Emirates Journal of Food and Agriculture. 2018. 30(4): 268-274
doi: 10.9755/ejfa.2018.v30.i4.1661
http://www.ejfa.me/

CAMEL MILK AND MILKING

Fermented camel milk: A Review on its bio-functional properties

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ABSTRACT

Camel milk is a healthy food used in many countries across the world for different health problems since long years. Fermented camel milk is proved to have some health benefits, proved or not, such as hypocholesterolaemic effect, antimicrobial activity, antioxidant activity, angiotensin I-converting enzyme (ACE) inhibitory activity, activity against diarrhea, anticancer activity. Camel milk originated lactic cultures also tested for probiotic potential and showed exciting results through *in vitro* experiments. Nowadays, researchers analyzing these properties with an aim to identify the unknown health benefits and emphasizing on the *in vitro*, *in vivo* and *in silico* experiments to bring out novelty in the market for the public.

Keywords: Camel milk; *Camelus dromedarius*; Fermented camel milk; Bio functional properties; Probiotics

INTRODUCTION

Milk is an important food for human consumption. Numbers of studies have been carried out on cow and buffalo milk. At reverse, studies on camel milk are limited despite of its nutritional significance. Camel milk is a blessing for people who are living in the high temperature regions and deserts. It has been reported that, camel milk is enriched with all the health essential nutritional constituents found in bovine milk (El-Agamy et al., 1998). Many authors reported the health benefits of fresh and fermented camel milk. Camel milk is used for the treatment of tuberculosis, asthma, dropsy, jaundice and leishmaniasis or kala-azar (Abdelgadir et al., 1998). To add in this, camel milk is having other health benefits such as a regulator of glycemia (Agrawal et al., 2007a), potential anti-carcinogenic (Magjeed, 2005), and anti-hypertensive (Quan et al., 2008). Camel milk has been also recommended for the children who are prone to allergic reactions due to bovine milk (El-Agamy et al., 2009).

Apart from these, camel milk is a medicament for intestinal and stomach diseases (Konuspayeva and Faye, 2011). In several countries across the world, fermented camel milk is produced by different communities. Fermented camel milk (FCM) has been proved as a therapeutic product. FCM has showed beneficial effects against the inflammation associated with obesity (Badkook, 2013). Various naturally fermented camel milk products like *Gariss, Chal, Shubat, Dhanaan, Airag, Butsalgaa, Arkhi, Tsagaa, Shmen* and *Yoghurt* are available in different countries like Turkmenistan, Kazakhstan, Mongolia, India etc. As very limited literature is available regarding the health benefits of fermented camel milk, this paper aimed to provide a brief review on the bio-functional properties of microflora in fermented camel milk, and their probiotic potential evaluated throughout the world (Abu-Taraboush et al., 1998). The presented bio-functional properties include hypocholesterolaemic effect, antimicrobial, antioxidant, angiotensin I-converting enzyme (ACE) inhibitory activity or antihypertensive effect, activity against diarrhea, anticancer activity, activity against Carbon tetrachloride (CCl4) and defense against lead (Pb) contamination.

MILK FERMENTATION- A NOVEL PROCESS

Fermentation is an ancient method to produce and preserve food. Microbial fermentation is one of the common practice of food processing and preservation. Fermentations provide a novel way to preserve food, to make a safe

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Received: 17 January 2018 ; **Accepted:** 30 March 2018

product, to destroy undesirable factors, to reduce the energy required for cooking, to improve the appearance and taste of some foods (Lopez, 1992). A novel product of fermentation, "lactic acid", has been proved to preserve important constituents present in milk, in a relatively more stable (Mehaia, 1993). It reduces the pH which affects the physical properties of casein and finally enhance the digestibility in human. Lactic acid also improves the usage of minerals like calcium and check the development of potentially injurious bacteria (McBean, 1999). Fermentation increases therapeutic values of milk (Svanberg and Lorri, 1997; Steinkraus, 2002). Naturally, camel milk is a blend of many bio-functional components. Camel milk contains all essential nutrients on which lactic acid bacteria can readily act and produce many bio-functional components which give health benefits to the consumer. Also reported that, an increase concentration in amino acids, fatty acids and organic acid were observed after fermentation of camel milk (Bahobail et al., 2014). Fermented camel milk in Kenya (*Suusac*) contains little amount of alcohol (1.1%) and dry matter (12.5%), fat (4.0%), and protein (3.0%) (Farah et al., 1990). Consumption of 1 kg of fermented milk may provide 766 kcal energy (Indra and Osorhaan, 1987).

