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Full Length Research Paper

Suitability of camel milk to transformation in Leben by lactic starter

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The aim of this work was to study the suitability of camel milk to transformation in Leben by lactic fermentation. Sixty strains of lactic acid bacteria (LAB) were isolated from camel milk. Strains were tested for their acidification activity, ability to use citrate, biomass yield, growth rate and Exopolysaccharide (EPS) production. Twenty-nine strains showed ability to use citrate. These strains were tested for their acidifying activity. Only seven strains present ΔpH ≥0.3 U and then were considered as rapid acidifier strains. Four strains produced EPS and two strains showed high acidification rate and high yield of biomass at the end of fermentation. The strain $SLCch_{14}$was the most acidifying strain (0.45 U) and had a high ability to produce an important biomass (0.98 g/l). $SCC1_{33}$ produced EPS and presented a satisfactory ability of acidification (0.35 U). These strains, tentatively identified by API 50 CHL, were divided into three groups*: Lactococcus lactis, Lactobacillus pentosus, Lactobacillus plantarum, Lactobacillus brevis and Pediococcus pentosaceus*. These strains were applied to prepare Leben from camel milk. In order to study the potentiality of the starter to ferment other types of milk–goat milk was chosen. The pH and total acidity of the final product were 3.93; 5.30 and 110; 43 D° for camel and goat respectively. The rheological study showed that Leben present a non-Newtonian and thixotropic behaviour.

Key words: Camel milk, goat milk, Leben, lactic acid bacteria, fermentation.

INTRODUCTION

Milk is the main source of nutrition for the neonate calves and provides all the essential nutrients for growth and development for example proteins, minerals, carbohydrates, fatty acids, growth factors, immune modulators, etc (El Hatmi et al., 2007). Camel milk is popular for its traditionally anti-infective, anti-cancer, anti diabetic and more generally as a restorative in convalescent patients. This can be attributed to some of its components as antimicrobial factors (Lactoferrin, lysozyme, Lactoperoxidase, immunoglobulin) (Konuspayeva et al., 2004). Camel milk was therapeutically used against dropsy, Jaundice, problems of the spleen, asthma, anaemia, and piles and other lung ailments and has proven beneficial in the treatment of tuberculosis (Al Hashem, 2009). Camel milk has been used fresh or fermented in different regions of the world. Traditional fermented camel's milk is widely consumed in Africa and in Middle Eastern countries (Ashmaig, 2009). It is produced by spontaneous souring of camel's milk. The daily residual fresh milk is poured into the milk container. The acidification develops after a few days due to natural lactic acid microflora (Guasch-Jané et al., 2005; Robert, 2008) and the contaminants from the processing containers.

In Sudan for example, because camels are reared under unstable, nomadic roaming, their milk is not always available for urban or village residents. The camel's milk

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being abundant in remote localities that is why the camel herders prepare *Gariss*, a fermented product, on which they sustain living for several months as the sole source of various nutrients (Dirar, 1993; Abdelgadir et al., 1998). In Kenya, both fresh and fermented camel milk is widely consumed by the pastoralist communities living in the arid and semi-arid regions of the country. The fermented camel milk (*Susaac*) is traditionally prepared by spontaneous fermentation of unheated milk in smoke-treated gourds. In Kazakhstan, milk consumption is present under raw milk and mainly fermented (*shubat*) and these products are considered as typical products linked to cultural identity. They are used traditionally for their medicinal and probiotic properties which may be interesting for the food industry (Konuspayeva et al., 2007).

In Tunisia, no practices of camel milk fermentation were known. Development of new fermented functional camel milk in Tunisia can be promised. In fact, during the last decade, the interest of industries and consumers for functional foods has been exponentially increasing. The use of milk with particular nutritional properties such as camel milk, alone or in combination with bacterial strains having probiotic properties and/or producing physiologically active metabolites, represents one of the technology options for manufacturing dairy functional beverages (Gomes and et al., 1998). The traditional method of milk fermentation results in a product with varying taste and flavour and often of poor hygienic quality. The transformation of camel milk by fermentation is not easy and more research for elucidating the process is needed. To improve the spontaneous traditional fermentation, controlled fermentation using mesophilic lactic acid bacteria, starter culture is a very important strategy for camel milk processing (Farah et al., 1990; Mohamed et al., 1990; Kamoun, 1995; Abu-Tarbous et al., 1996; Lhoste, 2004). With this in mind, the isolation of the microflora in camel milk as a basis for possible development of suitable starter cultures for fermented camel milk products is necessary. Thus, the objective of this work is to study the effect of autochthonous Lactic Acid Bacteria (LAB) isolated from camel milk to transform in Leben.

