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Highlights

S. cerevisiae with satisfactory GSH production was selected as the antioxidant agent.

Active films made from sodium alginate, sucrose and *S. cerevisiae* were developed.

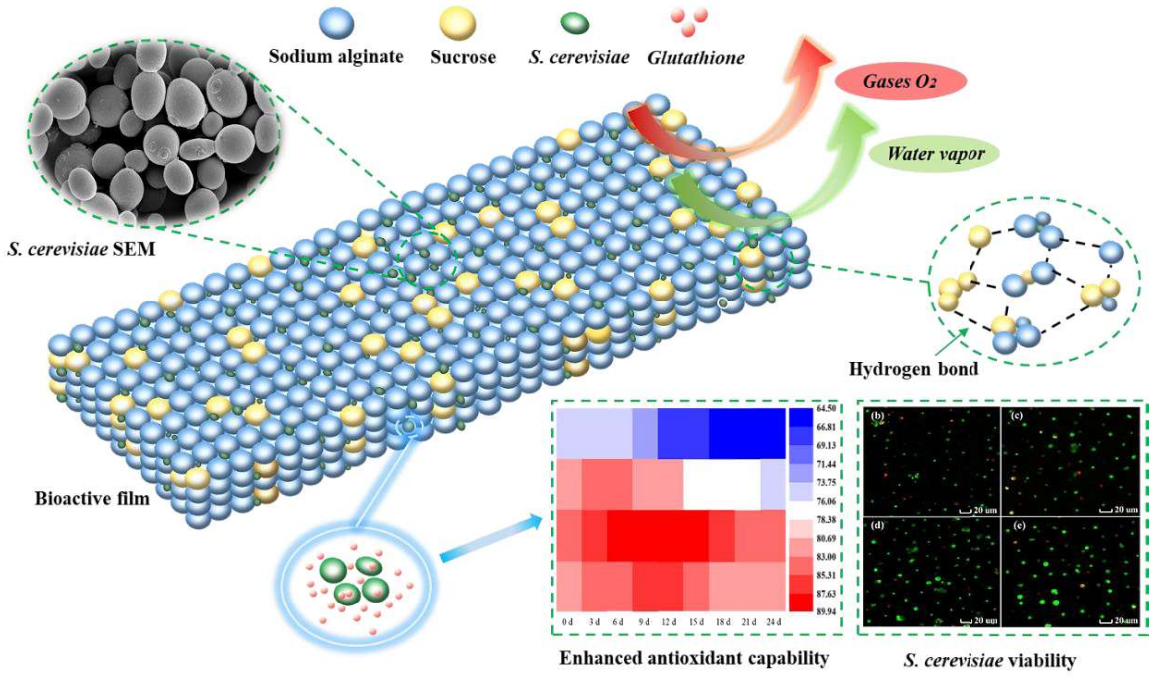
Sucrose significantly improved the mechanical property of the films.

Sucrose maintained the antioxidant property of the films during the storage period.

Active films improve the quality and pericarp browning of longan.

Abstract

A sodium alginate (SA) film incorporated with *Saccharomyces cerevisiae* (SE) and sucrose (SU) was fabricated to control the quality and pericarp browning of longan. The SE with satisfactory glutathione production was selected as the antioxidant agent. The scanning electron microscopy (SEM) results revealed that the SU-rich SA film could be used as an effective carrier to protect the cell integrity of SE. The FTIR and mechanical property results indicated that the SA-SE film with the incorporation of SU has good flexibility due to the existence of hydrogen bonds. Notably, the cell viability of the SE was significantly improved with the addition of SU, which positively affects the antioxidant property of the film during the storage period. Finally, the SA-SU-SE films obviously improved the quality and pericarp browning of longan. The SA-based film incorporated with SU and SE may be established as a novel antioxidant fruit packaging material.



Saccharomyces cerevisiae-encapsulated and sucrose-rich sodium alginate film: an effective antioxidant packaging film for longan preservation

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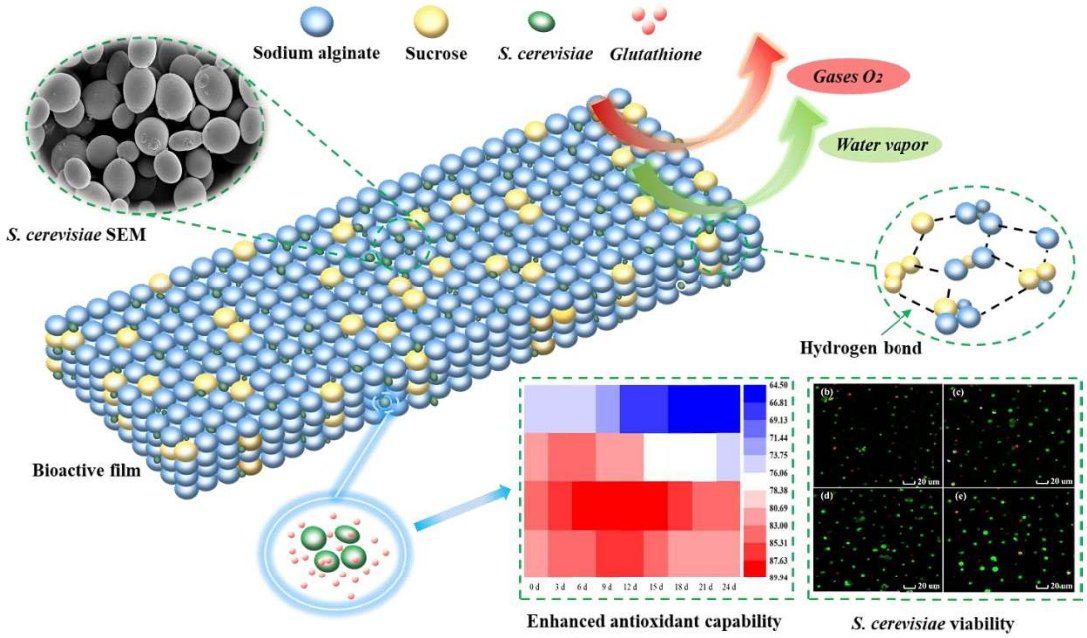
Abstract

A sodium alginate (SA) film incorporated with *Saccharomyces cerevisiae* (SE) and sucrose (SU) was fabricated to control the quality and pericarp browning of longan. The SE with satisfactory glutathione production was selected as the antioxidant agent. The scanning electron microscopy (SEM) results revealed that the SU-rich SA film could be used as an effective carrier to protect the cell integrity of SE. The FTIR and mechanical property results indicated that the SA-SE film with the incorporation of SU has good flexibility due to the existence of hydrogen bonds. Notably, the cell viability of the SE was significantly improved with the addition of SU, which positively affects the antioxidant property of the film during the storage period. Finally, the SA-SU-SE films obviously improved the quality and pericarp browning of longan. The SA-based film incorporated with SU and SE may be established as a novel antioxidant fruit packaging material.



Keywords: Bioactive film; *Saccharomyces cerevisiae*; Glutathione; Antioxidant; Longan fruit;

Introduction

Longan (*Dimocarpus longan Lour.*) is popular with consumers due to its unique flavor and high nutrient value. However, the shelf life and market value of the longan fruit could be significantly reduced due to post-harvest physiological changes, especially pericarp browning caused by enzyme[1]. Hence, it is essential to control the postharvest quality of longan fruit.