LACTIC BACTERIA (BIODIVERSITY AND GENERALITIES)

Lactic acid bacteria (LAB) are defined as Gram-positive cocci or rods with low-GC (guanine-cytosine) content. These are acid-tolerant, generally non-spore forming bacteria and associated by their common physiological and metabolic characteristics. Furthermore, LAB produces lactic acid as the main metabolic end-product at the end of the carbohydrate fermentation. LAB has been known for many health benefits and industrial importance. In general, the genera that comprise the LAB are *Lactococcus*, *Streptococcus, Lactobacillus*, *Leuconostoc*, *Pediococcus*, as well as the more peripheral *Lactobacillus, Aerococcus*, *Enterococcus*, *Oenococcus*, *Carnobacterium*, *Vagococcus*, *Tetragenococcus*, and *Weisella*; these belong to the order *Sporolactobacillus*, (Sonomoto, 2011). Information regarding the isolated species of lactic acid bacteria from raw and camel milk is explained in the forward sections.

Different species of bacteria are isolated from raw and fermented camel milk. The diversity in the microflora would be due to the different geographical, environmental and milk composition (Aziz et al., 2009).

Researchers from the various countries had examined raw camel milk for the isolation and identification of different microbes. *Enterococcus durans*, *Enterococcus faecium, Enterococcus faecalis, Lactobacillus paracasei, Lactococcus lactis* subsp. *lactis, Leuconostoc mesenteroide, Lactobacillus casei* subsp. *casei, Lactobacillus casei. Lactobacillus kefiri, Lactobacillus curvatus, Lactobacillus sakei* have been isolated from fresh camel bulk milk of both the species (*Camelus dromedarius* and *Camelus bactrianus)* and, also, from the sample of '*Shubat*' (Akhmetsadykova et al., 2015). Hamed and Elattar (2013) had isolated several lactic acid bacteria from raw camel milk obtained from Arabian Camels of Egypt. They determined one isolate *Lactobacillus plantarum, Lactococcus lactis, Enterococcus durans, Aerococcus viridians* and, in addition, isolated seven isolates of *Enterococcus faecium*. Benkerroum et al. (2003) and Jans et al. (2012) also exhibited the predominance of *Enterococci*, especially *Enterococcus faecalis*, in camel milk microflora in Morocco and in Kenya respectively. From raw camel milk, isolated lactic acid bacteria like *Lactobacillus fermentum* and *Lactobacillus plantarum* showed probiotic potential like absence of undesirable properties (Mahmoudi et al., 2016). In their study, Khedid et al. (2009) identified and isolated a variety of bacteria from raw camel milk derived from Morocco, including species such as *Lactobacillus helveticus* (10%), *Lactococcus lactis* subsp. *lactis* (17.5%), *Lactobacillus casei* subsp. *casei* (5.80%), *Streptococcus salivarius* subsp. *thermophilus* (9.20%) and *Lactobacillus plantarum* (5%). Same authors (Khedid et al., 2009) also isolated *Lactobacillus amylophilus* from camel milk which was already proved as a beneficial strain in the fermentation of crude starch to lactic acid and has a lot of applications in food industries (Naveena et al., 2004). In a recent study, probiotic *Lactobacillus* strains (*Lb. reuteri*-KX881777, *Lb. plantarum*-KX881772, *Lb. plantarum*-KX881779) isolated from camel milk found more promising rather than the other non-camel milk strain (*Lb. plantarum* DSM2468), during the comparative study (Ayyash et al., 2017).

