Utilization of chitosan as an antimicrobial agent for pasteurized palm sap (*Borassus flabellifer* Linn.) during storage

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Abstract The objective of this research was to assess the potential of chitosan for improvement the quality of pasteurized palm sap during storage. First, the effect of chitosan content on sensory attributes was investigated to select suitable concentration of chitosan for further study. Fresh palm sap was enriched with chitosan at various concentrations (0–2 g/L) and pasteurized at 80 °C for 10 min, consequently evaluated by consumers. It was found that samples added chitosan in the range of 0–1.00 g/L were considered acceptable. Thus, the addition chitosan in the concentration of 0–1.00 g/L was chosen for further study. The sample without chitosan addition was used as a control sample. Each selected sample was determined for their qualities during storage at 1 week interval. It was found that lightness and transmittance values of all samples tended to increase during storage. Lower PPO and invertase activity were observed in all chitosan-treated samples compared to control sample. Chitosan could minimize the loss of sucrose and the increase in glucose and fructose content during storage. In addition, an increase in chitosan concentration resulted in the increase in DPPH radical scavenging activity. Furthermore, the addition of chitosan could retard the development of microorganism during storage as demonstrated by lower microbial loads compared to control sample. It can be concluded that a combination of pasteurization with chitosan addition (0.50 g/L) and low temperature storage could preserve palm sap for approximately 6 weeks. Thus, the incorporation of chitosan in palm sap could be used as an alternative way to extend shelf life of pasteurized palm sap.

Keywords Palm sap · Chitosan · Pasteurization · Shelf life

Introduction

Palmyra palm (*Borassus flabellifer* Linn.) can be found in tropical countries such as Thailand, Malaysia, Indonesia, India, Myanmar, Sri Lanka and Cambodia. The most important product of palmyra palm is the sap, syrup and cake. The tapping process of palm sap involves the bruising of the interior of the developing inflorescences by means of a wooden mallet or tong, thereby stimulating sap flow. Sap is collected by cutting the outer end at the head of the inflorescences. Three to six inflorescences are tied together and inserted into a suitable container for sap collection, usually using an earthenware pot (in Sri Lanka) or a bamboo tube (in Thailand) (Davis and Johnson 1987). Fresh sap is sweet, oyster white colour and translucent, with nearly neutral pH (Gupta et al. 1980). The sap is sterile (free of microorganisms) while flowing in palmyra inflorescences. However, microorganisms are found in the sap which is coming from an environment during collecting process. Microbes are introduced into the sap by unsanitary tapping procedures and unsanitary collection. Consequently, the growth of microorganisms in the sap will be manipulated. Further contamination of sap occurs when the utensils are not completely cleaned and sanitized between sap runs, especially during summer season, which favours the rapid growth of microbial loads. Increased temperatures favour the rapid growth of microorganisms, thereby increasing their populations over time. These microorganisms use sugars in the sap as an energy source and result in fermentation of palm sap. The fermenting organisms are dominated by yeasts, particularly *Saccharomyces cerevisiae* and lactic acid bacteria (Chanthachum and Beuchat 1997). Since palm sap is rich in sugars (10–17 %) and, unless it is collected under hygienic conditions, rapidly

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fermentation and conversion reactions to acids and alcohols occur (Iwuoha and Eke 1996). To prevent fermentation, Kiam wood (*Cotylelobium lanceotatum* Carih.) or Payorm wood is commonly added to the collection receptacle because it can delay spoilage in palm sap by reducing microbial populations as well as keeping the quality of a product (Chanthachum and Beuchat 1997).