When it comes to the preservation and browning control of fruits, the active packaging materials are always attracted more attention than traditional materials due to they can interact positively with food and the environment so that improve the quality of the foods [2]. The active packaging generally consists of the film-forming polymer matrix and the natural active substance. Several natural polymers have been widely used as the film-forming matrix to develop active packaging materials [3]. Sodium alginate (SA) is an anionic polysaccharide that contains two structural units of 1–4 linked α-l-guluronic acid and β-d-mannuronic acid. it has been widely used as an effective active substance carrier for developing packaging materials because it has low price and good film-forming properties [4].

Various natural substances have been added to the film-forming polymer matrix to develop active packaging materials [5]. Recently, numerous natural bioactive agents made from microorganisms or their derivatives have received considerable attention in food application due to their effective biological activities and health benefits, such as antimicrobial activity, antioxidant activity, and anticancer [6]. The European Union Novel Food regulation (9258/1997 EEC) states that the microorganism used in food industry (including food products or food packaging) should be (Qualified Presumption of Safety) QPS [6]. Several studies have tried to use the some QPS microorganisms as the potential active substance to develop active packaging films or coatings [7, 8].

Saccharomyces cerevisiae as a QPS microbial with good biological activity has been widely applied in foods manufacture and medicine production industry [8-12]. Remarkably, *S. cerevisiae* produces glutathione (GSH) is a low-molecular-mass thiol, which has demonstrated antioxidant capability, anti-aging, and detoxification [13]. Moreover, several studies have reported that the GSH could effectively inhibit the enzymatic browning and enzyme activity in fruits [14]. Hence, *S. cerevisiae* cells with satisfactory GSH production could be used as a novel antioxidant agent to develop active packaging materials.

Actually, one of the main concerns of packaging materials containing microorganisms is the cell viability of the incorporating microorganism during the storage period, because the viability of the microorganism

affects their cell numbers or biomass production, which affects the bioactive property of the film [2]. Hence, the improvement of microorganism viability is critical for strengthening the bioactive property of the film. On the other hand, SA film as a common food preservation film has poor mechanical property and low oxygen permeability, which restrict its applications. Sucrose (α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside) is the world's most abundant free low molecular weight carbohydrate. Several studies have reported that sucrose could retain the cell viability of *S. cerevisiae* under harsh storage conditions and increase the biomass production of *S. cerevisiae* [15-17]. Moreover, studies have demonstrated that *su*crose could be used as a plasticizer to increase polymer chain mobility so that improve the mechanical property of the natural polymer film [18]. Therefore, sucrose could be added into the SA film containing *S. cerevisiae* to improve the bioactive and mechanical properties of the film.

However, to our best knowledge, no works regarding the use of sucrose to improve the bioactive and mechanical properties of SA-*S. cerevisiae* films for controlling the quality and pericarp browning of longan fruits. Hence, the present work aimed to fabricate the SA active films enriched with *S. cerevisiae* and sucrose. The effect of sucrose on the viability of *S. cerevisiae* in SA films was explored. The mechanical and biological properties of the films were also evaluated. Finally, the bioactive film was applied to control the quality and pericarp browning of longan fruits at ambient temperature (25 ± 1 °C).

2. Materials and methods

2.1. Materials

Saccharomyces cerevisiae (CICC 1048) was obtained from China Center of industrial Culture collection. All chemical agents including sodium alginate, sucrose, methanol, GSH standards, fluorescein diacetate (FDA) and propidium iodide (PI) were obtained from Nanjing Jiancheng Technology Co., Ltd. (Jiangsu, China).

2.2. Preparation of *S. cerevisiae* culture

The yeast was cultured in nutrient yeast extract peptone sucrose broth. The prepared yeast cultures were packed in an Erlenmeyer flask and incubated at 25 °C for 24 h. The *S. cerevisiae* culture was centrifuged at 5000 rpm for 10 min to obtain the precipitate. The obtained precipitate was washed two times with sterile normal saline and then centrifuged to prepare the *S. cerevisiae* pellet.

2.3. Determination of growth curve and GSH production

The growth curve was measured by noting the absorbance of *S. cerevisiae* cultures at 600 nm every 2 hours. The nutrient yeast extract peptone sucrose broth was served as the control. The intracellular

GSH production curve was measured using the 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) method. The content of GSH in the film was measured using the DTNB method. The *S. cerevisiae* pellet (0.05 g) was mixed with 4 mL of (40% *v/v*) menthol and cultured at 25 ℃ for 2 hours. Then, the supernatant was obtained by centrifuging the mixture at 6000 rpm, 4 ± 1 °C for 15 min. Tris-HCl buffer and DTNB solution were then added to 1 mL of the supernatant and reacted at 25℃ for 15 min. The absorbance value at 412 nm was measured using 2 mL of deionization water as a blank control. The absorbance value was substituted into Eq. (1) to calculate the GSH content. The GSH content was determined every 2 hours within 36 hours in this work.

$$y_1 = (x + 0.0228)/1.17 \quad (1)$$

Where x is the absorbance value at 412 nm. y_1: the content of GSH in the pellet (mg/100g *S. cerevisiae* pellet).

2.4. The fabrication of the films

SA powder (2.0% *w/v*) was dissolved in distilled water at 60 °C under continuous stirring to obtain an SA solution. The SA-*S. cerevisiae* solutions (SA-SE) were prepared by adding *S. cerevisiae* pellet into the SA solution at 30 °C. The concentration of *S. cerevisiae* in SA-SE solutions was 1×10^8 cells mL^{-1}.The different concentrations of sucrose (1.0% *w/v*, 3.0% *w/v*, and 6.0% *w/v*) were respectively added into the SA-SE solutions at 30 °C to prepare the SA-*S. cerevisiae*-1.0% sucrose (SA-SE-1.0%SU), SA-*S. cerevisiae*-3.0% sucrose (SA-SE-3.0%SU) and SA-*S. cerevisiae*-6.0% sucrose (SA-SE-6.0%SU) solutions. The films were obtained by casting all prepared solutions into the sterile plates and then drying at 30 °C for 12 h in a sterile oven.

2.5. Viability of *S. cerevisiae* in the films during the storage period

The bioactive film incorporated with *S. cerevisiae* was stored at 25 ± 1 °C and 75% relative humidity (RH) for 24 d, and the viability of the yeast was evaluated every three days by determining the colony-forming units using the plate-count method. Briefly, film samples (1 g) were aseptically transferred into 9 mL of the sterile saline solution and agitated to ensure that all yeasts were released into the solution. Finally, a microorganism solution of an appropriate concentration was spread on a yeast extract peptone dextrose (YEPD) medium.

Moreover, to check the cellular state of the yeast, the fluorescence of the films after storage for 24 days was also checked with a confocal laser microscope (Leica TCS SP5) at 480 nm excitation wavelength. The FDA was dissolved in acetone at a concentration of 10 μg/mL. PI was dissolved in distilled water at

a concentration of 5 μg/mL. Films were first stained with FDA and then with PI, both for 20 min at room temperature in the dark. The viable yeast cells were presented with green fluorescence; dead yeast cells were presented with red fluorescence.

2.6. Microstructure observation of the film and yeast cell

A scanning electron microscope (JEOL, JSM-6360) was used to observe the film morphology. The tested films were fixed on bronze stub using double-side adhesive and then sputtered with gold in a vacuum evaporator.

Cell morphology was observed by a cold field emission SEM (SU8200, HITACHI, Japan). The *S. cerevisiae* cultures were centrifuged at 4 ℃ and 2000 rpm for 10 min and then fixed with 5% glutaraldehyde. After fixation, the samples were rinsed with phosphate buffer (0.1 M). Subsequently, the samples were fixed with 1% ruthenium tetroxide and rinsed thrice in the same buffer. After the pretreatment, the samples were dehydrated with ethanol and then dried at the CO_2 critical point to obtain the dried samples. The dried samples were attached with an aluminum foil for ion sputtering.

