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The use of pasteurization to control microbial growth in rubber latex

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ABSTRACT

One cause of destabilization of fresh natural rubber latex is growth of microorganisms. This is commonly controlled by use of ammonia (NH_3) as a preservative. However, NH_3 is highly volatile and exposure to its fume can lead to health hazards. Moreover, the evaporation of NH_3 means that more NH_3 must be added to maintain the concentration level needed for the preservation. Hence, this research aimed to explore use of pasteurization to control microbial growth in rubber latex before it is spun to be viscid latex. To prevent heat destabilization of rubber latex, the latex was adjusted to pH 10 using 20% by weight of potassium hydroxide (KOH) solution. Pasteurization was done by immersing a plastic bottle containing 60 mL rubber latex in a hot water bath at (60˚C) for 15 min. Control of microbial growth was observed by measuring Volatile Fatty Acid (VFA) at different storage times (up to 13 h) and comparing this result to the number of colonies using plate count method. It was found that pasteurization could decrease the number of microorganisms presenting in the latex initially (10-10^5 times). However, the number of microorganisms in the pasteurized latex increased with storage time. VFA was also observed to increase with time. The Dry Rubber Content (DRC) of rubber latex before pasteurization (DRC of 22.20-34.10) had no effect on efficiency pasteurization. However, the number of microorganism presenting in the rubber latex before pasteurization affected efficiency of pasteurization significantly. For instance, when the number of microorganism in rubber latex was so large that its VFA was above 0.08 (the VFA number accepted by Thai Industrial Standards Institute 506), the efficiency of pasteurization was poor. On the other hand, if the number of microorganism was small and VFA was below 0.08, pasteurization could efficiently preserve the latex at room temperature up to 13 h.

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INTRODUCTION

Thailand is one of the largest exporters of natural rubber latex (NRL) products in the world and natural rubber latex is the raw material in the production of tires, toys, rubber gloves, and the other rubber products (Danwanichakul *et al.,* 2012). Natural rubber latex is a colloidal system which is stabilized by negative charges of proteins and phospholipids in the latex (Liyanage, 1999; Eng and Tanaka, 1993; Sansatsadeekul *et al.,* 2011). Unfortunately, loss of the stabilization of this colloidal system, known as destabilization of NRL, spontaneously occurs within a few hours. Therefore an addition of preservative into NRL is necessary. The destabilization was explained to be caused by (i) acids produced by decomposition of non-rubber component by microbial can produce acids and (ii) hydrolysis of lipids in the latex. The hydrolysis produces fatty acids which can replace the protein on the surface of the rubber particles and react with metal ions such as calcium and magnesium that are originally in the latex or from the compounds produced by enzymatic reactions in the latex (Kowuttikulrangsi, 2000).

At present, ammonia is the most common preservative used to preserve natural rubber latex (Santipanusopon and Riyajun 2009). Ammonia possesses several satisfactory preservative properties such as antibacterial, retarding chemical reaction of multivalent metal ions and being colourless (Lowe, 1960). Nevertheless, the disadvantages of ammonia are high volatility, strong odour, corrosive nature and its toxicity. Therefore, preservative systems for natural rubber latex have been developed by combining ammonia with other chemicals in order to reduce concentration of ammonia. The examples of these preservative systems are formaldehyde (Walter 1959), tetramethylthiuram disulphide (TMTD) mixed with zinc oxide (ZnO). However TMTD has problems with discoloring the latex and carcinogen nitrosamine generation (Boonsatit *et al.,* 2008). This work, hence, aimed to find alternative method to preserve NRL. As one cause of destabilization involves activity of microorganisms, pasteurization was selected. Pasteurization is a heating process to reduce the number of microorganism commonly used in milk. As milk is also a colloidal system and pasteurization employs low-temperature that can destroy spoilage organisms without destroying the original characteristics of the liquid being treated (Holsinger *et al.,* 1997), it was expected that pasteurization could be applied to the preservation of NRL. The study focused at the preservation of NRL before it is spun to be viscid latex.