MATERIALS AND METHODS

Origin of milk

Milk samples were collected from camels (*Camelus dromadarius)* and goats (*Capra heircus*) of the herd of the Institute of Arid lands (IRA Medenine). The samples were immediately cooled and brought to the laboratory in an isotherm container and being analyzed on arrival.

Microbiological analysis

All samples studied have undergone a preliminary treatment to obtain the dilutions according to standard NF V08-0IO. Milk samples (1ml) were diluted in buffered peptone saline mixed in stomacher bag. In order to quantify the various microbial groups, appropriate dilutions were plated: Aerobic total plate count was carried out on plate count agar (PCA) (Sharlau Chemie S.A), incubated at 30°C for 72 h. Yeast and moulds on Sabouraud Chloramphenicol (Pronadisa) and incubated at 25°C for 3 to 5 days. Total coliform were grown in Violet Red Bile Agar (VRBA) (AppliChem.Biochemica) in double layer. The plates were incubated at 30°C for 22 h.

Isolation of lactic acid bacteria (LAB)

LAB were isolated on Man-Rogosa-Sharp (MRS) (Pronadisa) agar and incubated at 30°C for 24 to 48 h in order to apply the conventional tests for identification. The type and form of colonies were studied after plating on solid medium (MRS) by a microscopic observation. All isolates were initially examined for Gram staining and catalase production. Only Gram-positive and catalase-negative isolates were considered. Citrate utilization, in the presence of carbohydrates, was studied on Simmons citrate medium (Fluka Biochemica). The presence of a blue coloration (even locally only on the surface) indicated a positive reaction.

Physicochemical analysis

pH was measured at 20°C with a pH-meter (Thermo-Orion). The Dornic acidity was determined by titrating 10 ml of homogenized fermented camel milk with N/9 NaOH in the presence of drops of phenolphthalein. Acidity was expressed in D° (NFV04-106, 1969).

Apparent viscosity

The viscosity was determined by applying a shear stress of 0.1 to 100 rpm at an oscillation frequency of 1 Hz for 1 min with a Brookfield type viscometer (model DV-E, MA, USA).Viscosity was expressed in centipoises (cP).

Biomass production

Strains were subcultured on MRS broth; 100 ml of the medium were inoculated with 10% of the active culture. Bacterial growth was monitored by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer (CECIL CE 2041/2000 Series) during 6 h. The difference between the initial OD and the OD at which cells were collected (ΔOD) was taken as an indication for the growth amount. The maximum growth rate was determined from the slope of the linear part of curve representing Log OD versus time. At the early stationary phase, 30 ml of culture were harvested by centrifugation (Sigma GmbH, Model 6K15, Gottingen, Germany) at 5000 g for 30 min at 4°C. The dry weight was determined after drying the pellet at 105°C for 24 h. The remaining 70 ml were used to study the separation of biomass by centrifugation and measurement of OD_{600} of supernatant (Ayad et al., 2004).

Exopolysaccharides production

The Cultures were streaked on modified MRS (m-MRS; glucose replaced with 100 g/l sucrose) (Van Geel-Schutten et al., 1999) and incubated at the optimum growth temperature for 24 h. Then they were tested for slime formation using the inoculated loop method (Knoshaug et al., 2000). Formed colonies were dragged up using a metal loop and the strains were considered positively slimy producer if the length of slime was above 1.5 mm (Ayad et al., 2004)

Table 1: Physico-chemical and microbiological properties of camel and goat milks.

