Effect of Waiting Time and Process Temperature on the Quality of Commercially Sterilized Flavored Liquid Milk

Pengaruh Waktu Tunggu dan Suhu Proses Terhadap Mutu Susu Cair Berperisa Steril Komersial

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Abstract. In Indonesia, commercially sterilized flavored liquid milk is regulated requiring minimum F_0 -value of 3.0 minutes. Due to batch thermal processing operation, a waiting time, period for product holding before sterilization process at room temperature, is necessary. Waiting time is identified as a critical factor due to potential increase in initial microbial load. Consequently, increase of waiting time might be associated with the need of higher F_0 -value. The objective of this study was to evaluate the effect of waiting time (0, 1, 2, 3, 4, 5, and 6 hours) and process temperatures (121.9, 122.6, and 123.9°C) for fixed processing time (10 minutes) on the selected quality parameters of commercially sterilized flavored liquid milk. Our result showed no significant increase on the number of microbes, pH, and viscosity of the milk after waiting time up to 6 hours. After sterilization, all products tested with waiting time up to 6 hours and processing temperature at 121.9°C (F_0 -value of 11.00-13.54 minutes), 122.6°C (F_0 -value of 15.49-17.01 minutes), 123.9°C (F_0 -value of 20.53-21.46 minutes) were organoleptically acceptable. However, decrease of vitamin B1 (6-7, 9.6-10.4, 22-23%), vitamin C (45-50, 100, and 100%) were observed at processing temperature of 121.9, 122.6, and 123.9°C, respectively. These findings shows that the use of processing temperature of 121.9°C (F_0 -value of 11.00-13.54 minutes) is consider to be the best alternative to produce commercially sterilized flavored liquid milk having waiting time up to 6 hours.

Keywords: commercially sterilized milk, Fo.value, sterilization, vitamin B1, waiting time of sterilization

Abstrak. Di Indonesia, susu berperisa steril komersial sebagai produk pangan steril komersial harus memiliki nilai F_0 minimum 3 menit. Pada proses termal yang dijalankan dengan sistem batch, waktu tunggu sebelum proses sterilisasi tidak dapat dihindarkan. Waktu tunggu didefinisikan sebagai periode waktu produk susu yang menunggu setelah proses pengisian pada botol dan sebelum proses sterilisasi pada suhu ruang. Waktu tunggu menjadi salah satu faktor kritis karena mempengaruhi jumlah mikroba awal. Sebagai akibatnya lama waktu tunggu diduga dapat memengaruhi kebutuhan nilai F_0 yang lebih tinggi. Penelitian ini dilakukan untuk mengevaluasi pengaruh waktu tunggu (0 sampai 6 jam) dan proses sterilisasi dengan suhu sterilisasi yang berbeda (121.9, 122.6, dan 123.9°C dengan waktu proses sterilisasi yang tetap (10 menit)) pada mutu susu cair berperisa steril komersial. Hasil penelitian menunjukan bahwa tidak ada perubahan signifikan pada jumlah mikroba, pH, dan viskositas selama waktu tunggu hingga 6 jam. Setelah sterilisasi, seluruh produk susu yang telah mendapatkan perlakuan waktu tunggu dan proses sterilisasi pada suhu 121.9°C (nilai F_0 11.00-13.54 menit), 122.6°C (nilai F_0 15.49-17.01 menit), 123.9°C (nilai F_0 20.53-21.46 menit) dapat diterima secara organoleptik. Meskipun demikian terjadi penurunan vitamin B1 (6-7, 10, 22-23%) dan vitamin C (45-50, 100, dan 100%) pada suhu proses sterilisasi 121.9, 122.6, dan 123.9°C secara berturut-turut. Dari hasil penelitian ini menunjukan bahwa suhu sterilisasi 121.9°C (F_0 11-13.54 menit) dan waktu tunggu 6 jam dapat menjadi pilihan yang terbaik dalam proses pembuatan susu cair berperisa steril komersial.

Kata Kunci: nilai F₀, susu steril komersial, sterilisasi, vitamin B1, waktu tunggu sterilisasi

Practical Application: This study provides an information to optimize batch operation for processing of commercially sterilized liquid flavored milk, especially with respect to waiting time. Waiting time is defined as a period of time needed for the product to be ready before the sterilization process at room temperature.

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INTRODUCTION

For safety reason, low acid foods (pH>4.6 and aw>0.85), hermetically sealed and stored at room temperature are required to be commercially sterilized. In Indonesia, commercially sterilized food is regulated by Decree of The Head of National Agency of Drug and Food Control of Indonesia number 24, 2016. The regulation required minimum F₀-value not less than 3.0 minutes (BPOM 2016). If the D-value for *Clostridium botulinum* at reference temperature of sterilization of 121.1°C is 0.25 minute, thus the minimum requirement of 3.0 minutes for F₀-value is equivalent to 12D process (Hariyadi 2017).

