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Shelf life extension of pasteurized coconut milk using chitosan as a natural preservative

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Abstract

This research aimed to seek a natural food preservative by applying chitosan to preserve quality and extend the shelf life of pasteurized coconut milk. Coconut milk containing different concentrations of chitosan (0, 1, 3, and 5%(v/v)) were pasteurized at 72°C for 20 min and stored at 4°C. The changes in fatty acid (FFA), peroxide value (PV), pH, colors, total viable count (TVC), and sensory quality were monitored throughout the storage time. The shelf life was then evaluated using an accelerated shelf life test. The pasteurized coconut milk added with chitosan had lower FFA content, PV, TVC, and degree of phase separation than that without chitosan. These results indicated the ability of chitosan to retard lipolysis and lipid oxidation, inhibit microbial growth, and improve the stability of pasteurized coconut milk. Pasteurized coconut milk with 3% chitosan received the highest sensory scores, and thus its shelf life was further investigated using the temperature quotient (Q_{10}) technique. Pasteurized coconut milk with 3% chitosan had a longer shelf life (7 days) than the control (2 days). Overall, chitosan can be used as a natural preservative to delay changes in the chemical, physical and biological quality of pasteurized coconut milk, while it did not show a negative effect on the coconut milk's organoleptic quality. The opportunity of using chitosan as a natural food preservative is achievable.

Keywords: Coconut milk, Chitosan, Natural preservative, Accelerated shelf life test

1. Introduction

Coconut milk has been used as a common ingredient for many kinds of Asian foods [1,2]. However, due to rapid quality deterioration, coconut milk has a short shelf life, even at refrigerated temperatures. The high fat, proteins, and carbohydrate contents of coconut milk support the growth of various bacteria, including *Bacillus* sp., *Microbacterium* sp., *Achromobacter* sp., *Micrococcus* sp., and coliforms [1]. It is also susceptible to chemical reactions, especially lipid oxidation, and lipolysis, which have been reported as major problems that alter coconut milk's desirable odor and flavor during storage [1]. In addition, coconut milk is an emulsion [2,3]; hence, changes in its appearance due to creaming or phase separation are also considered unacceptable [1].

Pasteurization is used for coconut milk to inhibit bacteria [1,4]. However, the treatment is insufficient to destroy all spoilage microorganisms and delay chemical reactions. Therefore, pasteurized coconut milk has a relatively short shelf life under refrigerated temperatures. To overcome these problems, applying natural antimicrobial and antioxidant agents is a promising way to inhibit unfavorable microbiological and chemical reactions, thereby maintaining qualities and extending the shelf life of pasteurized coconut milk.

Among natural substances, chitosan, a biopolymer-based polysaccharide derived from chitin, has been widely used in food, medical, and pharmaceutical industries [5,6] since it has numerous intrinsic activities such as antimicrobial, antioxidant, antidiabetic, and antitumor [5-8]. It is also biodegradable, sustainable, non-toxic, inexpensive, and environmentally friendly. Chitosan consists of D-glucosamine and N-acetyl-D-glucosamine linked by β-(1-4)-glycosidic linkage. It is soluble in a dilute acidic aqueous solution below pH 6 in which amino groups (NH_2) of chitosan accept hydrogen atom (H^+) and form protonated amine (NH_3^+) groups [9]. Chitosan in dilute organic acids exhibited broad-spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria [7,10]. The mechanism of action has been reported due to an electrostatic interaction between NH_3^+

groups of chitosan and anionic molecules on the bacterial cell surface, causing changes in membrane permeability and leakage of materials from inside the cell [5,10]. The antioxidant activity of chitosan has also been reported due to the presence of hydroxyl (OH) groups and amino (NH_2) groups in its monomer. The OH groups of chitosan react with active free radicals via a hydrogen atom abstraction, whereas the NH_2 groups exhibited free-radical scavenging activity [8,11]. Hence, chitosan converts free radicals to stable radicals and stops the oxidation reaction cycle. In addition, the NH_2 group of chitosan has lone electron pairs, which could chelate trace metals and prevent lipid oxidation at the initiation step [7,11].