Characteristic	Camel	Goat
Density	1.030 ± 0.01	1.028 ± 0.0023
pH	6.43 ± 0.1	6.74 ± 0.037
Acidity (°D)	17.00 ± 3.2	15.50 ± 1.44
Matter fat (g/l)	37.00 ± 4.92	32.00 ± 2.30
Dry matter (g/l)	124.34 ± 11.06	128 ± 1.15
Ash (%)	0.5 ± 0.1	0.85 ± 0.12
Total mesophilic aerobic bacteria (CFU/ml)	2.7310^{2} ± 0.7	$7.20\ 10^{5}$ ± 0.5
Yeast and molds counts (CFU/ml)	$1.74\ 10^{2}$ ± 1.3	$1.36\ 10^{3}$ ± 4.15
Lactic acid bacteria(CFU/ml)	$1.26\ 10^{3}$ ± 0.9	$9.0\ 10^{2}$ ± 1.15

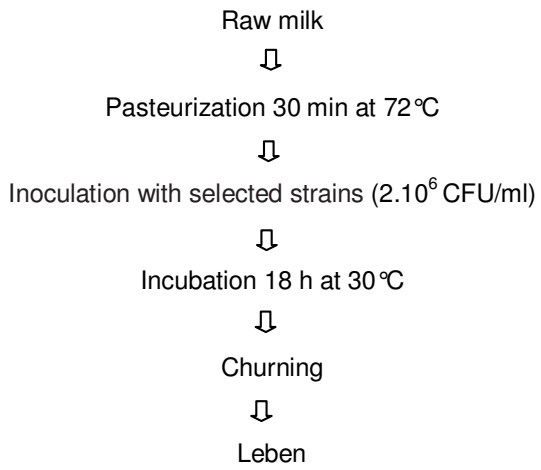

Figure 1. Diagram of Leben preparation.

Biochemical identification API systems

Fermentation of carbohydrates was determined using API 50 CHL, a standardized system, consisting of 50 biochemical tests for the study of carbohydrate metabolism by microorganisms. API 50 CH was used in conjunction with API 50 CHL medium for the identification of *Lactobacillus* and related genera strips according to the manufacturer's instructions (Biomerieux, Marcy l'Etoile, France) (Ghanbari et al., 2009). Briefly, 10 ml of pure water was dispensed with the strip placed in the incubation box, after the bacterial cultures had been introduced into the API 50 CHL system in API 50 CHL medium (5 ml). The set-up system was then incubated at appropriate temperature of 30°C for 24 and 48 h, after the wells were filled with the bacterial suspensions by the line mark with the addition of paraffin oil. Identification tables were prepared as (+/-) according to colour change in evaluation of results of API strips reaction. Numerical profiles of strains were identified adding positive values in indicative table. The API LAB PLUS database (Bio Merieux, France) was used for the interpretation of the results.

Leben preparation

Two strains were selected randomly, based on the difference of morphological characteristics to inoculate pasteurized camel or goat milk. The selected strains were inoculated in sterile tubes containing 10 ml of pasteurized camel and goat milk and incubated at 30°C for 24 h. These pre-cultures were used to inoculate 250 ml of pasteurized milk (Figure 1). After fermentation, the viscosity, pH, acidity and cell count were determined.

RESULTS AND DISCUSSION

Characteristics of milk

The objective of this study is to isolate a Lactic Acid Bacteria (LAB) from camel milk and their application in Leben preparation. Samples of camel milk were collected at different stage of lactation and different rearing methods. There was a difference of physicochemical characteristics of the camel and the goat milks (Table 1). Density of goat milk was lower than in camel milk, which could be explained by the different lactation stages and feeding (El Hatmi et al., 2006). Camel milk was more acidic than milk goat. This was due to the presence of vitamin C (ascorbic acid) (Farah et al., 1992) and high content of lysozyme (Barbour et al., 1984). Abu Lehia (1989) showed that the fat content was higher in camel milk than in goat milk with more long-chain fatty acids and unsaturated fatty acids. Camel milk was characterized by high amount of unsaturated fatty acids (40.1%) and particularly palmitoleic acid (C16:1). The total aerobic mesophilic flora concentration was lower in camel milk than in goat milk. Furthermore, yeasts and molds were present in amounts large in goat milk. This difference could be attributed to the antibacterial effect of camel milk (El Hatmi et al., 2006). In fact, the camel's milk has a bacteriostatic effect against both pathogens, suggesting that different antimicrobial systems occurring in camel's milk may be responsible for this inhibition (Benkerroum et al., 2004). Unlike the total aerobic mesophilic flora, lactic acid bacteria were more abundant in camel milk than in goat milk.

