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ETHANOL STABILITY OF ALXA BACTRIAN CAMEL MILK

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ABSTRACT

Ethanol stability of fresh milk samples from ten healthy 5-year old Alxa bactrian camels was investigated. Camel's milk samples precipitated upon addition of equal volume 75% ethanol, whereas fresh cow's milk precipitated at 77% ethanol addition. The effects of sodium and potassium on ethanol stability were also investigated. Addition of sodium chloride and potassium chloride resulted in considerable decrease in ethanol stability.

Key words: Alxa bactrian camel, camel milk, ethanol stability

There are three fine breeds of *Camelus bactrianus* in China, namely Xinjiang, Alxa and Sunite camel. Alxa can be further divided into Gobi camel and Desert camel based on their stature, physical feature and breed distinctions. Camels are distributed in more remote and backward areas in China, where quality of camel milk can not be tested. In the past, the ethanol test was used widely as a simple indicator of cow milk freshness. The ethanol stability test may provide a practical quality test for Alxa bactrian camel milk. However, the composition of camel milk and bovine milk differs considerably and thus ethanol stability also differs.

Milk ethanol stability (MES) was defined as the minimum concentration of added aqueous ethanol that gives rise to milk coagulation (Horne and Parker, 1979). MES was used as a simple, cheap, efficient and quick pass-or-fail test to detect milk sourness in many countries. Test was also applied to predict milk heat stability.

Horne and Parker (1981a) found that serum phase components govern the sigmoidal shape and position of the ethanol stability/pH profile. Moreover, these authors (1981a, b) confirmed that among serum phase components the ionic calcium concentration played an important role, a fact previously observed by Davies and White (1958). Salts (calcium, magnesium, phosphorus and citrate) were reported to influence ethanol/pH profile parameters (Donnelly and Horne, 1986; Horne, 1987). Other important variables to ethanol stability previously found were the ionic strength (Horne, 1987) and the pH (Horne and Parker, 1979; Horne, 1992). All this valuable information was obtained by changing the original micelle equilibrium in some way, e.g. pH or dialysis of milk.

The ethanol stability of bovine, caprine and goat milk have been studied and discussed extensively by Horne and Parker (1981a-d), Horne (1987, 1992), Chen and He (1993), Horne and Muir (1990), Gao (1992), Guo *et al* (1994), Horne and Parker (1982) and Guo *et al* (1998).

However, the information about the ethanol stability of camel milk is scarce in available literature. Therefore, it is necessary to investigate the stability of Alxa bactrian camel milk to ethanol in order to establish a practical standard alcohol test for camel milk industry.

Materials and Methods

Collection of samples

Sampling was done 90 days post partum. All the samples collected and transported on ice to the laboratory and were stored at -40°C until analysis. During testing samples taken at the same stage of lactation were thawed, pooled and portions taken for analysis. The control cow milk was sampled from Holstein, which were first birth and were in the 90 day of lactation, on the Inner Mongolia Agricultural University farm.

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Physical parameters analyses

Milk sample physical parameters were measured as follows: titrable acidity (TA) and specific gravity were determined according to the method of Association of Official Analytical Chemists (AOAC, 1990a).

Chemical analysis

Nitrogen was determined by the Kjeldahl method. A nitrogen conversion factor of 6.38 was used for calculation of protein contents of milk samples. Dry matter (DM) of the samples was determined gravimetrically after drying in a forced-draft oven at 105°C until a steady weight was achieved. Fat percentage was determined according to the method of Rose-Gottlieb and ash content was measured gravimetrically (Aggarawala and Sharma, 1961). Lactose content was determined by the difference of DM minus other solid components.

Ethanol stability test

Alcohol test was performed by mixing equal volumes (2 ml) of milk sample and ethanol solution (water/ethanol ranging from 10 to 100%, v/v) at room temperature (Monica *et al*, 2004; Guo *et al*, 1998). Effects of sodium and potassium on ethanol stability were determined by addition of sodium chloride or potassium chloride (w/v 2, 4, 6, 8, 10%,) to milk samples before the alcohol tests at room temperature. Effects of pH on ethanol stability was measured by adjusting the pH (ranging from 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4 and 7.6) of the milk sample by adding 2N HCl or NaOH (Guo *et al*, 1994). Effects of removal of Ca^{2+} on ethanol stability was measured by treatment with EDTA solution (ranging from 0.1, 0.2, 0.4 to 1.0ml, v/100v), 0.1 milligram Ca^{2+} could be chelated by 1 millilitre EDTA solution.

