

Development of Probiotic Soft Cheese with *Lactobacillus casei* as Adjunct Culture

Muhammad Waheed Iqbal*, Wanmeng Mu, Imran Mahmood Khan, Ali Mohsin,
AbdurRehman and Marwa YF Koko

State Key Laboratory of Food Science and Technology, School of Food Science and Technology,
Jiangnan University, 1800 Lihu Avenue, Wuxi, Jiangsu 214122, China
waheed2678@yahoo.com*; +86-13057312957

Abstract

Probiotics are living microorganisms that provide beneficial health effects by improving immune system and inhibiting the growth of pathogenic bacteria. They have been used in different food products such as cheese, which is the distinctive way to preserve milk. Different varieties of cheeses are developed all over the world. The present review was designed to the development of goat milk soft cheese with the addition of probiotics namely *Lactobacillus casei* and *L. acidophilus*. The probiotic *Lactobacillus casei* was used as adjunct culture while the isolated *L. acidophilus* from yoghurt is used for the alignment of stomach walls and different kind of stomach and digestible problems. This review emphasized on the Pakistani dairy industrial related challenges that have been producing quality goat milk for cheese production containing high amount of α -S₂ casein, rancid flavor and free fatty acids that are used for stomach alignment. By the preparation of probiotic soft cheese from goat milk, we can overcome the challenges and improve the quality of goat milk for human health.

Keywords: Probiotics, goat milk, *Lactobacillus casei*, cheese production, adjunct culture.

Introduction

Milk is considered as a nutrient fluid produced by the mammary glands of female mammal. It acts as the basic source of nutrients for young ones until they are capable of digesting various varieties of food. It is also used for making different milk products like cream, margarine, curd, frozen yoghurt, cheese, whey protein, dried milk powder, fortified milk and a lot of other food products and commercial items (Silva *et al.*, 2012). Milk is a physiologically and biologically complex fluid, which contains mainly water, protein lactose, fat and inorganic, compounds as its principle constituents. It is consider as food matrix of simple access and has generous quantity of nutrients, vitamins and minerals. Percentage of lactose, proteins, lipids and mineral salts in milk is 5%, 3.2%, 4%, and 0.7% respectively (DaCosta *et al.*, 2014). It is the secretion of memory gland of different mammals, which is obtain except the previous fifteen, or next five days of calving and other periods, which are important for making the milk colostrum free and consisting of the lowest recommended content of milk fat and milk solid, not fat. It is one of the most extravagantly studied human foods (Kvamsas, 2013). Milk from different species has its own characteristics. Buffalo milk is white liquid having very smooth texture, especially low in cholesterol and higher in calcium than cow milk, sheep and goat milk. Cow milk contains 43% more cholesterol, 58% and 40% less calcium and protein respectively than buffalo milk.

Cow milk is considered as a basic food for human giving essential nutritional components because it contains specific concentration and types of amino acids which are very close to amino acid requirement of human (Pembleton *et al.*, 2016). The bovine milk is less digestible as compared to goat milk. The people living in rural areas all over the globe obtain milk and flesh from goat. Although nearly 2% of the total milk is obtained from goats per year, they play a very significant role in health and financial well-being of humankind in several countries around the globe. The people who are hypersensitive to cow milk can easily use goat milk. Proteins causing allergic reaction present in cow milk are lower in goat milk. It is naturally homogenized so it does not cream out and is easier to digest (FAOSTAT, 2013; Garcia *et al.*, 2014). Chemical composition and acidity of raw cow milk used for the production of probiotic cheese and after adding probiotic its composition changed, the fat level increased just because of lactic acid bacteria or probiotics from lactic acid family decreased the pH level which is used to enhance the shelf-life of cheese (Yerlikaya and Ozerr, 2014). Goat milk is contributing a large amount for supportable development just because of its increasing market demand. Producers can get better yield and financial benefits from milk and other products obtained from goats. Milk composition of different species is shown in Table 1.

*Corresponding author

Table 1. Milk composition of different species (Source: Jensen, 1995).