STRAINS OF LAB ISOLATED IN DIFFERENT FERMENTED CAMEL PRODUCTS

Naturally fermented camel milk and products were examined by researchers for identifying variety of species from samples collected from different regions of the world. Mainly *Enterococcus* and *Lactococcus* genus seems dominating in camel milk and *'Shubat'* (Akhmetsadykova et al., 2015). Seifu et al. (2012) isolated 58% *Lactobacillus*, 25% *Lactococcus* and 17% *Enterococcus* from traditional Ethiopian fermented camel milk *'Ititu'*. Rahman et al. (2009) collected *'Shubat'* from seven Bactrian samples in China, found predominance of *Enterococcus* and *Lactobacillus* using biochemical tests. Lore et al. (2005) isolated the lactic acid bacteria from one of the traditional fermented camel milk product *'Suusac'* and reported the presence of homofermentative *Lactobacillus salivarius, Lactococcus raffinolactis, L. plantarum, Lactobacillus curvatus* and *Leuconostoc mesenteroides* subsp. *mesenteroides* as hetero-fermentative. Soleymanzadeh et al.

(2016) collected fermented camel milk product *"Chal"* (diluted fermented camel milk) from Iran, Turkman Sahra, Golestan province and isolated lactic acid bacteria like *Leuconostoc lactis, Lactobacillus plantarum, L. paracasei, L. kefiri, L. gasseri, Enterococcus faecium* and *Weissella cibaria* and identified by conventional and molecular methods. *'Hurunge'*, one of the famous traditional fermented camel milk product from China were investigated for lactic acid bacterial isolates and identified *Lactococcus lactis* ssp. *lactis, Lactococcus raffinolactis, Lactococcus lactis* ssp. *cremoris, Leuconostoc mesenteroides* ssp. *cremoris, Lactobacillus casei, Lactobacillus homohiochii*, *Lactobacillus plantarum*, *Lactobacillus acetotolerans* and *Lactobacillus kefiranofaciens* and few strains of yeasts also isolated like *Candida kefyr, Candida valida, Candida krusei Kluyveromyces marxianus* var. *lactis* and *Saccharomyces cerevisiae* (Quan et al., 2006). Maurad and Meriem (2008) isolated thirty-eight lactic acid bacteria (*Lactobacillus plantarum*-SH1 to SH38) from traditional butter which was prepared using *Shmen* (fermented camel milk) as a starter culture. Among these strains SH12 and SH24 showed promising probiotic potential, high acidifying rates, and proteolytic activity.

BIO FUNCTIONAL PROPERTIES OF FERMENTED CAMEL MILK

Fermented camel milk is used in the treatment of health problems like tuberculosis, dropsy, leishmaniasis or kala-azar and asthma in different regions of the world including Sudan, India, and Russia (Abdelgadir et al., 1998). Lactic acid bacteria produced peptides from milk proteins during fermentation and provide several health benefits to the consumers. Bioactive peptides like ACE-inhibitory peptides and anti-oxidative peptides were also derived from fermented camel milk and the comparative study also showed the maximum ACE-inhibitory in fermented camel milk rather than bovine milk (Moslehishad et al., 2013).

Hypocholesterolaemic effect

Fermented camel milk, particularly, *Gariss* containing *Bifidobacterium lactis* (BB-12) has been exhibited to possess a hypocholesterolaemic effect through *in vivo* experiment in rats (Elayan et al., 2008) and in the lowering of plasma and liver cholesterol levels (Ali et al., 2013). However, the hypocholesterol regulating mechanism of camel milk is still unclear. Various hypotheses were discussed by researchers (Li and Papadopoulos, 1998; Rao et al., 1981; Buonopane et al., 1992).

