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Research Article

Effect of Ginger Powder Addition on Fermentation Kinetics, Rheological Properties and Bacterial Viability of Dromedary Yogurt

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Abstract: This study aims to evaluate the direct use of ginger powder in dromedary's yogurt manufacturing by determining the kinetic acidification, the rheological parameters and the stability of the final product during 28 days of cold storage. The supplementation of dromedary milk with ginger powder at concentration ranging from 0.6 to 1% w/v, enhanced the growth of inoculated lactic acid bacteria, accelerated significantly the rate of pH reduction ($p<0.0001$) and reduced the time of fermentation to 50%. On another hand, its addition improved the consistence index K, decreased the flow behavior index n, increased the water holding capacity and enhanced slightly the viability of *Streptococcus salivarius ssp thermophilus* during cold storage. Thus, the supplementation of dromedary milk with ginger powder at concentration ranged from 0.6 to 1% w/v complements its healthy characteristics, produced acceptable yogurt and allows energy and time saving in the manufacturing process.

Keywords: Dromedary milk, *Lactobacillus delbrueckii ssp bulgaricus*, *Streptococcus salivarius ssp thermophilus*, yogurt, *Zingiber officinale*

INTRODUCTION

During recent years, an increasing interest has been developed in foods that contribute to a positive effect on health beyond their nutritional value (Martina-Diana *et al*., 2003). Among this food, much attention has been focused on camel milk. Truly, apart from the essential nutriments available from cow milk, fresh or fermented camel milk contains high concentration of bioactive substances (Shori and Baba, 2014c) which have great therapeutically virtues toward treatment of several diseases such as cancer, diabetes, jaundice, tuberculosis and asthma. Moreover, camel milk has been recommended to be consumed by children who are allergic to bovine milk because it lacks β-lactoglobulin and contains α−lactalbumin. All these medicinal properties make this milk attractive to some consumers and its production is gradually increasing (Omer and Hamed, 2010).

The exploitation of this milk with particular characteristics compared to cow's milk becomes an opportunity to diversify the dairy market. However, most of the dromedary milk is drunk fresh, because contrary to the other types of milk, it is often described as not easily transformed into dairy products (Attia *et al*., 2000), Hashim *et al*. (2008) and Farah *et al*. (1990) studied the preparation of fermented milk and reported that, camel milk produced a thin, flowing and very soft yogurt. Attia *et al*. (2001) also found that, during lactic fermentation process, dromedary milk showed a behavior different from that of bovine milk at the microbiological, biochemical and structural levels. This milk was shown to have greater resistance to bacterial growth leading to less active cultures and did not produce a curd structure but few dispersed small casein fragments at the surface and a film or firm gel at the bottom of the vessel.

Several solutions were proposed to improve texture of fermented dromedary milk products. The most commonly used methods include an increase in milk solids (Mortada and Omer, 2013) and addition of stabilizers such as gelatin or alginate (Hashim *et al*., 2008). Moreover, several recent studies showed the inclusion of medicinal herbal extracts into milk during fermentation such as *Allium sativum* and *Cinnamomum verum* (Shori and Baba, 2012a).

Ginger (*Zingiber officinale*) has been used as a spice for over 2000 years. Its roots and the obtained extracts contain spectra of biologically active compounds responsible for its various medical

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applications as an analgesic, antiemetic, antiulcer, antipyretic, prostaglandin suppression and cardio depressant among many others (Ajayi *et al*., 2013). Moreover, ginger has a considerable amount of starch (up to 40%, dry basis) with potential applications. The importance of starches is long recognized as they are an important source of energy (Ahmed *et al*., 2011) and it is one of the most frequently used thickening agents in yogurt production (Ibrahim, 2015). Latona *et al*. (2012) also reported that ginger is a good source of protein. So the addition of this plant could be a good source of growth factors for inoculated lactic acid bacteria and may offer the possibility of improving the texture of fermented dromedary milk.

The present study aims to evaluate the effect of adding ginger powder to dromedary's yogurt manufacturing by determining the chemical composition, the kinetic acidification, the microbial growth during fermentation and their viability during cold storage, the post-acidification and the rheological parameters of yogurts.