Species	Water%	Fat%	Protein%	Total solid %	SNF%	Lactose%	Ash%
Goat	87.50	4.30	3.53	13.50	7.70	4.28	0.87
Cow	86.61	4.14	3.58	13.19	9.25	4.96	0.71
Buffalo	82.76	7.38	3.60	17.24	9.86	5.48	0.78
Sheep	80.71	7.90	5.23	19.29	11.3	4.81	0.90
Camel	87.61	5.38	2.98	12.39	7.01	3.26	0.70

This need is elevating due to increasing population of human. There is a saying, “goat is the poor man’s cow” which fits truly (Golinelli et al., 2014). The second feature is that the experts are particularly interested in products such as cheese and curd from the milk of goat in several countries. Its usage has a great medicinal importance. It is composed of fat and protein, which is highly digestible as compared to cow’s milk. This combination is greatly beneficial in infant diets (both human and animal). Goat milk has a buffering quality that makes it useful in the treatment of ulcer patients. The people who are hypersensitive to cow milk can consume goat milk and it can be used as a milk replacer for other farm animals (Zachar et al., 2011). Golinelli et al. (2014) noted that various types of goat milk products are available such as frozen yoghurt, butter, skimmed milk or enriched milk, fermented and other types of frozen products like, curd, frozen yoghurt and powdered milk. Several goat and sheep milk cheeses are recognized in various countries. Cheese is a coagulated and concentrated product of milk being nutritious, versatile and expedient with range of flavors, textures, forms and varieties (Farkye, 2004; Ribeiro and Ribeiro, 2010). The diversity is due to more and more knowledge of cheese making technology as well as biochemistry and microbiology of cheese ripening. The application of goat milk has been increasing day by day. Cheese manufacturing from the goat milk has a social and economic effect due to their distinctive organoleptic and textural properties. Furthermore, goat cheese is highly nutritious due to their high protein content further improved by probiotic cultures. Single and mixed types of cultures of streptococci, lactobacilli and *Lactobacillus acidophilus*, which react as probiotics in fresh soft cheeses made from goat milk. Usually starter cultures give flavors, shape and texture of cheese and cease the multiplication of different types of pathogenic bacteria (Ribeiro and Ribeiro, 2010).

Cheese is a coagulated and concentrated product of milk, which is highly nutritious, versatile and expedient with range of flavors, textures, forms and varieties (Queiroga et al., 2013). The diversity is because of an elevating awareness of cheese making methods and biochemistry, as well as due to the study of cheese ripening at microbiological level.

The cheese manufacturing utilizes nearly one third of the total milk produced around the globe (Oliveira et al., 2012). Cheeses are classified based on origin, milk sources, manufacturing, appearance, composition, texture and ripening (Mehra and Kelly, 2006). Soft cheeses are manufactured through the goat milk by using probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* as adjunct culture (Udyarajan et al., 2007). In recent years, the public awareness is growing towards the diet related health issues and has increased the demand of food with different promoting effects. The Food and Agriculture Organization define probiotics as living organisms that on ingestion gives beneficial effects on the consumer’s health. All the probiotic strains belong to genera *Lactobacillus* and *Bifidobacterium*, which is associated with the gastrointestinal tract (Anal and Singh, 2007). Probiotic is a terminology known as “for life” and its definition is “living microorganisms that cause advantageous impact on the health of host by ameliorating its microbial control”. Presently, the definition of probiotics has been advanced as “live microorganisms that, when introduced in suitable concentration, grant a beneficial impact on the host health” (FAO/WHO, 2002). Lactic acid bacteria (*Lactobacillus acidophilus*) which is isolated from yoghurt samples are very significant probiotics, which have advantageous impact on the host digestive system. These are Gram-positive bacteria and can normally survive without oxygen but can also live in oxygen availability (Burns et al., 2012). Nowadays people are particularly conscious about their health. They demand healthy and disease resistant food that includes probiotics so the knowledge and interest about probiotics is elevating (Kailasapathy, 2009). The probiotics are significantly basic eatables because they exhibit nearly 65% of the global basic eatables trade centre and the probiotic trade canters are developing persistently. Beneficial microbes introduced in various eatables provide health benefits to consumer. Such product includes both dairy (cheese, curd, frozen yoghurt and dairy desserts) and non-dairy ones (Anal and Singh, 2007). Probiotic was claimed to have numerous health benefits for foods containing particularly *Lactobacillus casei* and *L. acidophilus*. They inhibit the gastrointestinal inflammation (Reid, 2008). They control infections occurring during pregnancy and obstacle of urinary tract infections. They inhibit the allergic diseases and other medical complications (Park, 2007).