2.7. Fourier transform infrared (FTIR) spectroscopy

FT-IR spectrum of the film was measured to evaluate the chemical structure of the bioactive film. The spectrum of the dried film sample was analyzed in the range of 525–4000 cm^{-1} with a Nicoletis50 infrared spectrometer (Perkine Elmer 16 PC spectrometer, Boston, USA).

2.8. Antioxidant property of the films

The antioxidant property of the film was evaluated by analysis the scavenging ability of DPPH radicals. Briefly, 9 mL of the film extract solution was mixed with 2 mL of DPPH solution (0.1 mM solution in ethanol) and incubated under the dark for 45 minutes. The absorbance of the solution at 517 nm was noted with a UV spectrophotometer. The value of DPPH scavenging activity was evaluated based on Eq. (2):

$$DPPH\ scavenging\ activity\ (\%) = (A_0 - A_1)/A_0 \times 100\% \quad (2)$$

Where A_1 and A_0 signify the absorbance of the DPPH solution mixed with or without film extract solution.

2.9. The barrier and mechanical properties

The water vapor permeability (WVP) of all samples was measured referred to the method reported by Yang Zhikun et al. [19]. Firstly, a centrifuge tube (15 mL) was filled with 10 mL of distilled water and covered with the fabricated film. After that, the centrifuge tube was placed in the dryer. The weight of the centrifuge tube was weighted every two hours. The following formula obtained the WVP:

$$WVP = (\Delta m \times \Delta P)/(A \times \Delta t \times x) \quad (3)$$

Where Δm represents the weight gain of the tube per unit time Δt (s); A is the area of exposed film; x is the thickness of the film, and Δp is the partial water vapor pressure difference on both sides of the film. The oxygen permeability (OP) of the film sample was determined with an automated oxygen permeability testing machine according to Zhai Xiaodong et al. [20].

Tensile strength (TS) and elongation percentage at break (EB) of the film samples (20 × 60 mm rectangular shapes) were conducted using a Texture analyzer (TA-XT2i, Stable Micro Systems Ltd, Surrey, UK) according to the ASTM method. The initial grip separation distance was 40 mm, and the cross-head speed was 1 mm/s. The TS and EB were obtained by the following formula:

$$TS = F/S \quad (4)$$

$$EB(\%) = \Delta l/l_0 \quad (5)$$

2.10. Visual appearance and transmittance

The visual appearance of the film was obtained with a scanner. The transmittance of films was measured at 200–800 nm wavelengths using a spectrophotometer.

2.11. Potential application on longan fruits

Fresh longan fruits (cultivar "Shixia") with healthy outer and uniform size were provided by local producers. The effect of browning control of longan fruits by the fabricated film was measured in this work. The longan fruits were washed with distilled water and then air-dried. The air-dried fruits were immersed in different film-forming solutions and air-dried again. The fruits only washed with distilled water served as the control. After that, all treated fruits were placed in a polyethylene tray and covered with polyethylene film, and then stored at 25 ± 1 °C and 75% RH for eight days.

2.11.1. Browning index

The pericarp browning of longan fruits was assessed by the analysis of the extent of total browning area on each fruit pericarp and evaluated by using the following scales: where 1 represents no browning; 2 represents slight browning; 3 represents ≤25% browning; 4 represents 25%~50% browning; 5 represents ≥50% browning. The browning index was calculated using the following Eq: ∑ (browning scale × percentage of corresponding fruit within each class).

2.11.2. Polyphenol oxidase (PPO) and peroxidase (POD) enzyme activity

The previously described method with minor modifications was used to determine the PPO and POD

enzyme activities [21]. Fruit pericarp was blended with a high-speed blender and mixed with phosphate buffer (0.2 M, pH 6.5). The supernatant was obtained by centrifuging the homogenate at 10,000 × g, 4 ± 1 °C for 15 min. For PPO activity, the obtained supernatant (50 μL) was pipetted into a solution containing 1 mL of catechol (0.1 M) and 1.95 mL of phosphate buffer (0.2 M) and then reacted in the dark. The absorbance of the reaction solution at 410 nm was noted. For POD activity, the supernatant (50 μL), 0.15 mL of guaiacol (0.01 M), 0.15 mL of H_2O_2, and 2.66 mL of phosphate buffer (0.2 M) were mixed to obtain the assay solution. After that, the assay solution was reacted in the dark for 25 min, and then the absorbance at 470 nm was noted. The unit (U) of PPO and POD activity defined as a change of 0.01 in absorbance per minute. The enzyme activity expressed as U g^{-1} of fresh weight (FW).

2.11.3. Weight loss

The weight loss (%) was determined based on the ratio of the decreased weight of longan fruits (W_d) over the original weight of longan fruits (W_o).

$$Weight\ loss\ (\%) = Wd/W_o \times 100 \quad (6)$$

2.12. Statistical analysis

Significant differences were analyzed with SPSS 19.0 software using one-way analysis of variance (ANOVA). The statistical level of significance was $P < 0.05$. All experiments were performed five times.

3. Results and discussion

3.1. Characterization and GSH content determination of *S. cerevisiae*

The micrographs (400×) of *S. cerevisiae* cells were oval or spherical (Fig. 1a). The colony morphology of *S. cerevisiae* was white, smooth, and sticky appearance (Fig. 1b). The growth curve of *S. cerevisiae* was also presented in Fig. 1c. The results showed that the lag phase of *S. cerevisiae* was within 4 h, the logarithmic phase was 6-18 h, and the stationary period was after 18 h. The GSH is a biologically active tripeptide with excellent antioxidant capability, which is the secondary metabolites of *S. cerevisiae* [13]. The intracellular GSH production curve of *S. cerevisiae* was presented in Fig. 1d. In the logarithmic growth phase (4-18 h), the intracellular GSH content rapidly accumulated, and the GSH content in the 18-h *S. cerevisiae* pellet was 497 ± 16.8 mg/100g. The intracellular GSH content reached the highest value (521 ± 26.0 mg/100g) after 24 hours. The intracellular GSH content in *S. cerevisiae* pellet did not obviously change within 18-36 hours. This result indicated that the feasibility of *S. cerevisiae* to produce GSH with sucrose as the main carbon source and the intracellular GSH content is related to the growth of yeast. Since the yeast strains in the logarithmic growth phase were most active and with higher

intracellular GSH content, the 18-h seed solution was selected to prepare the yeast pellet as the antioxidant agent.

3.2. Scanning electron microscopy

Fig. 2a presented that the cross-section of SA film was completely uniform and smooth. The cell morphology figure of *S. cerevisiae* was observed in Fig. 2b, all the *S. cerevisiae cells* are oval, and their average particle size was around 5~15 μm. The surface of the *S. cerevisiae* cell was smooth and flat. Some intact *S. cerevisiae* cells could be obviously observed in the cross-section of the SA-SE film (Fig. 2c). This result indicated that the *S. cerevisiae* cell could be well alive and dispersed in the SA polymer. As can be seen from Fig. 2d, the cross-section of 1.0%SU loaded SA-SE film was denser. This phenomenon might be caused by intermolecular hydrogen bonds between sucrose and the natural polysaccharide matrix [22]. Some irregular semi-crystalline structures was observed in cross-section of the film when the incorporating SU concentration increased to 3.0% *w/v* (Fig. 2e). Similar structure was observed in the cross-section of the SA-SE-6.0%SU film (Fig. 2f). Jiyuan Xu et al. [23] also found that the cross-section of chitosan became rough with the addition of xylooligosaccharides. Moreover, the average size of *S. cerevisiae* cells in all films was reduced. This is because the size of the microorganism will be changed under different stresses. Similar founding was also reported by Soto-Reyes, N et al [24]. It is interesting to note that the *S. cerevisiae* cell density in the film was obviously increased when the incorporating SU content was 3.0% *w/v* and 6.0% *w/v*. This could be due to the fact that sucrose as an effective protectant with small molecular structures could easily replace the water molecules removed during the drying process so that protect the yeast cell integrity [25]. This result revealed that the SA-based bioactive films incorporated with SU and *S. cerevisiae* were fabricated successfully in this work, which was expected to be a novel ideal carrier.