MATERIALS AND METHODS

Milk samples: Fresh dromedary milk was collected from Ibnou farm (Ghardaia, southern Algeria). The collected milk was then skimmed with three successive centrifugations at 2000 g for 15 min to achieve fat separation (Attia *et al*., 2001). Milk powder was purchased from a local market.

Starter cultures: Commercial freeze-dried mixed yogurt culture (DELVO-YOG, CY-222.DSL) composed of *Lactobacillus delbrueckii ssp bulgaricus* and *Streptococcus salivarius ssp thermophilus* were used as starter cultures.

The lyophilized culture was suspended in 12% reconstituted skim milk powder, previously autoclaved at 120°C for 15 min and incubated at 42°C for 4-6 h (El-Batawy *et al*., 2014).

Preparation of Ginger powder: Fresh ginger root (*Zingiber officinale Roscoe*) was purchased from a local market. Root was peeled, washed, weighed and sliced, then dried with a tray drier at 50°C until moisture content was less than 10% (weight basis). Dried sliced ginger was reduced to powder using an electric grinder (Nantaporn *et al*., 2010).

Chemicals analysis: Total solids, ash, total nitrogen and total non protein nitrogen of pasteurized dromedary milk (control and fortified milk) were determined according to standard methods, respectively by drying at 102±2°C, by incineration at 550°C and by the kjeldahl method (AFNOR, 1993).

Proximate composition of ginger powder was estimated with standard methods. Protein content was determined by the Lowry assay (Lowry *et al*., 1951) and ash content by incineration at 550°C.

Evaluation of proteolysis activity of ginger protease: Proteolysis activity of ginger protease was assessed after heat treatment of supplemented dromedary milk by measuring liberated free amino group (NPN). Samples for analysis were prepared by adding 5 mL of trichloroacetic acid (24%) to 5 mL of milk to obtain a final concentration of 12%. After 5 min of incubation at room temperature, the samples were filtered using Whatman No.2 paper (Attia *et al*., 2001). The NPN content was determined as mentioned below.

Preparation of fermented milk: Skimmed dromedary milk samples were divided into 6 equal portions. One of them was used as a control sample; while 5 other ones were used for prepare five different formulations of fermented dromedary milk fortified with ginger powder (0.25, 0.5, 0.6, 0.75 and 1% w/v).

All samples were heated at 63°C for 30 min, then cooled to incubation temperature and each milk sample was inoculated with 3% (10^6-10^7 cfu/mL) of *St. thermophilus* and *Lb. bulgaricus* 1:1 (Magdi *et al*., 2010) followed by incubation at 43°C until the pH reached 4.4.

Milk powder (the second control) was reconstituted with distilled water to 12% w/v and was prepared using the same procedures:

DM : Dromedary milk
CM : Cow milk

Fermentation kinetic: During fermentation, the pH and acidity values were determined every 1.5 h until the desired pH was achieved. The pH of the samples was measured using a pH meter (Hanna-instrument). The titratable acidity was measured according to AFNOR method and was expressed in Dornic degrees (1°D = 0.1 g lactic acid/L of milk). The pH variation was calculated as follows: Δ pH = pH_2-pH_1, where pH_2 and pH_1 are the pH values at time t_2 and t_1 respectively. The acidity variation was calculated via the same method.

The time required for the pH to reach 4.4 was defined as the fermentation time. Based on the results of preliminary studies, the milk-ginger's mixture was prepared using 0.6 % (w/v) of ginger powder to perform the following tests.

Culture growth: Bacterial counts were determined in dromedary milk, cow milk and dromedary milk fortified with 0.6% ginger powder, at each sampling time during fermentation process.

The number of *Lb. bulgaricus* was determined using MRS agar acidified to pH 5.4 with 100% glacial acetic acid then incubated at 37±1°C for 72 h under anaerobic condition (IDF, 1997). *St. thermophilus* was enumerated using M 17 (IDF, 1997) and subsequently incubated at 37±1°C for 48 h under aerobic condition (Supavititpatana and Kongbangkerd, 2011b).

Water-holding capacity: Water-Holding Capacity (WHC) was measured after 20h of cold storage

according to the method described by Supavititpatana *et al*., (2010a).