Even though chitosan's antimicrobial or antioxidant effects have been widely reported in various foods, its impact on the shelf life extension of pasteurized coconut milk has never been reported. This study, therefore, aimed to investigate the quality and shelf life of pasteurized coconut milk containing chitosan at chilled storage. The chemical, microbiological, physical, and sensory properties, as well as the shelf life of pasteurized coconut milk with chitosan, were determined in comparison to those of pasteurized coconut milk (with no chitosan).

2. Materials and methods

2.1 Materials

Coconut milk was prepared in the laboratory by pressing the coconut kernel with the hydraulic press machine. Chitosan powder was purchased from Marine Bio Resources Co., Ltd. (Samutsakhon, Thailand). Ascorbic acid and glycerol were purchased from NT Chemical (Bangkok, Thailand) and Merck Ltd. (Bangkok, Thailand). In this study, chitosan solution was prepared by dissolving the chitosan powder (0.5 g) in 100 mL of 1%(w/v) ascorbic acid solution containing 0.1%(w/v) of glycerol. The mixture was then shaken at 60°C in a water bath shaker at 180 rpm for 2 h. After leaving at 25°C for 24 h, the mixture was centrifuged at 2500 rpm for 5 min. The supernatant was collected and used as a chitosan solution. Other chemicals used in this study were of analytical grade.

2.2 Preparation of pasteurized coconut milk with chitosan solution

Chitosan solution was first prepared as described in section 2.1. Then, coconut milk containing different concentrations of chitosan solution (0 (control), 1, 3, and 5%(v/v)) were prepared before pasteurization at 72°C for 20 min. After that, each pasteurized coconut milk was packaged in a polyethylene bag, heat-sealed, and stored at 4°C. On days 0, 2, 5, and 7 of storage, the samples were tested to determine the chemical, microbiological, physical, and sensory properties in comparison to those properties of the control.

2.3 Chemical properties of coconut milk

Chemical properties, including free fatty acid (FFA) content (% of lauric acid), peroxide value (PV), and pH of samples, were determined during storage. According to the AOCS Official Method Ca 5a-40 [12], FFA content was investigated using titration. The volume of sodium hydroxide (NaOH:0.1 M) used to neutralize the FFA in samples was used to calculate FFA content (% of lauric acid), as shown in Equation 1.

$$\text{FFA content} = \frac{M_{NaOH} \times V_{NaOH} \times 0.200 \times 100}{W_{sample}} \tag{1}$$

where M_{NaOH} is the concentration of NaOH (N), V_{NaOH} is the volume of NaOH (mL), and W_{Sample} is the mass of the sample used (g).

PV was determined using the titration technique [13]. Briefly, the sample (5 g) was mixed with saturated potassium iodide (KI) solution (2 mL) and acetic acid-chloroform (2:1 v/v) solution (20 mL). The mixture was then incubated in a water bath at 95°C for 1 min. After adding saturated KI solution (4 mL), distilled water (10 mL), and 1% starch solution (1 mL), the mixture was titrated with 0.2 N sodium thiosulfate ($Na_2S_2O_3$) solution. The volume of $Na_2S_2O_3$ solution was used to calculate the PV (meq. Peroxide/kg oil) using Equation 2.

$$\text{PV} = \frac{M_{Na_2S_2O_3} \times V_{Na_2S_2O_3} \times 100}{W_{sample}} \tag{2}$$

where M is the concentration of $Na_2S_2O_3$ solution, V is the volume of $Na_2S_2O_3$ solution (mL), and W_{Sample} is the mass of the sample used (g).

pH value of samples was measured using a digital pH meter (Seven Compact, Mettler-Toledo (Thailand), Ltd., Bangkok, Thailand). All the measurements were carried out in triplicate.

2.4 Microbiological property of coconut milk

The total viable count (TVC) of samples was determined during storage using a standard plate count method [14]. A series of 10-fold dilutions of samples in 0.1% sterile peptone water was aseptically made. Bacteria were enumerated on days 0, 2, 5, and 7 of the storage periods using a pour plate technique. The Petri dishes were then incubated at 32°C for 48 h. The experiments were carried out in triplicate.