Antimicrobial activity

Fermented camel milk is having antimicrobial effects against different pathogens. In a study, in Iraq, antimicrobial activity of fermented camel milk was reported (Lafta et al., 2014). LAB isolated from Tunisian camel raw milk showed antibacterial activity against *Staphylococcus aureus, Listeria monocytogenes* and *Escherichia coli* also inhibited *Salmonella typhimurium* (Mahmoudi et al., 2016). *Mycobacterium tuberculosis* was effectively inhibited by camel milk (Sharma et al., 2014).

Yateem et al. (2008) collected raw camel milk (Arabian camel) samples from Kuwait and allowed them for spontaneous fermentation for one week through endogenous bacteria at room temperature. Furthermore, upon 16S rRNA gene sequencing the bacterial species were confirmed. *Lactococcus lactis lactis* antagonistic behavior was demonstrated by the production of 14 mm against *Salmonella sp.* and 20 mm inhibition zones against *E. coli* strains. As fermented camel milk contains different lactic acid bacteria, may produce peptides and bacteriocins which showed inhibitory activity against many pathogens including *Bacillus*, *Staphylococcus*, *Salmonella* and *Escherichia* reported by various researchers. Further discussion regarding the inhibitory activity was also presented in the section of studies on probiotic potential.

Antioxidant activity

It was reported that, during the gastrointestinal digestion or fermentation of bovine milk β-Casein, it produced radical scavenging bioactive peptides (Kansci et al., 2004). In the similar way, β−Casein (65 % of total caseins) fraction present in camel milk, produced antioxidant peptides (Jrad et al., 2014). Salami et al. (2011) reported the higher antioxidant activity of camel β-CN after enzymatic hydrolysis with chymotrypsin. Shori and Baba (2014) also reported the *in-vitro* antioxidant and antidiabetic activity of fermented camel milk which is supplemented with garlic extract. The researchers used traditional LAB such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* to ferment camel milk and analyze the activity. Antioxidant activity of LAB isolated from fermented camel milk *"Chal"* collected from Turkman Sahra, Golestan Province, Iran was determined, and it was found higher in camel milk as compared to bovine milk (Soleymanzadeh et al., 2016). Antioxidant activity of fermented camel milk was higher as compared to unfermented, and other dairy animals using *Lactobacilli acidophilus* culture (NRCC, 2013-2014). Similarly, higher antioxidant activity of camel milk fermented with *Lactobacillus rhamnosus* PTCC 1637 was reported as compared to the bovine milk. Peptides generation during fermentation of camel milk increased with the increase in proteolytic activity during storage which ultimately results in increase in antioxidant activity (Moslehishad et al., 2013). A recent comparative study reported that, camel milk fermented with indigenous camel milk probiotic lactic strains (*Lb. reuteri*-KX881777, *Lb. plantarum*-KX881772, *Lb. plantarum*-KX881779) showed maximum antioxidant activity as compared to the bovine milk (Ayyash et al., 2017).

Angiotensin I-converting enzyme (ACE) inhibitory activity or Antihypertensive effect