Results and Discussion

Chemical composition

The gross composition of camel milk and cow milk was showed in Table 1. The titrable acidity was denoted in terms of lactic acid content (g/100g). The contents of fat, protein, lactose, total solids and ash in camel milk were higher than cow milk. However, the values of acidity (%) and specific gravity were similar to cow milk.

Ethanol stability

The results of the present study confirmed that ethanol stability of camel milk was slightly lower than that of cow milk. The average value was 75±2.0% for camel milk compared with 77±1.0% for the control cow milk. This difference may be due to the lack of b-casein or low amount of b-casein in bactrian milk and high content of Ca^{2+} in camel milk.

Farah and Farah-Riesen (1985) suggested that two casein fractions homologous to bovine a-casein and b-casein, however, there was no protein bands homologous to bovine b-casein be clearly detected in dromedary camel milk. Naima *et al* (2005) reported that there was absence of b-casein in Algerian dromedary camel milk (Larbaa breed), whereas there was very low intensity of b-casein in camel milk (Targui breed). Zhang *et al* (2005) revealed that protein bands homologous to bovine b-casein was not clearly detected in Alxa bactrian camel milk.

Although casein fractions have been isolated in camel milk and were found to be homologous with bovine casein (Larsson and Mohamed, 1986), the balance between the different casein fractions was very different. The authors reported that there was a low amount of b-casein only about 5% of the total casein, compared with about 13.6% in bovine casein (Farah, 1993). Therefore, there was absence of b-casein or very low content of b-casein in camel milk. Steric stability of casein micelles is dependent on many factors, b-casein is only one factors (Walsra and Jenness, 1990), thereby, absence of b-casein could facilitate the ethanol-mediated coagulation (Horne, 1992).

Zadow *et al* (1983) reported that high content of Ca^{2+} may reduce the ethanol stability of cow milk. The present study results showed that the content of Ca for camel milk was higher (154.6mg/100g) than that of bovine milk (118.40mg/100g).

Effect of sodium and potassium

In order to investigate the effects of sodium and potassium on alcohol stability of camel milk, sodium chloride or potassium chloride was added to samples before the stability test was performed. Fig 2 revealed that ethanol stability of camel and

Table 1. Gross composition, titratable acidity and specific gravity of camel and cow milk (mean values ±sd).

	Camel milk	Bovine milk
Protein (%)	3.55 ± 0.04	3.15±0.08
Fat (%)	5.65 ± 0.12	3.90±1.03
Lactose (%)	4.24 ± 0.12	4.17±0.24
Total Solid (%)	14.31 ± 0.19	11.09±0.42
Ash (%)	0.87 ± 0.03	0.79±0.04
Acidity (%)	0.17±0.013	0.16±0.016
Specific Gravity	1.028±0.001	1.028±0.001

Data are means of triplicate determinations.

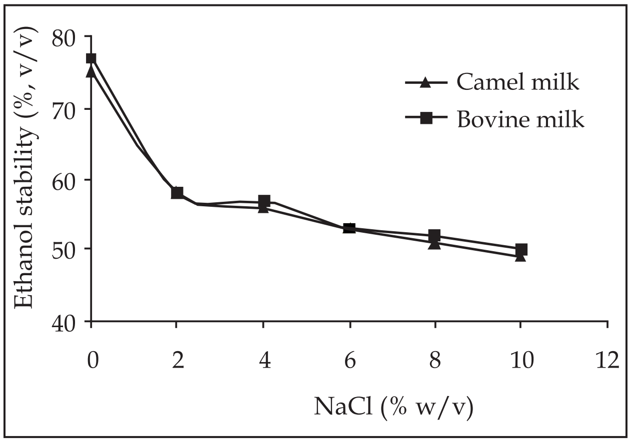

Fig 1. Effect of the addition of sodium chloride on ethanol stability of camel and bovine milk and their mean values.

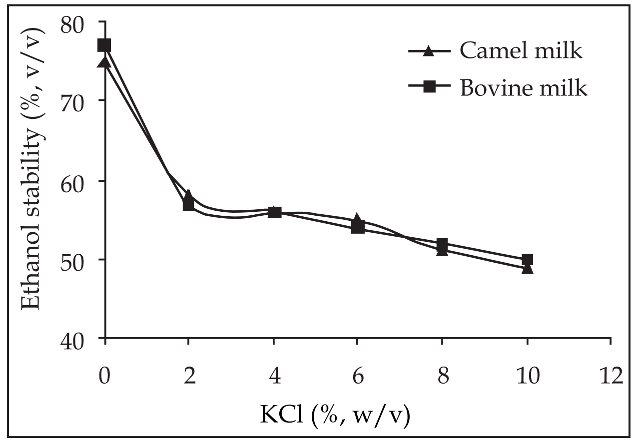

Fig 2. Effect of the addition of potassium chloride on ethanol stability of camel and bovine milk and their mean values.

