Physico-Chemical and Microbial Quality of Soft-White Cheese Made from Raw Milk with Different Somatic Counts

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Abstract— The study was conceptualized to assess the effect of somatic cell count on the quality of raw milk and soft-white cheese. Cow's milk was classified into normal (T1), trace (T2) and positive 1 (T3) based on the direct microscopic somatic cell count (SCC). Soft-white cheese was subjected to quality changes; such as physio-chemical compositions: pH, titratable acidity, moisture content, total solids, protein and fat recovery; and total microbial count. Milk samples were subjected to quality analyses prior to cheese manufacture. All samples passed the alcohol precipitation and clot-on-boiling tests. Significant differences were observed on the pH, titratable acidity, moisture, protein, fat and total solids contents of cheese made from T1, T2 and T3 milk. Cheese from normal milk had the lowest titratable acidity from day 0 until day 11 indicating its good keeping quality. The high number of microbial counts even on the 0 day of storage has been observed in soft-white cheese made from high number of somatic cell. The findings of the study indicate that milk with elevated SCC could compromise the typical sensory quality of the product and could pass the standard quality assessment; however, the quality of the resulting product would be inferior from normal milk. It is recommended that SCC determination be included in the quality assessment protocol in the farm.

Keywords— soft-white cheese, kesong puti, somatic cell count, cheese quality.

INTRODUCTION

Milk is known to be a high-value nutritional biological fluid mainly composed of water, proteins, fat, sugars, vitamins and minerals. Other important components existing naturally in raw milk are somatic cells (SCs). Somatic cells are inherently present in milk; since they are leukocytes (white blood cells). The presence of somatic cells in milk is used as an indicator of udder health since it defends the mammary gland from bacterial infection that causes mastitis.

The role of somatic cell in the udder can be considered as a positive effect, since somatic cells protects against bacterial infection called mastitis disease; however, in milk it can be viewed as a negative impact. Murphy *et al.* (2016) reported that at higher levels, somatic cells and bacteria are associated with increased activity of enzymes that causes damage to milk components which potentially results in product defects. Milk component alterations are attributed to changes in vascular permeability due to the inflammatory process and the damage of epithelial cells that are responsible for the synthesis of milk components, as well as changes in the enzymatic action of somatic cells or micro-organisms in the infected mammary gland (Kitchen, 1981).

Increased somatic cell count (SCC) or bacterial counts that influence the quality of processed dairy products depend on several factors like enzyme level, specificity, heat stability, processing and storage temperature, pH level, moisture content, and the presence of inhibitors and activators (Fairbairn and Law, 1986; Mottar, 1989; Sørhaug and Stepaniak, 1997; Datta and Deeth, 2001; Considine *et al.*, 2004; Ismail and Nielsen, 2010). The utmost negative consequences of the presence of somatic cells are related to shorter shelf life and less sensory content or undesirable organoleptic characteristics of the final product, due to enzymatic activities of somatic cells (Töpel, 2004).

Local studies on the influence of somatic cell on soft-white cheese *(kesong puti)* quality have not been given much attention. The primary objective of this paper is to determine the quality characteristics of soft-white cheese *(kesong puti)* made from milk with somatic cell counts higher than normal level.

MATERIALS AND METHODS

Milk Sample Collection

The collected milk sample was categorized as normal and subclinical mastitis infected milk based on the results of the California mastitis test (CMT) and direct microscopic somatic cell count. Milk samples from each type of milk (normal and subclinical mastitis infected milk) were further subjected to quality test using the alcohol precipitation test (APT) and clot-on-boiling test (COB).

Milk Quality Testing

Milk samples from each type of milk was well agitated; and an equal amount of milk and CMT reagent were poured into the CMT paddle then rotated counter clockwise. If CMT reagent and milk reacts and formed a slime type liquid to gel like content it indicates infection. The thicker the gel formation the greater mastitis infection; if the milk and the CMT reagent just mixed-up and does not reacts, it indicates that the milk was in its normal level. After CMT, SCC follows using direct microscopic somatic cell count. It verifies the number of leukocytes present in the milk as an internal measure of the udder health. Alcohol precipitation test (APT) and cot on boiling (COB) indicates the acidity of the milk. In APT, equal amount of milk and 68% ethanol were poured into a test tube then mixed-up. If the tested milk is of good quality, there will be no coagulation, clotting or precipitation; the presence of flakes or clots indicates poor milk quality. Same goes with COB, 5 ml of milk samples were poured into test tube then submerged into a boiling water bath for 5 minutes; if no coagulation occurs, it indicates that milk can withstand heating process at the time of testing.

