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Comparison of the Acidification Activities of Commercial Starter Cultures in Camel and Bovine Milk

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Abstract

Camel milk has been reported to be difficult to ferment due to anti-microbial properties. The present study tested eight commercial starter cultures for their ability to grow in camel milk. All investigated cultures were able to acidify camel milk and reached a final pH at a level similar to what was achieved in bovine milk, but the speed of acidification was generally lower in camel milk. This could be due to inhibitory substances in camel milk or due to reduced availability of nutrients. Experiments using mixtures of camel and bovine milk or supplementation with casein hydrolysates allowed us to distinguish between these possibilities. High acidification rates were obtained in camel milk mixed with bovine milk or supplemented with casein hydrolysate. This demonstrates that the cultures are not inhibited by camel milk and we conclude that the growth rates of these cultures in pure camel milk are limited by the rate of proteolysis.

Key words; acidification activity, fermented camel milk, dairy starter cultures, lactic acid bacteria, proteolysis

1. Introduction

Camels (*Camelus dromedarius*) are significant for many pastoralist communities of the dry zones of sub-Saharan Africa by providing milk, meat and transportation. More than half of the world's 28 million camels are found in the East African countries of Somalia, Sudan, Ethiopia and Kenya (FAO STAT, 2014). Camel milk has a gross composition similar to bovine milk. However, the relative composition, distribution and the molecular structures of the milk components are different and e.g. β-lactoglobulin is absent in camel milk. The sequence homology between milk proteins from camel and cow is in the range of 60 to 90 % (Kappeler, Farah, & Puhan, 1998).

It is commonly claimed that camel milk is technically more difficult to process into products than milk from other livestock and that it is only suitable for drinking (Al haj & Al Kanhal, 2010). Only few investigations have dealt with the possibilities of making camel dairy products through diligent adjustments in the technology. Some improvement of the production of butter (Berhe, Seifu, & Kurtu, 2013; Farah, Streiff, & Bachmann, 1989), cheese (Ahmed & Kanwal, 2004; Mehaia, 2006), and yoghurt (Ibrahem & El Zubeir, 2016; Hashim, Khalil, & Habib, 2009) have been described. Hence, there seems to be ample possibility to design and develop novel dairy products from camel milk.

Camel milk has been reported to be difficult to ferment because of the high content of anti-microbial components, thus, hindering acidification and curd formation (El-Agamy, Ruppanner, Ismail, Champagne, & Assaf, 1992). The relative concentration of lysozyme, lactoferrin, lactoperoxidase and immunoglobulins in camel milk is reported to be higher than for bovine milk (Elagamy, 2000; Kappeler, Ackermann, Farah, & Puhan, 1999; Konuspayeva, Faye, Loiseau, & Levieux, 2007).

Effective starter cultures are needed in order to produce value added fermented camel dairy products with extended shelf life. Currently, there are commercial starter cultures developed for bovine, sheep, and goat dairy industries. However, no data is available concerning the fermentation potential of such commercial starter cultures on camel milk. Therefore, the current research was undertaken to thoroughly characterize the acidification activities of commercial starter cultures in camel milk in comparison to bovine milk. This can ensure selection of better performing cultures and the optimization of incubation temperatures for fermentation of camel milk.

2. Materials and Methods

Pooled Camel milk (10 camels) and bovine milk (10 cows) samples were collected from Babile area and Haramaya University dairy farm in Ethiopia respectively. Eight lyophilized commercial starter cultures in 50-unit sachets were obtained from Chr. Hansen A/S (Denmark) (Table 1). The unit for starter cultures used by Chr Hansen A/S is defined as the activity of 100 ml of an active bulk starter culture and one unit of culture is suitable for the inoculation of 10 liters of milk.