3.3. FTIR studies

The FTIR spectrum of SA, SA-SE, SA-SE-1.0%SU, SA-SE-3.0%SU and SA-SE-6.0%SU films were presented in Fig. 3. For the neat SA film, the characteristic band at 1026, 1407, 1593, and 2929 cm^{-1} were assigned to the elongation of C-O groups, the asymmetric stretching vibration of COO- groups, and the CH stretching, respectively. The broad peak in the range of 3000–3667 cm^{-1} corresponding to the hydroxyl groups (-OH). The weak bands at 948 indicated the presence of uronic acid functional group. As observed in the spectrum of SA-SE, the incorporation of *S. cerevisiae* did not affect the FTIR spectra of the pure SA films. This result could be due to the fact that there are no interactions between the yeast

and the carrier material. Orozco-Parra, Mejía, and Villa [26] also reported that the addition of microorganisms did not affect the FTIR spectrum and crystalline structures of polysaccharide film. After adding 1.0% SU to SA-SE film, the FTIR spectrum of the SA-SE-1.0%SU film shows a combination of characteristics similar to that of the pure SA film, and the characteristic peaks corresponding to SA at 1593 cm^{-1} and 1407 cm^{-1} were slightly blue-shifted due to hydrogen bonding between SA and SU molecules. The decrease in the intensity of peak at 1593 cm^{-1} and 1407 cm^{-1} of SA-SE-1.0%SU film can be indicative of the greater degree of disorder in SA (Fig. 2d, 2e, and 2f) and could be related to the plasticizing effect of the SU on the SA matrix. Pępczyńska et al. [27] also reported that the addition of SU could decrease the intensity of the Amide I band of salmon gelatin film.

The peak in the SA-SE-3.0%SU film shift from 947 to 924 cm^{-1} was attributed to the Csingle bondO bonding stretch associated with plasticization. Noticeably, The O-C stretching band of SA-SE-3.0%SU film at 989-1027 cm^{-1} was double-peaked instead of the single peak in pure SA-SE film. The double peaks occurred because the sucrose formed stable hydrogen bonds at CH bends and with both 'O' of C-O-H and 'O' of anhydroglucose ring in SA molecules. Moreover, the characteristic peak corresponding to the hydroxyl groups (-OH) in the SA film was broadened and strengthened, indicating more hydroxyl groups (-OH) were formed in SA-SE-3.0%SU film due to the hydrogen bonding. This result indicated that the 3% SU concentration could form stronger bonds with SA than 1.0% SU. Similar founding was also reported by Pushpadass, Marx, and Hanna [28], who added SU into the starch-based film. No significant difference was observed in the FTIR spectrum of SA-SE-3.0%SU and SA-SE-6.0%SU films. Thus, it could be assumed that the addition of a certain content (3.0%~6.0%) of SU into SA film involved the formation of hydrogen bonds between the SA and SU, replacing some of the original strong bonds in the hydroxyl groups of pure SA.

3.4. *S. cerevisiae* cell number and antioxidant capability in stored films

The viability of the yeast is an essential parameter which had a significant influence on the property of the yeast films. The cell number of the *S. cerevisiae* in the film during storage at 25 ± 1 °C and 75% RH was presented in Fig. 4a. The initial cell number of *S. cerevisiae* in SA-SE film was 7.22 ± 0.01 Log CFU/g and it presented a decreasing trend through the storage period. The viable *S. cerevisiae* number in the SA-SE film was lower than that of SA-SE-SU films. This is because the presence of SU could effectively maintain the *S. cerevisiae* cell integrity during the drying process (Fig. 2). It is interesting to observe that the viability of *S. cerevisiae* in all SA-SE-SU films exhibited a trend of rising first and then

falling. This is because the yeast could rapidly proliferate by using sufficient nutrients in the film at the early stage of storage. However, with the increase of storage time, the yeasts in the film will produce large amounts of metabolites which are detrimental to cells and eventually cause cell death. The viable cell numbers in SA-SE-1.0%SU film reached the peak value (9.32 ± 0.4 Log CFU/g) at 6 d, and then gradually decreased. Remarkably, the SA-SE-3.0%SU and SA-SE-6.0%SU films could delay the arrival of the peak value. The peak value of viable *S. cerevisiae* cells in SA-SE-3.0%SU, and SA-SE-6.0%SU films was 10.20 ± 0.1 Log CFU/g and 9.41 ± 0.2 Log CFU/g, respectively. After storage for 24 days, the viability of *S. cerevisiae* in SA-SE-SU films was obviously higher over that of SA-SE film. This result indicated that the addition of SU could improve the viability of the yeast in the SA film. The viable cell numbers in the SA-SE-3.0%SU and SA-SE-6.0%SU films were higher over that of SA-SE-1.0%SU film, and no obvious difference was observed in SA-SE-3.0%SU and SA-SE-6.0%SU films. These results indicated that the appreciated concentration of SU could promote the growth of *S. cerevisiae* in the stored film. However, the excess SU content (~6% *w/v*) could not furtherly increase the cell viability of *S. cerevisiae*. This is because high concentrations of SU may alter the osmotic pressure in the polymer matrix, thereby inhibiting the growth of yeast [29].

The antioxidant capability of the active film is essential for controlling the oxidant damage of foods. The DPPH scavenging activity of the films during 24 days of storage period was presented in Fig. 4b. The DPPH scavenging activity of pure SA film was 8.6 ± 0.62%, and it hardly changed during the test period (Fig. S2). This could be due to the presence of hydrogen bond donating functional groups in SA that could also scavenge some free radicals [30]. Notably, the initial antioxidant property (0 d) of the SA film was obviously increased to 76.0 ± 1.1% by the addition of *S. cerevisiae*. This is due to the fact that *S. cerevisiae* itself could be used as an effective antioxidant [31]. Moreover, *S. cerevisiae* will produce some metabolites with antioxidant ability, especially glutathione (Fig. 1d). A similar result was also reported by Siying Li et al. [32], who observed that the exopolysaccharide produced by lactic acid bacteria could improve the antioxidant property of the film. The initial DPPH scavenging activity (0 d) of the SA-SE film was further strengthened with the addition of SU. No significant difference was observed in the initial antioxidant property of SU-rich SA films. This is because the cell number and integrity of *S. cerevisiae* were well-maintained during the film-forming process by the addition of SU (Fig. 1 and 3a) so that maintain the biological capability of *S. cerevisiae* cell. The antioxidant property of SA-SE film exhibited a decreasing trend during the storage period. It is interesting to observe that the antioxidant

property of sucrose-rich SA-SE film presented a trend of rising first and then falling during the storage period. After storage for 24 days, the DPPH scavenging activity of SA-SE-1.0%SU films decreased to 75.5 ± 1.0%, while the value of SA-SE-3.0%SU and SA-SE-6.0%SU were ~85.4% and ~81.6%, respectively. The changing trend in antioxidant property of the films during the storage period was consistent with that of the number of viable yeast cells in the films (Fig. 4a). Our previous research demonstrated that the antioxidant property of the film during the storage was related to the content of the active substance [4]. Thus, it could be concluded that the presence of SU maintains the number of *S. cerevisiae* cells with satisfactory GSH production in the stored film, which offer a great potential for maintaining the antioxidant property of the films during the storage period. While the mechanism between *S. cerevisiae* cell numbers and the antioxidant properties of the films is unclear, the active film with enhanced antioxidant properties is beneficial for reducing the food oxidative damage.