Production of Soft-white Cheese (kesong puti)

Fresh milk coming from different quality of milk was subjected to Ekomilk analyzer to determine its composition especially the fat content. The milk was then placed in a cheese vat added with 3.5% table salt (equivalent to the weight of the milk), and then stir until salt dissolves. The milk was filtered with a clean cheese cloth before pasteurization at 72°C for at least 15 seconds using a double boiler method followed by immediate cooling to 40°C. While stirring, rennet is added at a rate of ~0.03% (total volume) of milk. The stirring lasted for about a minute after the complete addition of rennet. The milk was covered and set aside for 20 minutes and consistently monitored for any sign of coagulation every 5 minutes. When milk has completely coagulated, the coagulum was cut by means of vertical and horizontal cheese knives and again leave the curd undisturbed for 5 – 10 minutes. While waiting, the cheese moulds with clean cheesecloth underneath were arranged in a basin. After 5 – 10 minutes after cutting the coagulum, the curd was slowly stirred for 10 – 15 minutes to separate the whey from the curd then set aside the curd for another 2 – 3 minutes followed by draining and removal of 40 – 50% of the whey. The curd with some whey was scoped or poured into the cheese moulds. The curd in the mould was then covered with clean cheese cloth and drained at room temperature for 2 – 3 hours or refrigerated for 4 – 6 hours. Afterwards, the cheese was cut according to desired blocks then packed each block with plastic wrapper, stored at a chilling temperature.

Physico-Chemical Quality and Microbial Count of Soft-white Cheese (Kesong puti)

The quality characteristics of soft-white cheese (*kesong puti)* made from normal and subclinical mastitis milk (SMM) were monitored for twelve (12) storage days. The physicochemical component includes pH level, titratable acidity, moisture content and total solids, protein content (Kjeldahl method) and fat (Ether Extract). All parameters followed the AOAC (2006) method. The microbial count method was adapted from FDA Bacteriological Analysis Manual (BAM, 2001) and 3M™ Petrifilm™ Aerobic Plate Count manual.

Statistical Analysis

The experimental design is simple ANOVA in CRD with two (2) Factors: type of milk as factor A and length of storage as factor B. Software used was Statistical Analysis System (SAS) release 9.4 (SAS Institute, Inc., Cary NC). All data gathered were analyzed using Analysis of Variance (ANOVA). Wherein, the type of milk has a specific level of the somatic cell for each soft-white cheese. Mean comparison was analyzed using Least Significant Difference (LSD) test at 5% level of significance.

RESULTS AND DISCUSSION

Milk Composition and Quality

The milk composition and characteristics of the cow's milk with varying somatic cell count is shown in Table 2. According to the FAO (2013), the cow's milk protein has the range of *3.2-3.4, averaging 3.3%; and cow's fat content has 3.1-3.3, averaging 3.3%. Islam et al. (2008) reported that the protein and fat of* Holstein cross is *3.11±0.76 and 4.28±0.92, respectively.*

In addition, Talukder *et al.* (2013) reported that the milk composition of Sahiwal – Friesian crossbred has a fat content of 4.27±0.12, solid non-fat with 9.03±0.14 and total solids of 0.15±13.30. With this, it can be concluding that all classes of milk were within the acceptable level of milk composition.

Table 1: Milk composition of cow's milk with varying somatic cell count

MILK COMPOSITION	NORMAL	TRACE	POSITIVE 1
Fat, %	4.04	4.05	4.63
Protein, %	3.19	3.45	3.36
Solid Non-Fat, %	8.45	9.15	8.89

Shown in table 2, the quality results of milk using California mastitis test, direct microscopic somatic cell count, alcohol precipitation test (APT) and clot on boiling (COB) of different milk quality. The CMT score corresponds to the qualitative quality of the somatic cell count in milk (Leslie *et al.* 2002); whereas the somatic cell count represents the quantitative measurement of udder health or the degree of infection. The CMT score and SCC of the three types of milk were: for normal milk the CMT score was negative with somatic cell count of 263,981.23 cells/ml; for trace type of milk the CMT score was trace having somatic cell count of 459,522.88 cells/ml; and positive 1 type of milk was scored 1 with somatic cell count of 971,190.20 cells/ml.

