EVALUATION STORAGE STABILITY AND ACID TOLERANCE OF PROBIOTIC IN COMMERCIAL MILK PRODUCTS

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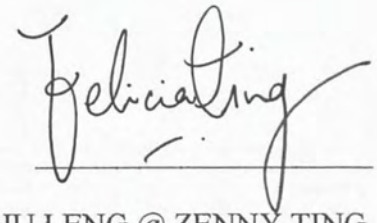

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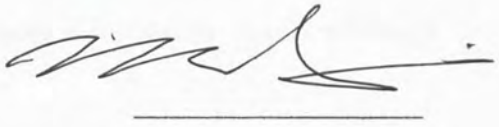

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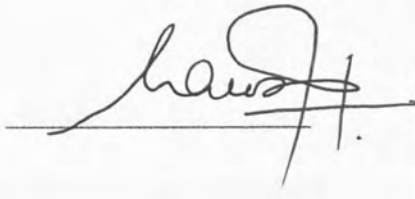

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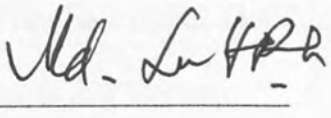

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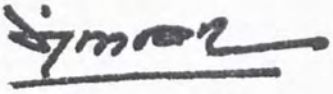

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ABSTRACT

The survival of the probiotic bacteria *Lactobacillus acidophilus* in selected culture milk selected commercial products, namely plain Vitagen, flavour Vitagen and flavour Nutrigen, was investigated. The probiotic bacteria in culture milk had been evaluated using the nutrient agar as growth medium. The survival of probiotic bacteria was determined based on the existence of colonies on top of the nutrient agar. The results showed that the viability cells of probiotic bacteria in each sample decreased with time during storage at 4°C over 4 weeks. The probiotic bacteria could not survive in very acidic environment (pH2.5) but survive well in the pH range 3.5 to 5.5. The surviving cells of probiotic bacteria were, however highest at the pH 5.5. Consequently, the probiotic bacteria can survive easily in the human stomach since the pH of stomach is in the range of pH 4-pH 6.

ABSTRAK

Kajian tentang kebolehan hidup bakteria probiotik iaitu *Lactobacillus acidophilus* dalam beberapa minuman susu kultur yang terdapat di pasaran telah dijalankan. Dalam kajian ini, sampel produk susu kultur daripada jenama Vitagen tanpa perisa , Vitagen berperisa dan Nutrigen berperisa telah digunakan. Bakteria probiotik dalam susu kultur telah dikaji dengan menggunakan nutrient agar sebagai medium pertumbuhan. Kebolehan hidup bakteria probiotik ditentukan berdasarkan kehadiran koloni di atas permukaan lapisan nutrient agar. Daripada keputusan yang diperolehi bagi kajian pengstoran dalam suhu 4°C selama 4 minggu berturut-turut, didapati sel bakteria probiotik dalam setiap sampel adalah menurun dengan masa. Kajian tentang keupayaan hidup bakteria probiotik dalam keadaan medium berasid yang mempunyai pH yang berbeza-beza, iaitu pH 2.5, pH 3.5, pH 4.5 dan pH 5.5 juga mendapati bahawa bakteria probiotik mampu hidup pada pH 3.5 ke pH 5.5 tetapi tidak pada pH 2.5. Walau bagaimana pun,bilangan sel bakteria probiotik hidup dengan lebih tinggi pada pH 5.5. Sehubungan itu bakteria probiotik mampu untuk hidup di dalam perut manusia memandangkan pH pada perut adalah diantara pH 4-pH 6.

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SHORT FORM AND SYMBOL

%	Percent
mL	Milliliter
Min	Minute
°C	Celsius
ND	Not detected
CFU	Colony-Forming Unit
g	Gram
LAB	Lactic Acid Bacteria
UHT	Ultra High Temperature

CHAPTER 1

INTRODUCTION

1.1 Probiotic

Probiotic organisms are increasing by incorporated into food as dietary adjuncts benefit to help maintain a healthy microbial gastrointestinal balance, with possible resulting benefits for the human health (Klaver *et al.*, 1993). Such microorganisms, predominantly incorporated, into fermented dairy products, are the probiotic bacteria *Lactobacilli* and *Bifidobacteria.*

So far the active use of yeasts as dietary adjuncts for humans has been limited, despite the occurrences of yeasts as an integral part of the microflora of many dairy related products (Klaver *et al.*, 1993). This may indicate that the potential of yeasts for incorporation into dairy products as probiotic agents. Therefore, yeasts must be included in the search for finding new starter cultures for fermented milk products.

Food fermentation has been used for centuries as a method to preserve perishable food products. The raw materials traditionally used for fermentation are as diverse as: fruits, cereals, honey, vegetables, milk, meat and fish. It is possible to obtain a large variety of different food products by selecting difference raw materials, starter cultures and fermentation conditions (Klaver *et al.*, 1993).

The subject might seem biased towards fermented dairy product. Cheese and cultured dairy drinks (yogurts, buttermilk, vitagen and so on) are produced by the acidic fermentations of milk by lactic acid bacteria.

