CHAPTER 1: INTRODUCTION

Polysaccharides are composed of saccharide subunits with molecular-weight of 2000 kilo dalton (K Da). The polymers are composed of more than 10 monosaccharides, which are joined with glycosidic linkage (Telefoncu, 1992; Pazur, 1970; Varki *et al.*, 2008 and Varki *et al.*, 1999).

In general there are two types of polysaccharide: intracellular polysaccharides (IPS) and extracellular polysaccharides (EPS). The former are produced by animals (glycogen), plants (starch, inulin), and microorganisms (glycogen) whereas the latter are produced widely by some bacteria and microalgae and less among of yeasts and fungi (Degeest and De Vuyst, 2000).

Lactic acid bacteria are able to produce several types of polysaccharides (Degeest *et al.*, 2001). Microbial exopolysaccharides occur as capsular or ropy type. Capsular exopolysaccharide (CPS) are covalently bounded to the cell surface whereas ropy exopolysaccharide are secreted as slim form (ropy) in cell environment. Bacteria may produce either capsular EPS or ropy EPS, or both (Yang *et al.*, 2000 & Broadbent *et al.*, 2003). The mesophilic lactic acid bacteria have much more widespread ability to form CPS than thermophilic heteropolysaccharide. The synthesis of the CPS or the ropy EPS however is almost dependent on the strain (Mozzi *et al.*, 2006).

Bacterial exopolysaccharides (EPSs) are long-chain polysaccharides consisting of repeating branched, units of sugar derivates which are mainly glucose (D-glucose), galactose (D-galactose), rhamnose (L-rhamnose), mannose, N-acetylglucosamine, N-acetylgalactosamine, D-glucuronic acid in various ratio (Welman and Maddox, 2003 & Vaningelgem *et al.*, 2004).

EPS behaves like protein molecule with a carbohydrate structure consisting of α- and β-linkages in different types (Lindhorst, 2007). The arrangement in the formation of

main carbohydrate molecules (D-galactose, D-glucose and L-rhamnose) present in different proportions determine α- and β-linkages in carbohydrate. In addition, EPS-producing lactic acid bacteria can produce not only one type of polysaccharide but also different types of polysaccharides due to fermentation conditions (Looijesteijn *et al.*, 2000). Furthermore, it is possible that the same strain is capable to produce high and low- molecular weight EPS fractions which do not differ in monomeric composition (De Vuyst and Degeest, 1999).

The EPS production is used as replacement for commercial stabilizers in yogurt manufacturing because it decreases syneresis, thus improving texture and viscosity (Martensson *et al.*, 2003). For instance ropy EPS produced by lactic acid bacteria resulted in higher viscosity and lower degree of syneresis in yogurt compared with non-EPS producing lactic acid bacteria (Bouzar *et al.*, 1997 & Folkenberg *et al.*, 2005). In contrast, several studies showed the amount of EPS produced by strains and the rheological features of fermented milk product (yogurt) have no direct relationship to each other (Cerning *et al.*, 1990; Marshall and Rawson, 1999). Furthermore the rheology of yogurt depends not only on the quantity of EPS present, but also on the structure and apparent molecular mass of the polymer and the physical state of the proteins, particularly caseins (Hassan *et al.*, 2003; Petry *et al.*, 2003& Welman and Maddox, 2003). In this regard, the increase in viscosity can be related to the changes in physical properties of the milk proteins due to lower pH (Aslım *et al.*, 2006).

The real functions of EPS for bacterial cells have not been completely clarified. EPSs not only play a role in the protection of the microbial cells against desiccation, phage attack and phagocytosis, antibiotics or toxic compounds, but also impart a number of human health benefits particularly immunostimulatory, antitumoral, antiulcer effects or cholesterol-lowering activity (Aslım *et al.*, 2006 and Lin & Chang Chien, 2007).