Table 2. Quality test of different classes of milk

QUALITY PARAMETERS	NORMAL	TRACE	POSITIVE 1
APT	Negative	Negative	Negative
COB	Negative	Negative	Negative
CMT Score	Negative	Trace	1
SCC cells/ml	263,981.23	459,522.88	971,190.20

The somatic cell count matched the guidelines in CMT scoring, interpretation and SCC range adapted from Ruegg (2005) from National Mastitis Counsel. All samples were negative in COB and APT regardless of CMT score and somatic cell count. This may imply that the somatic cells cannot be detected by the common platform tests and may give rise to further food safety and production problems. This matter can be incorporated to the mandated procedures set by Food and Drug Administration (FDA), Philippine National Standards (PNS), and National Dairy Authority (NDA).

Physico-Chemical and Microbial Quality of Soft-white Cheese (kesong puti) made from Different Somatic Count

The physico-chemical quality of soft-white cheese (kesong puti) made from milk with different somatic cell counts of 263,981.23 cells/ml for normal; 459,522.88 cells/ml for trace; and 971,190.20 cells/ml for positive 1 were presented in Table 3.

Table 3: Physico-chemical quality of soft-white cheese (kesong puti) made from milk with different somatic cell counts

Storage Days	pH			P Value	TITRATABLE ACIDITY			P Value
	Normal	Trace	Positive 1		Normal	Trace	Positive 1	
0	6.79^{a}	6.61^{b}	6.63^{b}	0.0002	0.0737	0.0740	0.0937	0.0735
5	6.73^{a}	6.48^{c}	6.65^{b}	0.0002	0.0600	0.0627	0.0670	0.8926
11	6.75^{a}	6.51^{b}	6.52^{b}	0.0001	0.0513^{b}	0.1123^{a}	0.0773^{b}	0.0124
Mean	6.76	6.53	6.60	-	0.0617	0.0830	0.0793	
	MOISTURE, %				**PROTEIN, %**			
0	67.86	66.86	69.37	0.2561	9.98	10.53	10.26	0.8992
5	62.10^{b}	61.17^{b}	64.87^{a}	0.0004	10.18^{b}	12.13^{a}	7.52^{c}	0.0028
11	59.41^{b}	64.38^{a}	62.50^{a}	0.0086	9.61^{b}	15.44^{a}	11.03^{b}	0.0138
Mean	63.12	64.14	65.58	-	9.92	12.70	9.60	-
	FAT, %				**TOTAL SOLIDS, %**			
0	16.68	15.90	16.06	0.6749	32.14	33.14	30.63	0.2561
5	21.30^{a}	21.85^{a}	18.02^{b}	0.0297	37.90^{a}	38.83^{a}	35.13^{b}	0.0004
11	23.90^{a}	20.85^{b}	19.08^{b}	0.0038	40.59^{a}	35.62^{b}	37.50^{b}	0.0086
Mean	20.63	19.53	17.72	-	36.88	35.86	34.42	-

Acidity of Soft-white Cheese (kesong puti)

The mean values of pH level of the soft-white cheese *(kesong puti)* shows relative significant different among the type of milk in each level of time storage. As for the TA, it was noted that only on the 11th day of storage, there had been significant difference among the type of milk with respect to time of storage. In pH, the highest value was detected on the 0 day of storage coming from normal type of milk with pH 6.79. It was generally observed that the normal type of milk was superior compared to trace and positive 1 type of milk. Furthermore, according to Aquino *et al.* (2011), the typical pH value of soft-white cheese *(kesong puti)* is at 6.81± 0.06. On the other hand, the TA value of soft-white cheese *(kesong puti)* made from normal milk obtained the lowest value (0.0737) compared to the soft-white cheese *(kesong puti)* made from trace (0.0740) and positive 1 milk (0.0937). The acidity of soft-white cheese *(kesong puti)* made trace milk had the highest mean value in TA among the three types of milk. This may be due to the presence of high somatic cell count that has altered the product's pH value. The decrease in the acidity of the soft-white cheese *(kesong puti)* is attributed to the rennet coagulation of milk into curd; due to association with para-k-casein base on electrostatic interaction (De Roos *et al.* 1997). Banks *et al.* (2003) discussed that rennet coagulation of milk is pH-dependent, with optimum activity for hydrolysis of the Phe105—Met106 in K-casein at pH 6.0. Decreasing the natural pH of milk from pH 6.7 to 6.0 results in solubilization of calcium phosphate and a decrease in the charge on the casein micelles, thus resulting to gel formation. This represent that the product becomes slightly acidic as the storage days increases making it prone to spoilage. Barbano *et al.* (1991) also did not observe significant differences in the pH values of milk with SCC varying from <106,000 to 1,300,000 cells/mL. However, Klei *et al.* (1998) observed that the milk pH increased significantly with increasing SCC.