Little consideration has been given to the growth kinetics and biochemical basis of yeast growth in milk and yogurt (Klaver *et al.*, 1993). The frequent occurrences of yeast in dairy related products indicate the ability of yeasts to survive and metabolise milk constitute. Yeasts might develop in milk as secondary flora, after yogurt culture bacteria growth. Lactic acid bacteria (LAB) ferment about 35% of the lactose in milk through hydrolysis to glucose. Only the glucose is changed into lactic acid, while the galactose moiety was related mainly by *Streptococcus termophilus* into the extra cellular environment (Klaver *et al.*, 1993).

The high concentration of galactose present (about 1%) was the main reason for the growth of galactose positive and non-fermenting yeasts in yogurt (Saarela *et al.*, 2000). Furthermore, the low pH of yogurt and ability of yeasts to utilize organic acids create a selective environment for yeasts growth (Saarela *et al.*, 2000).

According to literature the minimum dosage of probiotic cells per day for any beneficial effect on the consumer is consider to be 10^8-10^9 probiotic cfu/ml (Kurmann and Rasic, 1991) corresponding to an intake of 100-g product containing 10^6-10^7 cfu/ml per day. However, it has been reported (Kailasapathy and Rybka, 1997) that *L.acidophilus* and *B. bifidum*, present in bio-yogurt, are unstable. Their poor survival in yogurt is attributed to the low pH of the environment and low acid tolerance. Since yeasts have ability to utilize organic acid, thereby increasing the pH of the environment, growth of a probiotic yeasts in association with probiotic bacteria has been suggested.

This study reports on the ability of *S. boulardii* to growth in bio-yogurt and other dairy products in order to further investigate at a later stage the effect on the survival of the AB bacteria (*Lactobacillus acidophilus* and *Bifidobacteria*) during shelf life.

1.2 Objective

The objectives of this evaluation are;

1) To investigate the survival of probiotic bacteria in commercial culture milk product (vitagen) resembling to in-vitro test.
2) To evaluate the survival of probiotic bacteria in commercial product (vitagen) during storage stability.

CHAPTER 2

LITERATURE REVIEW

2.1 Fermented milk

These are numerous fermented milk products which are manufactured in many countries of the world (Campbell-Platt, 1987; Kurmann *et al.*, 1992), but few are of commercial significance. The exact origins of fermented milks making is difficult to establish, but fermented milks were produced some 10-15000 years ago as man's way of life changed from being a 'food gatherer' to a 'food producer' (Pederson, 1979). These include the domestication of animals such as the cow, sheep, goat, buffalo and camel. It is likely that this transition may have occurred at different times in different parts of the world. However, archaeological evidence shows some civilizations (e.g. the Sumarians and Babylonians in Mesopotamia, the Pharoes in north-east Africa and the Indians in Asia were well advanced in agricultural and husbandry methods, and in the production of fermented milks(Tamime, 1977).

It is possible that modern fermented milks production evolved as follows;

i) The methods of production manufacturing involved in constant use of the same vessels or the addition of fresh milk to an ongoing fermentation relying mainly on the indigenous microflora to sour milk.

ii) The heating of milk over an open fire to concentrate the milk slightly followed by seeding the cool milk (blood or ambient temperature) with previous day sour milk.

iii) The preparation of these products with the use of defined microorganisms since the early 1900s.

Thus, the nature of these products differed from one area to another depending on the microflora used and climatic conditions of the region.

The manufacturing stages of fermented milks are still a complex process combining; microbiology and enzymology, chemistry and biochemistry, and physics and engineering. These are a considerable degree of similarity in technological aspects, only some will be discussed.

2.1.1 Fermented milks with lactic acid

The milk with lactic acid bacteria is a popular product in the dairy industry. In general, most of the fermented milks (e.g. yoghurt, ymer, dahi, buttermilk or therapeutic products) are made using mixed starter cultures, but sometimes a single strain organism is

employed, for example, during the manufacture of Bulgarian buttermilk and acidophilus milk (Nahaisi, 1986).

2.1.2 *Acidophilus* milk

Unfermented milk products known as 'sweet acidophilus milk' was promoted in the 1970s in the USA. The product has the same flavour as normal milk because little acid develops under refrigerated storage. Fermented acidophilus milk can be made using a single starter culture of *Lb. acidophilus*. Strains isolated form the intestinal tract however, grows slowly in milk only increasing 5-fold in 18-24 h with a developed acidity of ≤ 0.8% (Marshall *et al.*, 1982b; Marshall, 1986).

Interest in these strains comes from the possible benefits for alleviation of intestinal and other disorders. Publications review the 'health-promoting' properties of *Lb. acidophilus* (Nahaisi, 1986; Welch, 1987; Gilliland, 1989; Gilliland and Walker 1990; Sellars, 1991). The role of *Lactobacilus* spp.(most likely a *Lb. acidophilus* with ability to colonize the vagina) and lactate-gel (a pharmaceutical preparation containing growth substrates of lactobacilli) in the treatment of bacterial vaginosis, on survival of HIV in the female genital tract, and on diarrhea and immunological complaints have been reported by many researchers (Andreshch *et al* ., 1986; Martin *et al.*, 1988; Holst and Brandenberg, 1990; Marteau *et al.*, 1990; Perdigon *et al.*, 1990, 1992; Klebanoff *et al.*, 1991; Cocinnier *et al.*, 1993).