bovine milk decreased sharply ($p<0.01$) from about 75 to 58% and 77 to 57%, respectively in the presence of 2% (w/v) sodium chloride in the samples. The stability was further decreased as sodium chloride concentration was increased up to 10% (w/v), and the ethanol stability reached 49 and 50% for camel and bovine milk samples, respectively. It was suggested that there was the same variation trendline between camel and bovine milk samples. The results confirmed that the maximum ethanol stability was decreased for camel and bovine milk by addition of sodium chloride to samples. The results were similar to reported by Horne and Parker (1981a).

On the other hand ethanol stability of camel and bovine milk was dramatically decreased by adding potassium chloride to samples (Fig 2). The average value of the stability was 75 and 77% before adding potassium chloride to camel and bovine milk. The ethanol stability was reduced dramatically to 49 and 50% on average for the camel and bovine milk samples with 10% KCl. The results showed that addition of K^+ to camel and bovine milk also decreases the ethanol stability.

The ethanol stability was decreased for camel and bovine milk by adding sodium chloride and potassium chloride. The results were not similar to goat milk (Guo *et al*, 1998). Reasons for the effect of Na^+ or K^+ on the ethanol stability of camel milk were not reported, Na/K ratio may account at least partially for the phenomena.

The value of Na/K ratio was 0.38 and 0.35 for Alxa bactrian camel and cow milk samples, respectively. The Na/K ratio of dromedary camel milk was 0.44, 0.34, 0.44, 0.48, 0.34 and 0.32, reported by Sawaya *et al* (1984), Abu-Lehia (1987), Mehaia and Al-Kahnal (1989), Mehaia *et al* (1995), Gorban and Izzeldin (1997) and Shamsia (2009), respectively. Therefore, the Na/K ratio of Alxa bactrian camel mik was similar to dromedary camel milk.

The Na/K ratio of Alxa bactrian camel mik was higher than that of goat milk (0.23, 0.27) as reported by Guo *et al* (1998) and Rincon *et al* (1994), at the same time the ratio of Na/K in camel milk was higher than that of goat milk as reported by Luo and Guo (1991). However, the ratio of Na/K in camel milk was similar to cow milk (0.38, 0.34 and 0.33) as reported by Guo *et al* (1998), Rincon *et al* (1994) and Young and George (2006). Therefore, there was no difference in Na/K between camel and bovine milk.

Effect of Ca^{2+}

Effects of Ca^{2+}on ethanol stability was measured by removal of Ca^{2+} treatment with EDTA solution. Fig 3 showed that the stability was increased for camel milk, while the level of Ca^{2+} was decreased by addition EDTA solution. The patter of ethanol stability/removel Ca^{2+} for camel milk was in agreement with that for cow milk. Horne and Muir (1990) reported that the calcium ion plays the dominant role in controlling the ethanol stability, and the level of free calcium controls the ethanol stability of the system. The authors reported that the ehtanol stability of bovine milk would be decreased by increasing the level of soluble Ca (Thom *et al*, 2004).

Effect of pH

The effects of pH on ethanol stability of milk samples was measured by adjusting the pH with 2N HCl or NaOH in the range 6.0 to 7.6 before adding ethanol solution. Fig 4 showed that the pattern of ethanol stability/pH curve for camel milk was

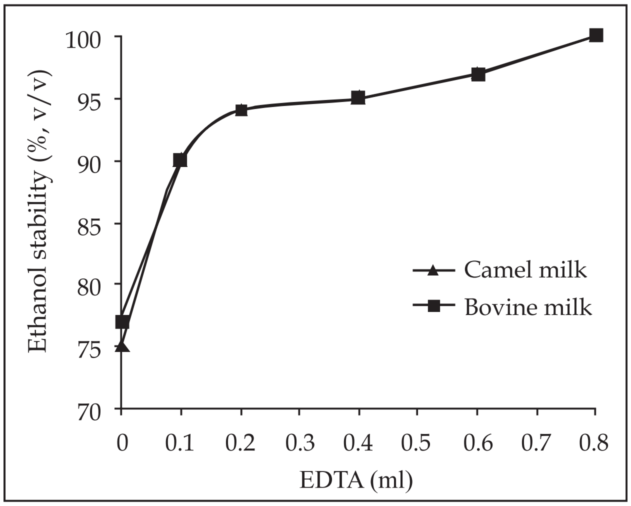

Fig 3. Effects of removal of Ca^{2+} on ethanol stability of camel and bovine milk and their mean values.