Composition soft-white Cheese (kesong puti)

The chemical composition in terms of moisture, protein, fat and total solids of soft-white cheese *(kesong puti)* are presented in Table 3. There had been significant relations (P<0.05) between the type of milk at each time of storage in all milk components. It was observed that soft-white cheese *(kesong puti) made* from positive 1 milk has the highest MC of 69.37% and lowest in TS with 30.63% in 0 day of storage, followed by soft-white cheese *(kesong puti)* made from normal milk with 67.86% (MC) and 32.14% (TS), and trace milk with 66.86% (MC) and 33.14% (TS). It can be noted the decreasing trend in MC and increasing trend in TS of soft-white cheese *(kesong puti)* made from normal and positive 1 type of milk. The changes in moisture content and total solids are continuous throughout the period because of the continual whey separation and casein coagulation through time. It happens because rennet (chymosin) and pH value (6.5 - 6.7); lower pH increases enzyme activity and neutralizes charge repulsion between micelles. The changes in moisture content can be related to microbial count; food, pH, and moisture is one of the primary requirement of microorganism for growth and given condition, microbial growth is more likely to occur, furthermore, according to Aquino *et al.* (2011), the MC (%) of soft-white cheese *(kesong puti)* is at 62.15 ± 7.3.

Milk fat and protein percentages varied because milks were obtained from different groups of cows, but it appeared that total protein was lower in soft-white cheese *(kesong puti)* and higher in fat content made from milk with SCC 263,981.23 cells/ml (Table 3). The protein content of the three types of milk had been significantly different amongst type of milk and storage time, excluding the 0 day. The highest protein content of soft-white cheese *(kesong puti)* made from normal milk was observed at 5th day of storage with 10.18%, while for soft-white cheese *(kesong puti)* made from trace and positive 1 milk was observed on the 11th day of storage with 15.44% and 11.03% respectively. This can be related to other milk components; several studies explained that casein micelle system is an excellent example of colloidal dispersion and repulsive forces hold the micelles in suspension until removed by some external influence. As the casein micelles are negatively charged, resulting in a zeta potential of approximately −20 mV, and this charge is reduced by approximately 50% on rennet treatment (Dalgleish 1984; Darling and Dickson, 1979; Green and Crutchfeld, 1971; Pearse, 1976). Therefore, the fluctuation in protein content of all treatment over time may be affected by moisture content, total solids and pH. It was mentioned earlier that whey proteins continuous to separate to casein micelles over time and can be seen in moisture content. In the study of Politis and Ng-Kwai-Hang (1988), the protein percentage increased as the SCC in milk increases; and Barbano *et al.* (1991) mentioned that low SCC milks (≤106,000) had a much lower level of proteolytic activity than the high (≥127,000) SCC milks. Similar with Klei *et al.* (1998), the proteolysis activity of cottage cheese made from high SCC milk was faster than that of low SCC in milk during refrigerated storage; and quality defects in cottage cheese that are related to high SCC in milk. O'Brien *et al.* (2001) observed that there had been considerably higher plasmin activity in milk with SCC of 300,000 to 370,000 cells/ml as compared with milk with SCC of 120,000 to 230,000 cells/ml. As manifested in the given data of the protein percentage of soft-white cheese *(kesong puti)*, the SCC level could really affect the quality and increase the probability of flavour defects. In addition, according to Aquino *et al.*, (2011), the protein content of soft-white cheese *(kesong puti)* is 12.04 ± 1.76.

The fat content of soft-white cheese *(kesong puti)* made from the three types of milk was noticed to have an increasing trend from the 0 day until the 11th day of storage. Soft-white cheese *(kesong puti)* made from normal milk has significant difference from positive 1 milk on the 5th day of storage (P<0.0297) and 11th day of storage (P<0.0038). The data may be related to moisture content (MC), whey loss, and total solids (TS), making the fat globule concentrated as the storage days increases. Politis and Ng-Kwai-Hang (1988) revealed that fat percentage decreased slightly as the SCC in milk increases. Parallel to the study of Klei *et al.* (1998), there had been no significant in percentage fat recovery among the cottage cheese made from different SCC. Nevertheless, this can also be related to the increasing trend in aerobic count; where fat can act as protectant and gives defensive advantage. Several studies confirmed the concept of fat as protectants including: Senhaji (1997) confirms the hypothesis that the heat protection phenomenon of fats is due to reduced water activity in the fat during heating; Sprong *et al.* (1999) also suggested that the fats would act as protectant against the thermal inactivation of *Salmonella*; and Juneja and Eblen (1999) investigated the effect of increasing fat levels on bacterial thermal inactivation by testing the survival of *Salmonella*.