MATERIALS AND METHODS

Materials

Fresh natural rubber latex (NRL) was obtained from rubber latex collection centers in eastern region of Thailand. Only the latex obtained within 2 h after harvesting were used. The latex was filtered with cloth to remove any debris before testing. Potassium hydroxide (KOH), Luria broth (LB) and Agar were supplied by Merck Ltd, Germany. Autoclaved distilled water was used when sterile condition was required for instance, when diluting natural rubber latex during plate counts of viable bacterial cells.

Selection of pasteurization temperature

Preliminary tests were conducted to select temperature appropriate to pasteurize natural rubber latex (NRL). As denaturation temperature of protein is the temperature that affects characteristics of milk (Srinivasan, 2002), the temperature that causes change in characteristics of NRL was studied. This was done by placing 2 bottles (plastic, capacity of 60 mL) containing NRL (60 mL) in stirred water bath. One bottle was closed (to prevent evaporation of water during heating and this set-up was used in the rest of this study) whereas another bottle was fitted with a thermometer and covered with aluminium foil. The water bath was heated using a hot plate (IKA, C-MAG HS7). Temperatures of water and one bottle of NRL were monitored. At the same time, the characteristics of NRL that show protein denaturation such as viscosity and consistency, in the closed bottle were observed.

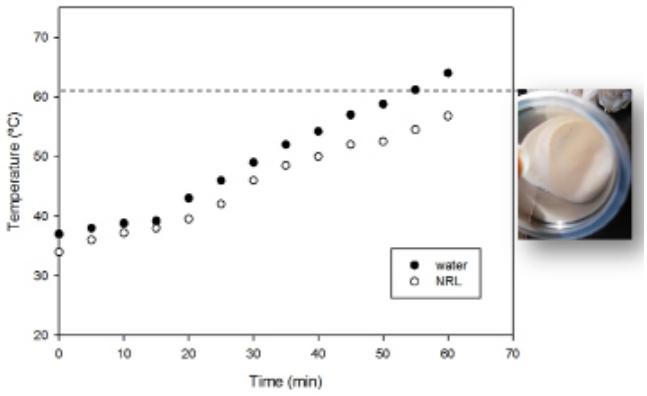

Figure 1 Temperature profile of natural rubber latex and water bath. Dash-line was observed temperature of natural rubber latex started to curdle

Figure 1 shows temperature of NRL and water during heating. According to the figure, temperature of water bath was similar to temperature of the latex (A plot of temperature of NRL against temperature of water with 1:1 scale gives a linear line with $R^2 = 0.9905$). This result suggests that the heat transfer from the hot water to the NRL inside the bottle was fast. By observing the NRL in the closed bottle, it was found that the latex appeared viscous when the water temperature reached 61.2°C. By the time the water temperature reached approximately 70°C, the latex coagulated.

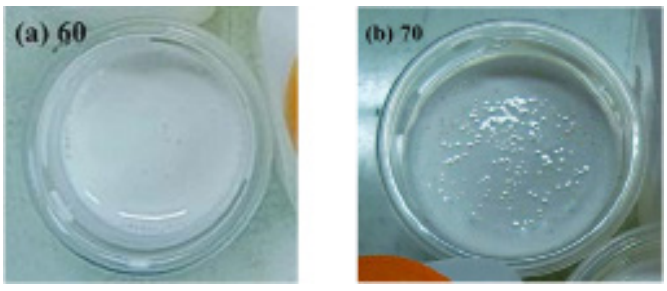

Figure 2 Texture of pasteurized rubber latex at difference temperature (a) 60°C and (b) 70°C

Figures 2(a) and (b) show surface textures of natural rubber latex heated until water temperature of 60°C and 70°C, respectively: significantly more bubbles were observed in the sample heated to 70°C. Clearly, 60°C is the maximum temperature that can be used to pasteurize natural rubber latex. As normally during pasteurization, a

sample is held at a constant temperature, heating time at which denaturation of protein occurs is interested. Hence, additional testing was done by heating closed bottles containing NRL in water bath (constant temperature of 60°C) in order to find the appropriate pasteurization time. The results showed that the longest time that natural rubber latex could be heated at 60°C before becoming curdled was 15 min. Hence, temperature and time of pasteurization used for further testing were 60°C and 15 min, respectively. Nevertheless, the latex pasteurized at this condition was observed to be more gel-like compared to the fresh latex. Hence, the latex had to be prepared in order to reduce heating effects on characteristics of the latex. The preparation is explained in the next section.

