Study on cow’s raw milk quality in the eastern Taiwan during hot season

Mei-Jen Lin(1)(3), Ying-Ching Li(1), Yu-Chi Lin(1) and Kuen-Jaw Chen(2)

ABSTRACT

The aim of this study was to investigate the quality of raw cow’s milk during hot season in Eastern Taiwan. Milk samples were collected from a dairy farm of Holstein at Hua Lien, Taiwan in July and August, 2007. Gross compositions, titratable acidity (TA), ethanol stability (ES), pH, total calcium (tCa), and ionic calcium (iCa) concentrations in milk were measured. All data were statistically analyzed as 3 categories, which were low ethanol stability (LES, < 70%), medial ethanol stability (MES, 70%~74%) and high ethanol stability (HES, > 74%), respectively. The percentages of LES, MES, and HES milk samples were 20.2%, 17.2%, and 62.6% of total 1,248 samples, respectively. Results showed that TA, total (iCa) and ionic calcium (tCa) concentrations of LES milk samples were significantly higher than other treatments, but pH value was significantly lower in LES milk. The fat, protein and solids-not-fat contents of LES milk were significantly higher than other treatments; however, milk yield of LES was lower. There was a negative correlation between ionic calcium concentration and ethanol stability. Therefore 20% LES milk was unstable in less than 70% ethanol solution, in summer milk in Eastern Taiwan. The LES milk also showed poor physicochemical properties with high ionic calcium concentration.

(Key Words: Cow’s raw milk, Ethanol stability, Gross compositions, Physicochemical properties)

INTRODUCTION

The aim of dairy industry is to pursue the highest production, the best milk quality and the lowest production cost to acquire the highest economic benefit. There are difference between milk composition and physicochemical properties of Holstein cows owing to different productivities, lactation stages, and seasons. Holstein cow’s milk yield decreased at the temperature between 21~27℃ and decreased significantly at the temperature above 27℃. Generally, the climate in summer in the South and East Taiwan area is too hot and humid for Holstein cow to reproduce and produce good quality milk. To
incorporate developing non-toxic agricultural products in Hua Lien, this study collected milk samples from a dairy farm at Hua Lien, the eastern Taiwan, during hot season (July and August, 2007). In terms of milk quality, casein micelles play a crucial role on enzyme-, acid-, heat-, or ethanol-induced coagulation of milk, which affects the characteristics of dairy products (Huppertz and Fox, 2006). The salts in milk and milk products play an important role on stability of casein micelles as well as nutritional and organoleptic characteristics of milk (Tsioulpas et al., 2007). There are several factors affecting the equilibrium of calcium salts in milk, such as pH, gross compositions, and structure of casein complex (Wang, 2005). The ionic calcium concentration (iCa) is one of the important factors affecting casein micelles stability in milk. There are many methods to measure the casein micelle stability in milk, including ethanol stability, heat stability, and rennet coagulation. Ethanol stability test was widely used as a simple, cheap, efficient and quick pass-or-fail method. This method is still in use in Taiwan, leading to rejection of the batch of milk if flocculation were formed with an equal volume of 70% (v/v) ethanol solution added. It is an easy method for monitoring milk stability during refrigeration. Therefore, the purpose of this study was to investigate the cow’s raw milk quality during hot season in the eastern Taiwan.

MATERIALS AND METHODS

Preparation of Milk Sample

Raw milk was sampled three times a week from a dairy farm at Hua Lien, Taiwan in July and August, 2007. Milk samples were collected from 30 individual of Holstein at each sampling, cooled to 4°C immediately, and transported to the laboratory within 24 hrs. In total, 1,248 samples were collected from 30 animals throughout the study.

Ethanol Stability Measurement

Ethanol stability measurement was used as an indication of protein stability in this study. A series of aqueous ethanol solutions were prepared at concentrations ranging from 2 to 98% at 1% intervals. Equal volumes of ethanol solution and milk sample were mixed in a Petri-dish for observing the precipitation formation. The maximum concentration of ethanol solution which did not precipitate the milk was recorded as the ethanol stability (ES) of the milk.

Ionic Calcium Concentration Measurement

The ionic calcium concentration (iCa) in milk was measured using a pH/ion analyser plus a calcium direct ION flow cells (ion selective electrode, ISE) at the room temperature.