To extend shelf life of palm sap, thermal process such as pasteurization usually applies. In Thailand, pasteurized palm sap was normally produced by heating at boiling temperature for approximately 60 min. It has been reported that thermal deterioration can take place during thermal processing and affect on the quality of product, especially colour, flavour and nutritional values. The most common chemical reactions that influence on the quality of heated sap are inversion reaction, Maillard reaction and Caramelisation (Apriyantono et al. 2002 and Ho et al. 2007). In order to reduce thermal degradation during production, it is necessary to minimize heating temperature and time. The use of natural antimicrobial agents could be applied and combined with the utilization of lower heating temperature and time to reduce thermal degradation of food properties (Potter and Hotchkiss 1995). The food industry perceives that consumers demand convenient, fresh, healthy products free of synthetic preservatives. As an alternative, the antimicrobial properties of natural compounds, such as chitosan, have been recognized as being safe for use in food preservation (No et al. 2007). Chitosan is mostly applied as a food additive or preservative, and as a component of packaging material, not only retard microorganism growth in food, also to improve the quality and shelf life of food (Edirisinghe et al. 2012; Leceta et al. 2013). Martín-Diana et al. (2009) studied the effect of chitosan addition on the quality of orange juice. It was found that the extension of the quality of the orange juice, especially in parameters such as browning and reduction of aerobic counts was observed. Higher chitosan concentrations maintained the quality better than lower concentrations from a quality point of view.

There is no documentation on the use of chitosan as a preservative agent in palm sap. The present investigation describes such a study. The objective of this research was to assess the potential of chitosan for improvement quality and safety of pasteurized palm sap during storage under low temperature.

Materials and method

Sample collection and preparation

Fresh palm sap was obtained from contacted farm in Phetchaburi province, Thailand. Palm sap was kept in an icebox during transportation to Faculty of Agricultural

Product Innovation and Technology, Srinakharinwirot University. Initially, palm sap was passed through a sheet cloth to remove the contaminants. After that, various concentrations of chitosan including 0.25, 0.50, 1.00, 1.50 and 2.00 g/L was added to the filtrate. The sample without chitosan addition was used as a control sample (0 g/L of chitosan). Then, the mixture of palm sap and chitosan was homogenised using a lab-scale homogenizer for 5 min and then pasteurized at 80 °C for 10 min. After that, pasteurized palm sap was packed in polyethylene containers and immediately cooled in ice water for 5 min. Thereafter, sensory evaluation was done in each sample. Mean score of overall acceptability (≥ 5) was used as a criterion to select the sample for further study. Afterward, each selected sample was monitored quality changes during storage under low temperature at 1 week interval.

Sensory evaluation of palm sap after pasteurization

Immediately after pasteurization of palm sap, sensory evaluation was performed. The consumers scored the acceptability of the samples using a 9-point hedonic category scale (where 1 = dislike extremely and 9 = like extremely), rating appearance, flavor, taste and overall acceptability. Thirty untrained panelists were asked to rate each sensory attribute. Only sample obtained the mean score of overall acceptability higher than 5 was selected for further study.

Physical properties measurement

The lightness of pasteurized palm sap was measured in term of L^* value (CIE system) by using a Hunter Lab colorimeter (ColorFlex EZ). The clarity of sample was estimated by measuring the transmittance at 650 nm using a spectrophotometer (Shimadzu, Kyoto, Japan) and expressed in term of percentage.

Chemical properties analysis

The pH value was measured at ambient temperature with a pH meter (Satorious, USA) which calibrated at pH 4.0 and 7.0. Total acidity was determined by titration with 0.01 N NaOH using a few drops of 1 % phenolphthalein solution as an indicator. The result was calculated as a percentage of citric acid (Ranganna 1986). Total soluble solid (TSS) was measured by using hand refractometer. The activity of polyphenol oxidase (PPO) was analyzed as described by Cano et al. (1997). The enzyme extracts for determination of PPO were made by homogenization of 10 g of each sample with 20 mL of 0.2 M sodium phosphate buffer (pH 6.5) in an ultrahomogenizer with external cooling, for 3 min with stop intervals each 30 s. After that, the mixture was centrifuged at 10,000 g with refrigerated centrifuge and collected the