Figure 3 Textures after pasteurization of rubber latex and rubber latex adjusted to pH 10

Preparation of rubber latex before pasteurization

After filtering using cloth, the pH of NRL was adjusted to pH 10 using 20% Potassium hydroxide (KOH) and a pH meter (EUTEC, pH 510). This pH adjustment was recommended by several researchers to increase NRL stability (MTEC; siamglove 2015). The pH adjustment was needed to help stabilizing NRL during pasteurization as the pasteurized latex appeared more gel-like than the fresh latex. Figure 3 shows that by adding 20% KOH, pasteurized latex appeared similar to the fresh latex both in terms of texture and viscosity (Figure 3). The stabilization function of KOH at pH 10 is because hydroxide ions increase hydrolysis extent of latex at pH 10 (Yang, 1998). More negative charges are then produced and could widen double layers in the latex, providing higher strength of repulsive forces (Sonsalub, 2014). In addition, increasing alkalinity of the latex may prevent the growth of some bacteria. Moreover, some Magnesium ions (Mg^{2+}) presenting in the latex and causing the destabilization may be transformed to Magnesium hydroxide ($Mg(OH^-)_2$) depositing and separating from the latex.

Pasteurization rubber latex

The NRL with pH adjusted (60 mL) was added to a 60 mL bottle and a lid was closed tightly. The filled bottles were submerged in a stirred water bath of 60°C for 15 min.

Characterization of pasteurized rubber latex

The parameters that may affect effectiveness of pasteurization and properties of NRL namely Total Solid Content (%TSC) and Dry Rubber Content (%DRC) were quantified. TSC is the percentage by weight of latex that is nonvolatile at a definite temperature. TSC was determined by drying 2±0.5 g of NRL in an oven at 70°C until constant weight (approximately for 16 h). DRC is defined as the percentage by weight of latex, which is coagulated by acetic acid. In order to measure the DRC, 5±0.5 g latex was coagulated by 2%wt acetic acid and was dried in an oven at 70°C until constant weight, (approximately 16 h). %TSC and %DRC determined according to ASTM D 1076.

Storage time of pasteurized rubber latex was determined by monitoring volatile Fatty Acid Number (VFA NO.) which was measured by titration method (3 replicates). In order to study effectiveness of the pasteurization, the numbers of vital microorganism present in freshly obtained NRL, adjusted pH NRL, and pasteurized NRL were measured using plate count (Chaikumpollert *et al.,* 2015). This was done by diluting each NRL sample with autoclaved distilled water in serial dilution range of 10^{-1} – 10^{-10}. Then each diluted samples (0.1 mL) was poured onto an agar plate (Luria broth) and colonies found on the plate were counted after 48 h of incubation at room temperature. The concentration of microorganism in the sample was reported as colony forming unit in one milliliter: cfu/mL.

Viscosities and mean particle sizes of fresh and pasteurized NRL were measured using Brookfield viscometer (provided by Sudarat Sonsalab, Thammasat University) and Beckman coulter delsa™ nano particle analyzer, respectively.

RESULTS AND DISCUSSION

Effect of pasteurization on microbial activity and storage time of pasteurized rubber latex

Table 1 illustrates numbers of vital microorganisms found in fresh NRL (N_0), pH adjusted NRL (N_{pH}) and pasteurized NRL (N_p). The number of microorganisms of each sample was divided by N_0 in order to observe effects of KOH addition and pasteurization on

Table 1 Microbial Population Count; The numbers highlighted were from NRL that was kept for a period of time (about 5 hours) before pasteurization was conducted.