Monitoring and control of good manufacturing practices are needed to ensure that commercial sterility has been achieved to ensure the safety and quality of milk. During in container sterilization process, milk that has gone through preliminary process is filled into bottle for further sterilization. Due to batch thermal processing operation, a waiting time, a period for product holding after filled into bottle and before sterilization process, is necessary. Waiting time, is one of critical factors due to potential increase of initial microbial count. Sunarlim Widaningrum (2005) reported that storing and pasteurized milk at room temperature $(27.5^{\circ}C)$ for 6 hours would increase total count bacteria from log 4398±1793 cfu/mL up to log 5486±1511 cfu/mL. Due to the possibility of increasing microbial count, increase of waiting time might be associated with the need of higher F₀-value. The higher F₀-value can be achieved by extending the sterilization time and/or applying higher temperature of processing. However, potential negative impact of higher F₀-value should be anticipated; especially with respect to some quality parameters; such as viscosity, specific gravity, pH and content of vitamin (B1 and C) content of milk due to potential overprocessing (Anandh et al. 2014).

Due to this condition, it is necessary to perform a study to evaluate the effect of waiting time length and sterilization temperatures on the selected quality parameters of commercially sterilized flavored liquid milk product. The quality parameters that will be assessed are organoleptic properties, pH, viscosity, content of vitamin B1 and C.

MATERIALS AND METHODS

Material

This study used a vanilla flavor liquid milk as a model. The liquid milk was filled in a 145 mL HDPE bottle. Tools used in this study were data logger TMI Orion PicoVACQ, micro pipet, petridish, scot bottle, thermometer, *Brookfield* Viscometer DV2T, HANNA pHmeter, beker glass, and High Performance Liquid Chromatography (HPLC) Alliance 2695.

Experimental set up

Before sterilization process, several preliminary processes performed on flavored liquid milk such as homogenization, pasteurization, and filled in a HDPE bottle. The bottled milks were taken as samples after filling process. The initial temperature of milk after bottling was 6-9°C. Before sterilization, the bottled milk samples were prepared and incubated at a room temperature 29-30°C for up to 6 hours. The samples were checked every hour, for product temperature, microbial count (i.e. total mesophilic, total spore, and termophilic), pH, and viscosity. All prepared bottled milks were then sterilized at temperatures of 121.9. 122.6, and 123.9°C for a fixed processing time of 10 minutes. Fixed processing time was applied due to the technical consideration associated with the operation of an industrial scale retort used (STOCK dft technology tipe SRX1300-4-HV-PS-WT, a rotary full water immersion retort, Stock 2017). The quality parameters were analyzed, such as organoleptic parameters, pH, viscosity, content of vitamin B1 and C. The repetition of analysis also carried out three times.

Calculation of F_0 -value and effect of temperature sterilization process

The F_0 -value is calculated based on heat penetration data. The heat penetration was carried out using data logger TMI Orion PicoVACQ. The F_0 -value was calculated by General methods using Microsoft excel by assuming that z value of *C. botulinum* spore is 10°C (Hariyadi 2017).

Temperature of liquid milk

Temperature of liquid milk was measured using a calibrated digital thermometer. The value is expressed as average of 5 measurements.

Mesophilic microbial count

Potato count agar was used to determine mesophilic microbe. The plate was incubated at $35\pm1^{\circ}$ C for 48 hours. After the incubation period, the calculated colonies are expressed as cfu/mL (BAM 1998).

Termophilic microbial count

The thermophilic microbial was determined by using Agate count plate agar. The plates were incubated at $55\pm1^{\circ}$ C for 48 hours. The calculated colonies were recorded as colony forming unit/mL (cfu/mL) (BAM 1998).

Total spore microbial count

Plate Count Agar was prepared to determine total spore microbe. Prior to inoculation, the liquid milk sample was heated at a waterbath at 80° C for 10 minutes. Incubation period of total spores was at $35\pm1^{\circ}$ C for 48 hours. Developed colonies were recorded as cfu/mL (BAM 1998).

pH value

The value of pH was determined by using calibrated digital pH meter (HANNA series HI-2211). pH meter was calibrated using buffer solution pH 4.00 and 7.00 (Umar *et al.* 2014).

Viscosity

Viscosity was measured by using Brookfield Viscometer DV2T, using spindle 1 for viscosity range 1-100 cP. The spindle speed rotation was set 60 rpm and milk temperature was set on 25°C. The values were recorded as centipoises (cP) (Bourne 2002).

