mixture effectively reduced respiration activity in longan fruits during storage.

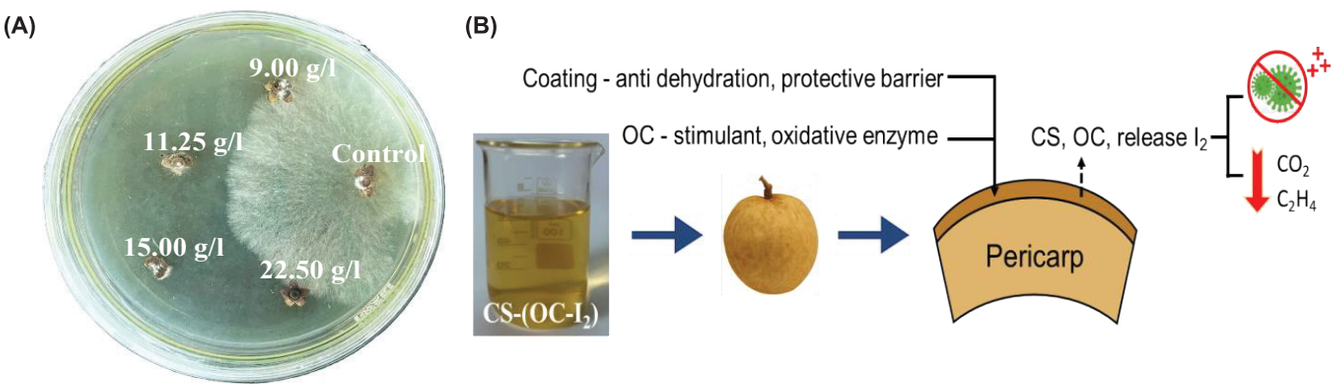

Fig. 7. Fungal growth of longan pedicels on PDA medium treated with chitosan-(oligochitosan-iodine) mixture at different concentrations after 5 days of incubation (A) and the mechanism of the chitosan-(oligochitosan-iodine) mixture action in longan preservation (B).

Fig. 8. Photographs of longan fruits treated with chitosan-(oligochitosan-iodine) mixtures after 0 (A) and 15 (B) days of storage at room temperature.

According to G. Miranda, et al. (2019) [47], fruit respiration tends to increase in the early stages of dehydration but decreases at later stages as solid content rises, thereby reducing respiration (provided the fruit is not spoiled). Consistent with this, after 20 days of storage, respiration in the treated samples rose slowly, likely due to increased weight loss (mainly water), which elevated TSS content in the fruits.

The development of fungi from longan pedicels is shown in Fig. 7A. After 15 days of storage, longan pedicels from each treatment were cultured on PDA medium to assess fungal growth. Following 5 days of incubation, the control sample exhibited extensive fungal growth with large colony diameters, whereas fungal growth on pedicels treated with the CS-(OC-I_2) mixture was nearly absent.

These results demonstrate that treatment with the CS-(OC-I_2) mixture effectively extended the shelf life of XCV longan fruits, up to 15 days at concentrations of 9.00 and 11.25 g/l, and up to 20 days at 15.00 and 22.50 g/l. The proposed mechanism of action of the CS-(OC-I_2) preservative is illustrated in Fig. 7B.

Photographs of XCV longan fruits treated with the CS-(OC-I_2) mixture during storage are presented in Fig. 8. The fruits in the control group showed clear signs of spoilage after 15 days, marked with a white "×". In contrast, the treated fruits retained their appearance, with no significant change in colour observed throughout the storage period.

4. Conclusions

In this study, the CS-(OC-I_2) mixture was successfully prepared at room temperature without the use of toxic chemicals. The mixture demonstrated effective film-forming and antimicrobial properties. When applied at concentrations of 9.00-22.50 g/l, it extended the shelf life of longan fruits to 15-20 days under ambient storage conditions. The production and application of CS-(OC-I_2) in postharvest fruit preservation represent a novel and promising approach for developing safe agricultural products. While the results are encouraging, further research, particularly sensory evaluation and on-farm postharvest trials, will be essential to confirm its practical applicability.

CRediT author statement

Du Duy Bui: Conceptualisation, Funding acquisition, Resources; Tho Phuoc Tran: Software, Visualisation; Kien Trung Chu: Investigation, Writing original draft; Project administration, Writing, Reviewing & Editing; Tuan Nghiem Anh Le: Data curation, Methodology; Giang Ngoc Doan: Supervision, Validation; Formal analysis.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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