Microbial Quality of Soft-white Cheese (kesong puti)

A high number of somatic cell count (SCC) in milk indicates severe infection which is directly linked to pathogenic microorganism present in milk (Sharma *et al.* 2011). And according to several authors (Vázquez *et al*. 2013; Reyher *et al*. 2012; Harjanti *et al*. 2018), *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Corynebacterium spp*. and other potential pathogens are *Pseudomonas* spp., *Bacillus* spp., and *Klebsiella* spp. Figure 1 shows the logarithmic aerobic count of soft-white cheese *(kesong puti)* made from normal milk with somatic cell count of 263,981.23 cells/ml; trace and positive 1, with somatic cell count of 459,522.88 cells/ml and 971,190.20 cells/ml. It was observed that soft-white cheese *(kesong puti)* made from normal milk have the lowest aerobic plate count on the 0 day of storage, followed by trace and positive 1. Rapid increased was observed from 0 to 11th in all type of milk, this may be due to the usual shelf life of soft-white cheese *(kesong puti)* which is not more than 11 days. The increasing microbial load of the soft-white cheese with high SCC can be linked to the common mastitis pathogens. Moreover, according to the Gulf Standardization Organization (GSO) standard, the acceptable microbiological load for soft-white cheese is 10^2-$<10^3$ cfu/ml. Food and Drug Administration (FDA) also has a similar acceptance of microbial load of 10^2 cfu. Complying with the national and international standards, the soft-white cheese from both trace and positive 1 milk are not acceptable even on the 0 day of storage. The contributing factor for microbial growth are the pH value, moisture content, protein and fat, it gave the ideal condition for microbial escalation. Consequences of having high microbial load would lead to food borne illness such as food intoxication, and infection. The World Health Organization has reported that food borne disease is a global burned which cause death up 600 million each year.

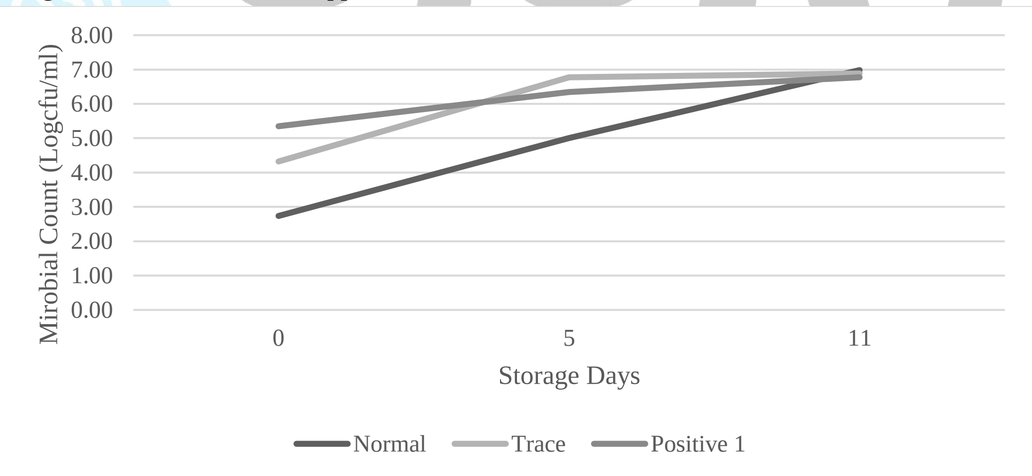

Figure 1: Aerobic Plate Count of soft-white cheese (kesong puti)

CONCLUSION

In conclusion, the results mainly confirmed that the soft-white cheese made from high somatic cell count shown significant difference with soft-white cheese made from normal somatic cell count. Therefore, when the somatic cell number is above a certain threshold, there are many probabilities that defects and product quality are deteriorating. Elevated number of somatic cell can be considered as milk quality indicator in milk and milk products. It also shows a high number of microbial counts even on the 1st day of storage as seen in soft-white cheese made from high number of somatic cell.

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