2.5 Physical properties of coconut milk

Visual observation of changes in the appearance of pasteurized coconut milk containing different concentrations of chitosan was carried out. Changes in Commission Internationale de l'Eclairage (CIE) color of samples were also determined during chilled storage. A chroma meter (Minolta CR-300 Series, Konica Minolta, Osaka, Japan) equipped with a D65 light source was carried out to obtain lightness (L^*), redness (a^*), and yellowness (b^*) values.

2.6 Sensory evaluation

The sensory evaluation was performed by a panel consisting of 10 male and female assessors from the Department of Food Technology, Chulalongkorn University. The panelists in this study have experienced the sensation of pasteurized coconut milk and clearly understand the quality of coconut milk. They were asked to evaluate the sensory properties, including color, odor, appearance, and the overall acceptance of the samples, using a 9-point hedonic scale [15]. Samples were identified as a three-digit code before serving randomly to the panelists. The highest score (9 points) indicated extremely desirable, whereas the lowest score (1 point) indicated extremely undesirable. Samples were classified as unacceptable when the score was lower than 5.

2.7 Shelf life study

The pasteurized coconut milk containing optimum chitosan concentration was selected based on its chemical, physical, microbiological, and sensory properties. These selected samples were expected to have less oxidation reaction, changes in appearance, and bacterial number but higher sensory scores than other samples. Once selected, the shelf life studies of the sample in comparison to that of the control (pasteurized coconut milk) were performed using accelerated shelf life testing, as described by Phimolsiripol, et al. [16], by storing the samples at different temperatures (25, 35, and 45°C) for 48 h. The shelf life of the samples was then calculated through the plots between ln FFA content (% of lauric acid) versus storage time (h). Coconut milk with less than 0.30% FFA content (% of lauric acid) is considered good quality [17]; hence 0.30% FFA content was used to indicate the end point of shelf life.

To predict the shelf life of samples at 4°C, a data plot between $\log_{10}$ (shelf life) ($\log_{10} t_s$) versus temperature (°C) was made to calculate the Q_{10} value and shelf life of a sample using Equations 3 and 4, respectively.

$$\left|\text{Slope}\right| = \ln\frac{Q_{10}}{10} \tag{3}$$

$$Q_{10}^{\frac{\Delta T}{10}} = \frac{t_{s1}}{t_{s2}} \tag{4}$$

where ΔT is the difference between T_1 (4°C) and T_2 (25°C). Whereas t_{s1} and t_{s2} are the shelf life (h) of the sample at T_1 (4°C) and T_2 (25°C), respectively.

2.8 Statistical analysis

Data in this study were reported as mean ± standard deviation (S.D.) from triplicate measurements. Statistical analysis was performed using IBM SPSS software (IBM SPSS Statistic Version 22). Duncan's new multiple range tests were used to identify differences between averages in CIE color, FFA content, and the number of bacteria in pasteurized coconut milk with different chitosan concentrations during chilled storage. All tests were performed using $p < 0.05$ to represent statistical significance.

3. Results and discussion

3.1 Effect of chitosan on chemical properties of pasteurized coconut milk

Changes in chemical properties, including FFA content, PV, and pH of pasteurized coconut milk containing different chitosan concentrations, were investigated on days 0, 2, 5, and 7 of storage.

As shown in Figures 1A and 1B, FFA contents and PVs of all samples increased with storage time. However, samples with higher chitosan concentrations had significantly lower FFA contents and PVs than those with lower chitosan concentrations on days 2, 5, and 7. Pasteurized coconut milk with 1, 3, and 5% chitosan had FFA contents and PVs of 0.27, 0.25, and 0.21%, and 34.09, 32.87, and 30.86 meq. peroxide/kg oil, respectively, on day 7 of storage. Whereas those of the control were 0.50% and 39.11 meq. peroxide/kg oil, respectively. FFA can be used in measuring coconut milk quality. The major saturated fatty acids in coconut milk are lauric, myristic, and palmitic. Coconut milk with elevated FFA emerges a rancid flavor due to lipid oxidation and lipolysis and generally becomes unacceptable [18,19]. According to the Thai Industrial Standards Institute (TISI), the FFA content (% of lauric acid) of coconut milk with good quality should not exceed 0.3% [17].