In food industry many polysaccharides and stabilizers are used for water binding, viscosifying, gel forming and thickening agent (Looijesteijn *et al.*, 2000). *Xanthomanas campestris* which produces Xanthan, was first used as a microbial EPS (De Vuyst *et al.*, 1998). There is an increasing interest in the production of EPS dairy cultures due to strong consumer demand for smooth and creamy yogurt products. EPS formation of lactic acid bacteria in fermented milk products and yogurt has a role as emulsifying and viscosifying agent or communicates favorable rheological properties. In addition, it was reported that EPS from food grade organisms, particularly lactic acid bacteria, has potential as food additives and functional food ingredients to both health and economic benefits (Welman and Maddox, 2003).

Yogurt is a fermented milk product produced by *Streptococcus salivarius* ssp. *thermophilus* (*Streptococcus thermophilus*) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (*Lactobacillus bulgaricus*) metabolic activity on lactose (Tamime and Marshall, 1997). In some countries, less traditional bacteria such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *lactis* are also added together with the main starter culture (McKinley, 2005).

In the present studies, three herbs, i.e *L. barbarum*, *M. grosvenori* and *P.guajava* leaves, and *G. mongostana* fruits were used in yogurt preparation to enrich the nutritional value of yogurt. The effects of herbs and *G. mangostana* water extracts on changes of pH and TTA, physicochemical properties (total solids, water holding capacity, syneresis) of yogurt was studied. The extent of EPS production in presence of herbs was also evaluated. The effects of herbs and *G. mangostana* water extract on growth of *Lactobacillus* spp. and *S. thermophillus* was studied by turbidity measurement and indirect viable cell counts.

CHAPTER 2: LITERATURE REVIEW

2.0 Literature review

Malaysia is one of the countries in Asia that is endowed with highly diverse biological resources. Natural phytochemical antioxidants, particularly in local fruits, have gained increasing interest among consumers and the scientific community (Rosa *et al.*, 2008). This is because epidemiological studies have reported that frequent consumption of fruits is associated with a healthy lifestyle (Mezadri *et al*., 2008).

Yogurt is a fermented milk prepared by specific lactic acid fermentation through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Kumar and Singh 2007 and Birollo *et al.*, 2000). Other lactic acid bacteria (LAB) that have a role in preparing yogurt are from the genera *Lactobacillus, Streptococcus*, *Leuconostoc,* and *Bifidobacterium* (Samona and Robinson, 1991) can be mixed with yogurt starters to produce fermented milks with specific desirable characteristics such as new flavor (Hartley and Denariaz, 1993 and Dellaglio, 1988). Furthermore, some lactic acid bacteria possess probiotic features i.e. "living microorganisms, which on consumption in certain numbers, exert health benefits beyond inherent basic nutrition" (Schaafsma, 1996).

Yogurt is a good source of minerals and vitamins (Hartley and Denariaz, 1993) and contain small amount of lipids (Yukuchi, Goto, Okonogi, 1992). In addition fermented milk and yogurt help to regulate the absorption of dietary nitrogen components in the body (Gaudichon *et al.,* 1994). Yogurt may also supply many lactic acid bacteria (Fernandes *et al.,* 1992 and Sanders, 1994) including S*treptococcus thermophilus* and *Lactobacillus delbruckii* ssp.

which are able to partially resist bile salts and gastric acidity of intestine prior to residing in the gastrointestinal tract (Bouhnik *et al.*, 1992 and Marteau *et al.*, 1997) where their growth suppresses the proliferation of pathogenic bacteria (Rowland, 1993 and Goldin, 1986).

2.1 *Momordica grosvenori*

M. grosvenori or Luo Han Guo is cultivated for its fruit in the southern part of China and is used for the treatment of pharyngitis or pharyngeus pain, and antitussive medicine in China and Japan. The fruit is also consumed for its anti-inflammatory, antioxidant, anti-diabetic and nephroprotective properties (Song *et al.*, 2006, 2007; Min-Hsiung Pan *et al.*, 2009). The glycosides extracted from *M. grosvenori* (MSE) have anti-carcinogenic activities demonstrated by its ability to inhibit a two-stage carcinogenesis test on mouse skin tumor cells (Takasaki *et al.*, 2003). Studies by Takeo *et al.* (2002) showed that the sweet elements of *Momordica* plants, i.e. the glycosides and particularly 11-oxo-mogroside V (See Figure 2.1), had strong oxidative modification of low-density-lipoprotein with anti-carcinogenic activity.