%DRC	Vital microorganism (cfu/mL)			Ratio	
	Fresh NRL, N_0	pH adjusted NRL, N_{pH}	Pasteurized NRL, N_p	N_{pH}/N_0	N_p/N_0
22.20	1.00×10^{8}	5.38×10^{10}	3.70×10^{5}	5.38×10^{2}	3.70×10^{-3}
24.56	1.00×10^{8}	8.00×10^{8}	1.52×10^{4}	8.00×10^{0}	1.52×10^{-4}
29.04	1.00×10^{8}	1.27×10^{11}	1.00×10^{7}	1.27×10^{3}	1.00×10^{-1}
29.61	9.90×10^{7}	1.26×10^{7}	3.20×10^{2}	1.27×10^{-1}	3.23×10^{-6}
30.53	8.00×10^{6}	5.80×10^{6}	1.00×10^{2}	7.25×10^{-1}	1.25×10^{-5}
32.19	9.90×10^{7}	2.00×10^{7}	5.60×10^{2}	2.02×10^{-1}	5.66×10^{-6}
33.85	3.20×10^{6}	2.00×10^{9}	4.00×10^{2}	6.25×10^{2}	1.25×10^{-4}
34.10	9.90×10^{7}	3.60×10^{8}	4.00×10^{2}	3.64×10^{0}	4.04×10^{-6}

the number of microorganisms. The results in Table 1 should be considered as two separate sets: (i) very fresh NRL (within 2 h after collection from tree) and (ii) not very fresh NRL (highlighted in the table). The latter sets of NRL were collected for a period of time (~ 5 h) before pasteurization was done; some bubbles which indicated destabilization could be observed. The numbers of vital microbial cells found in these sets of NRL confirmed that the latex was not fresh as large numbers of microorganisms were found. Moreover, VFA values were larger than 0.08, the VFA value acceptable by Thai Industrial Standards Institute 506 (See Figure 4).

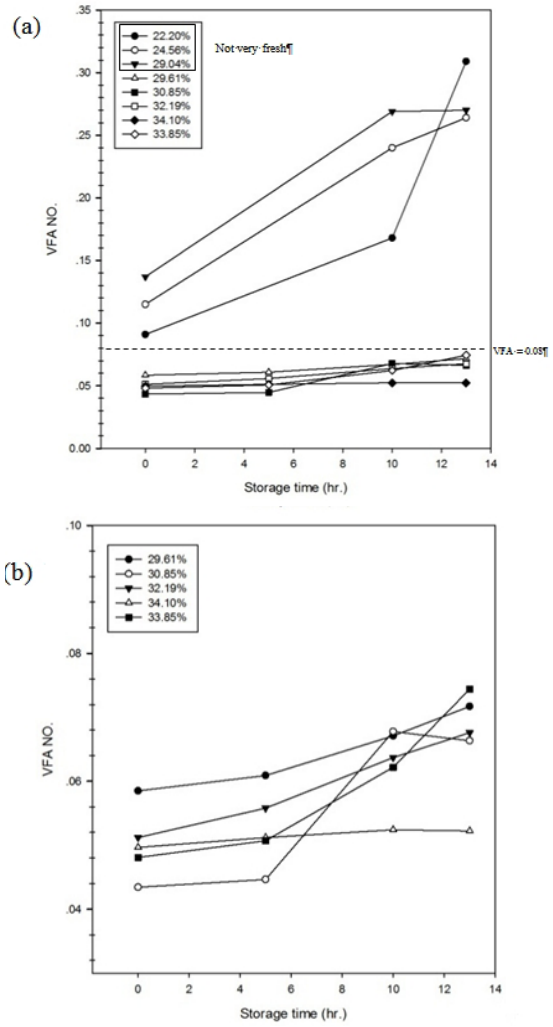

Figure 4 Volatile fatty acid number of pasteurized rubber latex at difference %DRC (a) all of the results and (b) high efficiency of pasteurized rubber latex with dash line required by buyers

