



UNIVERSITI PUTRA MALAYSIA

***PHYSICO-CHEMICAL AND MICROBIOLOGICAL QUALITIES OF RAW
GOAT MILK AND ANTIBACTERIAL CHARACTERISTICS OF ISOLATED
LACTIC ACID BACTERIA***

LAI CHIA YEE

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By

LAI CHIA YEE

**This is submitted to the School of Graduate Studies, University Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science**

November 2014

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Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for Degree of Master of Science

PHYSICO-CHEMICAL AND MICROBIOLOGICAL QUALITIES OF RAW GOAT MILK AND ANTIBACTERIAL CHARACTERISTICS OF ISOLATED LACTIC ACID BACTERIA

By

LAI CHIA YEE

November 2014

Chairman : Professor Fatimah Abu Bakar, PhD

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Raw goat milk is recognized as a nutritious food owed to its originality and medicinal values. In recent years, people consumed goat milk more frequently for its natural health promoting components. However, data for quality and safety of the locally produced raw goat milk are scarcely available. There is no official quality and safety standard to describe on raw goat milk. Only the total bacterial count is used as the main quality or safety measure. This is not adequate as there are numbers of food-borne related facts. The contamination of the milk proper storage temperature and duration should be accounted for public notifications due to food safety concerned. This study aimed to evaluate the microbiological quality and safety of stored raw goat milk prior to further processing steps. In platform tests, milk samples passed organoleptic test and Clot On Boiling test but were mostly (88.89 %) failed in alcohol test. The proximate composition, the water content of milk samples were relatively high while the remained contents of protein, fat, carbohydrates and ash are much lower than the reference values. In general, results obtained from physico-chemical analysis showed contents of local milk samples were partially in lined with reported literature due to subjective factors of breeds, geographical areas and feeds. There was no significant difference observed in pH values of milk samples in comparison to the reported data. Initial total plate count, coliform count and proteolytic count determined were 3.44 log cfu/ml, 1.87 log cfu/ml and 1.97 log cfu/ml respectively. Storage time showed significant effect on the bacterial counts ($p < 0.05$) of milk samples. Milk samples were kept and observed up to 12 hours under ambient temperature (3.95 log cfu/ml) had not exceeded guidelines limit of total aerobic count for food. The shelf-life of the milk samples were extended up to 10 days storage under refrigerated temperature of 4°C. A total of 150 Lactic Acid Bacteria (LAB) isolates were determined to a presumptive genus level and numbers were selected for species identification using the BIOLOG System. Among the isolated LAB species from goat milk samples, identified were *Lactobacillus buchneri*, *Lactobacillus brevis*, *Enterococcus casseliflavus*, *Enterococcus faecium* and *Weissella viridescen*. Bacteriocin Like Inhibitory Substance (BLIS) were obtained from the

identified LAB species and further verified on their antibacterial activity. There was at least one level of variables have significantly affect the antibacterial activity towards selected Gram-positive test bacteria, ATCC 19155 *Listeria monocytogenes*, *Staphylococcus aureus* and *Streptococcus spp.* Besides, BLIS goat milk LAB species had shown significant effect ($p\text{-value} < 0.05$) on antibacterial activity against selected Gram-negative test bacteria such as *Escherichia coli* (*E. coli*), *Pseudomonas spp.*, *Klebsiella spp.*, ATCC 14028 *Salmonella typhimurium* and *Serratia marcescens*. BLIS produced by *Lactobacillus brevis* and *Lactobacillus buchneri* isolated from the goat milk spoilage bacteria has shown potent inhibition against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*. These findings emphasized the importance of microbiological quality study of locally produced raw goat milk and screening of more novel LAB species with their antibacterial activities.



