EFFECT OF HONEY ON PROBIOTICS AND YOGHURT CULTURES

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ABSTRACT

Lactobacillus acidophilus, *Bifidobacterium lactis* and yoghurt cultures, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subp *bulgaricus* were cultured in skim milk with 1%, 3% and 5% honey. Samples were incubated at 37°C, 48 hours. Samples were collected at 6 hour intervals and examined for growth and pH. Besides, twelve percent nonfat dry milk containing 5% honey, 5% inulin and 5% raftilose were pasteurized and inoculated with *Lactobacillus acidophilus*, *Bifidobacterium lactis* and yoghurt cultures, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subp *bulgaricus*. The samples were collected at 6 hour intervals and examined for growth and pH. Growth and changes in physiochemical of each strain was also assessed after 8 hours of fermentation in the presence of above prebiotics and honey. The effect of honey increased with increasing concentration and was maximal at 5%. Growth of each strain was enhanced when they were grown in the presence of raftilose followed in a descending order by inulin and honey.

ABSTRAK

KESAN MADU TERHADAP PROBIOTIC DAN KULTUR PEMULA YOGURT

Lactobacillus acidophilus, Bifidobacterium lactis and yoghurt cultures, Streptococcus thermophilus and Lactobacillus delbrueckii subp bulgaricus dikulturkan ke dalam susu dengan 1%,3% and 5% madu. Sampel diinkubasikan pada suhu 37°C selama 48 jam. Kemudian sampel dikumpulkan pada setiap 6 jam untuk pemeriksaan pertumbuhan dan pH. Selain itu, 12% susu yang mengandungi 5% madu, 5% inulin dan 5% raftilose dipasteurkan dan diinokulasikan dengan Lactobacillus acidophilus, Bifidobacterium lactis dan kultur pemula, Streptococcus thermophilus dan Lactobacillus delbrueckii subp bulgaricus. Sampel dikumpulkan pada setiap 6 jam untuk permeriksaan pertumbuhan dan pH. Pertumbuhan dan perubahan fizikokimia setiap bacteria juga diperiksa selepas 8 jam penapaian dengan kehadiran madu dan sumber prebiotic. Kesan madu semakin menonjol dengan peningkatan kepekatan madu. Kesan madu adalah paling baik pada kepekatan 5%. Pertumbuhan setiap bacteria dipertingkatkan apabila mereka diinkubasikan dalam raftilose dan diikuti oleh inulin dan madu.

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ABBREVIATIONS

ANOVA	Analysis of Variance
DVS	Direct Vat Set
SFA	Saturated Fatty Acid
SPSS	Statistical Package of Social Science
USDA	United States Department of Agriculture

SYMBOLS

%	Percentage
<	More than
>	Less than
±	Plus minus sign
≤	Less than or equal to
°Brix	Degree Brix
°C	Degree Celcius
cfu	Colony Forming Unit
cP	Centipoise
g	gram
Kg	Kilogram
mL	miliLitre
min	Minutes
w/v	Weight per Volume

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CHAPTER 1

INTRODUCTION

For millennia, humans have utilized microorganisms in processing foodstuffs such as in milk fermentation and the production of alcoholic and non-alcoholic beverages. Many of these products are derived from the results of spontaneous lactic acid bacteria fermentation and through the influence of climatic conditions and local traditions in the handling of food. Fermented milk products such as yoghurt, buttermilk, kefir and koumiss are produced throughout the world (Torre *et al.*, 2003). Benefits from consumption of buttermilk and yoghurt have been known for centuries, but only in the 20th century the positive results from the consumption of fermented dairy products were related to probiotics present in these products (Tomasik & Tomasik, 2003). Yoghurt is considered a health-promoting food for its nutritional value and for its content of more than 10^7 cfu/ml living lactic acid bacteria (Chen *et al.,* 1999). Mesophilic lactic acid bacteria (mainly *Lactococcus* spp.) and thermophilic lactic acid bacteria (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) are traditionally used in fermented milk products like buttermilk and yoghurt.