Importance of goat milk

It is considered that milk is the main source for the neonates who cannot digest foods. In the early stage of lactation, it contains constituent called colostrums which contained antibodies necessary for babies until their immune system development and prevent them from diseases. As we know in the early stage, neonate babies just consume mother milks and typically, after 6-24 months they become habitual of other alternative sources of milk (Golinelli et al., 2014). Although milk can be obtained from several species of animals like cow, buffalo, camel, goat, and sheep, in which the cow milk considered the most important once, having a nutritional property because of its resemblance with human milk. According to the Dziuba and Deziuba (2014) milk has unique composition of water, solids, fat and non-fat solids (87, 13, 3.7 and 8.9).

Introduction to cheese

As we know that, each type of dairy products is very nutritious, healthy and useful to human beings because it contains milk constituents, in which cheese is very common dairy product produced almost in whole world. The recorded history shows that it is a traditional food product. It made from pressed each type of milk curd. Cheeses are of two types ripened and un-ripened cheese called as aged and fresh respectively (Silva et al., 2012). It has versatile in nature and a very delicious dairy product and have a 900 kinds of varieties including soft, medium and hard cheese having different flavors and forms. Different kinds of vitamins like A and B12, minerals like calcium, phosphorus, and zinc and rich in protein found in cheese. The most important nutrient calcium is mostly lack in our diet and it abundantly found in milk (Cruz et al., 2009). According to latest research 9/10 women and 6/10, men come under lack of calcium in their diet according to the recommended dose. The basic technology to produce all kinds of cheese is similar, relatively small deviations in processes during production resulting in large detected variation in the final cheese. Goat milk cheese is very rare and nutritious type of cheese that is soft in texture and white in color, palatable taste and enriched with vitamins (Ayar et al., 2009). Cheese can be made from the milk of any species, while cow's milk is mostly used in the US and Western Europe, now a day there are increasing concern in production of goat's milk cheese. In areas where fresh milk is available abundantly, cheese had successfully made from together anhydrous milk fat and recombine skim milk powder (Ayar et al., 2009).

Probiotics and its importance

Scientist named Elie Metchnikoff, who discovered that the usage of fermented milk could turn around putrefactive effects of the gut micro-flora, first knew the idea of probiotic.

Probiotics are live bacteria and yeasts that are beneficial for health, especially for digestive system of the body. We generally think that bacteria causes diseases, but as we know our body is full of bacteria, some bacteria are good for health called probiotics while the other ones that cause diseases are called pathogens. Probiotics has often called as "good" or "helpful" bacteria since they help to keep our gastro intestinal track healthy (Ross et al., 2005). Yerlikaya and Ozer (2014) described the characteristics of cheese by adding different probiotics in it, then checked its dry matter, fat, and salt percentages. The study showed that with the addition of probiotics they have less dry matter while high fat contents and less salt contents. Probiotics are present in different types of foods products such as yogurt, while prebiotics are found in whole grains, garlic, onions, bananas and honey. In addition, probiotics and prebiotics are added to different foods and act as dietary supplements. A variety of probiotic dairy products as well as cheeses with functional properties is available in the worldwide market (Ross et al., 2005; Castro et al., 2013). The identity, safety and health claims of probiotics have attracted a large amount of attention from different public and regulatory organizations (Valle et al., 2014).

Fig. 1. China Yakult with probiotics.



Figure 1 shows that recently China Yakult is very famous probiotic product in the form of fermented milk that contains about 6.5 billion of good bacteria called probiotics (*Lactobacillus casei*). This proved to be very healthy in China which is discovered by Dr. Minoru Sirota and he used it in dairy products called Yakult comes from "jahurto" an Esperanto word which means yogurt. This product is used for gastrointestinal alignment that enhances the digestion and maintains the bowel movement and also helps to reduce different kinds of toxins and infections used against immunity system in the body.

