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

3

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26 **Abstract**

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

38

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

41

42 1. Introduction

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

51 It is commonly claimed that camel milk is technically more difficult to process into products
52 than milk from other livestock and that it is only suitable for drinking (Al haj & Al Kanhal,
53 2010). Only few investigations have dealt with the possibilities of making camel dairy
54 products through diligent adjustments in the technology. Some improvement of the
55 production of butter (Berhe, Seifu, & Kurtu, 2013; Farah, Streiff, & Bachmann, 1989),
56 cheese (Ahmed & Kanwal, 2004; Mehaia, 2006), and yoghurt (Ibrahim & El Zubeir, 2016;
57 Hashim, Khalil, & Habib, 2009) have been described. Hence, there seems to be ample
58 possibility to design and develop novel dairy products from camel milk.

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

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

73 2. Materials and Methods

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

80 Standardized inoculums were prepared by resuspending a 50-unit sachet of culture in 500 ml
81 of autoclaved bovine milk. The resuspended cultures were distributed into 100 ml bottles and
82 frozen at -20 °C. Fermentation experiments were conducted in milk which had been
83 pasteurized at 65 °C for 30 minutes and cooled to the incubation temperatures. Inoculation of
84 250 ml portions of milk was done by adding 0.5 ml of the thawed inoculum. This is
85 approximately twice the standard inoculation rate compared to direct use of the lyophilized
86 culture. The increased rate of inoculation was used to compensate for the potential loss of
87 activity due to the extra freeze-thaw procedure.

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

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

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

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109 3. Results and discussion

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

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

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

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

143 As a conclusion, all cultures were able to acidify camel milk and reached a final pH at a level
144 similar to bovine milk, but the speed of acidification of all tested cultures was lower in camel
145 milk than the corresponding bovine milk. The delay in fermentation time of the cultures in
146 camel milk from cow milk was from 1:15 to 4:10 hours under the corresponding optimum
147 incubation temperatures. This study has shown that camel milk could be acidified
148 satisfactorily to the level that was achieved in bovine milk using commercial cultures. This
149 disproves the claims that camel milk cannot be satisfactorily acidified due to its antimicrobial
150 properties (El Agamy et al., 1992). A recent report Habtegebriel & Admassu (2016) also
151 indicated that it was possible to acidify camel milk to pH 4.3 using commercial cultures.
152 To analyse if the delay of the acidification in camel milk is caused by antimicrobial activities
153 in camel milk or if it is due to reduced availability of nutrients, we analyzed the acidification
154 in milk supplemented with casein hydrolysate and in a 50:50 blend of camel and bovine milk.
155 The acidification activities were tested using R-707 and Yoflex mild 1.0 at incubation
156 temperatures of 30 and 42 °C respectively. The acidification activities in the casein
157 hydrolysate supplemented camel milk were higher than in the non-supplemented camel milk
158 and similar to the supplemented bovine milk. Moreover, also blending of camel milk with
159 bovine milk improved the speed of acidification to a level similar to the acidification activity
160 in bovine milk (Table 4 and Figure 2).

161 There was no significant difference in time to pH 4.6 values among the 50:50 blend and
162 supplemented camel and bovine milk samples. For R-707 the time to pH 4.6 in camel milk at
163 30 °C was 8:10 hours. The fermentation time was reduced to 6:46 hours when supplemented
164 by casein hydrolysate and to 5:48 hours when blended with bovine milk. For Yoflex mild 1.0
165 the fermentation time was reduced from 9:08 hours in camel milk to 3:20 in supplemented
166 camel milk and 3:55 hours in the mixed milk.

167 This shows that addition of amino acids in the form of casein hydrolysate or addition of
168 bovine milk can alleviate the delay of fermentation in camel milk. Based on this result we can
169 conclude that antimicrobial activities are not responsible for the delay. Our conclusion is that
170 the proteolytic systems of the tested cultures are unable in camel milk to support a growth
171 rate as fast as in bovine milk. Although this conclusion is firmly based on the results of our
172 experiments, it is less obvious to explain why the rate of proteolysis is lower in camel milk.
173 Beta casein is the preferred substrate for the proteinases of lactic acid bacteria (Siezen, 1999)
174 and camel milk is rich in beta casein (Kappeler et al., 1998). The cause of the retardation is
175 therefore not obvious. It will be interesting to investigate why the beta casein of camel milk is
176 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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191

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236

- 1 Figure 1: Acidification curves of R-707, CHN-22 and STI-12 cultures in camel and bovine
- 2 milk incubated at their respective optimum temperatures.
- 3 Figure 2: Acidification curves of the R-707 culture incubated at 30 °C in camel, bovine,
- 4 50:50 blend and casein hydrolysate supplemented milk
- 5

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

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

2

3

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

Group	Culture	Camel milk V_{\max} (Δ pH/minute)			Bovine milk V_{\max} (Δ pH/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 strains)	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

6 Results are mean values of four analysis, means with the same letter across columns and rows within group are
 7 not significantly different ($p > 0.05$), CV (coefficient of variation) = 5.2, 6.5, 3.6 for Group I, II, and III
 8 respectively.
 9

