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Development of Probiotic Cultured Milk Drink

By

Weihene Epage Dhammika Upul Shantha

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Declaration

The work described in this thesis was carried out by me under the supervision of Dr. K K D S Ranaweera, Head, Department of Food Science and Technology, University of Sri Jayewardenepura, Nugegoda, Sri Lanka and Mr. J N Ruwan Kumara, Quality Assurance Manager, Fonterra Brands Lanka (Pvt) Ltd., Delgoda Road, Biyagama, Sri Lanka. I declare that this report or any part of the report has not been submitted, presented or accepted in any previous application for another degree.

.....
W E D Upul Shantha

We hereby certify that the above statement made by the candidate is true to the best of our knowledge and that this thesis is suitable for the submission to the University for the purpose of evaluation

.....
Dr. K K D S Ranaweera
Head
Department of Food Science and Technology
University of Sri Jayewardenepura
Sri Lanka.

Date.....

Fonterra
Dairy for life
Fonterra Brands Lanka (Private) Limited
RUWAN KUMARA
QUALITY ASSURANCE MANAGER

.....
Mr. J N Ruwan Kumara
Quality Assurance Manager
Fonterra Brands Lanka (Pvt) Ltd
100, Delgoda Road, Biyagama
Sri Lanka

Date.....

Affectionately dedicated

To

My Wife; Niluka, My Parents, and Teachers.

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ABSTRACT

Cow's milk is a good source of vital nutrients for humans. Fermentation further enhances the nutritional value of milk. The functional food market is growing at a high speed every year. Probiotics, or live beneficial microorganisms in food, which is a very popular aspect in functional food concept can be easily coupled with Fermented dairy products.

A Cultured Dairy Drink with Probiotics will be developed under the research project.

A number of health benefits have been claimed for probiotic bacteria such as *Lactobacillus acidophilus, Bifidobacterium spp., Lactobacillus casei.* Because of the potential health benefits, these organisms are increasingly incorporated in to dairy foods. However to claim as a probiotic product having health benefits it required to maintain a certain amount of viable bacteria count of above 10^6.

A study was carried out to develop a probiotic cultured milk drink and to investigate its physical, chemical and microbiological properties.

In the production of Probiotic Cultured Milk Drink, Milk fat and milk solid non fat levels were standardized by adding required amounts of raw milk and skimmed milk powder. Then the raw milk and skimmed milk powder mixture was homogenized at 200 psi using a laboratory homogenizer and pasteurized by keeping in a water bath at 95 °C for 3 minutes. After that the mixture was cooled to 42 °C and inoculated with the starter culture (ABT 3) and keep in incubation room at 42 °C for 7 hours. Parallel to above, a mixture of sugar, stabilizer, presrvatives, colour and flavour was dissolved by adding water. Then the dissolved mixture

was pasteurized at 200 psi and cool to 4 °C. Subsequently the pre-dissolved and pasteurized ingredient mixture was added to the incubated product and mixed well. Then the final product was filled in to bottles and transferred to the cool room at 4±1 °C.

Final product samples were evaluated for organoleptic properties by 30 panelists using a 9 point hedonic scale. The sample with 0.05% stabilizer, 10 mg/kg colour and 0.03% flavour was selected as the best. Initial *Lactobacillus acidophilus* count was tested with increase of amount of innoculum in the formulation and fount out that the initial count of 10^8 when used 0.025%. Developed product was analyzed for crude protein, fat, ash and total solids and the results were 3.5% protein, 2.4% of fat, 0.7% of ash, and 20.3 of total solids.

The product was analyzed for pH, acidity and the *Lactobacillus acidophilus* count over a period of 30 days. pH was decreased from 4.1 to 3.92. The acidity was increased from 0.62 to 0.82. Although a significant decrease in pH with concomitant increase in acidity was observed during the shelf life at cold storage, the values were with in the acceptable levels.

During the cold storage of 30 days at 4±1 °C, a decrease of *Lactobacillus acidophilus* count was observed. It has been decreased from 24 x 10^7 of initial count to 2 x 10^7 at the end of the shelf life. The product can be claimed as probiotic in terms of *Lactobacillus acidophilus* given that the count has remained above the 10^6 level at end of the shelf life.

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CHAPTER ONE

1.0 Introduction

The functional food market is growing at an astonishing rate every year. Foods that contain some health-promoting component(s) beyond traditional nutrients are generally considered as functional foods. To become "functional", foods can be modified by various ways. One very important aspect here is to improve food in a way that they will facilitate the balance of gut microflora and increase the number of health beneficial microorganisms in the gut.