Milk protein is a rich source of ACE-inhibitory peptides (Jang and Lee, 2005). Prevention of hypertension was one of the reported benefit of fermented camel milk (Quan et al., 2008). *Lactobacillus helveticus* 130B4, isolated lactic acid bacterium from traditionally fermented camel milk has been reported to produce a novel ACE-inhibitory peptide Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp from China (Quan et al., 2008). The strain (*Lb. helveticus*) also has been experimented to derive ACE-inhibitory peptides from bovine milk proteins (Minervini et al., 2003). Moslehishad et al. (2013) reported that, as camel milk is rich source of proline residues, so upon its fermentation with *Lactobacillus rhamnosus* PTCC 1637, it produced novel antioxidant peptides and ACE-inhibitory peptides in camel milk as compared to the bovine milk. National research centre on camel (NRCC) reported generation of higher ACE- inhibitory activity in fermented camel milk as compared to unfermented camel milk, -and in the other dairy animals using *Lactobacillus acidophilus* and SARAS dahi (SARAS Dahi- Fermented milk from a company SARAS, Jaipur, India) as a starter cultures (NRCC, 2013-2014). Solanki et al. (2017) isolated novel ACE-inhibitory peptides (IPP and VPP) from fermented camel milk and concluded that camel milk seems promising ingredient for the functional food development. Similarly, Ayyash et al. (2017) also examined the ACE-inhibitory activity of fermented camel milk with camel milk based probiotic *Lactobacillus* strains (Lr.K777, Lp.K779 and Lp.K772) reported higher ACE-inhibitory activity in fermented camel milk rather than bovine milk under the same conditions. Yahya et al. (2017) studied the antihypertensive activity of fermented skim camel milk on spontaneously hypertensive rats (SHR). Unfermented skim camel milk and fermented skim camel milk prepared using fermentation by *Lactobacillus helveticus* (LMG11445) and *Streptococcus thermophilus* (ATCC 19258) were analyzed for antihypertensive activity in SHR rats in terms of short term (measurements taken on 0, 2, 4, 8 and 24 hours after single oral administration) and long-term administration effect (measurements were taken before administration and continued for every five days up to total twenty-one days). Results revealed the hypotensive effect on systolic blood pressure and diastolic blood pressure (in both short term and long term) with the high concentration of fermented skim milk.

Activity against diarrhea

Camel milk is used to treat the diarrhea due to viral infection (like Rota virus). Mona (2010) conducted an animal study on diarrheal rats and concluded that camel milk had higher amount of sodium and potassium which helped to stop diarrhea in rat models. They concluded that, the fermented camel milk is a unique food which provided high nutrition and various therapeutic benefits. In Kazakhstan, *shubat*, which is a national drink, showed promising antiviral property. Freshly prepared or conserved *shubat* was used for the study and concluded that the *shubat* exhibited virus-inhibiting or virucidal properties against viruses like ortho- and paramyxovirus. These properties became unchanged up to the end of its shelf life due to the presence of metabolic products of LAB and yeasts present in *shubat* (Chuvakova et al., 2000).

Anticancer activity

Limited literature is available on fermented camel milk for an anticancer activity. In a recent study, proliferation inhibition, indicated as an anticancer indicator, of water soluble extracts (< 3 kDa) of fermented bovine and camel milks against MCF-7, Caco-2 and HELA carcinoma cell lines was examined. It was exhibited that, the proliferations of MCF-7, Caco-2 and HELA cells was more inhibited during the treatment with water soluble extracts from camel milk rather than with bovine milk fermented by all present strains Lr.K777, Lp.K779 and Lp.K772 except Lp.DSM. It is concluded that, the high anti-proliferation activity of fermented camel milk prepared using Lr.K777, Lp.K779 and Lp.K772 may be attributed to the greater competition capability of peptides derived from fermented camel milk rather than those from fermented bovine milk. Determination of positive correlation between proliferation inhibition and ACE-inhibition suggested that peptides derived from fermented camel milk have multifunctional bioactivity (Ayyash et al., 2017).

Activity against Carbon tetrachloride (CCl4)

Different literatures reported the harmful effects of Carbon tetrachloride (CCl4) on human health i.e. xenobiotic effects. In a recent study of Hamed et al. (2017), who used fermented camel milk (prepared by using *Lactococcus lactis* subsp *cremoris*) to evaluate the cardio-preventive effects against the toxic effects of acute exposure to CCl4 on heart tissue of mice. Study significantly provide evidences against the reduction of CCl4 induced heart oxidative damages by reduction in toxicity markers and increasing the activities lactate dehydrogenase (LDH), alanine aminotransferase (ALT), creatine kinase MB (CKMB), Troponin I and creatine kinase (CK) activities.

Defense against lead contamination

Fermented camel milk prepared using specific strains of lactic acid bacteria reported to provide protection against lead (Pb) contamination. Lead toxicity can cause problems like cancer, anemia and saturnism. Fermented camel milk product *'shubat'* could decrease the availability of lead (Dallak, 2009; Al-Hashem, 2009; Loiseau et al., 2009, Akhmetsadykova et al., 2013).