When acidophilus milk was first launched in different Western markets, the product was not well received by the consumers (even by the health conscious). The reason could be associated with lack of belief in a correlation of 'acidophilus' products with 'good health' but the poor quality control during production with respect to microbiological quality and organoleptic properties may also have contributed. Improvement in the processing conditions and the blending of *Lb. acidophilus* with other starter organisms has resulted in an increase in market share (Marshall, 1986).

A general method for production of acidophilus milk is as follows: milk (whole or skimmed) is heated to 95°C for 60 min, cooled to 37°C and held for 3-4 h, re-heated to 95°C for 10-15 min, cooled to 37°C and inoculated with 2-5% bulk starter culture. The processed milk is incubated for up to 24h or to 1% lactic acid, cooled to 5°C, packaged and finally transferred to the cold store (Chandan, 1982).

However, modern methods of streamlining this process are: (i) the milk is homogenized at 14.5 MPa of pressure, heated to 95°C for 60 min, cooled to 37°C and inoculated with DV1 culture; the incubation time is 12-16h or to ~ 0.65% lactec acid; and (ii) the UHT process of 140-145°C for 2-3s would provide a milk where the proliferation of undesirable contaminants is avoided (Chandan, 1982; Alm, 1983). Mixed ropy and non-ropy strains of *Lb. acidophilus* may also be used, for example, in the former USSR for the production of moskowski (Kurmann *et al.*, 1992).

The retail product should contain 5 x 10^8 cfu ml^{-1} *Lb. acidophilus* at the time of comsumption (i.e. 14-21 days after manufacture). To ensure such quality, it is critical to cool the product at ~0.65% lactic acid and maintain it at ≤ 5°C during distribution and retailing (Tamime and Robinson, 1988).

The stability of the microflora in acidophilus milk may also be achieved by: (i) the addition of yeast extract or 'V medium' to milk to improve the growth of *Lb. acidophilus* (Alm, 1981); (ii) fortification of the milk proteins with concentrated skimmed milk (Alm, 1983); and (iii) the use of mixed inoculate of *Lb. acidophilus* and yoghurt starter culture to stabilize cell numbers and improve the flavour (Alm, 1982). Such approaches rely on careful selection of *Lb. acidophilus* strains, the acidity should be monitored so that survival of *Lb. acidophilus* is assured for at least 14 days (Gillian and Speck, 1977; Hull *et al.*, 1984; Robert *et al.*, 1984; Johnston *et al.*, 1987; Robinsan, 1987).Alternatively, the addition of high cell numbers of *Lb. acidophilus* to pasteurized milk before packaging will provide viable cell counts > 2 x 10^6 cfu ml^{-1} at the end of 2 weeks shelf-life (Tamime *et al.*, 1995a).

Other milks may also be used, and good-quality acidophilus milk has been produced from buffalo's milk with viable cell of 6.4-8.1 x 10^8 cfu ml^{-1} at pH ~4.0 (Rao and Gandhi, 1988). Mixed cultures of *Lb. acidophilus* and *Lb. paracasei* subsp. *paracasei* grown in soy milk substrate had high number of *Lb. acidophilus*, but the addition of *S. thermophilus* reduced the final count (de Valdez and de Giori, 1993). An alternative means pH providing the consumer with viable *Lb. acidophilus* is as a spray-dried blend

preparation. The powder is made from acidophilus milk, tomato juice and sugar, and the product has a viable cell count of 22.9 x 10^7 cfu g^{-1}, representing a survival rate of 14.8% (Prajapati *et al.*, 1986).

2.2 Probiotic

Probiotics are viable bacterial cell preparation or foods containing viable bacterial cultures or components of bacteria cells that have beneficial effects on the health (Fuller, 1987). Many of these probiotics are lactic acid bacteria. Probiotic lactic acid bacteria are useful in the treatment of disturbed intestinal microflora and increased gut permeability, which are characteristic to many intestinal disorders (Lee and Salminen, 1995). Example include acute rotavirus diarrhea. Other intestinal dysfunction, subjects with food allergy, subjects with colonic disorders, and patients undergoing pelvic radiotherapy. In such disease states altered intestinal microflora, impaired gut mucosal barrier, and intestinal inflammation may be present. Other properties of specific lactic acid bacteria include modification of intestinal microflora and its metabolic products, such as short-chain fatty acids and antimicrobial components (Lee *et al.*, 1999).

Successful probiotic bacteria are able to survive gastric conditions and colonize the intestine, at least temporarily, by adhering to epithelium. Well documented probiotic strains are clearly characterized and clinically documented. Such probiotic microorganisms with demonstrated probiotic properties appear to be promising

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