3.5. *S. cerevisiae* cellular state in the films

To check the cellular state of the *S. cerevisiae* in the film, the fluorescence of the stored films after storage for 24 days was presented in Fig. S1. The green fluorescence was the predominant color in all figures, indicating most *S. cerevisiae* could be alive in the developed film during the room temperature storage. This could be in accord with the fact that *S. cerevisiae* is a model organism with higher resistance to harsh environmental conditions. It was easy to observe that the red point numbers in Fig. S1a were obviously higher than that in other figures, indicating that the viable *S. cerevisiae* cell concentration in SA film was obviously lower than that in SA-SE-SU films. It is interesting to observe the cell density in the SA-SE-3.0%SU was higher than that of other SA-SE-SU films. This result was consistent with plate counts result (Fig. 4a). Oluwatosin, Tai, and Fagan-Endres [25] also found that certain content of sucrose could effectively improve the final cell density of *Lactobacillus Plantarum* during the storage. These results indicated that the addition of SU to SA film could improve the viability of the *S. cerevisiae* cell during the storage period.

3.6. Mechanical property

The tensile strength of the SA film exhibited a little change after the addition of *S. cerevisiae* (Fig. 5a). Similar result was also reported by Soukoulis et al. [33], who added the *Lactobacillus rhamnosus* into the starch-protein film. The TS of the SA-SE film significantly decreased with the incorporation of SU (P <0.05), and it furtherly decreased with the gaining concentration of SU. When the incorporating SU concentration exceeds 3.0% *w/v*, the TS of the film hardly decreased anymore. By contrast, the EB of

the film obviously increased with the incorporation of SU ($P < 0.05$), and it reached the highest value (37.6 ± 2.4%) when the SU concentration was 3.0% *w/v* (Fig. 5a). No significant difference was observed between SA-SE-3.0%SU film and SA-SE-6.0%SU films ($P > 0.05$). This result accord with the fact that sucrose as a plasticizer could increase the polymer chain mobility so that it strength the extensibility of the film [18]. Similar result was also studied by Fadini et al. [34]. This result showed that certain content of SU could be used to improve the flexibility of SA-SE film, and the SA-SE-3%SU exhibited the optimal extensibility.

3.7. Barrier property

Fig. 5b showed the water barrier property of the tested films. The addition of *S. cerevisiae* presented little influence on the WVP of SA film. Li Siying et al. [24] also observed that the WVP of the cassava starch/carboxymethylcellulose edible films altered little with the incorporation of lactic acid bacteria. Notably, it significantly decreased with the accession of SU. However, no significant difference was observed in the WVP value of the SA-SE-SU films ($P > 0.05$). Regarding the OP of the films (Fig. 5b), it altered little with the incorporation of *S. cerevisiae*. The OP of the SA-SE film obviously decreased to 19.1 ± 0.51 $cm^3 \cdot um \cdot m^{-2} \cdot d^{-1} \cdot Kpa^{-1}$ when the incorporating SU was 1.0% *w/v* ($P < 0.05$), and it reached the lowest value (14.5 ± 1.1) when the SU content was 3.0% *w/v*. However, the OP of the 6.0% *w/v* SU loaded film increased. This result could be due to the addition of high concentration sucrose may decrease interaction between the polymer chains so that it facilitates the migration of oxygen [35]. This result indicated that the addition of the appropriate amount (1.0~3.0% *w/v*) of sucrose positively affected the barrier property of SA-SE film.

3.8. Optical property

The visual appearance of all the developed films was presented in Fig. 5c. The pure SA film was light and transparent, and it became light-yellow with the incorporation of *S. cerevisiae.* The appearance of the SA-SE film was hardly altered by the addition of SU, and no significant difference was found in the appearance of all SU-rich SA-SE films. Similar result was also observed in the transmittance of the films (Fig. 5d). The transmission of all the film was > 75.0% at 600 nm, and the pure SA film presented the best transparency. The transparency of the SA at 600 nm slightly decreased to ~81.1% by the incorporation of *S. cerevisiae*, and it further slightly decreased to ~78.2% with the incorporation of SU. All the films presented the similar UV-barrier property. This result indicated that all the fabricated films in this work presented the satisfactory appearance and transmittance.

3.9. Application for longan fruits

3.9.1. Browning index (BI) and enzyme activity

The pericarp browning of longan is a decisive parameter for its edible quality and market value. PPO and POD are the key enzymes that cause the pericarp browning of longan [36]. Therefore, the effects of different treatments on the PPO and POD activities of longan fruits were checked in this work. As can be been in Fig. 6a, the PPO activity of all fruits increased firstly and decreased thereafter. The PPO activity of the untreated and SA-treated fruits was rapidly increased within the first 4 d. The PPO activity of SA-SE and SA-SE-SU treated fruits achieved a maximum value at 2 d, and it slowly decreased thereafter. At the end of storage, the PPO activity of untreated fruits was 582 ± 25 U g^{-1}FW while the value of SA-SE-3.0%SU was only 400 ± 35 U g^{-1}FW, and no significant difference was found in SA-SE-SU films ($P > 0.05$). Similar founding was also presented in the POD activity of longan fruits (Fig. 6b). The untreated and SA-treated fruits exhibited a sharp increase in POD activity in the initial four days. The peak value of POD activity in untreated and SA-treated fruits were 1075 ± 24 U g^{-1}FW and 976 ± 20 U g^{-1}FW, respectively. The POD in SA-SE and SA-SE-SU treated fruits were increased at the beginning of storage, reached the maximum value at two days of storage, and then decreased gradually from the second day to the eighth day of storage. At the end of storage, the POD activity in untreated and SA treated fruits were 782 ± 45 U g^{-1}FW and 715 ± 40 U g^{-1}FW while the value of SA-SE-SU films was between 456 and 475. The POD activity between SA-SE treated samples and SA-SE-SU treated samples was not significant ($P >0.05$). At the beginning of storage, the difference of BI values between untreated fruits and treated fruits were not significant ($P > 0.05$) (Fig. 6c). The significantly lower BI value was observed in the 4-d longan fruits treated with SA-SE and SA-SE-SU films over that of untreated 4-d longan fruits ($P < 0.05$). The SA treatment can also reduce the browning of peel to some extent. This could be in accord with the fact that the SA could act as a natural barrier to control the exchange of oxygen so that reduce the browning on the fruit peel. After 12 d of storage, the fruits treated with *S. cerevisiae*-encapsulated and sucrose-rich sodium alginate film-forming solutions presented the significantly lower BI value than that of control fruits. By analyzing the results of PPO, POD, and BI, the potential mechanism of inhibiting the pericarp browning of longan fruits by SA-SE-SU treatment might be due to that SA-SE-SU film as a barrier could effectively reduce the oxygen exchange (Fig. 6b) so that decrease the oxidative reaction rates of the longan fruits. On the other hand, the presence of GSH produced by *S. cerevisiae* with proven enzyme inhibition ability in the active film could slowly release

into the surface of the fruits so that inhibit the PPO and POD activities (Fig. 6a and 6b). Similar founding was also reported by Shi Shengyou et al. [37], who observed that chitosan/nano-silica coating treatment could effectively reduce the browning index of longan fruits by inhibiting the enzyme activities. Generally, an average BI values of more than three were deemed unsuitable for marketing [38]. Hence, the obtained results revealed that the addition of *S. cerevisiae* and sucrose to SA could improve the market value of longan fruits by reducing the browning index of the pericarp.