Overall organoleptic properties

Overall organoleptic properties of the commercially sterilized liquid milk were evaluated by the 10-trained panelists currently employed by the industry. The task of the panelist was simply to determine whether the products were organoleptically acceptable or not, based on overall judgment on color, odor and taste of flavored liquid milk.

Content of vitamin B1 and vitamin C

Measurement of vitamin B1 and vitamin C were done using HPLC (HP series 1100 with C-18 column and UV-Vis detector (254 nm for vitamin B1 (Lalic *et al.* 2014) and 245 nm for vitamin C (Dionex 2016), analyses). Sample injection volume was 100 mL, the mobile phase was buffer methanol (55:45), and flow rate of mobile phase was 0.5 mL/min (Lalic *et al.* 2014).

RESULT AND DISCUSSION

Effect of waiting time before sterilization to liquid milk quality

The first stage of this study was to evaluate the effect of waiting time on the increase of initial microbial count, changes in pH, and viscosity of milk. Before sterilization process, several preliminary processes performed on flavored liquid milk such as homogenization, pasteurization and filled in a HDPE bottle. The bottled milk samples were prepared and incubated at a room temperature 29-30°C for up to 6 hours. The initial temperature of milk after bottling was 8.96°C. Along with the increasing waiting time (1, 2, 3, 4, 5, 6 hours), temperature of liquid milk continued to increase, reaching 24.30°C after 6 hours of waiting time at room temperature (29-30°C).

Effect of waiting time and its corresponding increase of temperature on the increase of all types of microbes, i.e mesophilic, total spore and termophilic are shown in Figure 1. Waiting time up to 6 hours is associated with increase of mesophilic (from 0 up to 9.20 cfu/mL,) total spore (from 0 up to 5.00 cfu/mL) and thermophilic counts (from 0 up to 3.60 cfu/mL). Yuanita *et al.* (2013) reported that good quality milk contained 4.0 log cfu/mL of microbes at most.

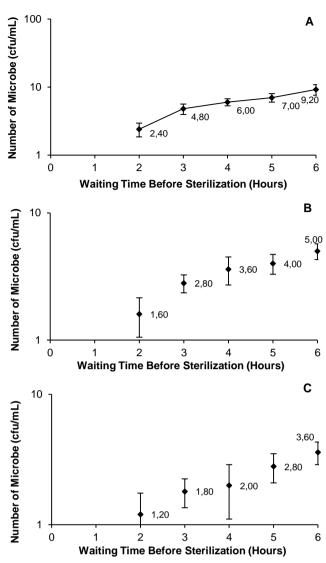


Figure 1. Effect of waiting time on the increase of microbial counts: mesophilic (a) total spore (b) and thermophilic (c)

Furthermore, according to FDA (2011), pasteurized milk (HTST) is considered to have good quality if it contains standard plate count <20.000 cfu/mL and coliform <10 cfu/mL during storage period. Increase of the microbial loads was less than 1 log and still comply with microbiological limit set by FDA for pasteurized milk (Figure 1).

Elrahman *et al.* (2013), Nababan *et al.* (2015), and Cahyaningtyas *et al.* (2016) reported that microbial activity could be related to changes in physicochemical properties like pH and viscosity. Figure 2 shows that waiting time up to 6 hours will change the pH and viscosity of flavored liquid milk but still within the acceptable standard for good quality milk.

Figure 2(a) indicates that pH of the milk slightly decreased during the waiting time up to 6 hours. pH of milk product decreased from 7.28 to 6.94. It complied with Indonesian national standard of good quality milk (6-7.5) (BSN 1998). Decreased in milk pH may be influenced by the increasing activity of microorganisms, such as acid producing bacteria which transform sugar (lactose) into lactic acid (Buckle *et al.* 1987).

Moreover, Increase in acidity will cause milk casein to coagulate (Ismanto *et al.* 2013). Microorganism in milk might decreased the quality and damaged the physico-chemical properties of milk, such as odor, texture, appearance, taste, viscosity. Increased in viscosity while waiting time up to 6 hours is shown on Figure 2(b). However, change of viscosity from 6.70 to 7.90 cP was not significant and remain complies with one of dairy industry standard for good quality milk (Memisi *et al.* 2014).