According to Table 1 addition of KOH increased the numbers of vital microbial cells in not very fresh NRL samples as shown by N_{pH}/N_0 . This could be because pH 10 lies within the optimum growth pH range of most bacteria typically found in NRL (e.g. *bacillus licheniformis* has optimum pH for growth of 8.5-9.0 (Narimasa, 1973)). However, no clear trend of change in the number of vital microbial cells was observed when KOH was added to the very fresh NRL. Considering N_p/N_0, it could be seen that pasteurization could decrease the number of vital microbial cells and the reduction has been reported to be due to inactivation of microbial cells by pasteurization (Boonsatit *et al.,* 2008). By comparing the N_p/N_0 of the very fresh NRL and the not very fresh NRL, it could be concluded that efficiency of pasteurization clearly depended on the number of microorganisms presenting before the pasteurization. The efficiency of pasteurization observed with the not very fresh NRL was considerably less than that observed with the very fresh NRL. This may not be because the wild microorganisms in latex were particularly resistant to the heat action, but the curdling of pasteurized rubber latex may prevent the microorganisms from the heating (Parton *et al*., 2007). Effects of DRC on efficiency of pasteurization were not obvious. Storage time of pasteurized rubber latex was studied by monitoring VFA at different storage times and the results are shown in Figure 4. VFA values of the very fresh NRL lay below 0.08, the VFA value acceptable by Thai Industrial Standards Institute 506, for up to 13 h. Although VFA values of the not very fresh NRL were larger than 0.08 at all time periods, the increase in VFA values was at a faster rate compared to the increase in VFA values observed with the very fresh NRL. This confirmed that the efficiency of pasteurization in improving storage time of NRL depended greatly on the quality of fresh NRL. Although, the storage of pasteurized NRL is not promising compare to the storage time obtained with other preservation methods. The pasteurization could be further developed to improve its efficiency. For instance, it has been reported that sterilization at high pressure could be done at high temperature and could preserve milk colloid stability (Ven, 2007). Hence, pasteurization of NRL could be done at high pressure at higher temperature to improve the preservation efficiency. Moreover, disadvantages of the pasteurization may also be neglected in production of NRL products that required minimal chemicals addition.

Table 2 Characteristic of natural rubber latex.

Properties	Fresh NRL	Ammonia NRL	Pasteurized NRL
TSC	36.99	NA*	36.89
DRC	34.10	NA*	35.08
Viscosity 6Hz(cP)	25.0	NA**	533.3
Viscosity 60Hz(cP)	10.8	NA**	110.0
Mean particle size	12.2	16.6	12.3

* Ammonia addition insignificantly affects TSC and DRC (Sethurej and Mathew, 1992)

**Not found in literatures.

Effect of pasteurization on characteristics of pasteurized rubber latex

Table 2 lists characteristics of fresh NRL, low ammonia NRL and pasteurized NRL. Clearly, pasteurization did not affect DRC, TSC and VFA No. However, viscosity of the pasteurized latex was much greater than those of low ammonia NRL and fresh NRL. Nevertheless, viscosity of concentrated rubber latex was in the same range as the viscosity of the pasteurized latex (80-100 cP, Sirisomboon and Chowbankrang, 2015). This suggests that available NRL production process could manage the viscous pasteurized latex.

The literature reported that NRL with large particle sizes had low viscosity and the viscosity increased with increasing total solids

(Sridee, 2006). As pasteurization did not affect mean particle size (Table 2), the high viscosity observed with the pasteurized NRL could be due to changes occurred in an aqueous phase. As aforementioned, these changes may be prevented or lessened by pasteurization at high pressure.

CONCLUSIONS

Pasteurization could reduce the number of vital cells in NRL and could be used to preserve NRL. The efficiency of pasteurization in preserving NRL depended significantly on the number of microorganisms originally found in NRL before pasteurization. If VFA value of starting NRL was higher than 0.08, the acceptable VFA value, pasteurization could slightly reduce the number of microorganisms but could not lower VFA value. In addition, the rate of destabilization could not be controlled. However, if VFA value of starting NRL was below 0.08, NRL could be preserved by pasteurization up to 13 h.

However, viscosity of the pasteurized latex was much greater than those of fresh NRL and low ammonia NRL. Further work should be done to improve this. The pasteurization condition should also be further modified to enable pasteurization at higher temperature or for longer time without significant change the NRL's characteristics. The higher temperature and longer pasteurization time should improve the pasteurization efficiency as a preservation method of NRL.

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