Rheological measurements: The rheological parameters were measured after 20 h of storage using a rheometer model HAAKE. VT 550. The flow curves were determined at a linear gradient shear rate from 0.5 to 1000/s, within 200 s. the description of the rheological behavior was performed using the rheological model of Ostwald-de Waele ($\tau = Ky^n$).

Stability during storage: evolution of pH, acidity and bacterial viability: Yogurt samples were stored at 4°C and the pH, acidity values and bacterial counts were determined after 14 and 28 days of storage as mentioned previously. The viable cells were calculated as follows:

% viability = (CFU at n weeks of storage/Initial CFU) ×100 (Riazi and Ziar, 2008)

Statistical studies: All results are presented as means of two independent replicates. Statistical analysis was performed using one way Analysis of Variance (ANOVA, Minitab, version 16).

RESULTS AND DISCUSSION

Chemical composition of dromedary milk: The average chemical composition of dromedary milk samples used for yogurt processing (Table 1) were in the range of values reported by Siboukeur (2005), Attia *et al*. (2001), Eissa *et al*. (2011) and El-Agamy (2000) (8.9-14.3% total solid, 2.5-4.5% protein, 0.25% NPN and 0.7-0.95% ash).

Supplementation of dromedary milk with ginger powder increased significantly ($p<0.05$) the total solid, the amount of protein, non-protein nitrogen and ash. The increase of these components in fortified dromedary milk is due to the ginger powder, while the increase of NPN amount could be attributed to the ginger powder protein and to the presence of a cysteine protease (Zingibain) active during heat treatment (63°C) and form a free amino group (Hashim, 2011 and Nafi *et al*., 2013). These results were similar to the results of Ko (1989) who also found that, the addition of microbial protease or papain to soy milk increases considerably the amount of non-protein nitrogen.

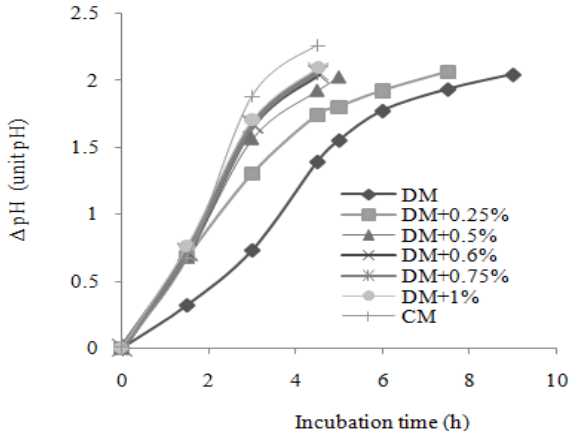
Chemical composition of ginger powder: The protein content of the ginger powder used in this study was 43.89±0.4980% which is higher than the 34.13% reported by Latona *et al*. (2012), while ash content is approximately the same (7.200±0.7211%).

Evaluation of proteolysis activity of ginger protease: The amount of liberated NPN realized by the action of ginger protease was 0.09%±0.002, 0.31%±0.007, 0.35%±0.007, 0.40%±0.007 and 0.44%±0.014 for dromedary milk supplemented with 0.25; 0.5; 0.6; 0.75 and 1% of ginger powder respectively. It can be stated that the rate of liberation of NPN increased significantly ($p<0.0001$) with the increase in the concentration of ginger powder.

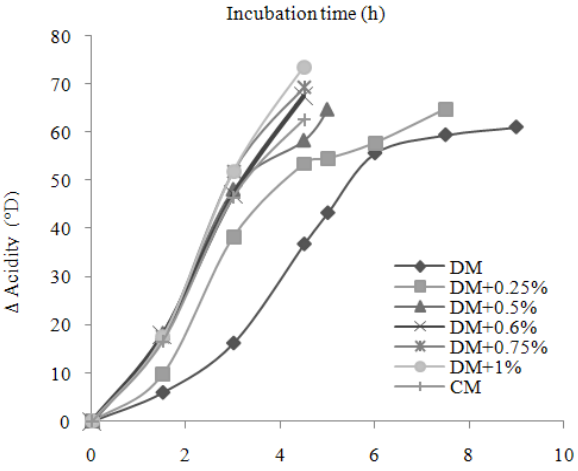
These results were similar to those of Ko (1989), who also found that, the addition of microbial protease or papain to soy milk increases considerably the amount of non-protein nitrogen.