supernatant for analysis enzyme activity. PPO activity was assayed using aliquots (0.075 mL) of extract and 3.0 mL of a solution of 0.07 M catechol in 0.05 M sodium phosphate buffer (pH 6.5). The reaction was measured with the spectrophotometer at 420 nm. The invertase activity was determined according to Mao et al. (2007). The assay was started by addition 100 μ l of 120 mM sucrose into the mixture containing 50 μ l of sample and 50 μ l of 1 M sodium acetate (pH 7.5). The reaction was stopped at 60 min by boiling the mixture for 3 min. The concentration of glucose liberated was determined with the DNS method (Miller 1959). The type and concentration of sugar were determined using HPLC (Agilent 1100 series) with a Zorbax Carbohydrate column and refractive index detector. The mobile phase was the solution of acetonitrile and water (80:20), pumped at a flow rate of 1.5 ml/min. The samples were prepared by making appropriate dilutions with distilled water. All sample solutions were passed through a 0.45 μ m nylon syringe filter to remove particulates prior to HPLC analysis. D-glucose, D-fructose and sucrose were used as the external standards. The calibration curve of each sugar was plotted between peak areas and concentrations (Stuckel and Low 1996). Quantification of polyphenol content in each sample was carried out according to the method of Balange and Benjakul (2009). The appropriate diluted sample (0.5 ml) was mixed with 0.5 ml of distilled water. Thereafter, 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of 2 % sodium carbonate solution were added. The mixture was mixed thoroughly and placed in dark for 40 min. After that, the absorbance was recorded at 725 nm and polyphenol content was calculated from the standard curve of gallic acid. DPPH radical-scavenging activity was determined by DPPH assay, as described by Binsan et al. (2008) with a slight modification. Appropriate diluted sample (1.5 ml) was added to 1.5 ml of 0.15 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 60 min. The absorbance of the resulting solution was measured at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The blank was prepared in the same manner, except that distilled water was used instead of the sample. A standard curve was prepared using Trolox and the antioxidant activity was expressed in term of micromole TE/g sample.

Determination of microbial load

Microbial loads including total viable count (TVC), mold and yeast count (MYC) and lactic acid bacteria (LAB) count were analysed at 1 week interval. At the specified time intervals, each sample was aseptically taken and serially diluted in 0.1 g/100 g peptone water for microbial counts. Pour plating on Plate Count Agar (Merck KGaA, Darmstadt, Germany) was performed for TVC, overlaid with the same medium, and

the plates incubated at 35–37 °C for 1–2 days. Spread plating on Potato Dextrose Agar acidified with 10 g/100 g tartaric acid (Merck KGaA, Darmstadt, Germany) was performed for MYC and the plates incubated at 20–25 °C for 5 days. LAB count was also analysed using the pour plate technique on MRS agar and the plates were incubated at 37 °C for 2 days.

Statistical analysis

All analysis and measurements were performed in triplicates. The experimental design for physical and chemical properties was a completely randomized design (CRD). Data was subjected to analysis of variance (ANOVA). Analysis was performed using a SPSS package.

Result and discussion

Sensory evaluation

The influence of chitosan addition on the sensory attributes in comparison with control sample was showed in Table 1. All sensory attribute scores tended to decrease with increasing chitosan concentration. Significant changes on all sensory attributes between control sample and samples added 0.25 and 0.50 g/L chitosan were not observed by the panelists. Significant difference in all sensory attributes between control sample and chitosan-treated samples was detected when concentration of chitosan reached 1.00 g/L, suggesting that high concentration of chitosan caused an increase in bitterness. Martín-Diana et al. (2009) revealed that a reduction in general acceptability was observed in orange juice samples with increasing chitosan concentration. Also, concentrations >1 g/L were scored as unacceptable for the sensory panel due to an increase in bitterness. Thus, samples added chitosan in the range of 0–1.00 g/L was selected for further storage study.

Change in lightness and clarity of pasteurized palm sap during storage

Color and clarity of juice are one of the most important parameters for justment of buying. The active hazes formed by phenolic-protein complexes are an important factor and responsible for the turbidity of pasteurized palm sap during storage (Naknean et al. 2010; Phaichammnan et al. 2010). Initially, the addition of chitosan in the concentration of 0.25 and 0.50 g/L was not affected the lightness and transmittance values ($P \geq 0.05$) while a decrease in lightness and transmittance values was found in sample added 1.00 g/L chitosan ($P < 0.05$). The result indicated that when the concentration of chitosan was below 0.50 g/L, it can soluble and greatly disperse in palm sap, while when the concentration reached

Table 1 Sensory values of palm sap added with various concentration of chitosan after pasteurization