Technological properties of lactic acid bacteria

The strains were isolated and purified from camel milk. They were characterized using conventional methods of microbiology: The morphological study, the catalase test, Gram stain and the use of citrate. Only the Gram positive,

Table2. Fermentation profiles of lactic acid bacteria used for Camel and goat milk fermentation.

Strain	SLC_{ch6}	$SCC_{1,7}$	SLC_{ch1}	$SCC_{1,13}$	$SCC_{1,33}$	$SCC_{1,15}$	$SCC_{1,24}$	$SCC_{1,6}$	$SCC_{1,8}$	$SCC_{1,2}$
Glycérol	+	-	w	w	+	w	-	w	w	+
L-Sorbose	-	-	-	-	w	-	-	-	-	-
D-Sorbitol	-	-	-	-	-	+	-	+	-	-
Amygdaline	-	+	+	w	w	+	-	+	-	-
Esculine	+	+	w	w	+	+	w	w	+	w
D-Mélézitse	-	-	-	-	-	-	-	+	-	w
Amidon	-	w	+	w	+	-	w	-	w	w
Identification	*L. lactis ssp lactis1*	*Lb plantarum*	*L. lactis ssp lactis1*	*L lactis ssp lactis1*	*L.lactis ssp lactis1*	*Lb pentosus*	*L. lactis ssp lactis1*	*Pediococcus pentosaceus*	*Lb plantarum*	*Lb brevis*

+, positive; w**,** weakly positive; **-,** negative after 48 h of incubation at 37°C. All strains fermented : L-Arabinose, D-Ribose, D-Xylose, D-Galactose, D-Glucose, D-Fructose, D-Mannose, N-AcetylGlucosamine, Arbutine, Salicine, D-Celibiose, D-Maltose, D-Lactose, D-Melibiose, D-Saccharose, D-Trehalose, D-Tagatose, Non fermented: Xylitol, Glycogene, Inuline, Methyl-αD-Mannopyranoside, Methyl-αD-Glucopyranoside, Inositol, Dulcitol, Methyl-βD-Xylopyranside, D-Adonitol, L-Xylose, Erythirol, D-Arabinose.

catalase negative and citrate positive were selected. In microscopy, the cells had different shapes coccobacillaire, cocci and bacilli, forming small chains of varying length, pairs or in clusters and were immobile. In first step, lactic acid bacteria (LAB) were tested for citrate utilization. The citrate was used by LAB to produce aroma compounds (diacetyl and acetoin) through citrate and lactose catabolism. Citrate can be metabolized by lactic acid bacteria and is considered to be the principal precursor of diacetyl (butter flavor) in fermented dairy products. Citrate is transported inside cells via citrate permease. Then cleaved to acetate and oxaloacetate by citrate lyase, and oxaloacetate is decarboxylated to pyruvate by oxaloacetate decarboxylase. Diacetyl is important for the organoleptic quality of dairy products, such as cottage cheese, butter, and fermented cream (Boumerdassi et al., 1996). Among 62 strains of lactic acid bacteria, 29 were citrate positive.

Ten strains chosen according to the difference in cell morphology were identified using API galleries. Regarding the carbohydrates fermentations the strains were divided in two groups (Table 2). The first ones dominated by regular rods ($SCC1_8$, $SCC1_7$, $SCC1_{15}$, $SCC1_2$) which fermented mostly Amygdaline, Sorbitol, Esculine and Glycerol, were tentatively identified as *Lb. plantarum, Lb. pentosus* and *Lb. brevis*. The second group was coccoid in shape (SLC_{ch14}, SLC_{ch6}, $SCC1_{13}$, $SCC1_{33}$, and SCC12). They fermented mostly, glycerol, esculine, amygdaline and amidon. They were tentatively identified as *L. Lactis1* and *Pediococcus pentosaceus*. Earlier studies have been reported the presence of the *Lactobacillus plantarum* and *lactobacillus brevis* in Sudanese fermented camel milk (Ashmaig et al., 2009). Sun et al. (2010) isolated the *Lactobacillus plantarum* and *Lactococcus lactis* from traditional fermented milk in Mongolia. In order to select a starter culture for lactic fermentation of both types of milk (camel and goat), the strains were characterized on the basis of acid production ability. The acidity increased during the fermentation time and there was variability in acidification rate between the different strains used to inoculate milk (Figure 2). The strain is considered fast, medium and slow when ΔpH reached 0.4 U for 3, 3 to 5 and > 5 h respectively (Ayad et al., 2004). This is applicable using cow's milk as a substrate. In our case, only strains with ΔpH ≥ 0.3 U after 6 h were kept for the next steps considering the antimicrobial activity of camel milk. Thus, the strains selected are: $SCC1_{33}$, $SCC1_8$, $SCC1_7$, $SCC1_{15}$, $SCC1_6$, $SCC1_{24}$ and SLC_{ch14} (*L. Lactis, L. plantarum, L. pentosus* and *P. pentosaceus).*