Total Calcium Concentration Measurement
The EDTA titration method was used for total calcium concentration (tCa) measurement. A 5 mL milk sample was added with 1 mL ammonia buffer solution and 0.02 mL calmagite indicator. The mixture was titrated against 0.01 M EDTA for tCa concentration measurement.

\[
\text{% calcium} = \left( \frac{0.004 \times \text{mL of 0.01 M EDTA}}{5} \right) \times 100
\]

\[
\text{mM calcium} = \frac{10}{4} \times \left( \frac{0.004 \times \text{mL of 0.01 M EDTA}}{5} \right) \times 100
\]

\[= 2 \times \text{mL of 0.01 M EDTA}\]

**Titratable Acidity and pH Measurement**

Titratable acidity (TA) was measured according to the Chinese National Standard method (CNS C6057, Taiwan, 1972). The pH test was carried out with a pH meter (Suntex SP-701 pH/mV/Temp meter, Taiwan) at room temperature.

**Gross Compositions Measurement**

Gross compositions were measured with the Milkoscope (Expert-2059, Germany) for analysing fat, protein, lactose and solids-non-fat (SNF) contents.

**Statistical Analysis**

According to the Chinese National Standard (CNS), the pass-or-fail standard of ethanol stability is 70%, which means the batch of milk will be rejected if flocculation were formed with an equal volume of 70% (v/v) ethanol solution added. However, diary industry sets the pass-or-fail standard of ethanol stability at 75%. Therefore, all data were statistically analyzed as 3 categories, which were low ethanol stability (LES, < 70%), medial ethanol stability (MES, 70%~74%) and high ethanol stability (HES, > 74%), respectively. Duncan's multiple range test and the least-square means were used for determining the significant levels between means and among treatments. All analyzes were performed as the general linear model (PROC GLM) of SAS.

**RESULTS AND DISCUSSION**

Figure 1 presents the distribution of milk samples of various ethanol stabilities (N =1,248). The percentages of low ethanol stability (LES), medial ethanol stability (MES), and high ethanol stability (HES) milk samples were 20.2%, 17.2%, and 62.6%, respectively. Ethanol stability measurement was used as an indication of protein stability, which represented that a milk with higher ethanol stability showed a higher casein micelle stability.

The average ethanol stability (ES) of LES, MES and HES milk samples were 61, 73, and 80%, respectively. The average ionic calcium concentration (iCa) of LES, MES, and HES milk samples were 3.09, 2.44, and 2.35 mM, respectively. The average total calcium concentration (tCa) of LES, MES and
Figure 1  Distribution of various ethanol stabilities in this study.
LES: low ethanol stability, < 70%. MES: medial ethanol stability, 70%~74%. HES: high ethanol stability, > 74%. N=1,248 samples.

MES and HES milk samples were 0.14, 0.13, and 0.13%, respectively. The average pH value of LES, MES, and HES milk samples were 6.63, 6.71, and 6.72, respectively (Table 1). Results of physicochemical properties of milk samples showed that iCa, tCa, and TA of LES milk samples were significantly higher than other treatments; but pH value was significantly lower in LES milk.

The total calcium content of cow’s milk is about 30 mM. Calcium in milk is partitioned between soluble and colloidal (dispersed) phases. Colloidal calcium which is associated with the casein micelles is about 20 mM; the soluble calcium which is in soluble phases is about 10 mM. The ionic calcium, about 2 mM in milk, has a pronounced effect on casein micelle stability and dairy processing characteristics (Lin, 2002). Chavez et al. (2004) reported that the addition of iCa increased ethanol unstability. The ethanol stability of milk, i.e., the stability of milk proteins to precipitation by ethanol, is also pH dependent. A typical ethanol stability pH-profile is sigmoidal when stability increasing with pH (De Kruif, 1999). The pH-dependence of the ethanol stability of milk may be attributed to pH-induced shifts in the mineral balance in milk, especially in the level of soluble calcium (Lin, 2002; Wang, 2005; Lin et al., 2006; Tsioulpas et al., 2007).

Calcium chloride addition reduced pH and ethanol stability, increased ionic calcium. Lin et al. (2006) reported that readjusting the pH range from 6.50 to 7.05 before calcium addition reduced the ionic calcium concentration, which indicated that the iCa did not recover to its original value. Moreover, milks from individual cows showed wide variations in their ionic calcium concentration.

In this study, Table 1 showed that tCa concentration was positively related to milk protein contents, which is in agreement with the results of Chow and Hu (1997). Chen (2002) also reported that the titratable acidity was affected by milk protein, somatic cell counts and parity.