Treatments/sensory attributes	Flavor	Taste	Appearance	Overall acceptability
Control	7.15	7.23	7.07	7.12
Chitosan 0.25 g/L	7.05	7.11	7.03	7.07
Chitosan 0.50 g/L	6.80	7.75	7.05	7.02
Chitosan 1.00 g/L	5.45	5.26	6.24	5.56
Chitosan 1.50 g/L	5.02	4.56	5.23	4.89
Chitosan 2.00 g/L	4.23	3.24	4.46	4.02

1.00 g/L, it was incompletely dissolved, leading to the reduction in transmittance value. Change in lightness and transmittance values during storage was shown in Fig. 1. A continuous increase in lightness and transmittance values was observed in all samples, except control sample and sample added 0.25 g/L chitosan, during storage. The undissolved particles, the protein from sap itself and the polyphenolic compounds as well as insoluble complexes caused by the interaction between protein and polyphenol might agglomerate and precipitate, leading to the increment in lightness and transmittance values (Kermasha et al. 1995; Siebert et al. 1996). The increase in lightness and transmittance values reached a maximum with 4 and 5 weeks and then tended to be decreased until the end of storage for control sample and sample added 0.25 g/L chitosan, respectively. Results suggested that, the suspended solid such as yeast and bacteria, giving a milky white appearance, might be developed as evidenced by the increase in microbial load during storage. In addition, change in lightness and transmittance values during storage was affected by chitosan addition ($P<0.05$). Higher lightness and transmittance values were found in all chitosan-treated samples compared to control sample. The highest increase in lightness and transmittance values was detected in sample added 1.00 g/L chitosan. This

result could be explained by the clarification effect described in fruit juices associated to chitosan (Chatterjee et al. 2004; Rungsardthong et al. 2006; Martín-Diana et al. 2009; Domingues et al. 2012). Chitosan acts as a coagulating agent and is effective especially at higher concentration.

Change in pH and total acidity of pasteurized palm sap during storage

Change in pH and total acidity of pasteurized palm sap during storage was shown in Fig. 2. Initially, all chitosan-treated samples had a higher pH and lower total acidity than control sample and the pH increased significantly ($P<0.05$) with increasing chitosan concentration. This effect could be due to the capacity of chitosan to reduce acidity based on its acid-binding properties (Imeri and Knorr 1988; Martín-Diana et al. 2009). When the pH is lower than 6.5, chitosan carries a positive charge along its backbone (Einbu and Varum 2003). The pH of all samples decreased with increasing storage times while the total acidity increased ($P<0.05$). Marked decrease in pH and increase in total acidity were observed in control sample after 3 weeks of storage. This result was related to the growth of microorganisms. Regarding to effect of chitosan addition, the decrease in pH and increase in total acidity of all

Fig. 1 Change in lightness (L*) (a) and transmittance values (b) of pasteurized palm sap as affected by chitosan addition during storage

chitosan-treated samples were lower than control sample throughout storage. This result indicated that chitosan could decrease the growth of microorganism in pasteurized palm sap during storage, resulting in the reduction in acid formation (Edirisinghe et al. 2012).

Change in TSS of pasteurized palm sap during storage

TSS decreased during storage from an initial value of approximately 17.10 °Brix to 13.50, 15.00, 16.60 and 15.00 °Brix for control sample and samples added 0.25, 0.50 and 1.00 g/L chitosan, respectively. The decrease in TSS during storage may be due to the utilization of sugars by growth of microbes. All chitosan-treated samples maintained high TSS throughout the storage. This is because of the antimicrobial properties of chitosan. Additionally, it can be seen that, lower TSS in sample added 1.00 g/L chitosan was found when compared to those added 0.25 and 0.50 g/L chitosan. This result was probably related to the coagulation properties of chitosan, particularly at high concentration. Cesar et al. (2012) also reported that a decrease in TSS was found after clarification of acai juice by using high concentration of chitosan.