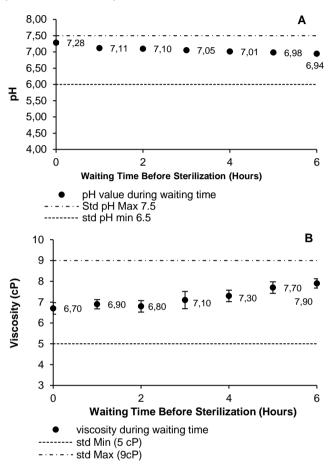


Figure 2. Effect of waiting time on the change of pH (a) and viscosity (b) of flavored liquid milk

Effect of sterilization process on flavored liquid milk quality

The sterilization process and calculation of F_0 -value

All liquid milk products treated for waiting time (up to 6 hours) were sterilized at temperature of 121.9, 122.6, and 123.9°C. They were evaluated for their quality. Based on the data calculation, various F_0 -values during sterilization process for 10 minutes at 3 different temperatures were presented in Figure 3. The F_0 -value increased as the sterilization temperature increased. For process temperature of 121.9, 122.6, and 123.9°C, the F_0 -value measured were 11.00-13.54 minutes, 15.49-17.01 minutes, 20.53-21.46 minutes, respectively. As Indonesian regulation, commercially sterilized flavored liquid milk is regulated minimum F_0 -value of 3.0 minutes, this F0 has met requirement. Along with the high sterilization temperature, change in product quality

should be minimized. In general, evaluation of flavored liquid milk quality could be associated with changes in physicochemical properties like color, viscosity, pH, and vitamin (Ling *et al.* 2015).

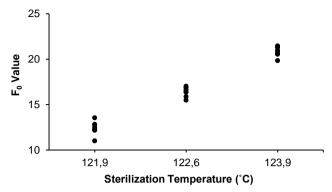


Figure 3. The value of F₀-value obtained by commercially sterilized flavored liquid milk during sterilization process for 10 minutes at 3 different temperatures (all products treated for waiting time (up to 6 hours))

Characterization of flavored liquid milk quality with different F₀-value

Organoleptic properties, pH and viscosity of favored liquid milk

All product tested, products with waiting time up to 6 hours and processing temperature of 121.9° C (F₀–value of 11.00-13.54 minutes), 122.6° C (F₀–value of 15.49-17.01 minutes), 123.9° C (F₀–value of 20.53-21.46 minutes) were organoleptically acceptable by the 10 trained panelists currently employed by the industry.

The value of pH and viscosity are two of important characteristic of flavored liquid milk. Effect of waiting time up to 6 hours and processing temperature on the change of pH and viscosity was shown at Figure 4.

Sterilization at different temperature significantly affected the pH of flavored liquid milk (P<0.05) (Figure 4(a)). However, for products from the same sterilization temperature, increased of waiting time up to 6 hours have no significant effect on pH (P>0.05). At sterilization temperature 121.9°C, pH value was 6.73, 122.6°C was 6.7 and at 123.9°C pH value was 6.6. Chavan et al. (2011) and Takeda et al. (2015) explained that heating of milk at temperature above 100°C causes decrease in pH due to organic group formation as a result of lactose degradation. Sterilization process with higher F₀-value (15-70 minutes) decreased pH value from 6.6 to 6.2 (Takeda et al. 2015). However, according to the standard of flavored liquid milk, the pH range obtained (6.0-7.5) is in compliance with the Indonesian national standard.

Effect of waiting time up to 6 hours and processing temperature at different temperatures on the change in viscosity of flavored liquid milk (Figure 4b). Change in viscosity of flavored liquid milk was not significant (P>0.05) and falls within the range of industry standard of 5-9 cP. In comparison, viscosity of flavored liquid milk after heating is higher than that of milk viscosity before heating. This is due to stabilizer which was used for the formulation of flavored liquid milk. The addition stabilizer is aimed to maintain the stability of emulsion, the binding of flavor and water, and to improve the texture, consistency, and stability of liquid in a food product (Tuazon 1996). Moreover, it might extend the shelf life since it is able to prevent the separation of milk fat and milk protein deposition (Sidley 2014).

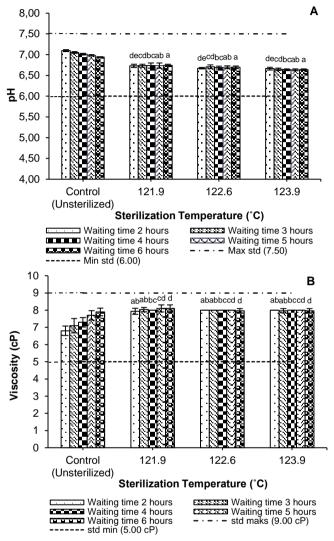


Figure 4. Effect of waiting time up to 6 hours and processing temperature on the change of pH (A) and viscosity (B) of commercially sterilized flavorred liquid milk

The stabilizer used in this study is based on cellulose and carrageenan. The combination of this stabilizer would be active after heating which was marked by the increasing viscosity. Cellulose was hydrocolloid commonly used in food industry as stabilizer and was able to prevent precipitation of milk casein (Cai *et al.* 2018). Moreover, carrageenan is polysaccharide made of algae. Pulkkkinen *et al.* (2014) mentioned that heating would increase in viscosity and product stability.