One approach exists to increase the number of health-promoting organisms in the gastrointestinal tract. The first is the oral administration of live beneficial microorganisms. These microorganisms, called probiotics, have been selected mostly from lactic acid bacteria and bifidobacteria that form a part of the normal intestinal microflora of humans, since these organisms are indigenous to the colon, because of the numerous health benefits claimed for them.

The main health benefits of probiotics are -

- Increased digestion
- Alleviation of digestive disorders
- Enhanced assimilation and synthesis of nutrients (including vitamins, minerals and protein)
- Improved absorption of calcium
- Protection against E.coli infections
- Improved immune function
- Improved lactose tolerance and digestibility of all milk products
- Reduction of vaginal infections and yeast infections
- Increased anti-carcinogenic (anti-cancer) activity
- Reduction/prevention of peptic ulcers caused by H. pylori bacteria
- Reduction in bad cholesterol
- Reduction of acne

Ready- to – Serve beverages (RTS) are popular among all ages but the nutritional quality of most is questionable. Preparation of a RTS based on milk, which is fermented using a selected culture would provide nutrition and promote the utilization of raw milk available in Sri Lanka.

The origins of fermented or cultured dairy products probably date back to the dawn of civilization. It is possible that serendipity, chance of contamination, favorable environmental and climatic conditions together played a role in the development of many of the cultured dairy products we know of today. From the current scientific understanding of food preparation and preservation, two basic reasons for converting milk into cultured dairy products can be ascribed. The first and the foremost reason is to preserve the high-quality nutrients present in the readily perishable fluid milk in the form of relatively more stable products. The second major reason is to provide variety in food by inducing changes in body, texture, colour, flavour, and nutritive properties.

With the expansion of the functional food market, it clear that there is an important niche for the pro-, pre and synbiotic approaches because of the strong commercial interests in providing these supplements to both humans and animals. Therefore these concepts are combined with almost every area in the food industry. This gave rise to the idea of using them with fermented foods especially fermented dairy products. The production of probiotic cultured milk drink is a good example.

Cow's milk is a good source of calcium which is incomparable with the calcium fortified beverages in which the calcium is hardly solublized, making it unavailable for the consumer.

Fermentation of the beverage would further upgrade the product by flavor and nutrition. Also, easily digestible to the consumer with lactose intolerance. It has also been found that acidification induced by fermentation demineralize casein micelles which increase the solublization of calcium collidal phohphates. Scientific research has proved that casein phosphopetides improve the absorbance of Zinc and Calcium in the food.

1.1 Objectives of the Study

1. To develop suitable ingredient formulation for milk drink with probiotic culture.
2. To study the physico- chemical and microbial properties in relation to shelf life.
3. To study the change of selected probiotic bacteria count in the product during its shelf life.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Probiotics

There is a long history of health claims concerning living microorganisms in food, particularly lactic acid bacteria in a Persian version of the Old Testament states that "Abraham owed his longevity to the consumption of sour milk." in 76 BC the Roman historian Plinius recommended the administration of fermented milk products for treating gastroenteritis.

Since the advent of the microbiology era, some investigators (e.g., Carre, Tissier, and Metchnikoff) attributed such health effects to shifts of the intestinal microbial balance.

The Russian Nobel Prize winner and father of modern immunology, Elie Metchnikoff, working at the Pasteur Institute in Paris, first conceptualized Probiotics in 1908. He believed that the flora in the lower gut was having an adverse effect on the host and that consuming soured milk could ameliorate these adverse effects. In support of this he cited the observation that Bulgarian peasants consumed large quantities of soured milk and also lived to a great age. He isolated what he called the 'Bulgarian bacillus' from soured milk and used this in subsequent trials. This organism was probably what became known as *Lactobacillus bulgaricus* and is now called *L. delbrueckii sub sp bulgaricus* that is one of the major organisms used to ferment milk and produce yoghurt. He hypothesized that the growth of this *Lactobacillus* in the gastro-intestinal tract would displace other putrefactive bacteria, reduce the concentrations of toxins in the gut and thus improve health.

In 1906 Henry Tissier, a French pediatrician, recommended the administration of bifidobacteria to infants suffering from diarrhea, claiming that bifidobacteria supersede the putrefactive bacteria that cause the disease. He showed that bifidobacteria were predominant in the gut flora of breast-fed infants (Tissier, 1984).

Although the concept of probiotics was introduced in the early 20th century, the term was not coined until the 1960's. The definition of the term has evolved through the years