Standardized inoculums were prepared by resuspending a 50-unit sachet of culture in 500 ml of autoclaved bovine milk. The resuspended cultures were distributed into 100 ml bottles and frozen at -20 °C. Fermentation experiments were conducted in milk which had been pasteurized at 65 °C for 30 minutes and cooled to the incubation temperatures. Inoculation of 250 ml portions of milk was done by adding 0.5 ml of the thawed inoculum. This is approximately twice the standard inoculation rate compared to direct use of the lyophilized culture. The increased rate of inoculation was used to compensate for the potential loss of activity due to the extra freeze-thaw procedure.

When milk was supplemented with casein hydrolysate, a level of 0.5 % (w/v) was reached by adding 1/20 of the volume of 10 % (w/v) casein hydrolysate (Sigma–Aldrich nr. 22090) dissolved in water. The stock solution had been autoclaved prior to use. Fermentations were conducted at 30 and 37 °C for the cultures R-704, R-707 and CHN-22; at 30, 37, and 42 °C for the cultures RST-743 and XPL-2; and at 37 and 42 °C for the cultures Yoflex mild 1.0, YF-L904 and STI-12. Acidifications were followed for 18 hours using an iCinac instrument (Alliance Instruments, Frepillon, France) which measures the pH, oxidation reduction potential and temperature of the culture simultaneously. The iCinac probes were first calibrated as per the manufacturer manual using buffers 4 and 7 supplied from the same company. The experiment was repeated two times and analysis was done in duplicate.

V_{max} and time to pH 4.6 were the parameters used to characterize the acidification activities of the starter cultures. V_{max} is the maximum acidification speed of pH drop per minute during the fermentation course. High acidification activity is equivalent to a high V_{max} and a short time to pH to 4.6. The V_{max} and time to pH 4.6 values are extracted from the acidification curves. Statistix 10.0 was used for data analysis. A three way full factorial design was used for the experiment taking V_{max} and pH to 4.6 as response variables. Least significant difference at (α = 0.5) was used for the mean comparison. The data were categorized into three groups and analyzed separately. Group I comprised of the mesophilic starter cultures (R-704, R-707 and CHN-22), group II comprised of mixed strains of

thermophilic and mesophilic cultures (RST-743 and XPL-2), and Group III comprised of thermophilic starter cultures (STI-12, Yoflex mild 1.0 and YF-L904).

3. Results and discussion

Tables 2 and 3 give the V_{max} and time to pH 4.6 of the eight investigated starter cultures in camel and bovine milk. Selected acidification curves obtained with those cultures are given in Figure 1.

There were significant differences ($p<0.05$) in the acidification activities of the cultures between camel and bovine milk and within the different incubation temperatures (Tables 2 and 3). The V_{max} and pH to 4.6 of group I cultures (R-704, R-707 and CHN-22) showed higher acidification activities at 30 than 37 °C in camel milk. Moreover, the acidification activities in bovine milk were higher than in camel milk at their corresponding incubation temperatures (Tables 2 and 3). The acidification curves for R-707, CHN-22 and STI-12 are presented in Figure 1. Similar acidification trends were observed for all three cultures of group I: incubation temperature of 30 °C was optimum and bovine milk was superior in acidification activities to camel milk. Thus, incubation temperature of 30 °C is recommended for the fermentation of camel milk using R-704, R-707 and CHN-22 starter cultures. The time to reach pH 4.6 in camel milk incubated at 30 °C was 8:10, 12:35 and 12:40 hours for R-707, CHN-22 and R-704 ,respectively. Therefore, R-707 is the best for the fermentation of camel milk among the three mesophilic starter cultures.

V_{max} values of RST-743 and XPL-2 under group II (Tables 2 and 3) cultures showed in camel milk highest acidification activities at 42 °C. There were no significant differences in v_{max} values of XPL-2 and RST-743 between 30 and 37 °C in camel milk. For RST-743 no significant difference in time to reach pH 4.6 was observed among the three incubation temperatures in camel milk. This may be attributed to the mixed strains of the culture that covers the mesophilic and thermophilic growth temperature ranges. Generally, higher acidification activities were observed in bovine milk than their corresponding values in camel milk. The acidification activity was higher in RST-743 than XPL-2 at the optimum incubation temperature.