Table 1: Chemical composition of dromedary milk

Samples	Components %			
	Total solid	Protein	Total NPN	Ash
DM	10.05±0.14	3.57±0.05	0.26±0.007	0.92±0.04
DM+0.25%	10.34±0.15	3.67±0.05	0.44±0.014	0.93±0.05
DM+0.5%	10.56±0.13	3.78±0.02	0.76±0.028	0.95±0.03
DM+0.6%	10.70±0.10	3.83±0.04	0.83±0.021	0.96±0.05
DM+0.75%	10.8±0.14	3.89±0.05	0.90±0.007	0.97±0.03
DM+1%	11.06±0.40	4.0±0.05	1.13±0.500	0.99±0.02



(a) Rate of pH variation



(b) Rate of titratable acidity variation

Fig. 1: Kinetic of acidification of dromedary milk, dromedary milk fortified with ginger powder at different concentration and cow milk

Fermentation kinetic: The dromedary milk fermentation shown a slow acidification rate (Fig. 1a) and required double time of fermentation as compared to cow milk. This result was in agreement with the one of Attia *et al*. (2001), who stated that the activity of the starter culture in dromedary camel milk was characterized by a longer lag phase than the bovine milk.

The enrichment of dromedary milk with 0.25, 0.5, 0.6, 0.75 and 1% w/v of ginger powder enhanced significantly the acidification rate ($p<0.0001$). As shown in Fig. 1a, during the first 3 h of incubation, there was a faster rate of pH reduction in all fortified dromedary milk and it was approximately 2 times higher as compared to dromedary milk alone. Whereas, during the following 1.5 h of incubation, it was more enhanced in dromedary milk fortified with 0.5, 0.6, 0.75 and 1% in comparison to the dromedary milk fortified with 0.25% of ginger powder.

This faster rate of pH reduction decreased the pH of dromedary milk supplemented to 4.4 after 4.5 h of incubation at 43°C, while the incubation of dromedary milk alone was prolonged and required 9 h of incubation to reach this pH. So, the enrichment of dromedary milk with ginger powder at a concentration superior to 0.5% decreases significantly the fermentation time of dromedary yogurt ($p<0.01$) to 50%.

The titratable acidity variation of all samples showed a similar trend as observed in the rate of pH reduction (Fig. 1b). The variation observed during the first 4.5 h of incubation was approximately 1.5 to 2 times higher in fortified dromedary milk than in dromedary milk alone.

As shown in Fig. 1a, the time required for the pH to reach 4.4 of dromedary milk enriched with ginger powder at concentration superior to 0.5% was the same for the fermentation of cow milk.

This decrease in fermentation time of supplemented dromedary milk could be due to the higher amount of non-protein nitrogen present in fortified milk. This is in agreement with previous study of Dave and Shah (1998), who found that the addition of growth factors, such as non-protein nitrogen to cow milk, enhances the acidification rate and reduces the fermentation time by 25-75%.

Supplementation of dromedary milk with 0.6, 0.75 and 1% w/v of ginger powder has shown a similar kinetic of acidification and a similar coagulation time (4.5 h), so the concentration of 0.6% w/v was selected for the following tests.

The viable counts of starter culture during fermentation: During the first 4.5 h of fermentation process, the growth kinetic of yogurt culture showed a similar trend as observed in the rate of pH reduction (Fig. 1a) and the inoculated lactic acid bacteria showed slow rate of growth in dromedary milk alone and quicker rate of growth in dromedary milk supplemented with ginger powder and cow milk (Fig. 2).