These results indicated that the SA-SE and SA-SE-SU treatments exhibited the satisfactory effect on inhibiting the PPO, POD activities, and pericarp browning of longan fruits, and the SA-SE-3.0%SU treatment presented the optimal effect.

3.9.2. Weight loss

The weight change is an important indicator to assess the quality of longan. Weight loss of the longan fruit was presented in Fig. 6d. The weight loss of longan samples gradually increased with the increasing storage time. Compared with untreated longan fruits, the weight loss of all treated fruits obviously decreased. The SA-SE-SU treated fruits achieved the lowest weight loss value at the end of storage, and no significant difference was found in SA-SE-SU treatments ($P > 0.05$). This decreased weight loss in SA-SE-SU treated fruits could be due to that the enhanced water vapor barrier property of the SA-SE-SU film could offer great potential in reducing the loss of moisture from fruits [39].

3.9.3. Appearance of longan fruits

The appearance of the stored-longan fruits was presented in Fig. 6e. At the beginning of storage, all longan fruits are full and without damage, and the pericarp of the fruits are bright color and healthy. No obvious difference was found in the pericarp of all longan fruits. By contrast, the pericarp of all fruits exhibited browning to some extent after storage for eight days. These changes could be due to the synthesis of brown pigments in the longan pericarp during enzymatic browning. The color of all untreated fruits was changed to dark brown, indicating severe browning and poor quality. The browning degree of SA-treated fruits was lower than that of untreated fruits, which is consistent with the result of BI (Fig. 6c). Remarkably, the SA-SE and SA-SE-SU treatments effectively reduce the browning degree in longan pericarp, and SA-SE-SU films showed the optimal effect. This valid inhibition effect could be in accord with the fact that the *S. cerevisiae*-loaded films with excellent antioxidant properties (Fig. 4b) effectively inhibit the enzyme activity in longan pericarp (Fig. 6a and 6b) so that decrease the browning degree and maintain good appearance quality. Li Siying et al. [32] also observed that the probiotic load films could

reduce the browning of fruits. This result indicated that SA-SE-SU films might be applied for controlling the pericarp browning of longan fruits.

4. Conclusion

S. cerevisiae-encapsulated and sucrose-rich sodium alginate film were developed successfully in this work. The SEM, FTIR, and mechanical properties revealed that SA-SE film with the incorporation of SU has good flexibility due to the existence of hydrogen bonds. The addition of SU to the SA-SE film improved the WVP and OP of the film. Remarkably, the viable cell number of *S. cerevisiae* in the SA-SE active film was obviously improved with the addition of SU. This improvement of *S. cerevisiae* viability positively affected maintaining the excellent antioxidant property of the stored film. A threshold of 3.0% *w/v* was established as the effect of SU concentration was not significantly above that concentration. Finally, the SA-SE-SU packaging material effectively improved the quality and pericarp browning of longan fruits by inhibiting the PPO and POD enzyme activities. The SA-SE-SU film could be used as a novel antioxidant packaging material for food preservation.

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Supplementary data

Some supplementary materials to this article were added.