Values of V_{max} for Yoflex mild.10 and YF-L904 under group III did not show significant difference between the incubation temperatures of 37 and 42 °C in camel milk. Similarly, values of pH to 4.6 for YF-L904 and STI-12 under group III did not show significant difference between the incubation temperatures of 37 and 42 °C in camel milk (Tables 2 and 3). However, Higher V_{max} value of STI-12 was observed at 42 °C than 37 °C in camel milk. Similar to the mesophilic starter cultures, the thermophilic cultures showed slower acidification activities in camel milk than bovine milk. STI-12 was the best among the thermophilic starter culture for the acidification of camel milk at 42 °C.

As a conclusion, all cultures were able to acidify camel milk and reached a final pH at a level similar to bovine milk, but the speed of acidification of all tested cultures was lower in camel milk than the corresponding bovine milk. The delay in fermentation time of the cultures in camel milk from cow milk was from 1:15 to 4:10 hours under the corresponding optimum incubation temperatures. This study has shown that camel milk could be acidified satisfactorily to the level that was achieved in bovine milk using commercial cultures. This disproves the claims that camel milk cannot be satisfactorily acidified due to its antimicrobial properties (El Agamy et al., 1992). A recent report Habtegebriel & Admassu (2016) also indicated that it was possible to acidify camel milk to pH 4.3 using commercial cultures.

To analyse if the delay of the acidification in camel milk is caused by antimicrobial activities in camel milk or if it is due to reduced availability of nutrients, we analyzed the acidification in milk supplemented with casein hydrolysate and in a 50:50 blend of camel and bovine milk. The acidification activities were tested using R-707 and Yoflex mild 1.0 at incubation temperatures of 30 and 42 °C respectively. The acidification activities in the casein hydrolysate supplemented camel milk were higher than in the non-supplemented camel milk and similar to the supplemented bovine milk. Moreover, also blending of camel milk with bovine milk improved the speed of acidification to a level similar to the acidification activity in bovine milk (Table 4 and Figure 2).

There was no significant difference in time to pH 4.6 values among the 50:50 blend and supplemented camel and bovine milk samples. For R-707 the time to pH 4.6 in camel milk at 30 °C was 8:10 hours. The fermentation time was reduced to 6:46 hours when supplemented by casein hydrolysate and to 5:48 hours when blended with bovine milk. For Yoflex mild 1.0 the fermentation time was reduced from 9:08 hours in camel milk to 3:20 in supplemented camel milk and 3:55 hours in the mixed milk.

This shows that addition of amino acids in the form of casein hydrolysate or addition of bovine milk can alleviate the delay of fermentation in camel milk. Based on this result we can conclude that antimicrobial activities are not responsible for the delay. Our conclusion is that the proteolytic systems of the tested cultures are unable in camel milk to support a growth rate as fast as in bovine milk. Although this conclusion is firmly based on the results of our experiments, it is less obvious to explain why the rate of proteolysis is lower in camel milk. Beta casein is the preferred substrate for the proteinases of lactic acid bacteria (Siezen, 1999) and camel milk is rich in beta casein (Kappeler et al., 1998). The cause of the retardation is therefore not obvious. It will be interesting to investigate why the beta casein of camel milk is less accessible than the beta casein of bovine milk.

177

178 **3.1. Conclusion**

179 Eight commercial starter cultures were tested and all were able to acidify camel milk and
180 reach a final pH at a level similar to bovine milk. However, the speed of acidification was
181 generally lower in camel milk than bovine milk. We have demonstrated that the difference in
182 speed in the two types of milk is due to difference in proteolysis rather than the presence of
183 inhibitory substance in camel milk. R-707 was found to be the best mesophilic culture and
184 STI-12 the best thermophilic culture for camel milk fermentation

185

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Figure 1: Acidification curves of R-707, CHN-22 and STI-12 cultures in camel and bovine milk incubated at their respective optimum temperatures.