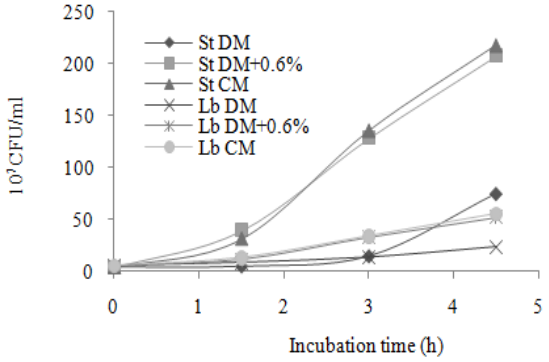


Fig. 2: Changes in viable cell count of starter culture during 4.5 h of incubation

This slow rate of growth of lactic acid bacteria observed in dromedary milk compared to cow milk was reported in a previous study of Attia *et al.* (2001), who suggest that the growth of yogurt culture in camel milk is delayed due to the presence of some inhibiting agents which extends the gelation process.

As shown in Fig. 1 and 2, dromedary milk fortified with ginger powder seemed more favorable for the growth of inoculated lactic acid bacteria than to the dromedary milk alone. Indeed, during the first 3 h of incubation, the amount of *St. thermophilus* was approximately 10 times higher in dromedary milk supplemented than that in dromedary milk alone, respectively. After 4.5 h of incubation, it was also approximately 3 times higher, while the amount of *Lb. bulgaricus* was approximately 2 times higher in fortified milk than that in dromedary milk alone during all fermentation process. Consequently, the pH of dromedary milk fortified with 0.6% of ginger powder reached 4.4 and the incubation was stopped, while the incubation of dromedary milk alone was extended to 9 h until the desired pH was achieved.

The difference between these milks in the rate of growth of starter culture could be due to the higher amount of non-protein nitrogen present in supplemented dromedary milk. Similar results were reported by Lucas *et al*. (2004) and Supavititpatana (2011), who found that the increase in the level of peptide and free amino acids enhanced the growth and acidification activity of *St. thermophilus* lead to enhance the level of formic acid and CO_2 which stimulates the growth of *Lb. delbrueckii ssp. bulgaricus*. Moreover, Wasinee *et al*. (2009) revealed that the addition of *Aspergillus ssp* protease to cow milk makes peptide from milk protein promoting rapid growth of yogurt culture.

When the pH of dromedary milk reached 4.4 (after 9 h of incubation), the amount of *St. thermophilus* attained 38 10^8 cfu/mL and the amount of *Lb. bulgaricus* attained 15.4 10^8 cfu/mL in dromedary milk alone (data not shown). At the same final pH, the counts of yogurt bacteria were higher in this milk in comparison to the dromedary milk fortified with ginger powder and cow milk. This can be related to the prolongation of fermentation time, from 4.5 to 9 h. Similar results were reported by Lucas *et al*. (2004) and Zhao *et al*. (2006), who also found that the addition of casein hydrolysates to cow milk reduces the fermentation time and decreases the number of final inoculated lactic acid bacteria.

The mains population was represented by *St. thermophilus* in all samples. These results were in agreement with Abu-Tarboush (1996), who revealed that the Streptococci were always more numerous than the Lactobacilli during fermentation of camel milk and cow milk at 42°C.

Stability during storage:

Evolution of pH, acidity and bacterial counts: The development of pH and acidity throughout storage shows a gradually increase in acidity and decrease of pH for all samples (Table 2).

The development of these parameters throughout the first 14 days storage shows a significant difference ($p<0.01$) in all samples, after a slight increase in the acidity ($p<0.05$) and decrease in pH was observed.

This result agree with those of McCann *et al*. (2011) and Lucas *et al*. (2004), who reported that the noticeable decrease of pH during the first week of storage is expected as the lactic acid bacteria continue to grow and produce lactic acid. As the growth of lactic acid bacteria is inhibited by a low pH, the rate of pH reduction would be slowed down significantly during further storage period.

At the end of storage, the highest acidity was obtained by the dromedary yogurt supplemented while the lowest one was obtained by dromedary yogurt, (Table 2). Espirito Santo *et al*. (2012) found similar results for yogurt supplemented with passion fruit peel powder.

The viable counts of starter culture during 28 days of refrigerated storage: During the whole self-life, *Lb. delbrueckii ssp bulgaricus* and *St. salivarius ssp thermophilus* counts showed a gradual decline for all samples. This gradual decline was significantly different ($p<0.05$) in all yogurts at the same weeks and during 28 days of storage. This result is in agreement with previous studies of Farahat and El-Batawy (2013); Shori and Baba (2012a) and Passephol and Sherkat (2009), which indicated that the decrease in lactic acid bacterial counts may be due to the sensitivity of these bacteria to acid developed along the storage period.