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

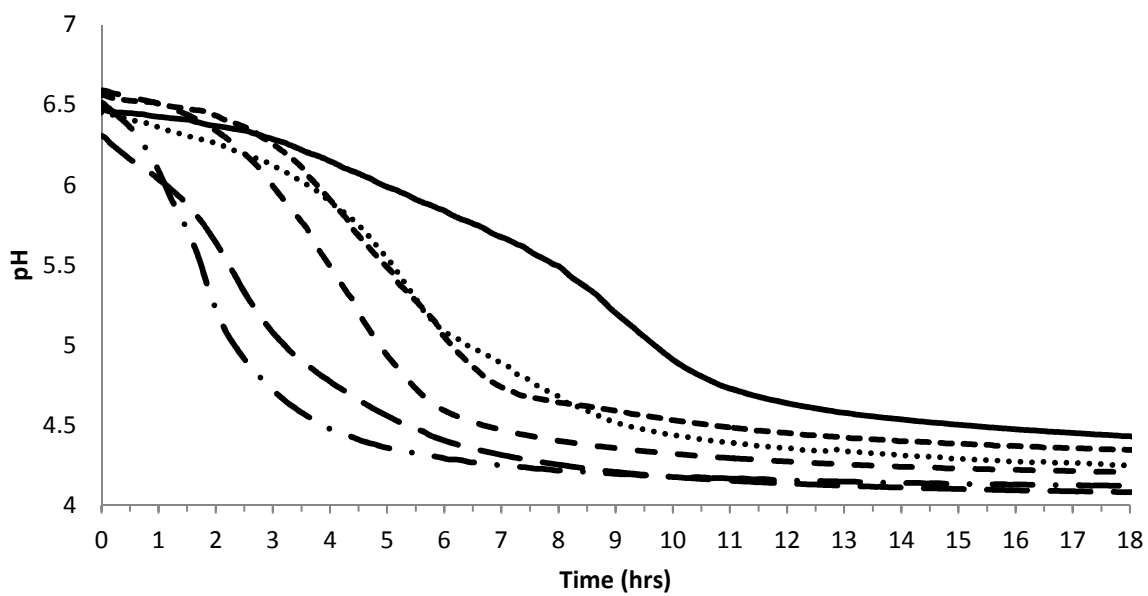
Group	Culture	Camel milk Time to pH 4.6 (h:min)			Bovine milk Time to pH 4.6 (h:min)		
		30 °C	37 °C	42 °C	30 °C	37 °C	42 °C
I (mesophilic cultures)	R-704	12:40 ^c	16:48 ^b		8:25 ^{de}	9:35 ^d	
	R-707	8:10 ^{de}	16:05 ^b		5:55 ^f	7:35 ^{ef}	
	CHN-22	12:35 ^c	21:15 ^a		9:10 ^{de}	19:45 ^a	
II (Mixture 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	8:54 ^{de}	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

12 Results are mean values of four analysis, means with the same letter across columns and rows within group are
 13 not significantly different ($p > 0.05$), Coefficient of variation (CV) = 7.4, 8.6, 7.7 for Group I, II and III
 14 respectively.
 15
 16
 17
 18
 19
 20

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

Culture	Milk	$V_{\max}(\Delta\text{pH/minute})$	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

23 Results are mean values of four analysis, means with the same letter across columns within culture are not
 24 significantly different ($p>0.05$), coefficient of variation (CV) = 5.8 and 7.1 for V_{\max} of R-707 and Yoflex mild
 25 1.0 respectively, CV= 5.6 and 5.4 for pH 4.6 for R-707 and Yoflex mild 1.0 respectively.
 26

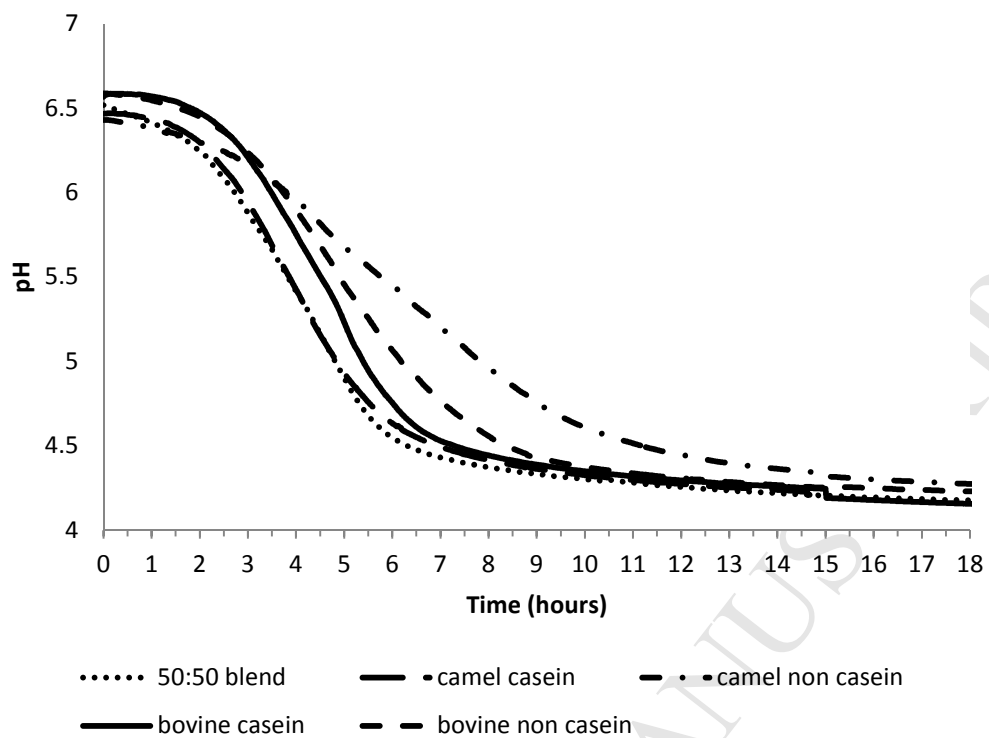


..... R-707 camel 30°C - - - R-707 bovine 30°C — CHN-22 camel 30°C
- - - CHN-22 cow 30°C - • - STI-12 camel 42°C - • - STI-12 bovine 42°C

ACCEPTED MANUSCRIPT

1

2



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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