Change in PPO activity of pasteurized palm sap during storage

One of main problems with juice production is the assurance of color stability. Control of enzymatic browning during processing and storage is important to preserve the original appearance and nutrition of juice. Enzymatic browning in palm sap products is caused by the action of polyphenol oxidases (PPO, EC1.14.18.1), which catalyzes oxidation of phenolic compounds containing two o-dihydroxy groups to the corresponding o-quinone. Several methods have been

used to attain the inhibition of PPO activity in juice. For example, heat inactivation, the addition of chemicals and pH adjustment were widely used (Icier et al. 2008). In this experiment, the application of heat treatment and chitosan addition on PPO activity was investigated. It was found that PPO activity was partially inhibited after processing as evidenced by the reduction in PPO activity in all samples. PPO activity of control sample decreased by approximately 40 % from the original value measured in fresh palm sap. Similar observation was found in pasteurized strawberry puree (85 °C, 10 min) (Gössinger et al. 2009). However, the results do not conform with the work of Serradell et al. (2000) who described a loss of PPO activity of more than 90 % after heating (70 °C, 30 min). Wesche-Ebeling and Montgomery (1990) notices a loss of relative PPO activity of more than 99 % by a heat treatment of 80 °C for 6 min. The differences among different studies might be due to the diversity of PPO sources, as well as processing condition used. In this study, PPO presented in palm sap could not completely inactivate by a heat treatment of 80 °C for 10 min. A partial inactivation of PPO after pasteurization was found in pasteurized palm sap as reported by Taiapaiboon (2004) and Loetkitsomboon (2004). After chitosan addition, lower residual PPO activity was observed in all chitosan-treated samples when compared to control sample ($P<0.05$). An increase in chitosan concentration was effective to reduce PPO activity. It is generally recognized that chitosan acts as a complexing agent and also reacts with protein in palm sap, resulting in the reduction in PPO activity (Sapers 1993; Sapers et al. 2001). During storage, a continuous decrease in PPO activity was observed in all samples, the residual PPO activity of control sample was approximately 9 % after 6 weeks of storage, followed by 13 %, 8 % and 0.6 % for samples added 0.25, 0.50 and 1.00 g/L chitosan, respectively. The decrease in PPO activity during

Fig. 2 Change in pH (a) and total acidity (b) of pasteurized palm sap as affected by chitosan addition during storage

storage was also reported by Park et al. (2002), who observed that PPO activity continuously decreased in carrot juice during storage at 4 °C. Gui et al. (2006) also observed that decreases in PPO activity of heated-apple juice during storage. A possible explanation was that chitosan can act as an antibrowning agent and form complex with PPO, leading to the reduction in the PPO activity. In addition, the changes in pH and total acidity are also responsible for the decrease in PPO activity. Normally, the optimum pH for PPO activity is between 5 and 7 (Sapers et al. 2001). Thus, an increase in acidity as affected by the activity of microbes could reduce the PPO activity in palm sap, particularly in control sample. Moreover, it can be seen that the greatest decrease in PPO activity was found in sample added 1.00 g/L chitosan. The probable reason could be due to the coagulating properties of chitosan, especially at high concentration, resulting in the highest hazes sedimentation. This result indicated that higher concentration of chitosan could greatly reduce PPO activity, however more sedimentation of undissolved particle may effect on the acceptance of consumer.

Change in invertase activity of pasteurized palm sap during storage

Invertase is an enzyme that catalyzes the hydrolysis of sucrose into glucose and fructose. The occurrence of invertase in palm sap was due to its present naturally and also synthesised by microorganisms (Taiapaiboon 2004). Initial invertase activity of fresh palm sap was 45.21×10^{-3} unit/min/g. After pasteurization, a decrease in invertase activity was observed in all treatments. This could be explained by the denaturation of invertase enzyme caused by thermal process. Regarding to effect of chitosan addition, an increase in chitosan concentration caused a decrease in invertase activity. The interaction between chitosan and enzyme might be responsible for the reduction in invertase activity. The chitosan and protein interacts each other through the electrostatic interaction (Rao et al. 2011). Change in invertase activity of pasteurized palm sap during storage was demonstrated in Fig. 3b. For control treatment, the invertase activity slightly decreased in the first 3 weeks of storage and increased thereafter (Fig. 3b). The increase in invertase activity was probably due to some species of yeast secrete invertase enzyme. It is generally known that the primary sources of invertase are from yeasts such as *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis* and fungi such as *Aspergillus oryzae* and *Aspergillus niger* (Pancoast and Junk 1980; Singh et al. 2006). This finding corresponded well with that for microbial load. In contrary to PPO, invertase exhibits relatively high activity over a broad range of pH (3.5–5.5) (Amaya-Delgado et al. 2006; Andjelkovic et al. 2010), thus high acid condition created by microorganism could not greatly inhibit the invertase activity. Conversely, a sharp