Probiotics are defined as ' preparation of a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation and colonization) in the host and by exert beneficial health effects in the host' (Itsaranuwat *et al.*, 2003). Incorporation of probiotics into conventional food products contribute extra-

nutritional benefits to the products, depending on the strains of the organism used (Sabikhi & Mathur, 2002). The health and nutritional benefits of probiotics are many and varied, including anticarcinogenic effects (Ouwehand *et al.,* 2002; Davidson *et al.,* 2000), antimicrobial activity (Klaver *et al.,* 1993; Hughes & Hoover, 1995; Awaisheh *et al.,* 2005), reduction of serum cholesterol levels and colon cancer risks (Chou & Hou, 2000; Sabikni & Mathur, 2002) and lactose intolerance (Alkalin *et al.,* 2004)

The health benefits of probiotic bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium* spp. have led to their incorporation into dairy foods such as yoghurts, infant formula and other fermented dairy products. Incorporation of bifidobacteria has been shown enhance the therapeutic value of yoghurt (Adhikari *et al.,* 2000). Bifidobacteria grow slowly in milk and the usual practice is to add yoghurt starter bacteria to enhance the fermentation process for making probiotic yoghurt (Akalin *et al.,* 2004). Most of the yoghurts contain *Lactobacillus acidophilus* alongside with the bifidobacteria. Hence, the colloquial designation of them is called as Acidophilus/ Bifidus (A/B) milks or yogurts (Samona & Robinson, 1994).

In recent years, the majority of yoghurt marketed in Australia, the USA, Japan and Europe has included probiotic bacteria (Talwalkar *et al.,* 2004). Besides, a wide variety of novel products containing probiotics have been developed and marketed in the European countries as well. They are food supplements or fermented milk with food formulations mostly used as delivery vehicle for probiotic bacteria and dietary supplements in the form of capsules and other formulations conceived to be consumed by healthy individuals (Fasoli *et al.,* 2003).

Intestinal Lactic Acid Bacteria (LAB) are closely associated with the human health because LAB is an important biodefense factor in preventing colonization and subsequent proliferation of pathogenic bacteria in the intestine (Oh *et al.,* 2000). In other words, Lactic Acid Bacteria (LAB) have two major functions which are not only to achieve acidification, curdling and production of flavour compounds but also inhibiting the outgrowth of pathogens and spoilage organisms (Marth & Steele, 1998). Improving the quality of lactic acid starters is of great importance in the dairy industry for the manufacture of cheeses and fermented milks. Their survival is generally measured by the capacity of cells to form colonies on agar medium (Fernanda *et al.,* 2000). An optimal balance of microbial organisms in the intestine is suggested to be an important aspect of maintaining good health (Hekmat & Mcmahon, 1992; Sun & Griffiths, 2000; Lourens-Hattingh & Viljoen, 2001).

According to Milo (2002), prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of species of bacteria in the colon. Growth of *Bifidobacterium* in the intestine is often related to the presence of specific growth factors, 'bifidus factors' as bifidobacteria is known to be a fastidious organism. Numerous researchers have reported that bifidobacteria grows poorly in milk, and therefore requires specific growth factors (Chick *et al.,* 2001; Alkaline *et al.,* 2004; Lian *et al.,* 2003). Oligosaccharides are well-known growth promoters for bifidobacteria.

Oligossaccharides are a group of short chain non-digestible polysaccharides that occur naturally in food. They are typically defined as glycosides that contain between 3 to 10 sugar moieties and are characterized by the type and sequence of the monosaccharides moieties present (Kajiwara *et al.,* 2002). Because of their prebiotic

properties, oligosaccharides have received much recent attention as functional food ingredients (Shin *et al.*, 2000). The Fructooligosaccharides (FOS) are naturally occurring, complex carbohydrates, the consumption of which increases the population of bifidobacteria in the colon. If a specific oligosaccharide (prebiotic) is found to stimulate a defined strain of *Bifidobacterium* (probiotic), then a dietary constituent contining both the pre- and the probiotic should be given the name 'synbiotic' (Itsaranuwat *et al.*, 2003). Therefore, bifidobacteria have been widely incorporated in various food products containing prebiotics and are among the leading candidates for use as a probiotic or dietary adjunct (Lian *et al.,* 2003).