Figure 2: Acidification curves of the R-707 culture incubated at 30 °C in camel, bovine, 50:50 blend and casein hydrolysate supplemented milk

1 Table 1: Description of the starter cultures used in the study

Culture	Taxonomy	Description
R-704	*Lactococcus lactis subsp. lactis* *Lactococcus lactis subsp. cremoris*	Mesophilic homo-fermentative O-culture
R-707	*Lactococcus lactis subsp. lactis* *Lactococcus lactis subsp. cremoris*	Mesophilic homo-fermentative O-culture
CHN-22	*Lactococcus lactis subsp. cremoris* *Leuconostoc pseudomesenteroides* *Lactococcus lactis subsp. lactis biovar diacetylactis* *Lactococcus lactis subsp. lactis* *Leuconostoc mesenteroides*	Mesophilic aromatic LD-culture (produces flavor and $CO_{2)}$
RST-743	*Lactococcus lactis subsp. lactis* *Streptococcus thermophilus*	Blend of mesophilic and thermophilic cultures
XPL-2	*Lactococcus lactis subsp. cremoris* *Lactococcus lactis subsp. lactis* *Lactococcus lactis subsp. lactis biovar diacetylactis* *Leuconostoc species* *Streptococcus thermophilus*	Blend of mesophilic aromatic LD and thermophilic cultures (produces texture, flavor and $CO_{2)}$
Yoflex mild 1.0	*Lactobacillus delbrueckii subsp. bulgaricus* *Streptococcus thermophilus*	Thermophilic yoghurt culture
YF-L904	*Lactobacillus delbrueckii subsp. bulgaricus* *Streptococcus thermophilus*	Thermophilic yoghurt culture
STI-12	*Streptococcus thermophilus*	Homofermentative thermophilic culture

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Table 2: Comparison of acidification activities of commercial starter cultures inoculated into camel and bovine milk.

Group	Culture	Camel milk V_{max} (ΔpH/minute)			Bovine milk V_{max} (ΔpH/minute)		
		30 °C	37 °C	42 °C	30 °C	37 °C	42 °C
I (Mesophilic cultures)	R-704	-0.0051^{f}	-0.0023^{i}		-0.0082^{c}	-0.0069^{d}	
	R-707	-0.0080^{bc}	-0.0047^{fg}		-0.0099^{a}	-0.0093^{ab}	
	CHN-22	-0.0060^{e}	-0.0033^{h}		-0.0080^{c}	-0.0042^{g}	
II (Mixture of mesophile and thermophile starains)	RST-743	-0.0066^{e}	-0.0060^{ef}	-0.0079^{d}	-0.0081^{d}	-0.0117^{b}	-0.0166^{a}
	XPL-2	-0.0042^{g}	-0.0052^{fg}	-0.0069^{de}	-0.0080^{d}	-0.0099^{c}	-0.0117^{b}
III (Thermophilic cultures)	Yoflex mild 1.0		-0.0067^{g}	-0.0071^{g}		-0.0116^{d}	-0.0157^{bc}
	YF-L904		-0.0073^{fg}	-0.0081^{f}		-0.0148^{c}	-0.0161^{b}
	STI-12		-0.0081^{f}	-0.0093^{e}		-0.0157^{bc}	-0.0173^{a}

Results are mean values of four analysis, means with the same letter across columns and rows within group are not significantly different ($p > 0.05$), CV (coefficient of variation) = 5.2, 6.5, 3.6 for Group I, II, and III respectively.

Table 3: Comparison of the time to reach pH 4.6 of commercial starter cultures inoculated into camel and bovine milk.