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**KUALITI FIZIKO-KIMIA DAN MIKROBIOLOGI SUSU KAMBING ASLI
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Susu kambing mentah diiktiraf sebagai makanan berkhasiat yang terhutang kepada keaslian dan nilai-nilai perubatan. Dalam tahun-tahun kebelakangan ini, orang minum susu kambing lebih kerap demi kesihatan komponennya yang semula jadi. Walau bagaimanapun, data tentang kualiti dan keselamatan susu kambing mentah yang dalam negara adalah jarang. Standard yang rasmi untuk kualiti dan keselamatan susu kambing mentah tidak wujud. Terdapat hanya jumlah kiraan bakteria yang umum digunakan sebagai kualiti atau keselamatan ukuran utama. Namun, ini adalah tidak mencukupi disebabkan fakta-fakta yang berkaitan dengan bawaan makanan. Pencemaran dengan suhu dan tempoh penyimpanan susu adalah penting untuk makluman awam disebabkan oleh keselamatan makanan yang berkenaan. Kajian ini bertujuan untuk menilai kualiti mikrobiologi dan keselamatan susu kambing mentah yang disimpan sebelum langkah-langkah proses yang selanjutnya. Dalam ujian platform, sampel susu lulus ujian organoleptik dan Clot Pada didih ujian tetapi kebanyakannya (88.89 %) tetapi gagal dalam ujian alkohol. Komposisi anggaran, kandungan air sampel susu agak tinggi manakala kandungan lain seperti protein, lemak, karbohidrat dan abu adalah jauh lebih rendah daripada nilai rujukan. Secara umum, keputusan yang diperolehi daripada analisis fiziko-kimia adalah sebahagiannya setara dengan kajian-kajian rujukan kerana faktor-faktor subjektif baka, kawasan geografi dan suapan yang berlainan. Tidak terdapat perbezaan yang ketara diperhatikan pada nilai pH sampel susu berbanding dengan data yang dilaporkan. Jumlah kiraan plat awal, kiraan koliform dan kiraan proteolitik yang ditentukan adalah 3.44 log cfu/ml, 1.87 log cfu/ml dan 1.97 log cfu/ml masing-masing. Masa penyimpanan menunjukkan kesan yang ketara ke atas kiraan bakteria ($p < 0.05$) daripada sampel susu. Sampel susu telah disimpan dan diperhatikan sehingga 12 jam pada suhu ambien (3.95 log cfu/ml) adalah tidak melebihi had garis panduan daripada jumlah kiraan aerobik untuk makanan. Jangka hayat sampel susu telah dilanjutkan sehingga 10 hari penyimpanan di bawah suhu sejuk dari 4°C. Sebanyak 150 Asid Laktik Bakteria (LAB) pencilinan telah ditentukan untuk tahap genus yang berdasarkan sangkaan dan nombor telah dipilih untuk mengenal pasti spesies menggunakan Sistem BIOLOG. Antara spesies LAB terpencil daripada sampel susu

kambing, yang dikenal pasti ialah *Lactobacillus buchneri*, *Lactobacillus brevis*, *Enterococcus casseliflavus*, *Enterococcus faecium* dan *Weissella viridescen*. Bakteriosin Seperti Bahan yg melarang (BLIS) telah diperolehi daripada spesies LAB dan seterusnya disahkan pada aktiviti antibakteria mereka. Terdapat tahap sekurang-kurangnya satu pembolehkan telah ketara memberi kesan kepada aktiviti antibakteria terhadap bakteria ujian Gram-positif dipilih, ATCC 19155 *Listeria monocytogenes*, *Staphylococcus aureus* dan *Streptococcus spp.* Selain itu, BLIS susu kambing spesies LAB telah menunjukkan kesan yang penting (p - nilai < 0.05) pada aktiviti antibakteria terhadap dipilih kerosakan Gram-negatif bakteria ujian seperti *Escherichia coli* (*E. coli*), *Pseudomonas spp.*, *Klebsiella spp.*, ATCC 14028 *Salmonella typhimurium* dan *Serratia marcescens*. BLIS dihasilkan oleh *Lactobacillus brevis* dan *Lactobacillus buchneri* yang didapati daripada bakteria kerosakan susu kambing telah menunjukkan perencatan yang kuat terhadap *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* dan *Salmonella typhimurium*. Penemuan ini menekankan kepentingan kajian kualiti mikrobiologi susu kambing mentah yang dihasilkan dalam negara dan mendapatkan spesies LAB yang novel dengan aktiviti antibakteria mereka.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BLIS	Bacteriocins like inhibitory substance
CFU	Colony forming unit
DMRT	Duncan's multiple range test
EMB	Eosine methylene blue agar
FDA	Food and drug administration
h	Hour
HCl	Hydrochloric acid
LAB	Lactic acid bacteria
M	Molar
MH	Mueller hinton agar
MRS	de Man, Rogosa and Sharpe agar
min	Minute
mL	Milliliter
μ L	Microliter
rpm	Revolutions per minute
TCA	Trichloroacetic acid
TSA	Tryptic soy agar
v/v	Volume over volume
w/v	Weight over volume

CHAPTER 1

INTRODUCTION

Consuming milk and products could be one of the routine practices upholding in Malaysia's population. In Malaysia, the total milk production of small-scale dairy farms may not fully support the local market needs (Barmaiyi *et al.*, 2014; Alyaqoubi *et al.*, 2014; Lye *et al.*, 2011). The local milk production may lack concern, especially for goat milk and yet it is one of the potential industries to develop. Goat milk production is a growing industry and presents a significant part of the economy in many countries, especially in Asia of India and China (Pal *et al.*, 2011). Goat milk delivers nutrients like cow milk where there are reviews of present data suggesting that it owns even greater benefits over cow milk (Ribeiro and Ribeiro, 2010; Ceballos *et al.*, 2009). Goat milk works as one of the important medicinal food and used as a nutritional source for infants and children as well (Haenlein, 2004). It has been clearly proven that consuming of goat milk improves the state of health and wellness of the human body, reduces the risk of developing disease especially allergies (Park and Haenlein, 2008). Because of the medicinal values and natural nutrient, raw goat milk serves as a healthy food or drink among Malaysian.