Table 2 presents the average milk components and milk yield of LES, MES and HES milk in this study. The average fat content of LES, MES and HES milk were 3.81, 3.09, and 2.91%, respectively. The average protein content of LES, MES and HES milk were 3.20, 3.08, and 3.06%, respectively. The
Table 1  Physicochemical properties of milk samples of various ethanol stabilities

<table>
<thead>
<tr>
<th>Treatments(^1)</th>
<th>(^2)ES (%)</th>
<th>(^3)iCa (mM)</th>
<th>(^4)tCa (mM)</th>
<th>(^5)TA (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>LES (&lt; 70%)</td>
<td>61 ± 6(^a)</td>
<td>3.09 ± 0.59(^a)</td>
<td>33.0 ± 2.9(^a)</td>
<td>0.14 ± 0.02(^a)</td>
<td>6.63 ± 0.09(^b)</td>
</tr>
<tr>
<td>(N = 252)</td>
<td>(N = 249)</td>
<td>(N = 249)</td>
<td>(N = 252)</td>
<td>(N = 249)</td>
<td></td>
</tr>
<tr>
<td>MES (70~74%)</td>
<td>73 ± 1(^b)</td>
<td>2.44 ± 0.41(^b)</td>
<td>30.7 ± 2.8(^b)</td>
<td>0.13 ± 0.02(^b)</td>
<td>6.71 ± 0.07(^a)</td>
</tr>
<tr>
<td>(N = 215)</td>
<td>(N = 215)</td>
<td>(N = 212)</td>
<td>(N = 212)</td>
<td>(N = 209)</td>
<td></td>
</tr>
<tr>
<td>HES (&gt; 74%)</td>
<td>80 ± 3(^c)</td>
<td>2.35 ± 0.45(^c)</td>
<td>30.1 ± 2.9(^c)</td>
<td>0.13 ± 0.02(^b)</td>
<td>6.72 ± 0.12(^a)</td>
</tr>
<tr>
<td>(N = 778)</td>
<td>(N = 781)</td>
<td>(N = 781)</td>
<td>(N = 779)</td>
<td>(N = 775)</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>3.37</td>
<td>0.47</td>
<td>2.88</td>
<td>0.02</td>
<td>0.11</td>
</tr>
</tbody>
</table>

\(^1\) LES: low ethanol stability, < 70%; MES: medial ethanol stability, 70%~74%; HES: high ethanol stability, > 74%. \(^2\)ES: ethanol stability. \(^3\)iCa: ionic calcium concentration. \(^4\)tCa: total calcium concentration. \(^5\)TA: titratable acidity, Mean±SD.

\(^a,b,c\) Means within a column with different superscripts are significantly different (P < 0.05).

Table 2  Gross compositions and milk yield of milk samples of various ethanol stabilities

<table>
<thead>
<tr>
<th>Treatments(^1)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>(^2)SNF (%)</th>
<th>Lactose (%)</th>
<th>Milk yield (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LES (&lt; 70%)</td>
<td>3.81 ± 0.89(^a)</td>
<td>3.20 ± 0.16(^a)</td>
<td>8.71 ± 0.46(^a)</td>
<td>4.58 ± 0.37(^a)</td>
<td>16.66 ± 2.44(^b)</td>
</tr>
<tr>
<td>(N = 252)</td>
<td>(N = 252)</td>
<td>(N = 252)</td>
<td>(N = 252)</td>
<td>(N = 23)</td>
<td></td>
</tr>
<tr>
<td>MES (70~74%)</td>
<td>3.09 ± 0.90(^b)</td>
<td>3.08 ± 0.18(^b)</td>
<td>8.51 ± 0.48(^b)</td>
<td>4.54 ± 0.28(^a)</td>
<td>18.81 ± 4.99(^a)</td>
</tr>
<tr>
<td>HES (&gt; 74%)</td>
<td>2.91 ± 1.00(^c)</td>
<td>3.06 ± 0.18(^b)</td>
<td>8.51 ± 0.47(^b)</td>
<td>4.56 ± 0.30(^a)</td>
<td>20.21 ± 4.55(^a)</td>
</tr>
<tr>
<td>(N = 781)</td>
<td>(N = 781)</td>
<td>(N = 781)</td>
<td>(N = 781)</td>
<td>(N = 122)</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.96</td>
<td>0.17</td>
<td>0.47</td>
<td>0.31</td>
<td>4.35</td>
</tr>
</tbody>
</table>

\(^1\) LES: low ethanol stability, < 70%; MES: medial ethanol stability, 70%~74%; HES: high ethanol stability, > 74%. \(^2\)SNF: solids-not-fat, Mean±SD.