The peroxide value of food products should not be more than 35 meq. peroxide/kg oil to avoid rancid odor [13]. Based on these standards, the results showed that adding chitosan could retard the chemical reaction and maintain the chemical properties of pasteurized coconut milk during storage. The concentration of FFA in coconut milk with chitosan has been relatively maintained during storage compared to the control. This was due to the inhibition of the growth of microorganisms by chitosan, which prevented the formation of microbial lipase by microorganisms and decelerated lipolysis [1]. The PV of coconut milk with chitosan was also relatively maintained during the chilled storage due to the antioxidant effect of chitosan to retard lipid oxidation [7]. Siripatrawan, et al. [8] reported that the NH_2 group of chitosan could convert free radicals into stable macromolecule radicals; hence, chitosan could act as a radical scavenger to stop the further reaction of free radicals. In addition, chitosan could also act as a metal chelator, thus, preventing the initiation step of lipid oxidation [7].

Changes in pH values of pasteurized coconut milk were displayed in Figure 1C. The pH value of control decreased significantly throughout the storage from 6.31-5.39. The decrease in the pH of coconut milk may be attributed to the breakdown of proteins and fats into free amino acids and free fatty acids [20,21]. In contrast, only a slight decline in pH values was observed in samples with 1-5% chitosan. This also supported that chitosan could inhibit the growth of microorganisms and retard their production of acidic byproducts in pasteurized coconut milk during storage.

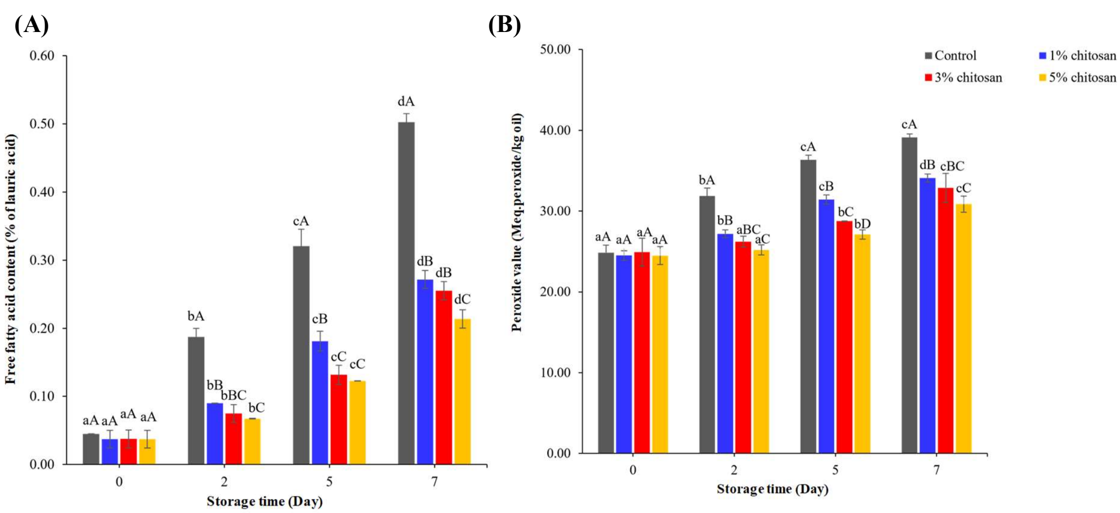

Figure 1 Changes in FFA content (A), and peroxide value (B) of pasteurized coconut milk treated with different chitosan concentrations during storage. Lowercase letters were used to indicate statistical analysis of data at different storage times. Uppercase letters indicate statistical analysis between chitosan concentrations in pasteurized coconut milk. Means with the same letter are not significantly different ($p \geq 0.05$).