decrease in invertase activity was noticed in all chitosan-treated samples, except sample added 0.25 g/L chitosan, within the first 4 weeks. Subsequently, invertase activity slowly decreased until the end of storage. The decrease in invertase activity could be attributed to the interaction between chitosan and invertase enzyme (Martin-Diana et al. 2009). The invertase activity of sample added 0.25 g/L chitosan continuously decreased until 5 weeks of storage and increased thereafter. The increase in invertase activity might be due to the activity of microorganisms during storage as mentioned previously. This study indicated that a considerable activity of invertase enzyme still retained in pasteurized palm sap and would play an important role in sucrose degradation, causing the increase of reducing sugar. Thus, the utilization of chitosan was effective to reduce the activity of enzyme in pasteurized palm sap during storage.

Change in type and concentration of sugar of pasteurized palm sap during storage

Generally, palm sap consisted of mainly sugars such as sucrose, glucose and fructose. The highest proportion of sugar is sucrose (Naknean et al. 2010). Changes in sucrose, glucose and fructose content during storage of pasteurized palm sap were presented in Fig. 4. Initially, the addition of chitosan at various concentrations was not influenced on the sucrose, glucose and fructose content ($P \geq 0.05$). Results indicated that as the storage time increased, the sucrose content of samples added 0.50 and 1.00 g/L chitosan decreased gradually. However, control sample and sample added 0.25 g/L chitosan showed a sharp decrease in sucrose contents, especially after 3 and 4 weeks of storage, respectively. Conversely, the increment in glucose and fructose content was observed in all samples during storage. Result suggested that, the inversion of sucrose took place during storage, leading to the formation of fructose and glucose. In addition, the loss of sucrose content was perhaps caused by microbes that utilized the sugars (Yusof et al. 2000). Several authors have investigated the change in sucrose, glucose and fructose content of processed juice such as apple juice concentrate (Babsky et al. 1986), banana juice (Yousaf et al. 2010) and sugar cane juice (Singh et al. 2006; Yusof et al. 2000) during storage. They revealed that the increase in reducing sugar and decrease in sucrose content were also found during storage.

Among all treatments, the control sample rendered the highest reducing sugar and lowest sucrose content. Hydrolysis of sucrose was normally promoted by the action of invertase and high acid condition. From the result, the lowest pH and highest invertase activity were observed in control sample. High invertase activity found in control sample favoured sucrose inversion which is responsible for loss of sucrose. During storage, the loss of sucrose in pasteurized

Fig. 3 Change in residual PPO activity (a) and invertase activity (b) of pasteurized palm sap as affected by chitosan addition during storage

Fig. 4 Change in sucrose (a), glucose (b) and fructose (c) content of pasteurized palm sap as affected by chitosan addition during storage

palm sap could be minimized by the addition of chitosan in the concentration of 0.25–0.50 g/L. Chitosan might form complex with invertase enzyme, resulting in the reduction in enzyme activity as mentioned previously. This result was concomitant with lower invertase activity during storage in chitosan-treated samples compared to control sample. Moreover, chitosan acts as an antimicrobial agent as confirmed by lower proliferation of spoilage microbes. Thus, lower acid formation made by spoilage microorganisms, especially yeasts and lactic acid bacteria (Pancoast and Junk 1980), was observed in chitosan-treated sample. In addition, sample added 1.00 g/L chitosan exhibited lower sucrose, fructose and glucose content than those added 0.50 g/L chitosan. The decrease in reducing sugar and total sugar during clarification of juice by using high concentration of chitosan was reported by Cesar et al. (2012).