\(^a,b,c\) Means within a column with different superscripts are significantly different (P < 0.05).

average SNF content of LES, MES and HES milk were 8.71, 8.51, and 8.51%, respectively. In addition, the average milk yield of LES, MES and HES milk were 16.66, 18.81, and 20.21 kg, respectively (Table 2). Results of gross compositions showed that fat, protein and SNF contents of LES milk were significantly higher than other treatments; but the lactose contents were not significantly different. The milk yield of LES was lower than MES and HES milk.

There are many factors affecting gross compositions, such as season, feeds, lactation stages and genetic factors (Chen, 2002; Chow and Hu, 1997; Hung, 2008). Table 2 showed that ethanol stability increased, but milk protein content decreased in summer. Milk protein and milk yield was greatly affected
The Figure 2 shows the ethanol stability and ionic calcium concentration of milk samples at various ethanol stability levels. The average ES of LES, MES, and HES milk samples were 61 ± 6, 73 ± 1, and 80 ± 3%, respectively. The average iCa of LES, MES and HES milk samples were 3.09 ± 0.59, 2.44 ± 0.41, and 2.35 ± 0.45 mM, respectively. ES in samples with less than 2.35 mM iCa is consistently higher than 80%. Results indicated that the iCa in LES milk was significantly higher, but ES of LES milk was significantly lower than other treatments.

Tsioulpas et al. (2007) reported that casein micelles are stabilized through their surface negative charge which is affected by the amount of bound Ca. They postulated that the higher the concentration of ionic calcium is in the system, the more Ca is available for binding; moreover, high iCa causes the negative charge to reduce and promotes instability at lower concentration of ethanol.

The relationships between ES and iCa are shown in Figure 3. There was a significant negative relationship between iCa and ethanol stability in milk (Lin, 2002; Wang, 2005; Tsioulpas et al., 2007). The ES and iCa of milk samples with various ethanol stabilities were 42~88% and 1.30~4.45 mM, respectively. An earlier study found a significant linear relationship (r = -0.76, P < 0.05) between free Ca$^{2+}$ and ES using the same electrode system. The free Ca$^{2+}$ values in the study ranged between 1.0 and 3.4 mM (Lin, 2002).
CONCLUSIONS

The titratable acidity, total and ionic calcium concentrations in low ethanol stability (LES) milk samples were higher than other treatments, but pH value lower than other treatments. Fat, protein and solids-not-fat contents in LES milk were significantly higher than other treatments; however, milk yield of LES milk was lower. There was a negative correlation between ionic calcium concentration and ethanol stability. Therefore, there was 20% LES milk, unstable in less than 70% ethanol solution, in summer milk in the Eastern Taiwan. The LES milk also showed poor physicochemical properties with high ionic calcium concentration.

ACKNOWLEDGEMENTS

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REFERENCES


東台灣熱季期間乳牛生乳品質之探討

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摘要：本研究目的主要探討台灣於熱季期間乳牛生乳之品質。自 2007 年 7~8 月間，於花蓮縣某一乳牛場進行採樣，所有乳樣皆進行乳組成分、滴定酸度、酒精安定性、pH 值、總鈣及游離性鈣離子濃度之分析。依酒精安定性將試驗結果分為低酒精安定性（LES，< 70%）、中酒精安定性（MES，70~74%）及高酒精安定性（HES，> 74%）三組，進行統計分析。試驗所得乳樣共計 1,248 個樣品，LES、MES 和 HES 之比例分別為 20.2%、17.2% 和 62.6%。結果顯示，LES 組滴定酸度、總鈣及游離性鈣離子濃度顯著高於其他處理組，但 pH 值則顯著較低。乳組成分以 LES 組乳脂肪、乳蛋白質和無脂固形物含量顯著高於其他處理組；然而乳產量則以 LES 較低。綜合上述，以 LES 組乳樣之酒精安定性最差，且游離性鈣離子濃度最高；游離性鈣離子濃度與酒精安定性呈負相關，隨酒精安定性越高，游離性鈣離子濃度則越低。

（關鍵語：乳牛生乳、酒精安定性、乳組成分、理化性狀）