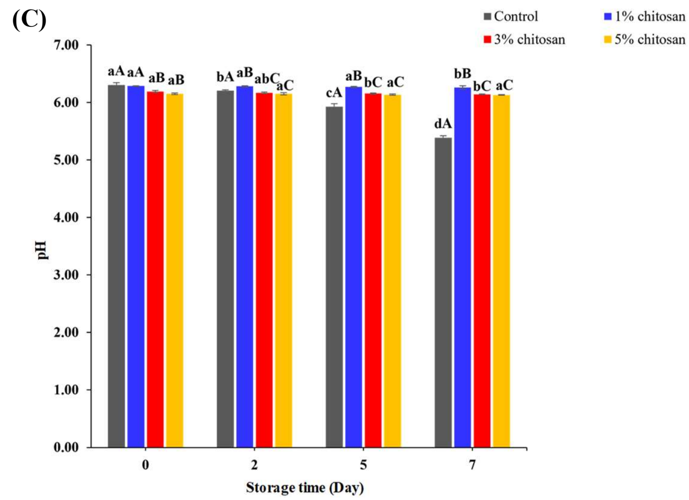

Figure 1 (continued) Changes in pH (C) of pasteurized coconut milk treated with different chitosan concentrations during storage. Lowercase letters were used to indicate statistical analysis of data at different storage times. Uppercase letters indicate statistical analysis between chitosan concentrations in pasteurized coconut milk. Means with the same letter are not significantly different ($p \geq 0.05$).

3.2 Effect of chitosan on the microbiological property of pasteurized coconut milk

TVC of control and pasteurized coconut milk with 1-5% chitosan during storage for 0, 2, 5, and 7 days were represented as log CFU/mL (Figure 2). The TVC of control increased sharply from 3.93 to 6.03 log CFU/mL at the end of chilled storage. On the contrary, the TVC of pasteurized coconut milk with 1, 3, and 5% chitosan at the end of the chilled storage was 5.25, 4.95, and 4.54 log CFU/mL, respectively, lower than that of the control. These results suggested that a higher chitosan concentration was more effective than the lower ones in inhibiting the growth of bacteria. According to the APCC standard [22], the TVC of good-quality coconut milk products should not exceed 5 log CFU/mL, implying that pasteurized coconut milk with 0 and 1% chitosan were not suitable for consumption after storage for 2 and 5 days, respectively. Whereas ones with 3 and 5% chitosan were considered good quality until 7 days of storage. Chitosan has a positive charge, which leads it to interact with bacterial cell surface through electrostatic interaction [5]. Through this interaction, alteration in the outer membrane and the cytoplasmic membrane's functions could occur, initiating the loss of inner cell materials, which eventually leads to cell death [5,8,23].

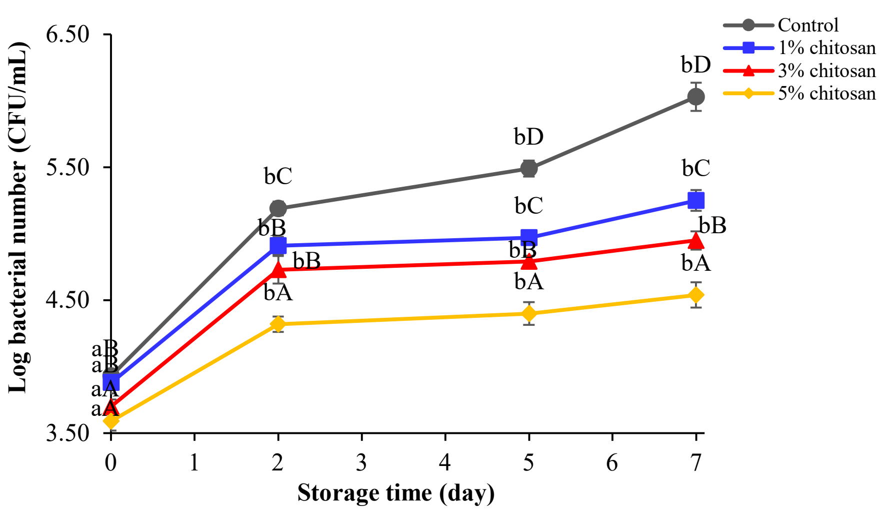

Figure 2 Bacterial number in pasteurized coconut milk treated with different chitosan concentrations during storage. Lowercase letters were used to indicate statistical analysis of data at different storage times. Uppercase letters indicate statistical analysis between chitosan concentrations in pasteurized coconut milk. Means with the same letter are not significantly different ($p \geq 0.05$).