different compared with that for cow milk which was typically a sigmoidal. The pattern of ethanol stability/pH curve was similar to that observed by Horne (1992). Ethanol stability of camel milk was increased, when pH increased from 6.0 to 6.6, and was higher than that of cow milk. The stability was similar between camel and bovine milk samples at the pH (6.8), whereafter, the stability of camel milk was increased, while pH increased from 6.8 to 7.6. However, the stability of camel milk was lower than that of cow milk at this pH.

The most obvious candidate among the serum phase components is phosphate, which has such a transition in this pH range. The doubly ionized form of phosphate is much more effective calcium sequestrant (Horne and Muir, 1990). Increasing the pH of the milk thus will reduce the free calcium level and therefore increases ethanol stability, as observed. In contrast, decreasing the pH of the milk will enhance the free calcium level and leads to a decrease in ethanol stability.

The difference of ethanol stability/pH curve between camel and cow milk may be due to the absence of b-casein or very low content of b-casein. On the side, the difference of ethanol stability/pH curve between camel milk and cow milk may be related to the size of casein micellar. Zhao (2006) revealed that the size of casein micellar in Alxa bactrian camel milk was larger than that of cow milk. The authors reported that the casein micellar size distribution of camel milk was significantly broader than that of cow milk and showed a greater number of large particles, and the average diameter of casein was 320 and 160 nm for camel and cow milk, respectively (Larsson-Raznikiewicz and Mohamed, 1986; Farah and Ruegg, 1989).

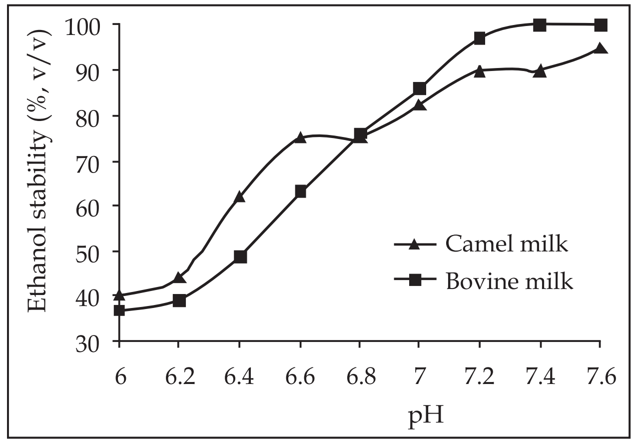

Fig 4. Ethanol stability/pH of camel and bovine milk.

Conclusions

The results of the present study confirmed that ethanol stability of Alxa bactrian camel milk was 75%, which is slightly lower than that of cow milk. The ethanol stability of bactrian camel milk was decreased with addition of sodium chloride and potassium chloride. Reasons for the effect of pH on the ethanol stability of camel milk are not clear. Further studies are needed in order to clarify the mechanism that the effect of pH on ethanol stability for camel milk.

References

Abu-Lehia (1978). Composition of camel milk. Milchwissenchaft 42:368-371.

Aggarawala AC and Sharma RM (1961). A Laboratory Manual of Milk Inspection. 4th ed. Asia Publishing House, Bombay, India.

AOAC (1990a). Acidity of milk (Titrimetric method). In: Official Methods of Analysis, No. 947.05. Association of Official Analytical Chemists Inc. Virginia, USA.

Chen YL and He YL (1993). Studies on the low-acidity alcohol test positive bovine milk. Journal of Jiangxi Agricultural University 10:62-65

Donnelly WJ and Horne DS (1986). Relationship between ethanol stability of Bovine and natural variations In milk composition. Journal of Dairy Research 53:23-33.

Farah Z and Farah-Riesen M (1985). Separation and characterisation of major components of camel milk casein. Milchwissenschaft 40:669-671.

Farah Z (1993). Composition and characteristics of camel milk. Journal of Dairy Research 60:603-626.

Farah Z and Ruegg MW (1989). The size distribution of casein micelles in camel milk. Food Microstructure 8:211-216.

Gao DX (1992). Studies on the relationship between titratable acidity and alcohol stability of caprine milk. Proceedings of the 1st Congress on Animal Products Between the Strains. C.X. Luo and C.W. Lin Eds, Nanjing, P.R. China. pp 111-118.