At the end of storage, the viability of *Lactobacillus delbrueckii ssp bulgaricus* was found higher in all samples in comparison to the viability of *St. salivarius ssp thermophilus*, because generally *Lb. delbrueckii ssp bulgaricus* can tolerate low pH conditions better than *St. thermophilus* (Wasinee *et al*., 2009). However, it's clear that, the number of lactic acid bacteria in all yogurts at the end of storage were higher (Table 2) than the recommended minimum levels (10^6-10^7 cfu/mL) (Farahat and El-Batawy, 2013).

The addition of ginger powder significantly ($p<0.01$) enhanced the viability of *St. salivarius ssp thermophilus* during storage time. Similar results were reported by Zhao *et al*. (2006), who revealed that the addition of casein hydrolysates to cow milk enhances the viability of these strains.

Rheological characteristics: As shown in Table 3, the supplemented dromedary yogurt seems to be more viscous with three times higher consistency index ($p<0.0001$) and lower flow behavior index ($p<0.05$) than the dromedary yogurt respectively.

The increase in consistency index and the decrease in flow behavior index of dromedary yogurt fortified with ginger powder could be due to the increase in total solid. Similar result was reported by Penna *et al*. (2006). Furthermore, ginger has a considerable amount of starch (up to 40%, dry basis) with potential applications (Ahmed *et al*., 2011) and the ability of starches to thicken gel and hold water has been exploited in yogurt manufacture (Ibrahim, 2015 and Najgebauer-Lejko *et al*., 2007).

Water-Holding Capacity (WHC): WHC measurements showed significant differences ($p<0.001$) between all yogurt samples. The higher WHC was obtained from yogurt sample made using cow milk (36.81%±0.50) and the lower WHC was obtained from

Table 2: The stability of dromedary yogurt with or without added ginger powder and cow yogurt during refrigerated storage for 28 days

	14 days of storage			28 days of storage		
	DM	DM+0.6 %	CM	DM	DM+0.6%	CM
pH	4.25±0.02	4.16±0.02	4.17±0.0	4.19±0.01	4.13±0.02	4.13±0.0
Acidity (°D)	96.78±1.7	113±1.3	87.57±0.6	100.1±0.9	117.9±2.5	94.33±3.2
% viability of *St.*	37.85±0.7	56.80±1.4	48.45±1.6	14.30±0.1	18.50±3.1	13.25±0.6
% viability of *Lb.*	65.85±1.3	42.25±5.4	55.80±2.5	38.25±4.5	13.05±1.9	25.20±5.0

Table 3: Rheological parameters of dromedary yogurts

Yogurt samples	Consistency index K ($Pa.s^n$)	Flow behavior index n	R^2
Dromedary alone	91.94±6.61	0.6127±0.011	0.9684±0.014
Dromedary supplemented	247.6±11.46	0.4840±0.049	0.9781±0.021

yogurt sample made using dromedary milk alone (21.86%±0.81), supplemented dromedary milk with ginger powder has significantly increased WHC ($p<0.05$); it attained 23.70%±0.1344 which is 1, 84% higher than that of control yogurt.

Hence, it can be stated that the addition of ginger powder to dromedary yogurt increased significantly the water holding capacity compared to control yogurt. This difference in WHC of the yogurts may be attributed to the starch present in ginger. Similar results were reported by Lobato-Calleros *et al*. (2014), who suggested that the addition of starch increases water-holding capacity as well as yogurt stability during storage.

CONCLUSION

The supplementation of dromedary milk with ginger powder at concentration ranging from 0.6 to 1% w/v enhanced the growth of yogurt bacteria, accelerated significantly the rate of pH reduction ($p<0.0001$) and reduced the time of fermentation from 9 h to 4.5 h. On another hand, its addition improved the consistence index K, decreased the flow behavior index n, increased the water holding capacity and enhanced slightly the viability of *Streptococcus salivarius ssp thermophilus* during cold storage. Thus, the addition of this medicinal plant to dromedary milk complements its healthy characteristics, produces acceptable yogurt and allows energy and time saving in the manufacturing process.

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