3.3 Effect of chitosan on physical properties of pasteurized coconut milk

The physical properties of coconut milk, including appearance and color, affect consumers' decision to purchase the product. Coconut milk is an emulsion, and phase separation and color change are considered defects. In this study, control, and pasteurized coconut milk with 1-5% chitosan exhibited phase separation on days 2, 5, and 7 of chilled storage. However, samples with chitosan exhibited less degree of phase separation than that of control (result not shown) throughout the storage period. Previous studies have reported that chitosan can be used as an emulsifier for food emulsions and can promote the formation of multilayer, oil-in-water, and water-in-oil emulsions [24,25]. Glycerol used for dissolving chitosan can also function as an emulsifier, but its concentration in pasteurized coconut milk is relatively low compared to chitosan [26]. Therefore, the effect of glycerol could be disregarded, and chitosan is responsible for the emulsification of the coconut milk.

Changes in color parameters of pasteurized coconut milk are shown in Table 1. On day 0, the b^* value of pasteurized coconut milk was significantly increased with the chitosan concentration. This suggested that chitosan affects the original color of pasteurized coconut milk. During 7 days of chilled storage, all samples' L^* and b^* values decreased and increased, respectively, with time. It has been reported that the Maillard reaction occurred when chitosan was heated with xylan, xylose, maltose, or protein, turning color from light yellow to brown [27]. Therefore, chitosan can cause more Maillard reactions during the pasteurization of coconut milk due to the presence of amino acids and reducing sugar [28].

3.4 Effect of chitosan on sensory properties of pasteurized coconut milk

The sensory quality of the pasteurized coconut milk with different chitosan concentrations is present in Figure 3. Likeness scores in color, appearance, odor, and overall acceptance of samples decreased with storage time. However, except for the color score, there was no correlation between chitosan concentration and the appearance, odor, and overall acceptance scores of samples. As shown in Figure 3, control, and pasteurized coconut milk with 1% and 3% chitosan had a higher color score than pasteurized coconut milk with 5% chitosan. Therefore, chitosan negatively affected the color of the coconut milk due to its' Maillard browning reaction during pasteurization when present at high concentrations. During 7 days of storage, pasteurized coconut milk with 3% and 5% chitosan received a higher appearance and odor score than control and pasteurized coconut milk with 1% chitosan. This result implied that chitosan was able to maintain stability and retard lipid oxidation in pasteurized coconut milk which preserved its' sensory properties during storage. In this study, pasteurized coconut milk with 3% chitosan received the highest overall acceptance score; hence, this sample was selected for a shelf life study in the following experiment.

Table 1 Changes in L^*, a^*, and b^* of pasteurized coconut milk treated with different chitosan concentrations during storage.

Chitosan concentration (%)	Day	L^*	a^*	b^*
0% (Control)	0	$81.65^{dB1}\pm0.15$	$-0.20^{aA}\pm0.04$	$3.07^{aA}\pm0.04$
	2	$81.03^{cC}\pm0.04$	$0.10^{bA}\pm0.01$	$3.11^{aA}\pm0.01$
	5	$80.66^{bC}\pm0.04$	$0.06^{bA}\pm0.01$	$3.22^{bA}\pm0.01$
	7	$79.20^{aA}\pm0.14$	$0.15^{bA}\pm0.02$	$3.26^{bA}\pm0.04$
1%	0	$81.50^{dB}\pm0.21$	$-0.15^{aB}\pm0.03$	$3.45^{aB}\pm0.02$
	2	$80.27^{cA}\pm0.01$	$0.03^{bB}\pm0.01$	$3.52^{bB}\pm0.04$
	5	$79.80^{bA}\pm0.02$	$0.03^{bB}\pm0.01$	$3.54^{bB}\pm0.01$
	7	$79.34^{aA}\pm0.09$	$0.09^{cB}\pm0.02$	$3.78^{cB}\pm0.01$
3%	0	$81.54^{bB}\pm0.01$	$-0.17^{aAB}\pm0.00$	$3.69^{aC}\pm0.00$
	2	$81.30^{bD}\pm0.02$	$-0.12^{bC}\pm0.01$	$3.71^{aB}\pm0.01$
	5	$80.37^{aB}\pm0.02$	$-0.13^{bC}\pm0.01$	$3.84^{bC}\pm0.02$
	7	$80.12^{aB}\pm0.12$	$-0.07^{cC}\pm0.01$	$3.86^{bC}\pm0.02$
5%	0	$80.93^{cA}\pm0.05$	$-0.16^{aAB}\pm0.01$	$4.02^{aD}\pm0.02$
	2	$80.83^{bcB}\pm0.01$	$-0.11^{bC}\pm0.00$	$4.15^{aC}\pm0.02$
	5	$80.67^{bC}\pm0.02$	$-0.16^{aD}\pm0.00$	$4.19^{aD}\pm0.01$
	7	$80.31^{aB}\pm0.18$	$-0.08^{cC}\pm0.01$	$4.22^{aD}\pm0.05$