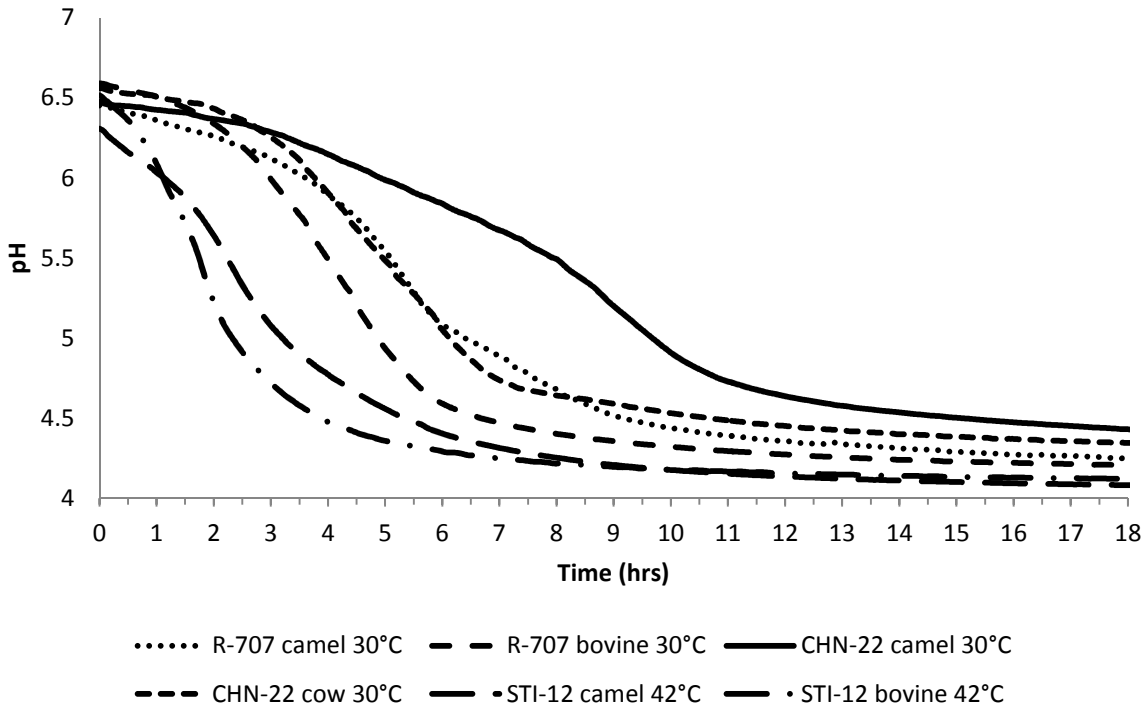
Group	Culture	Camel milk Time to pH 4.6 (h:min)			Bovine milk Time to pH 4.6 (h:min)		
		30 °C	37 °C	42 °C	30 °C	37 °C	42 °C
I (mesophilic cultures)	R-704	12:40^{c}	16:48^{b}		8:25de	9:35^{d}	
	R-707	8:10de	16:05^{b}		5:55^{f}	7:35^{ef}	
	CHN-22	12:35^{c}	21:15^{a}		9:10de	19:45^{a}	
II (Mixture of mesophile and thermophile strains)	RST-743	7:55^{ef}	7:52^{ef}	7:23^{f}	7:40^{f}	5:05^{g}	4:50^{g}
	XPL-2	13:40^{b}	15:08^{a}	9:58^{d}	11:20^{c}	8:54de	7:30^{f}
III (Thermophilic cultures)	Yoflex mild 1.0		8:30^{a}	8:27^{a}		4:30^{cde}	3:45^{ef}
	YF-L904		8:42^{a}	8:37^{a}		4:39^{cd}	4:03^{def}
	STI-12		5:32^{b}	5:10^{bc}		4:18^{def}	3:35^{f}

Results are mean values of four analysis, means with the same letter across columns and rows within group are not significantly different ($p > 0.05$), Coefficient of variation (CV) = 7.4, 8.6, 7.7 for Group I, II and III respectively.