This acidifying power was due not only to the fermentation of lactose into lactic acid, but also the proteolysis of caseins. The proteolytic activity of dairy lactic acid bacteria is essential for the bacterial growth in milk and involved in the development of organoleptic properties of different fermented milk products (Axelsson, 1998; Christensen et al., 1999). Acidification and proteolytic activity are difficult to dissociate and the differences of acidifying power between the different strains were certainly related to a difference in the initiation of proteolytic activity (Baati, 2000).

Exopolysaccharides production

Lactic acid bacteria have the ability to synthesize and excrete during their growth, extracellular sugar polymers called polysaccharides or exopolysaccharide (EPS), which can improve the texture and viscosity of the final product (Smith and Underwood, 1998). In general, the presence of polysaccharides in fermented products such as yogurt can increase the homogeneity of the product and make its presentation more enjoyable (Ruas-Madiedo, 2002). The texture of fermented milk depends also in the interactions between bacteria and the different proteins (spatial conformation, interaction, pH, ionic strength) (Özer et al., 1998). Our results showed that

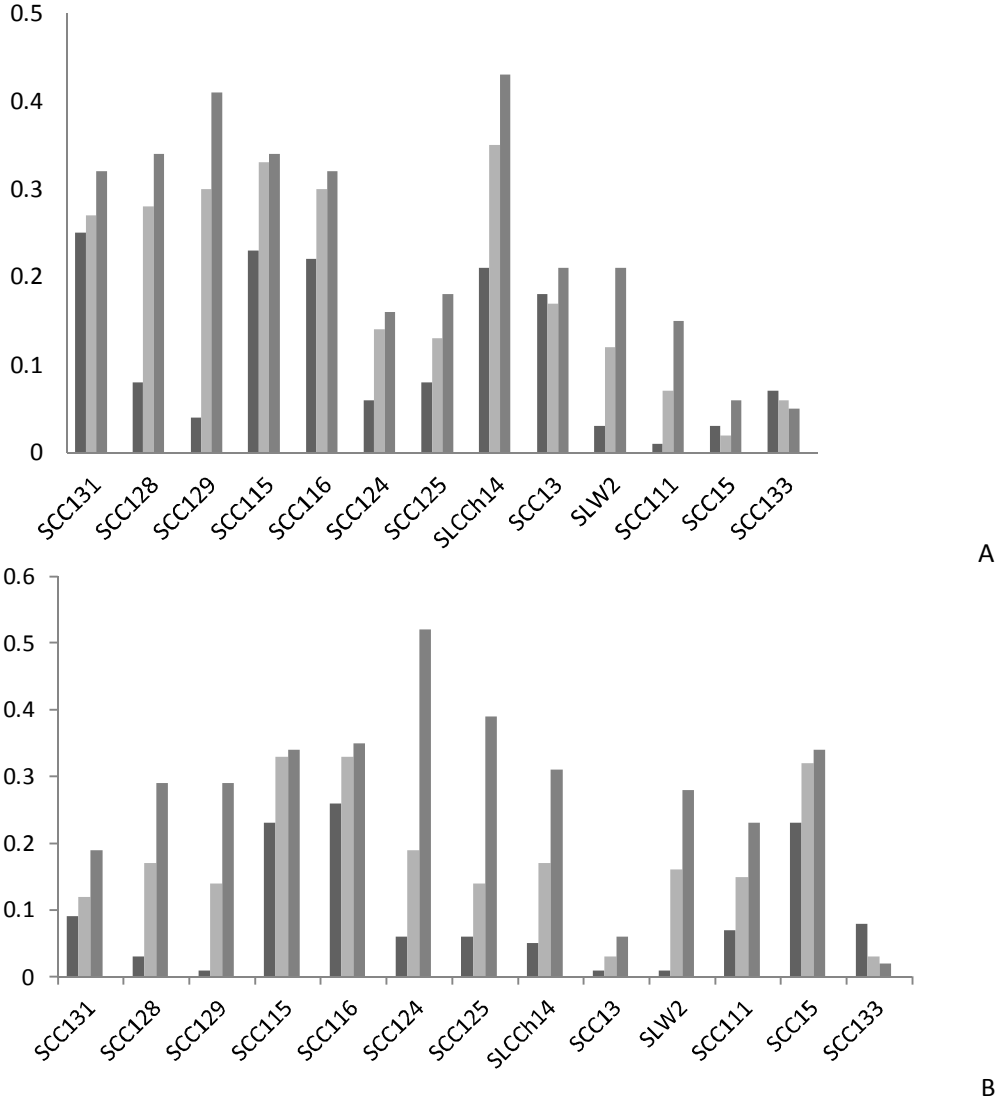

Figure 2. Evolution of ΔpH during the fermentation of camel (A) and goat (B) milk after 2H (■), 4H (■) and 6H (■) inoculated with different lactic strains and incubated at 30°C.

Table 3: Growth characteristics of the starters.

Strain	ΔOD_{600}*	Biomass (g/l)	μ_{max} (h^{-1})	EPS	OD_{600} Supernatant
SCC 1_{24}	0.448	0.53	0.052	+	0.003
$SCC1_{29}$	1.136	0.81	0.132	-	0.008
$SCC1_{28}$	0.67	0.69	0.071	+	0.019
$SLCch_{14}$	1.935	0.98	0.131	-	0.02
$SCC1_{31}$	0.864	0.20	0.080	+	0.106