Lowercase letters were used to indicate statistical analysis of data at different storage times. Uppercase letters indicate statistical analysis between chitosan concentrations in pasteurized coconut milk. Means with the same letter are not significantly different ($p \geq 0.05$).

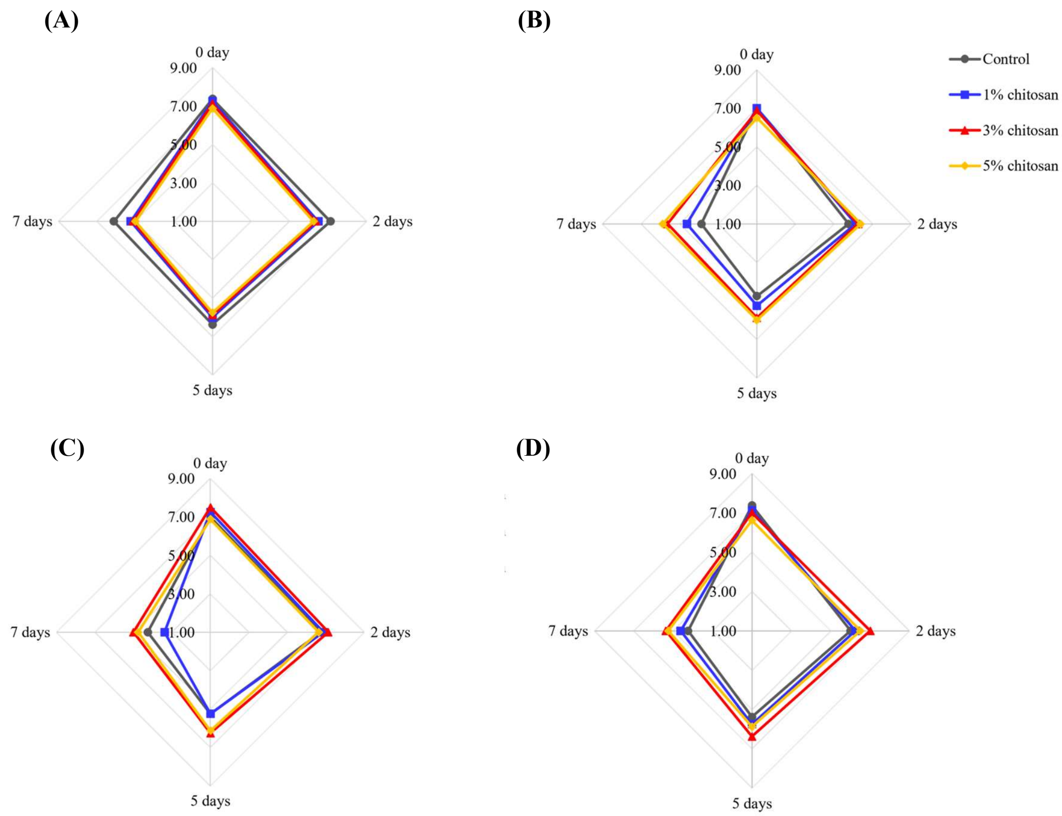

Figure 3 Sensory scores of colors (A), appearance (B), odor (C), and overall acceptance (D) of pasteurized coconut milk treated with different chitosan concentrations during storage.