Honey is made by bees and their raw material for nearly all the world's supply of honey is nectar produced in the nectarines of flowers. Honey is natural syrup containing primarily fructose (38.5%) and glucose (31.3) as the major carbohydrates, ranging from 65%-80% of the total soluble solids. Other sugars in honey include maltose (7.2%), sucrose (1.5%), and various oligosaccharides (4.2%) (de Rodríguez *et al.,* 2004). It is only natural that honey be known foremost as a sweetener, being composed principally of sugars (Goltz, 2001). There are a variety of organic acids such as acetic, butyric, citric, formic, gluconic, lactic, malic, pyroglutamic and succinic acids. The average pH of honey is 3.9 (Chick *et al.,* 2001).

In the long history of man, honey was used for thousands of years before cane or beet sugar. In the early history, man was almost entirely dependent upon honey for sweetness. There are, however, still a number of commercial and domestic products where honey is superior to sugar, because of flavor, texture, keeping qualities and other factors. Therefore, honey is a carbohydrate food which has occupied an important place in human nutrition since prehistoric times and is considered not only as a

sweetener but also as a healthy and wholesome food with curative properties (Shamala *et al.*, 2000). Not always do people know for certain all the sources of the honey that have been extracted. The colour of the flower has little or no relevance to the colour of the honey derived from the nectar. A honey flow normally yielding light coloured honey may be mixed in the hive with another honey that perhaps from a minor nectar source. Qualitatively, it is probable that the colour of honey must be at least due, in part, to some form of pigmentation of an organic nature (Goltz, 2001). Some well known floral honeys will differ in colour, depending upon such as variables as plant location, variety and such environmental conditions as temperature, soil, moisture and light (Zamora & Chirifie, 2006). There are varieties of oligosaccharides in honey that may function as prebiotics. Information is presently lacked on the ability of lactic acid bacteria and bifidobacteria to metabolize honey. Although the effects of oligosaccharides on colonic bacteria have been investigated, there are relatively few reports on the effects of oligosaccharides on lactic acid bacteria and bifidobacteria in yoghurt during storage. Thus, the objective of this study is to investigate the effect of honey that may serve as prebiotic in supporting the growth as well as the viability of the yoghurt cultures and probiotics during refrigerated storage.

The specific objectives of the study were:

1. To determine the growth of *B.lactis*, *L.acidophilus* and yoghurt cultures in milk with the presence of honey.
2. To evaluate potential of honey as a prebiotic in comparison with inulin and raftilose in probiotic yoghurt.
3. To determine the effect of honey on the growth of probiotics and yoghurt cultures in comparison with inulin and raftilose during fermentation of yoghurt and storage at 4^0C.

CHAPTER 2

LITERATURE REVIEWS

2.1. Functional Food

Nowadays, the consumer pays a lot of attention to the relation between food and health. Continuously increasing consumer health consciousness and expenditure are socio-economic factors responsible for the expanding worldwide interest on functional foods (Mattila-Sandholm *et al.,* 2002). Food products are designed for taste, appearance, cost and convenience of the consumer. The design of food products that confer a health benefit is a relatively new trend, and recognizes the growing acceptance of the role of diet in disease prevention, treatment and well-being (Sangeetha et *al.*, 2005). When designing foods for gut health, the specific ingredients may include micro-organisms (probiotics), non-digestible carbohydrates (dietary fiber and prebiotics) and bioactive compounds (e.g. phenolics).

Functional food can be defined as 'any food that has a positive impact on an individual's health, physical performance or state of mind in addition to its nutritional content' (Shah, 2001). Functional foods, designer foods, pharma foods and nutraceuticals are synonyms for food that can prevent and treat diseases (Milo, 2002). Functional food development is subject to much more scientific standards and complexity than traditional food development. The first generation of functional foods

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