STUDIES ON PROBIOTIC POTENTIAL

Probiotics are good bacteria. Probiotics are famous for health benefits (FAO/WHO, 2002). In dairy products, well balanced combinations of starter cultures and probiotics (*Lactobacillus, Bifidobacterium*,) are widely used in fermented foods (Vinderola et al., 2000; Lourens-Hattingh and Viljoen, 2001). In vitro tests were carried out for the screening of probiotic potential of lactic acid bacteria or starter culture. These *in vitro* tests are based on the host's gut environment which is created in laboratory. Camel milk and fermented camel milk was also referred as a source of probiotics in literature. Yateem et al. (2008) isolated lactic acid bacteria from naturally fermented camel milk, and studied their probiotic potential.

In a study of Maurad and Meriem (2008), the isolation of thirty-eight stains of *Lactobacillus plantarum* strains (SH1 to SH38) was achieved from traditional butter which was previously prepared using the camel milk (*shmen*) as starter cultures for fermentation in the region of Algeria (Sahara). Mutlag et al. (2013) collected 35 samples of raw camel's milk and isolated gram-positive cocci/rods and catalase negative bacteria. They termed them as presumptive lactic acid bacteria for the further study. These presumptive lactic acid bacteria (*Enterococcus, Lactobacillus, Lactococcus)* showed clear zone of inhibition against *L. monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 29213, bacteriocin production, great potential to survive bile conditions, sensitivity to amoxicillin, ampicillin, chloramphenicol and bacitracin. Cell free extract sensitive to proteolytic enzymes indicates the proteinaceous nature (i.e. bacteriocins) of inhibitors. After feeding to the albino mice, results showed the prevention of adhesion of *Staphylococcus aureus* and inhibition of *E. coli* in gastrointestinal tract.

Abbas and Mahasneh, (2014) isolated 400 isolates from fresh and fermented camel milk out of them thirty-four isolates were presumed as lactic acid bacteria from *Lactobacillus* species (termed as M1 to M34) such as *Lactobacillus brevis* (6%), *Lactobacillus fermentum* (12%), *Lactobacillus rhamnosus* (18%), *Lactobacillus plantarum* (23%), -and *Lactobacillus paracasei* ssp *paracasei* (41%). Upon *in vitro* analyses of probiotic potential these bacteria showed influencing probiotic potential. Only *Lactobacillus plantarum* isolates M1, M2, and M4 showed best survival at pH 3.0, but all the isolates showed tolerance to NaCl, temperature resistance, and bacteriocin activity (pathogen inhibition) against *B. cereus*, MRSA, *E. coli* ATCC 25922, and *thyphimurium* ATCC 14028. In another study, Benmechernene et al. (2013) had isolated thirteen *Leuconostoc* strains from four different camel milk samples collected from different areas of Saharan in South Western Algeria. Out of them, two isolates (*Leuconostoc mesenteroides subspecies mesenteroides* (B7, Z8)) were tested for probiotic potential. Recently, a study conducted by Saljooghi et al. (2017), on microbiological, organoleptic and biochemical changes in probiotic fermented camel milk prepared using probiotic culture ABT-10 (Chr. Hansen, Denmark) showed the survival of lactic acid bacteria (minimum 10^6 CFU/mL) and acceptable sensory qualities of fermented camel milk till the ninth day of storage at the refrigeration temperature. So, from the above studies we can say that, as there are *in vitro* tests determined the probiotic potential of camel milk or fresh camel milk originated bacteria but still some *in vivo* studies are required to confirm it in best way.

CONCLUSION

Traditionally, camel milk is served as a biomedicine for many health problems throughout the world. Fermented camel milk containing bioactive peptides possess multi functionality against the several health benefits determined through *In vitro* studies. However, several promising *In vivo* studies must be performed to evaluate the mechanisms and health benefits of fermented camel milk. Camel milk isolated lactic cultures possess strong potential to be known as starter cultures and probiotics in the current era, further validation is needed through *in vivo* studies.

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