Change in polyphenol content of pasteurized palm sap during storage

Polyphenol compounds are one of the most important groups of compounds in plant. In addition, they can be found in plant sap such as maple sap and palm sap (Deslauriers 2000; Cote 2003; Theriault et al. 2006). The presence of polyphenol compounds in palm sap was due to they occurred naturally and dissolved from Kiam wood or Payorm wood during collection (Phaichammnan et al. 2010). Recently, polyphenol compounds have received much attention since many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovascular diseases, stroke and certain forms of cancer. These protective effects have partly been ascribed to the antioxidant properties (Balasundram et al. 2006). In addition, the interaction between protein and polyphenol can form insoluble complexes and could grow to a large colloid size or haze (Kermasha et al. 1995; Siebert et al. 1996; Phaichammnan et al. 2010). Change in polyphenol content of pasteurized palm sap during storage was shown in Fig. 5a. There was no significant difference in initial polyphenol content among samples ($P \geq 0.05$). A significant decrease in polyphenol content was found for all samples during storage ($P < 0.05$). The reduction in polyphenol content was not significant difference between control sample and sample added 0.25 and 0.50 g/L chitosan during storage. It has been proposed that the polyphenol compounds contribute to haze formation by interacting with proteins through mechanisms involving prior polymerization or oxidation, leading to the formation of high molecular-weight polymeric complexes (Beveridge and Tait 1993). Lee et al. (2007) also found a decrease in polyphenol content in banana juice during storage. The greatest decrease in polyphenol content was found in sample added 1.00 g/L chitosan. This result

could be explained by the clarifying properties of chitosan. Chitosan has been found to be effective coagulating agent in aiding the separation of suspended particles from beverages. Shahidi et al. (1999) reported that chitosan has a good affinity for polyphenol compounds. In addition, Cesar et al. (2012) reported that chitosan, as a clarifying agent, complexes with polyphenol inducing flocculation and sedimentation in acai juice.

Change in DPPH radical scavenging activity of pasteurized palm sap during storage

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical and has been commonly used to evaluate free radical scavenging ability. When a hydrogen atom or electron was transferred to the odd electron in DPPH, the absorbance at 517 nm decreased proportionally to the increases of non-radical forms of DPPH (Brand-Williams et al. 1995). This assay is routinely practiced for assessment of free radical scavenging potential of an antioxidant molecule. Change in DPPH radical scavenging activity of pasteurized palm sap during storage was demonstrated in Fig. 5b. Initial DPPH radical scavenging activity of pasteurized palm sap increased with increasing chitosan concentration. The lowest DPPH radical scavenging activity was detected in control sample. Polyphenol compounds presented in palm sap are responsible for antioxidant activity. Moreover, antioxidant activity of chitosan has been reported. Siripatrawan and Harte (2010) reported that the scavenging mechanism of chitosan is related to the fact that free radical can react with the residual free amino (NH_2) groups to form stable macromolecule radicals, and the NH_2 groups can form ammonium (NH_3^+) groups by absorbing a hydrogen ion from the solution. Thus, the addition of chitosan could promote an increase in DPPH radical scavenging activity. A continuous decrease in DPPH radical scavenging activity was found in all samples during storage. The sample contained 0.50 g/L chitosan exhibited the highest DPPH radical scavenging activity during storage. A sharp decrease in DPPH radical scavenging activity within the first week was observed in sample added 1.00 g/L chitosan. The reduction in DPPH radical scavenging activity during storage could be attributed to the continuous precipitation between polyphenol compound and chitosan.

Change in microbial loads of pasteurized palm sap during storage

The microorganisms are found in the sap coming from environment during collecting process (Gupta et al. 1980). The fermenting organisms are dominated by yeasts, especially *Saccharomyces cerevisiae* and lactic acid bacteria. The reduction in microbial load was observed after pasteurization. However, some spoilage microorganisms may be survived

Fig. 5 Change in polyphenol content (a) and DPPH radical scavenging activity (b) of pasteurized palm sap as affected by chitosan addition during storage

and developed, leading to limited shelf life. Thus, chitosan, a natural antimicrobial agent, was introduced to retard the spoilage of product. For control sample, the TVC was found to be 3.30×10^1 cfu/ml after pasteurization while MYC and LAB count were not detected. The TVC, MYC and LAB count of all chitosan-treated samples were not detected after pasteurization. Result suggested that, the combination between chitosan and pasteurization is effective to reduce the microbial growth. In addition, chitosan concentration affected the microbial loads of pasteurized palm sap, resulting in a positive effect for extension of the shelf life. There was an increase in TVC, MYC and LAB count of all samples during storage (Table 2). Lower TVC, MYC and LAB count was found in all chitosan-treated samples during storage compared to control sample. Chitosan (up to 0.50 g/L) reduced microbial loads with no further reduction using concentration from 0.50 up to 1.00 g/L. As Thai Community Product