$SCC1_{16}$	0.737	0.06	0.073	+	0.115
$SCC1_{25}$	1.322	0.79	0.123	-	0.024
$SCC1_{15}$	1.381	0.88	0.073	-	0.094

* **ΔOD_{600},** difference between the initial optical density and optical density after 6 h of culture; **+,** EPS producing strains; **-,** non EPS producing strains.

only 4 strains (SCC 1_{24}, $SCC1_{8}$, $SCC1_{33}$, and $SCC1_{16}$) were able to produce EPS (Table 3).

Biomass production and growth rate

A starter is a microbial preparation of high cell density;

Table 4. Characteristics of fermented milk.

Strain	Milk	pH_f	Acidity (°D)	UFC/ml
$SLCch_{14}$	Camel	3.93	95	$1.61\ 10^7$
	Goat	4.00	110	$1.75\ 10^7$
$SCC1_{31}$	Camel	4.72	61	$4.30\ 10^7$
	Goat	5.30	43	$4.65\ 10^7$

pH_f and $Acidity_f$ measured after inoculation of camel and goat milk with a perspective strains.

therefore, it is necessary to select the starters to have significant biomass in the end of culture. A monitoring of OD was performed during strains culture on MRS broth. This allowed estimating the maximum growth rate μ_{max}.

The fermentation broth was centrifuged and the pellet was dried in order to determine biomass. The difference between the initial optical density (OD_{600}) and the OD_{600} at which cells were collected (ΔOD_{600}) as well as the dry weight of strains were used to reflect the growth amount (Table 3). Based on the biomass, cultures were divided into 3 groups: major yields when biomass ≥ 1.30 mg/L, an average yield when the formed biomass ranged from 0.6 to 1.29 mg/L, poor performance when the biomass was <0.6 mg/L (Ayad et al., 2004). Strains $SCC1_8$, $SLCch_{14}$, $SCC1_{24}$ and $SCC1_{15}$ were characterized by a high value of ΔDO_{600} and an important growth rate. The strains $SCC1_{24}$, $SCC1_{33}$ and $SCC1_{16}$ presented a weak biomass and growth rate.