3.5 Effect of chitosan on the shelf life of pasteurized coconut milk

Shelf life of pasteurized coconut milk with 3% chitosan and control was first studied at different temperatures (25, 35, and 45°C) for 48 h. The changes in FFA with time were found to be the first-order reaction. In this study, the FFA content was selected as a critical factor, and 0.30% was used as an endpoint to indicate the shelf life of pasteurized coconut milk. As shown in Table 2, with the substitution of FFA content at 0.30%, the calculated shelf life of pasteurized coconut milk containing 3% chitosan was 102.61, 40.87, and 30.09 h, respectively, at 25, 35, and 45°C. At the corresponding temperatures, the shelf life of the control was 60.90, 33.92, and 24.83 h. In order to predict the shelf life of the sample at 4°C (storage temperature used in this study) using the temperature quotient (Q_{10}) technique, the logarithmic values of shelf life (Table 2) were plotted versus tested temperature [29,30], as shown in Figure 4. The Q_{10} concept has been reported as an increase in reaction or spoilage rate when the temperature is raised by 10 degrees [29]. In the present study, the Q_{10} value for pasteurized coconut milk with 3% chitosan and control were 1.30 and 1.22, respectively (Table 2). Using these Q_{10} values, the predicted shelf life of pasteurized coconut milk with 3% chitosan was 178 h (approximately 7 days), higher than 92 h (approximately 3 days) of control when stored at 4 °C. The prediction agrees with the microbiological property of the sample since pasteurized coconut milk with 3% chitosan was considered good quality until the end of chilled storage (7 days). However, the shelf life of the control was examined at 2 days in this study for safety reasons.

Table 2 Shelf life at 25, 35, and 45°C of control and pasteurized coconut milk treated with 3% chitosan.

Treatment	Temperature (°C)	t_s (h)	$Log_{10}t_s$	Q_{10}
Control	25	60.90	1.78	1.22
	35	33.92	1.53	
	45	24.83	1.39	
3% Chitosan	25	102.61	2.01	1.30
	35	40.87	1.61	
	45	30.09	1.48	

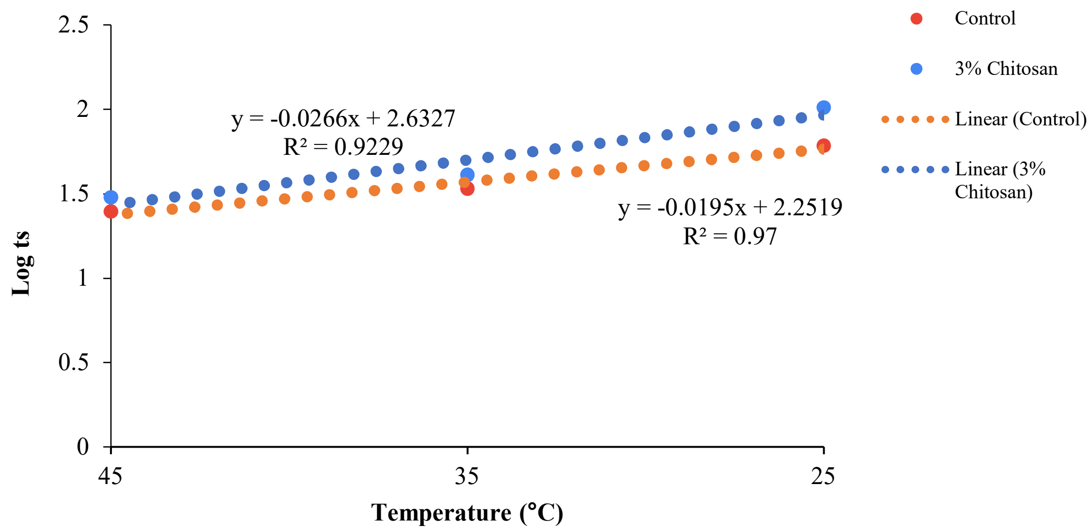

Figure 4 Data plot between log $_{10}t_s$ versus temperature of control and pasteurized coconut milk treated with 3% chitosan.

4. Conclusion

This research used chitosan to retard chemical reactions and inhibit bacterial growth in pasteurized coconut milk during chilled storage. The changes in FFA content, PV, and bacterial number over the storage period correlate well with chitosan concentration. Pasteurized coconut milk with 3% chitosan had the highest appearance, odor, and overall acceptance scores throughout the storage. The shelf life at 4°C of pasteurized coconut milk with 3% chitosan was 7 days longer than that of the control. Overall, chitosan can be used as a promising natural preservative to maintain the qualities and extend the shelf life of pasteurized coconut milk.

5. Acknowledgements

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