Characteristics of genus *Lactobacillus*

Lactobacilli is normally known as gram positive, non-spore forming and non-flagellated rods shaped or coccobacilli type of bacteria. Some of its types are aero tolerant and which used oxygen with the help of enzyme called flavoprotein oxidase; on the other hand some are anaerobic which can survive without oxygen. At pH 5.5-5.8 the growth of *Lactobacilli* was maximum (Cabuk and Harsa, 2015). This genus divided into three categories based on the type of their fermentation which may be (1) homo-fermentative (which form almost 85% lactic acid during fermentation from glucose units), (2) facultative hetero-fermentative (which form only 50% lactic acid and significant amounts of acetic acid, ethanol and carbon dioxide) while (3) Obligate hetero-fermentative (which produces acetic acid and CO_2). Obviously, *Lactobacilli* are dispersed into several species and invent its application in the food industry. *Lactobacillus* sp. has been very beneficial for gastro intestine of human being. It has a great ability to change the lactose into lactic acid and that used for those people who are lactose intolerance. It inhibits the inadequate growth of microorganisms by decreasing the pH of intestine/stomach and also useful for other strains producing bacitracin and other metabolic products in which CO_2 , H_2O_2 deacetylate which interact bacterial protein called arginine (Ouweh and Vesterlund, 2004).

Selection criteria for probiotics

Probiotic are health benefits for human and have generally recognized as safe (GRAS) and have low risk of diseases. The probiotic organisms have ability to tolerate and grow in *in vivo* environment of the required site of administration therefore must be capable to bear high viscosity of both conjugated and de-conjugated bile pigment and their low pH (Mahoney and Henriksson, 2003). Organisms that can tolerate acid and bile, having anti-carcinogenic properties are sensitive to oxygen and have ability to change gut micro-flora of human subjects (Liong and Shah, 2004). Some types of the probiotic organisms used in this study include *Bifidobacterium lactis* and *Lactobacillus acidophilus* which are mostly used in different types of food products in yoghurt and cheeses. Probiotic was selected based on its health effects, superior stability and quality survival through the GI tract (Coman *et al.*, 2012). In addition to that, the foods having the probiotic bacteria must preserve the characteristic sensory criterion of the basic food. Adjunct culture *Lactobacillus casei* was also used in the soft cheese. For the preparation of probiotic soft cheese, high-density cultureable organism should be used for cheese vat inoculation to maintain quality and its required characteristics when producing product and have long ripening period (Clancy *et al.*, 2006).

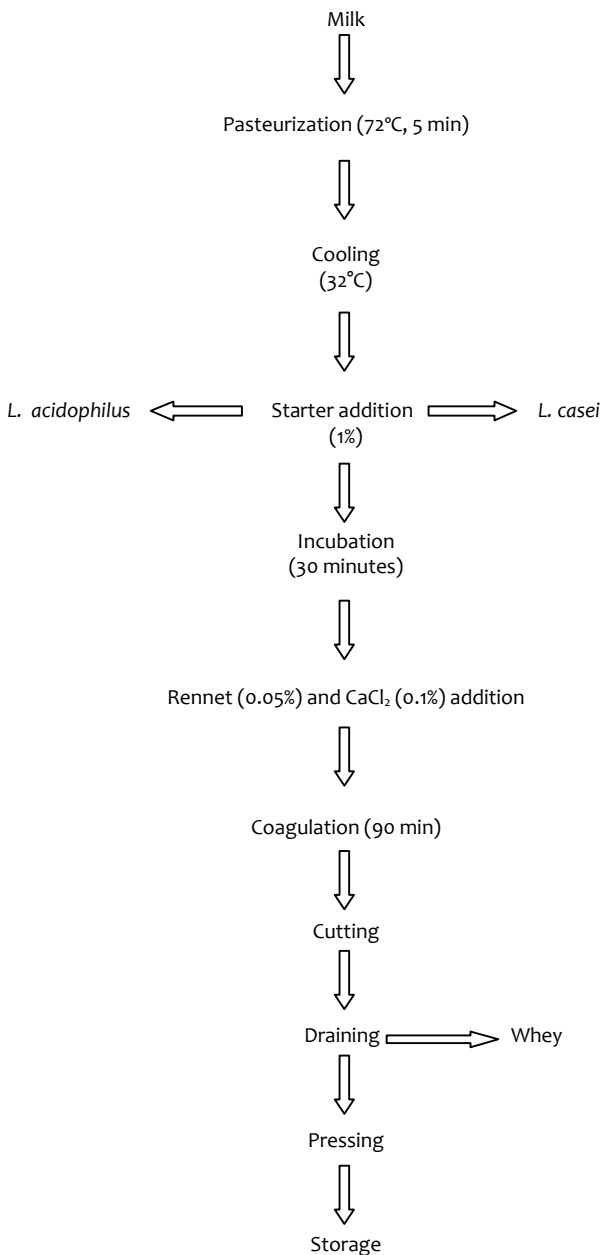
Application of probiotic bacteria in foods