Indeed, the production of small quantities of biomass could be an inconvenient for the industrial use of these strains. However, this low yield could be explained by the loss of biomass during centrifugation and this was due to the production of exopolysaccharides that prevent the separation of bacterial cells and culture medium. This was visualized in the OD values of supernatant (Table 3). According to El-Soda et al. (2003), a good separation of biomass was represented by an OD_{600} ranging between 0 and 0.1. The majority of strains had an OD_{600} <0.1 reflecting a good separation of biomass. Only two strains $SCCl_{33}$ and $SCCl_{16}$ had values greater than 0.1. As mentioned earlier, this was due to the production of EPS which prevent separation during centrifugation. On the basis of these results, two lactic strains were chosen: $SLCch_{14}$ and $SCCl_{33}$. The strain $SLCch_{14}$ was citrate + and presented an important ΔOD_{600}, a significant biomass at the end of culture, the largest growth rate and an important acidity power. The strain $SCC1_{33}$ was kept for further work because of his texturing power. Thus, both strains $SCC1_{33}$ and $SLCch_{14}$ were applied on camel and goat milk fermentation.

Application of strains in the preparation of Leben from camel and goat milk

Both strains ($SCC1_{33}$ and $SLCch_{14}$) were precultured in milk and used for Leben preparation. Results regarded pH, acidity and cell count at the end of fermentation (Table 4). As expected, the strain $SLCch_{14}$ presented more important acidity power then $SCC1_{33}$ but both strains were more acidifying with camel milk than goat milk which can be explained by their adaptation to the microenvironment of camel milk since the strains were isolated from camel milk. The previous work on camel fermentation was reported by Ziadi et al (2011) by applying a starter C1 isolated from traditional leben; the acidity and pH of camel and goat milk was of order 80D° and 77D°, respectively and a pH of 3.7 and 4 respectively. This result was less important than that found in this work. $SLCch_{14}$ strain had a lower pH but a low bacterial concentration by comparing to $SCC1_{33}$. This can be explained by the fast growth of $SLCch_{14}$ leading to an important acidification rate. This acidification was caused by the accumulation of lactate, which has an inhibitory effect on the growth of bacterial cells causing cell lysis.

The evaluation of rheological properties of fermented milk was performed by measuring the apparent viscosity and controlling the pseudoplastic behavior. The variation of apparent viscosity (ηapp) versus shear rate (γ) for fermented camel and goat milks, showed variable viscosity depending on shear rate (Figure 3). Thus Leben is a non-Newtonian liquid. Viscosity decreased with increasing of shear rate (charge) and increased with shear rate decrease (discharge) which shows that Leben is a shear-thickening fluid (Figure 3). This shear thinning behavior is due to the progressive breakdown of aggregates formed between milk caseins by the action of the decrease in pH. The values of apparent viscosity were more important for goat milk than for camel milk. This was due to the difference between the physico-chemical compositions of both types of milk. Indeed, goat milk has a higher dry matter and a different composition of fat (Karray and Attia, 2005). For both types of milk, contrary to what was expected, Leben prepared by applying strain SLC_{ch14} presented an important viscosity than the one prepared by $SCC1_{33}$ which was an EPS producing strain. This variation can be explained by the fact that the apparent viscosity of fermented milk does not only depend on the intrinsic viscosity of exopolysaccharides produced by lactic acid bacteria strains used, but also the firmness of the coagulum. The correlation between EPS and viscosity is very complex, since it is influenced by concentration, molecular mass and environmental pH (Vaningelgem et al., 2004). This result was confirmed by