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Figure 1

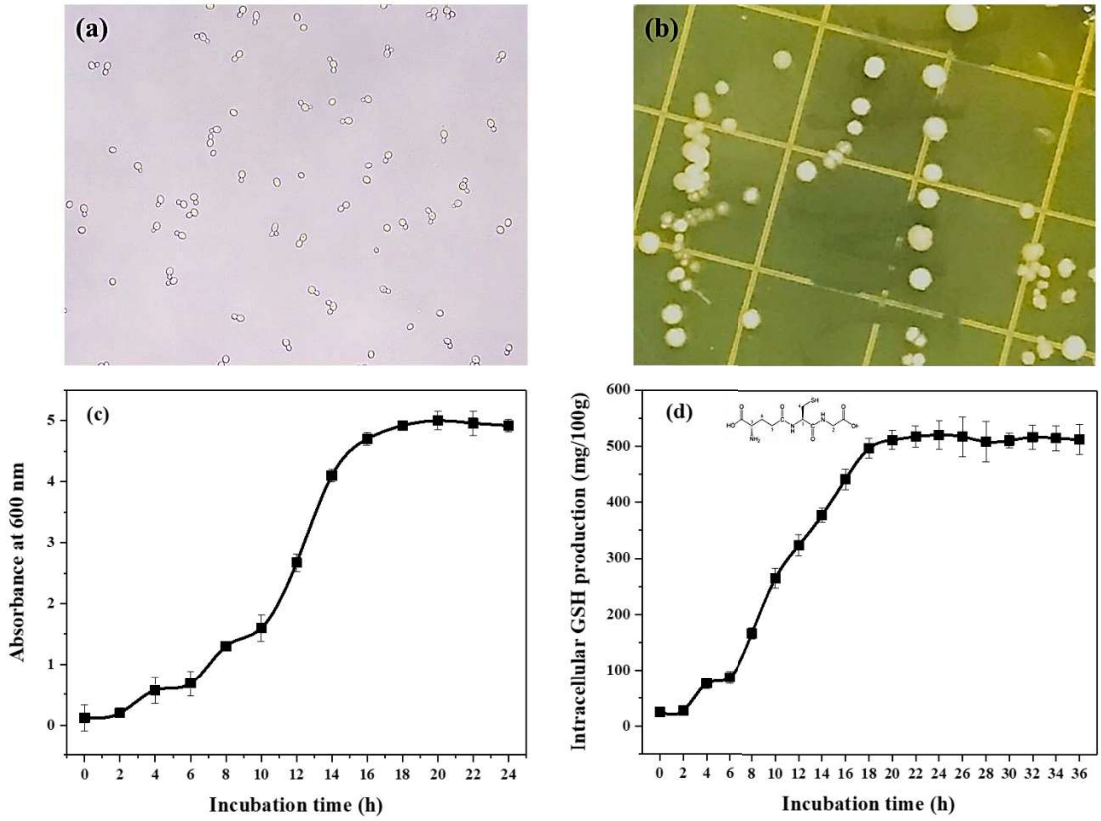

Figure 2

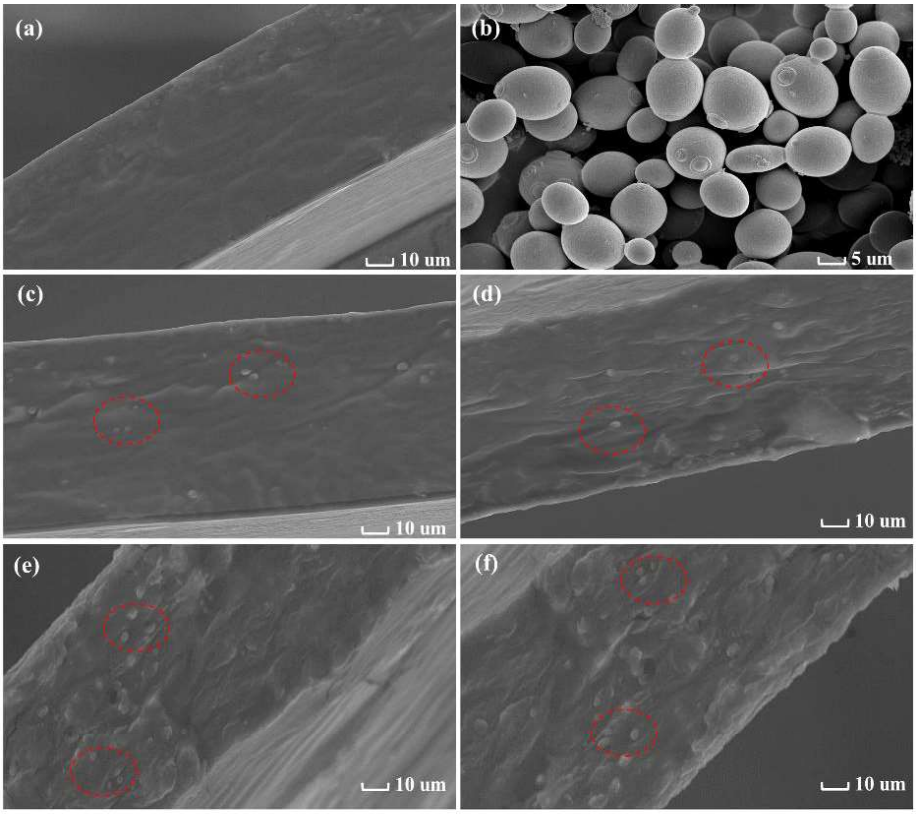

Figure 3

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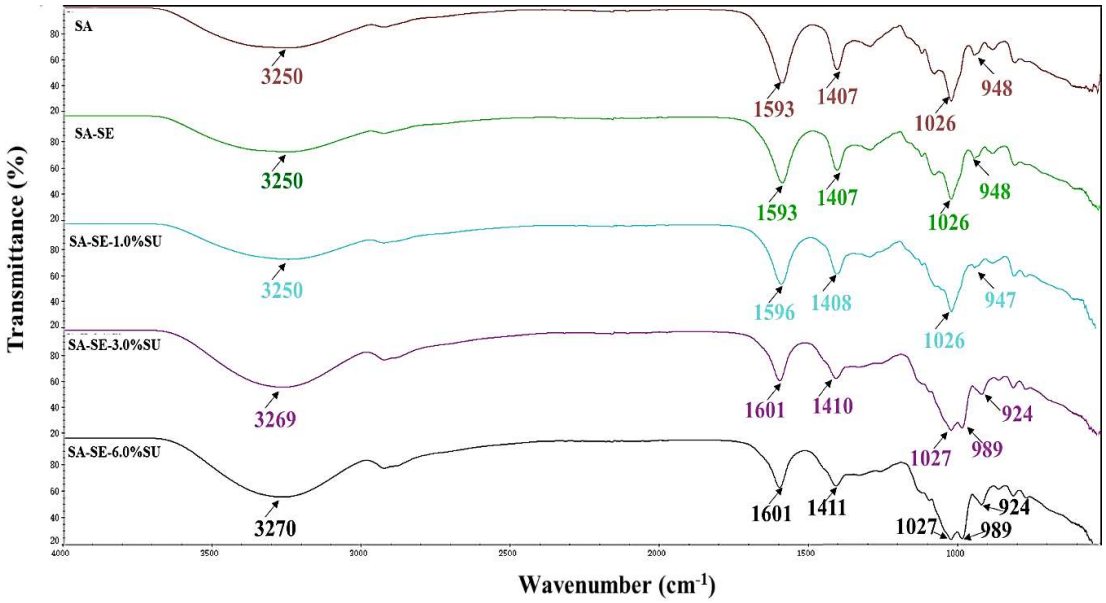