Many of health benefits been discovered while consuming live bacteria containing products these may include the symptoms of lactose intolerance, anti-carcinogenic properties, blood cholesterol reduction, improvement in immunity and improve the flavor of the product ((Da Costa *et al.*, 2014). Probiotic cultures that are used must have properties that are viable. To achieve these characteristics storage condition should be modified. It has been suggested that approximately 10^9 cfu per day of probiotic microorganisms is necessary to elicit health effects (Ross *et al.*, 2005). Cheese has been the most popular system may undergo H_2O_2 and thus in the final product low viable probiotic count produce. Previous studies show that the final product contained low count of successfully viable probiotic bacteria (Phillips *et al.*, 2006). Yoghurt has low buffering capacity than cheese. Soft cheese is a type of ripened cheese but it has less long ripening period so to attain suitable and required quality of cheese probiotic strain selection in ripening and shelf life should be carefully examine.

Probiotic soft cheese manufacturing

Starter cultures were activated in maintenance broths (either MRS or M 17) at 30°C for 24 h (Fig. 2). After activation they were centrifuged (15000 rpm/5 min/ 4°C) in order to obtain pellet. Pellet was inoculated in 10% skim milk powder (30°C , 24 h). Goat milk was pasteurized (72°C for 5 min) and cooled down to 32°C . At this temperature, starter culture activated in skim milk powder was added at 1% ratio (30 mL starter culture for 3000 mL milk). Cheeses was produced by using starter culture (*Lactobacillus acidophilus*) as control and the other by using yoghurt starter culture + adjunct culture (*Lactobacillus acidophilus* + *L. casei*) by varying the quantity of *Lactobacillus casei* according to treatment plan. For the coagulation of the milk and the elimination of the whey, 30 min after the starter culture addition, rennet enzyme (0.05%) and CaCl_2 (0.1%), for firm structure were added. The milk was coagulated for 90 min. The coagulum was cut and pressed overnight cheese was salted in 15% saline solution. Cheeses were ripened in this solution in plastic containers at 4°C for 30 d (Mehaia, 2002). The best example in food manufacture is cheese manufacturing dating from 6000-7000 BC. By reducing water activity and salt addition, fermentation of lactic acid and starter and non-starter secretion of antibiotics, important milk constituents can be preserved. Fresh consumption of cheeses is usual that is reproduced by acid and their preparation is also simple. Before the consumption of Rennet cheese, it is always ripened by the complex biochemical reaction. Production of basic texture and flavor in cheese is due to biochemical reactions that occurred during curd ripening (Fox and McSweeney, 2004).

Fig. 2. Probiotic soft cheese from goat milk with adjunct culture (*Lactobacillus casei*)



United States is the largest cheese producers, producing 30% of the world producers followed by Germany and France. Cheese production increases over past few decades in Australia. France is the biggest producer of cheese worldwide. The United States however is a biggest producer of cheese but mostly production is for the domestic market as a functional food. Functional foods are those that are containing probiotic bacteria and these foods are gaining popularity and likeness over the world. Soft cheese development with goat milk provide great prospect.

To improve health and food quality these cheeses provide great potential and introduce a variety of probiotic foods. Manufacturing and ripening are two stages for the preparation of cheddar cheese. pH, salt, cheese micro-flora and moisture are the levels that largely regulate the ripening process. Characteristic flavor and texture of cheese is developed during ripening and manufacturing steps can determine the finished cheese quality and nature to a variety of extent.

Conclusion

Goat milk has important nutritional, functional, technological and biological characteristics. It is more acceptable to human digestive system as compared to cow and buffalo milk. Goat milk is widely available in our country and thus can be converted into value added dairy products such as a wide range of cheese varieties due to its health benefits. Probiotic soft cheese is delightful, easy to make, nutritional and cost-effective, so it can be prepared on commercial scale from goat milk. Adjunct culture acts as food supplement with probiotic bacteria that provides certain benefits to human health. Through this study, it was proved that *Lactobacillus casei* can be used as an adjunct culture to enhance the sensory characteristics of probiotic soft cheese.

Acknowledgements

This review is based on knowledge gained during my research and working with projects financially supported by the Higher Education Commission (HEC) Pakistan, to the Department of Food Nutrition and Home Sciences, National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Punjab, Pakistan.

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