The studies about quality and food safety of local raw goat milk are rarely found. There is no available standard of raw goat milk quality and safety for consumption. In the local market, a total plate count (TPC) less than 106 cfu/ml used as a guideline or standard by Milk Collection Centers (MCC) as a Price Incentive Program (Boniface, 2012; Chye *et al.*, 2004). The milk selling price calculated based on the bacterial count in which a high microbial load may pose economic loss to local dairy farmers. Like cow milk, goat milk production is referring the same standard. The general total bacterial count regardless of milk types used as the main quality and safety assessment may not adequate to be a proper guideline. Besides, lack details and knowledge about microbiological quality and safety raw goat milk could lead to an uncomfortable issue of food contamination. Storage time and conditions of raw goat milk after milking are important factors to control the milk quality and safety. Improper storage conditions will lead to a significant change in the raw goat milk quality and safety. People consume raw goat milk in direct after milking and some may store it for some time. They may not have an appropriate idea in the way of keeping raw goat milk, including the appropriate temperature and duration. The common factors responsible for raw milk contamination, include the condition of the udder during lactation, the environment outside the udder and the equipment for handling and storage (Chambers, 2002).

Chilling of raw goat milk is a common practice to keep the milk fresh and prevent the growth of non-psychrotrophic bacteria. Yet, prolonged storage of raw goat milk under cold temperature may not safe. According to Yamazi *et al.*, 2013, the total loads of mesophilic bacteria, coliforms bacteria, *Escherichia coli* and psychrotrophic bacteria of milk stored for 48 hours or longer were relatively higher than the storage for 24 hours or less. A potential growth media of milk with low-temperature favors the growth of psychrotrophic bacteria that enter the milk from other sources such as dirty equipment and the environment outside that pose risk of contamination indirectly. An initial low psychrotrophic population of total plate count grows fast in milk and become the dominant bacteria after storage of a couple of days. Proper storage temperature and duration account major food safety concerned for public notifications.

Similar to other milk, raw goat milk is carrying comparable bacteria species but some may vary. There are studies revealed that lactic acid bacteria (LAB) are one of the famous bacteria found in goat milk, including the genera of *Lactobacilli* (5.13 log cfu/ml), *Lactococcus* (6.57 log cfu/ml), *Leuconostoc* (3.51 log cfu/ml), and *Enterococcus* (2.47 log cfu/ml) (Nikolic *et al.*, 2008; Tamagnini *et al.*, 2006; Alonso-Calleja *et al.*, 2002). LAB exert an important relationship between the quality and safety of the milk. Groups of LAB designated as GRAS (Generally Recognized as Safe) since decades ago. LAB do not pose any health risk to man and thus usually considered as “food grade” bacteria to be selected as protective probiotic. Therefore, isolation and screening of LAB from raw goat milk have always been the preferable way to obtain natural biodiversity of useful cultures with commercial values (Asgmaig *et al.*, 2009 and Sun *et al.*, 2010).

People nowadays demand for new flavors, best quality and health concerned food from time to time. There is an obvious need to search for novel ways and new food preservation agents of natural origins. LAB are commonly used as a safe and effective starter culture in fermented foods, especially in fermented dairy products. Marketers aim for new products require the use of microbial strains with novel properties. Screening the novel LAB strains from natural biodiversity or genetic modification of existing strains is important for the innovation to increase the variety of dairy products (Abd El Gawad *et al.*, 2010). Thus, isolation of wild LAB from local raw goat milk, not exposed to any industrial selection is potential to be used in making new food products. The safe, new and active strains of LAB are desired in production of local fermented food products such as fish sauces, shrimp pastes and tempoyak other than dairies (Mohd Adnan and Tan, 2007).

LAB have been comprehensively studied due to their beneficial and functional characteristics. Different strains of LAB isolated in dairy food processing environments based on their acid production rate, proteolytic activity, production of antimicrobial compounds and carbon dioxide (Lindgren and Dobrogosz, 1990). During fermentation, LAB are playing an essential role for organoleptic characteristics, acidification and production of aromatic compounds (El-Ghaish *et al.*, 2011). Local LAB could have considerable potential as starters or other significant roles in various food products. The isolation and selection of local LAB are insufficient and more works in different aspects should be carried out. This study was undertaken to screen the LAB that exhibit antibacterial activity from local raw goat milk samples. Bacteriocins produced by LAB during growth is having a competent antibacterial effect that making themselves dominant in a pool of microflora. Bacteriocins are proteinaceous antibacterial compounds which ribosomally synthesized and are usually active against genetically related species and some Gram-positive bacteria (De Vuyst and Vandamme 1994 and Klaenhammer, 1988). The search for new strain of LAB that having antibacterial ability in current study are crucial for the development of biologically safe local dairy products. In addition, the finding was thought to significantly contribute to the increasing demand for goat dairy product. An updated database of quality and safety of locally produced goat milk, isolation of LAB strains with antibacterial ability are fundamental for the food product development in Malaysia.

The objectives of this study are:

1. To determine the physico-chemical properties and microbiological quality or safety of locally produced raw goat milk.

2. To isolate and identify lactic acid bacteria strain from locally produced raw goat milk samples.
3. To screen and evaluate the potency of antibacterial activity among isolated and identified lactic acid bacteria strains.



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