Figure 4

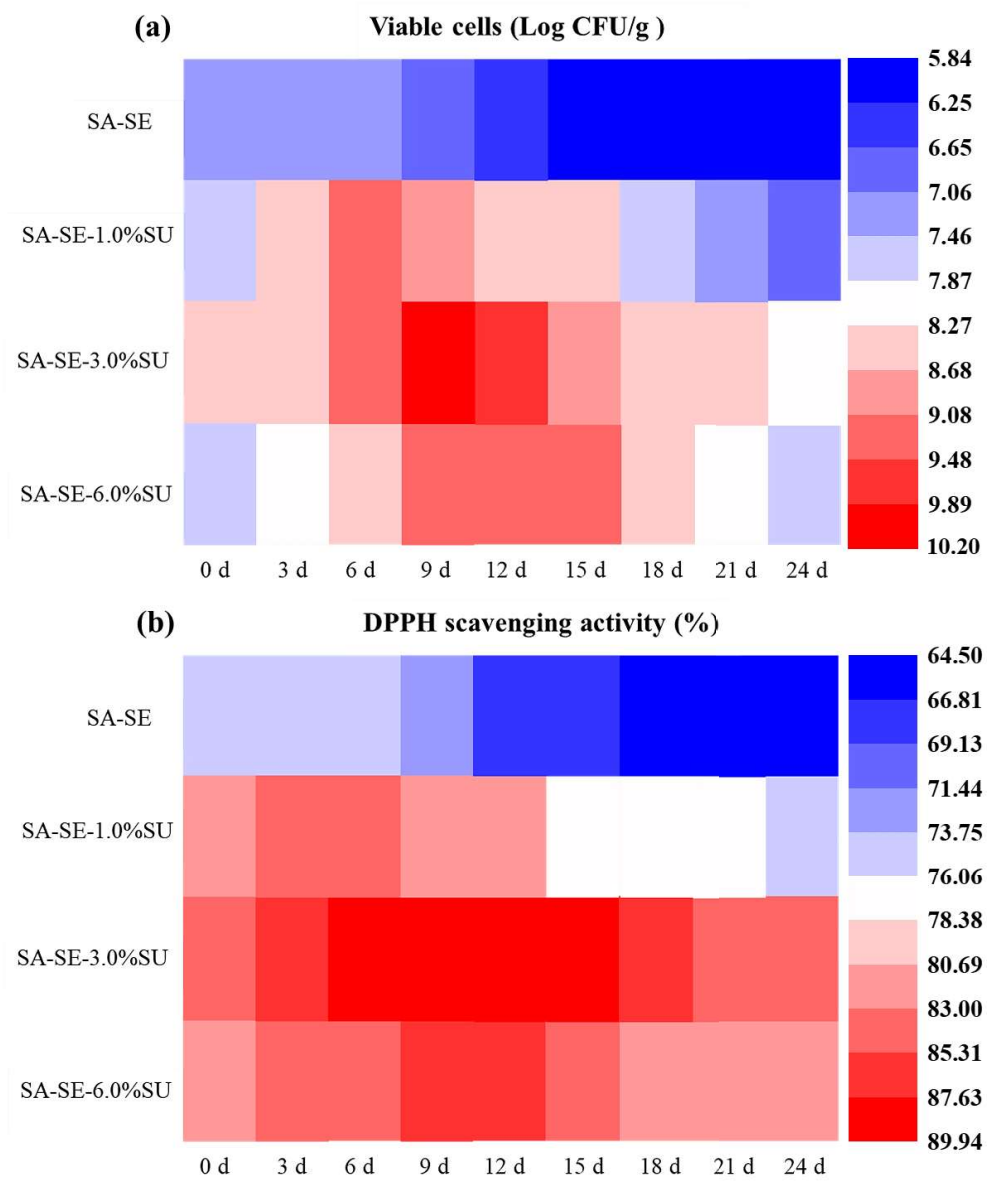

Figure 5

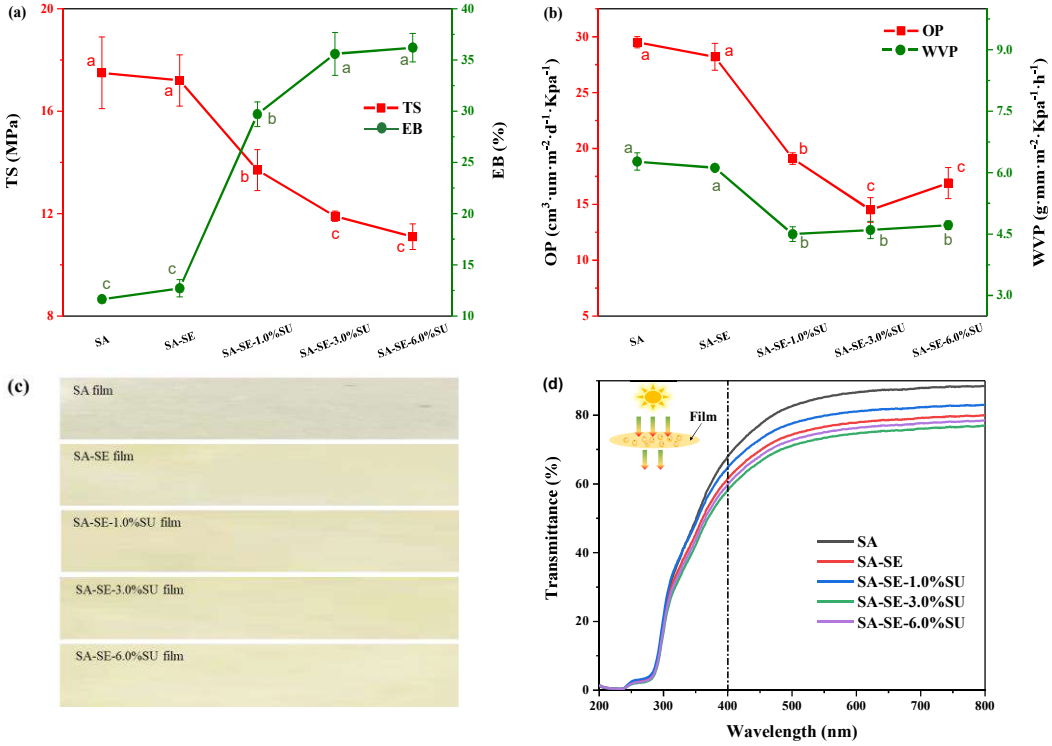

Figure 6

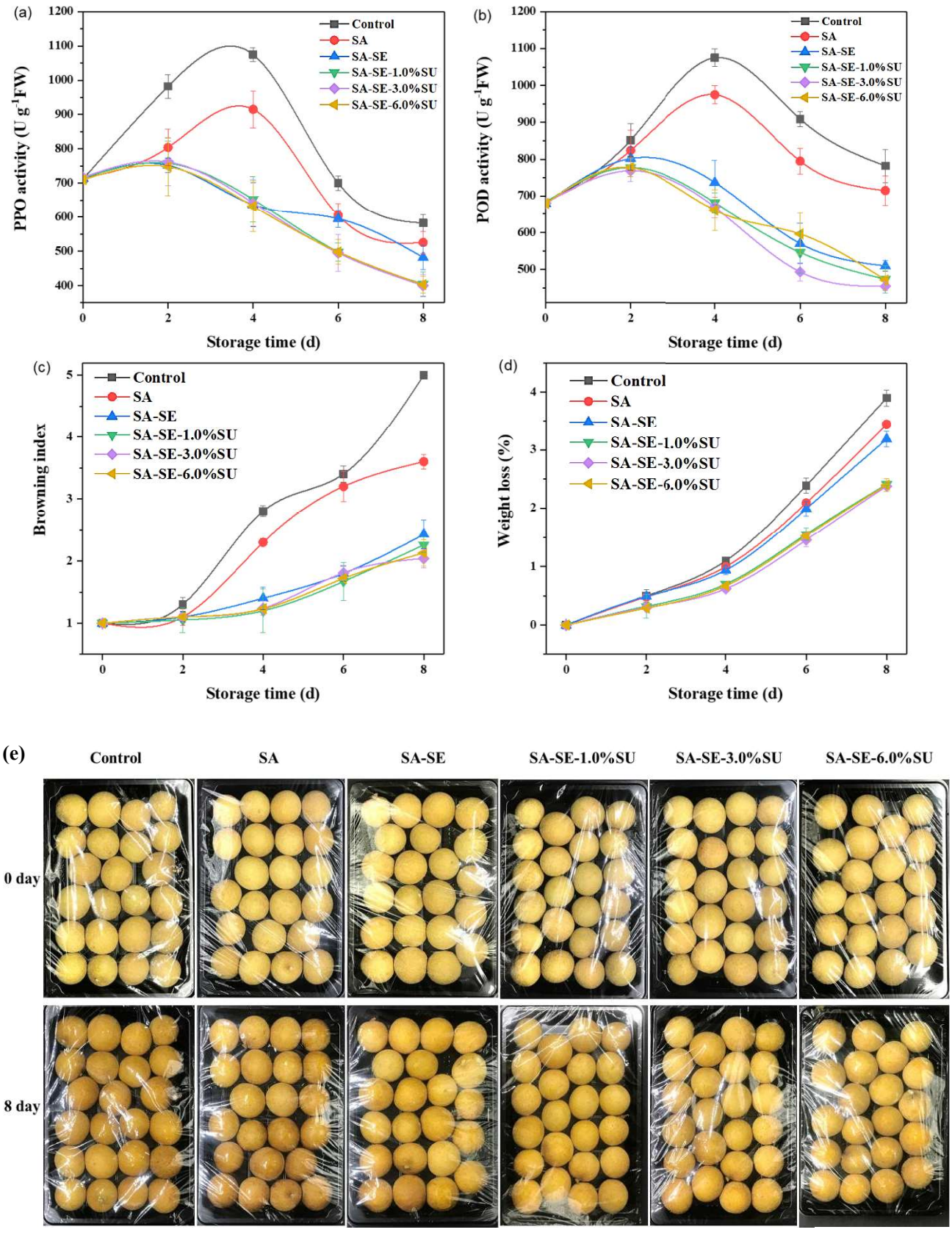

Figure. 1. The micrographs (400×) (a), colony morphology (b), growth curve (c) and intracellular GSH production curve (d) of *S. cerevisiae.*

Figure. 2. The scanning electron microscopy (SEM) microscopies of the cross section of SA (a), SA-SE (c), SA-SE-1.0%SU (d), SA-SE-3.0%SU (e) and SA-SE-6.0%SU films (f) and *S. cerevisiae* cell (b).

Figure. 3. FTIR spectrum of SA, SA-SE, SA-SE-1.0%SU, SA-SE-3.0%SU and SA-SE-6.0%SU films.

Figure. 4. The viable cells number of *S. cerevisiae* in the films (a) and the antioxidant capability of the films during the storage period (b).

Figure. 5. The mechanical property (TS and EB) (a), barrier property (WVP and OP) (b), visual appearance (c) and transmittance (d) of the films.

Figure. 6. The PPO (a), POD (b) activities, browning index (c), weight loss (d) and appearance quality (e) of stored-longan fruits treated with control, SA, SA-SE, SA-SE-1.0%SU, SA-SE-3.0%SU, and SA-SE-6.0%SU.

Conflict of Interest

Xiaobo Zou declares that he has no conflict of interest. Zhikun Yang declares that he has no conflict of interest. Jiyong Shi declares that he has no conflict of interest. Xiaodong Zhai declares that he has no conflict of interest. Xiaowei Huang declares that she has no conflict of interest. Zhihua Li declares that he has no conflict of interest. Yunyun Gong declares that she has no conflict of interest. Melvin Holmes declares that he has no conflict of interest. Megan Povey declares that she has no conflict of interest. Jianbo Xiao declares that he has no conflict of interest. Mingrui Li declares that he has no conflict of interest.

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