Gorban AMS and Izzeldin OM (1997). Mineral content of camel milk and colostrum. Journal of Dairy Research 64:471-474

Guo MR, Kindstedt PS, Politis I, Li Z and Cheng T (1994). Alcohol stability of caprine milk. Journal of Dairy Science 77(Suppl.):13.

Horne DS (1987). Ethanol stability of casein micelles-a hypothesis concerning the role of calcium phosphate. Journal of Dairy Research 54:389-395.

Horne DS (1992). Ethanol Stability. In Advanced Dairy Chemistry, Vol. 1 (Ed. PFFox). London: Elsevier Applied Science Publisher. pp 657-689.

Horne DS and Parker TG (1979). The pH sensitivity of the ethanol stability of in dividual cow milks. Netherlands Milk Dairy Journal 34:126-130

Horne DS and Parker TG (1981a). Factors affecting the ethanol stability of bovine milk. I. Effect of serum phase components. Journal of Dairy Research 48:273-284

Horne DS and Parker TG (1981b). Factors affecting the ethanol stability of bovine milk. II. The origin of the pH transition. Journal of Dairy Research 48:285-291.

Horne DS and Parker TG (1981c). Factors affecting the ethanol stability of bovine casein micelles: 3. Substitution of ethanol by other organic solvents. International Journal of Biological Macromolecules 3:399-402.

Horne DS and Parker TG (1981d). Factors affecting the ethanol stability of bovine milk. IV. Effect of forewarming. Journal of Dairy Research 48:405-415.

Horne DS and Parker TG (1982). Some aspects of the ethanol stability of caprine milk. Journal of Dairy Research 49:459-468.

Horne DS and Muir DD (1990). Alcohol and heat stability of milk protein. Journal of Dairy Science 73:3613-3626.

Larsson-Raznikiewicz M and Mohamed MA (1986). Analysis of the casein content in camel (*Camelus dromedarius)* milk. Swedish Journal of Agricultural Research 16:13-18.

Luo CX and Guo MR (1991). Changes in some minerals of goats milk during lactation. China Dairy Industry 19:195-200, 214.

Mehaia MA, Hablas MA, Abdel-Rahman KM and El-Mougy SA (1995). Milk-Composition of Majaheim, Wadah and Hamra Camels in Saudi-Arabia. Food Chemisty 52:115-122.

Mehaia MA and Al-Kahnal MA (1989). Studies on camel and goat milk proteins: Nitrogen distribution and amino acid composition. Nutrition Reports International 39(2):351-357.

Monica S Chave, Livia M Negri and Miguel A (2003). Taverna and Alejandra Cuatrin. Bovine milk composition parameters affecting the ethanol stability. Journal of Dairy Research 6:201-206.

Naima Alim, Fabio Fondrini, Ivan Bonizzi, Maria Feligini and Giuseppe Enne (2005). Characterisation of Casein fractions from algerian dromedary (*Camelus dromedarius*) milk. Pakistan Journal of Nutrition 4(2): 112-116.

Rincon F, Moreno R, Zurera G and Amaro M (1994). Mineral composition as characteristic of goat milk from the identification of animal origin of raw milk. Journal of Dairy Research 61:151-154.

Sawaya WN, Khalil JK, Al-Shalhat A and Al-Mohammad H (1984). Chemical composition and nutritional quality of camel milk. Journal of Food Science 49:744-747.

Shamsia SM (2009). Nutritional and therapeutic properties of camel and human milks. International Journal of Genetics and Molecular Biology 2:052-058.

Thom Huppertz, Sjoukje Grosman, Patrick F Fox and Alan L Kelly (2004). Heat and ethanol stabilities of high-pressure-treated bovine milk. International Dairy Journal 14:125-133.

Walstra P and Jenness R (1990). Dairy Chemistry and Physics. Wiley, New York. pp 234-237

Young W Park and George FW Haenlein (2006). Handbook of Milk of Non-Bovine Mammals. Australia: Blackwell Publishing Ltd, First Edition.

Zadow JG, Hardham JF, Kocak HR and Mayes JJ (1983). The stability of goat milk to UHT processing. Australian Journal of Dairy Technology 38:20-23

Zhao DianBo (2006). Studies on the Chemical Composition and Chemical-physical Properties of Alxa Bactrian Camels Milk in Inner Mongolia. Master's Thesis. ETH No 101153, China.

Zhang H, Yao J, Zhao D, Liu H, Li J and Guo M (2005) Changes in chemical composition of Alxa Bactrian camel milk during lactation. Journal of Dairy Science 88:3402-3410.