Vitamin B1 and vitamin C

The initial amount of Vitamin B 1 and vitamin C were 1.15 mg/100 mL and 0.011 mg/100 mL,

respectively. After pretreatment and before sterilization process, Vitamin B1 and vitamin C were 1.125 mg/100 mL and 0.009 mg/100 mL. This loss of 2% and 18% (respectively) was due to several preliminary processes performed on flavored liquid milk such as homogenization and pasteurization before sterilization.

Asadullah et al. (2010) and Cifelli et al. (2010) mentioned that significant decrease in vitamin B1, B2, B3, B6, B12 and folic acid during heating process depends on the type of milk processing and temperature. Pasteurization process may decrease the nutrients such as folic acid, vitamin B12, vitamin C, B6 and B1 contained in milk, up to 10%. UHT process at temperature of 130-140°C, there might be a decrease in the level of vitamin B of 10%, folic acid of 15% (Holdsworth, 1992), and the highest decline in vitamin C of 25% (Gliguem and Aragon 2005). Lalic et al. (2014) mentioned that UHT process decreases Thiamin level from 0.47 to 0.44 mg/L and vitamin C loss of 10% or a decrease from 2.0 to 1.8 mg/L. Some vitamins were sensitive to heat, fat soluble vitamin such as vitamin A (in the presence of oxygen), D, E, and beta-carotene also water soluble vitamin such as Vitamin C, B1, B2 under acidic condition (Awuah et al. 2007). In general, the highest decrease in the level of vitamin C in non-acidic food occurs during heating process. In the process of aseptic milk, the degradation of vitamin C rarely occurred, while lipid, carbohydrate, and other minerals were not affected.

Effects of sterilization process on vitamin C and vitamin B1 content is presented at Figure 5. The study found that sterilization process at 3 different temperatures condition was significantly correlated with decrease vitamin B1 and C level (P<0.05). Increased of waiting time increased up to 6 hours did not give significant impact (P>0.05) on content of vitamin B1. Sterilization temperature at 121.9, 122.6 and 123.9°C affected the content of vitamin B1 significantly, with 6-7.5, 9.6-10.4, and 22-23% reduction from their initial amount, respectively. Effect of sterilization process on vitamin C content is even more severe. Sterilization temperature of 121.9, 122.6 and 123.9°C has caused decrease on vitamin C content as much as 45-51.3, 100, and 100%, respectively.

CONCLUSION

This study showed that increased of the number of mesophilic, thermophilic, and total spore and changed the physico-chemical properties (pH and viscosity) during the waiting time before sterilization process (0 hour up to 6 hours) were not significantly affected the quality of the products to be sterilized. All of sterilized products tested, with waiting time of up to 6 hours and processing temperature at 121.9°C (F₀–value of 11.00-13.54 minutes), 122.6°C (F₀–value of 15.49-17.01 minutes), 123.9°C (F₀–value of 20.53-21.46 minutes) were organoleptic acceptable. However, decrease of vitamin B1 (6-7, 9.6-10.4, 22-23% respectively) and

vitamin C (45-50, 100, and 100%) content were observed at processing temperature of 121.9, 122.6, and 123.9°C, respectively. These findings shows that among the processing temperature studied, temperature of 121.9°C (F_0 -value of 11.00-13.54 minutes) is considered the best alternative to produce commercially sterilized flavored liquid milk having waiting time up to 6 hours.

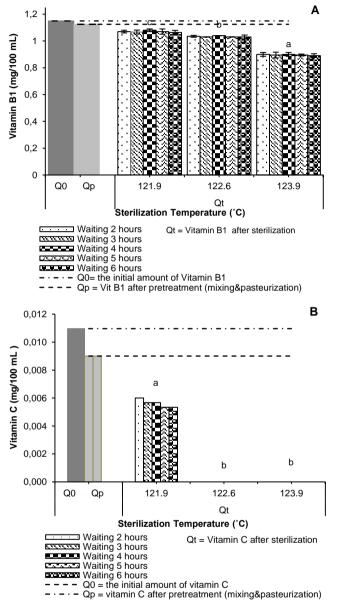


Figure 5. Decrease in vitamin B1 (a) and vitamin C (b) at different sterilization temperature

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