Standard (2003) indicates that TVC and MYC in pasteurized palm sap shall not be more than 5×10^2 cfu/ml and 100-cfu/ml, respectively. According to these criteria, the shelf life of samples as considered from microbiological standard was approximately 3 and 4 weeks for control sample and sample added 0.25 g/L chitosan, respectively, while samples added 0.50 and 1.00 g/L chitosan were assured for at least 6 weeks of storage. The antimicrobial application of chitosan has received considerable attention (No et al. 2002), with only attempts on juices investigated (Roller and Covill 1999; Kumar et al. 2009; Martín-Diana et al. 2009). Chitosan has been reported to inhibit the growth of microorganisms due to its chelating property. The chelating property of chitosan allows it to bind to the essential nutrients and metal ions, making them unavailable to microorganisms resulting in the inhibition of their growth rate. However, the exact mechanism for antimicrobial activity of chitosan is not fully understood

Table 2 Changes in microbial loads (cfu/ml) of pasteurized palm sap during storage

Storage time (week)/chitosan content (g/L)	Total microbial count (cfu/ml)				Mold and yeast count (cfu/ml)				Lactic acid bacteria (cfu/ml)			
	0	0.25	0.50	1.00	0	0.25	0.50	1.00	0	0.25	0.50	1.00
0	3.30×10^1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1	8.30×10^1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	1.23×10^2	3.50×10^1	nd	nd	6.30×10^1	nd	nd	nd	4.10×10^1	nd	nd	nd
3	2.75×10^2	7.40×10^1	nd	nd	9.60×10^1	5.80×10^1	nd	nd	1.03×10^2	4.40×10^1	nd	nd
4	6.53×10^2	3.15×10^2	3.10×10^1	nd	3.73×10^2	9.10×10^1	3.20×10^1	3.30×10^1	4.10×10^2	8.30×10^1	nd	nd
5	1.04×10^3	5.36×10^2	5.40×10^1	4.30×10^1	6.48×10^2	3.42×10^2	5.30×10^1	4.90×10^1	7.50×10^2	1.82×10^2	3.40×10^1	3.70×10^1
6	4.22×10^3	1.05×10^3	1.19×10^2	1.13×10^2	2.15×10^3	7.53×10^2	9.40×10^1	9.10×10^1	1.03×10^3	4.12×10^2	7.70×10^1	7.10×10^1

nd not detected

but many researchers have proposed different mechanisms. Altering the cell permeability due to the interactions between positively charged chitosan and negatively charged cell wall leading to leakage of intracellular components is one of the proposed mechanisms. Additional mechanism is the binding of chitosan with eukaryotic DNA leading to the inhibition of the mRNA via interference in protein synthesis (Devlieghere et al. 2004; Edirisinghe et al. 2012; Fang et al. 1994; Kong et al. 2010; Leceta et al. 2013; Papineau et al. 1991; Raafat and Sahl 2009; Sudarshan et al. 1992; Young et al. 1982). Moreover, the increase in microbial loads can be associated with the increase in acidity and reducing sugar and decrease in pH, TSS and sucrose during storage of pasteurized palm sap.

Conclusion

This study presents the possible use of chitosan as a natural preservative ingredient to extend the shelf life of pasteurized palm sap. Palm sap treated by using the combination between chitosan addition (0.50 g/L) and pasteurization was assured for at least 6 weeks of storage, whereas microbial load was reduced below standard level. High concentration of chitosan (1.00 g/L) can be also used to prolong the shelf life of pasteurized palm sap, however more sedimentation and the increment in bitterness might be considered as a quality defect of product, causing in unacceptability. Thus, this study recommends the use of chitosan at concentrations approximately 0.50 g/L to extend quality of pasteurized palm sap. In addition, further study related to sensory evaluation during storage should be conducted.

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