Table 4: Acidification activities of R-707 and Yoflex mild 1.0 in camel, bovine, 50:50 mix and casein hydrolysate supplemented milk

Culture	Milk	V_{max}(ΔpH/minute)	Time to pH 4.6 (h:min)
R-707	Camel	-0.0080^{b}	$8:10^{a}$
	Camel+0.5% casein	-0.0097^{a}	$6:46^{b}$
	Bovine	-0.0099^{a}	$5:55^{b}$
	Bovine+0.5% casein	-0.0094^{a}	$6:34^{b}$
	50:50 blend	-0.0092^{a}	$5:48^{b}$
Yoflex mild 1.0	Camel	-0.0071^{c}	$9:08^{a}$
	Camel+0.5% casein	-0.0207^{a}	$3:20^{b}$
	bovine	-0.0157^{b}	$3:45^{b}$
	Bovine+0.5% casein	-0.0230^{a}	$3:32^{b}$
	50:50 blend	-0.0134^{b}	$3:55^{b}$

Results are mean values of four analysis, means with the same letter across columns within culture are not significantly different ($p>0.05$),coefficient of variation (CV) = 5.8 and 7.1 for V_{max} of R-707 and Yoflex mild 1.0 respectively, CV= 5.6 and 5.4 for pH 4.6 for R-707 and Yoflex mild 1.0 respectively.



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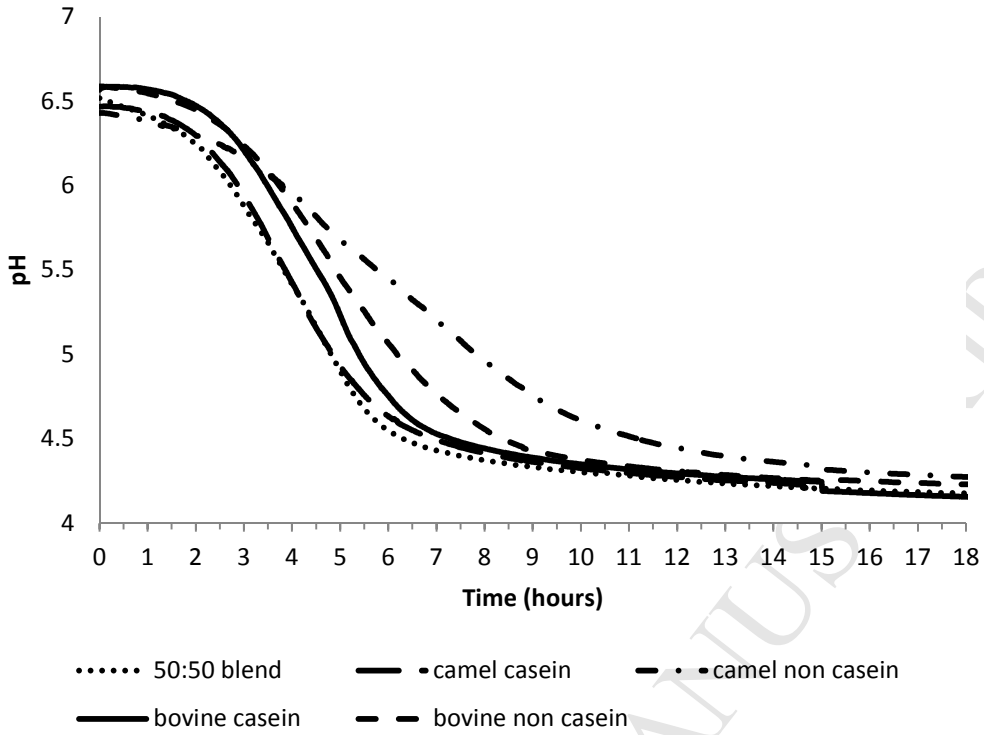

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Highlights

- ✓ Camel milk shows fermentation difficulties
- ✓ Acidification speed of 8 commercial cultures were relatively lower in camel milk
- ✓ Casein supplementation or blending improved the slow speed in camel milk
- ✓ The delayed speed is due to insufficient proteolysis than the inhibitory substances