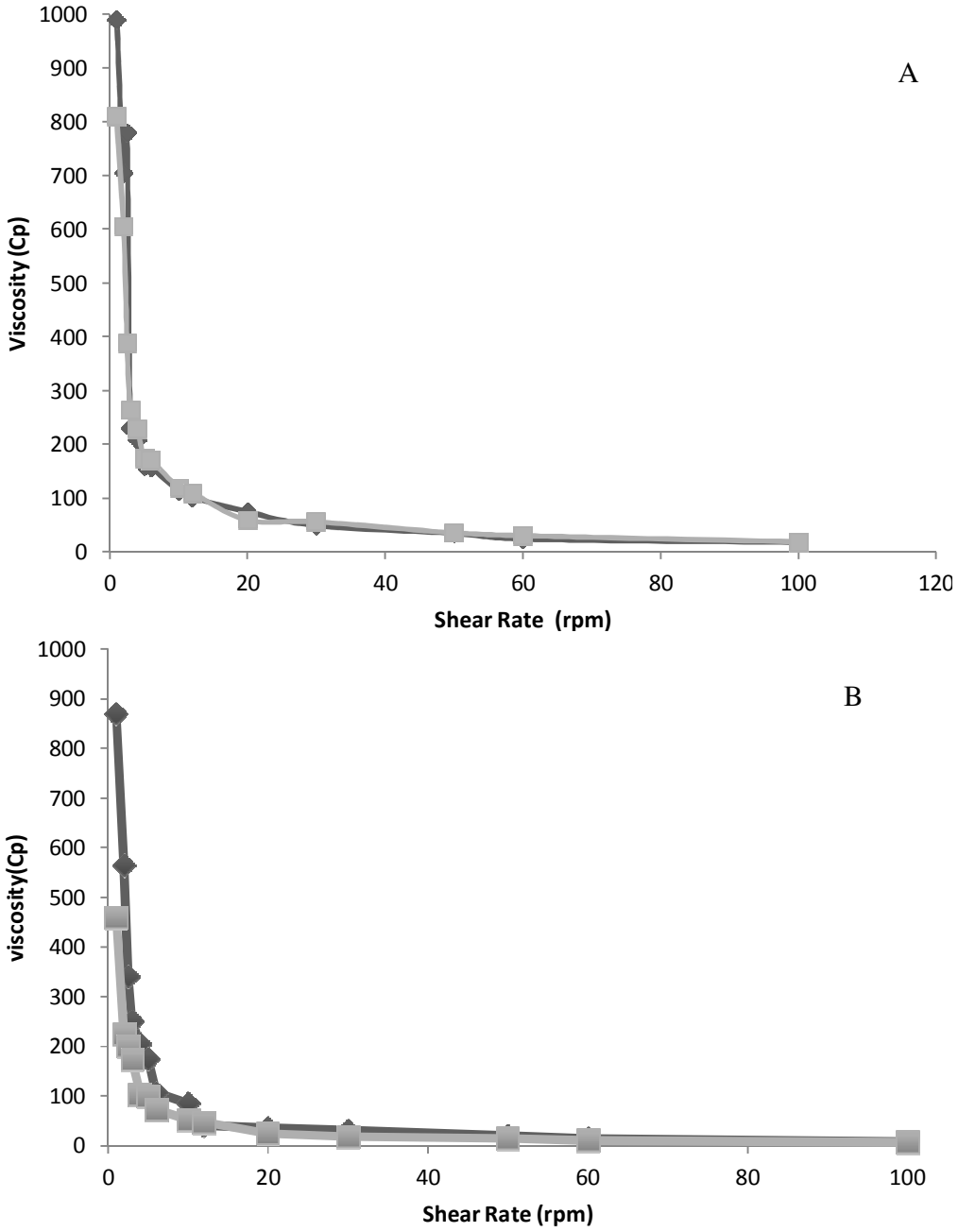

Figure 3. Flow curve of goat's and camel milk for the two strains $SLCCH_{14}$ (A) and $SCC1_{33}$ (B): charge (▲), discharge (■).

Chougrani et al., (2009) who said that the role of two genera in yoghurt manufacture can be summarized as milk acidification and synthesis of aromatic compounds (Serra, et al., 2009; Sahan, et al., 2008).

Conclusion

The present study described the technological potential of different strains of Lactic Acid bacteria isolated from camel milk and their use as starters for "Leben" preparation. Based on the overall evaluation of the obtained results, the strain $SCLch_{14}$ *(Lactococcus Lactis)* was more acidifying and had the ability to produce an important biomass, $SCC1_{33}$ *(Lactococcus Lactis)* was a strain producing EPS and acceptable acidifying power. Both strains showed a good separation power. The rheological study of the various prepared Leben confirms that Leben is a non-Newtonian fluid, shear thinning and thixotropic. This work may have important implication to put in the market fermented milk based of camel and goat milk. A sensory analysis and the monitoring stability of the pro-

duct during storage are possible for the continuation of this work. The molecular identification of the two strains should be made.

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