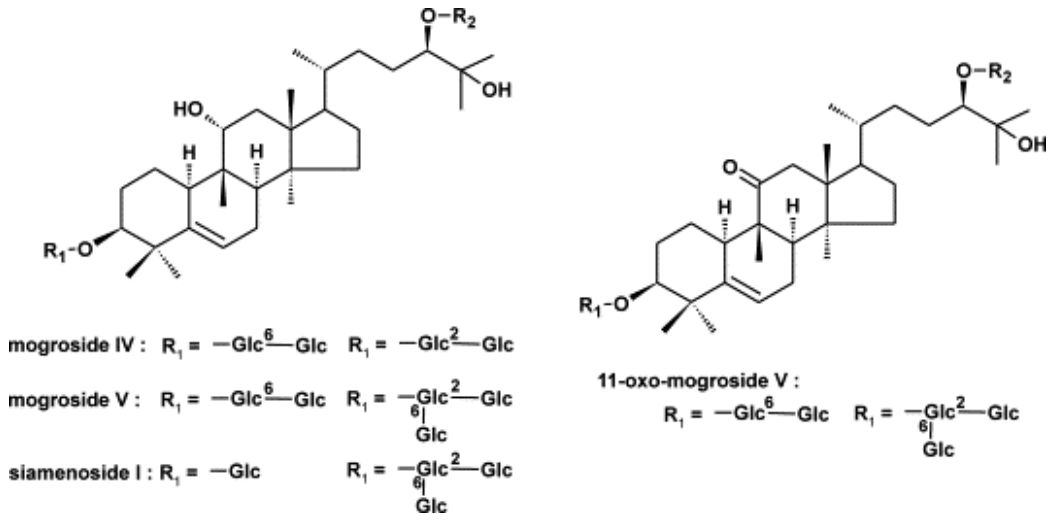


Figure 2.1: Structures of sweet glycosides isolated from the fruits of *M. grosvenori.*

Obesity and diabetes are important underlying causes of morbidity worldwide. Overconsumption of sugars and fat may be contributors to these conditions (Huffman and West, 2007), and identification of low-calories sweeteners could have beneficial effect on world public health. The active sweetness components of *M.grosvenori* are mogrosides and they are members of the family of triterpene glycosides (Lee, 1975; Takemoto *et al.*, 1983 and Yoshikawa *et al.*, 2005). Mogroside V in particular (Makapugay *et al.*, 1985) is estimated to be 250–400 times the sweetness of sucrose (Takemoto *et al.*, 1983 and Matsumoto *et al.*, 1990). Mogrosides II to IV compounds possess a triterpene backbone and are attached with two to six glucose units (Chang, 1996). The glycosidic bonds in these Mongrosides are not able to be broken by either human digestive degradation or the action of intestinal microorganisms, thus making these compounds having neither caloric nor glycemic properties (Suzuki *et al.*, 2005). *M. grosvenori* is classified as generally regarded as safe (GRAS) and is used as a tabletop sweetener or food ingredient. Despite many generations use of Lo Han Kuo fruit and its extracts by the Chinese as sweetener this plant safety is not yet supported by comprehensive toxicological studies (Fry, 2001).

Picture 2.1: *M. grosvenori* fruits

http://www.gd-wholesale.com/userimg/29/1587i1/luo-han-guo-plant-extract-mogroside-367.jpg

2.2 *Psidium guajava*

P. guajava can be cultivated in Asia with tropical and subtropical climate. Although it prefers dry climates, *P. guajava* adapts to different climatic condition easily (Rios *et al.,* 1977 and Stone, 1970). *P. guajava* tree is considered small at maximum 10m height and it has thin, smooth or uneven and peeling bark. Leaves are opposite with 5-15cm long, short-petiolate, the blade oval with prominent pinnate veins. The flowers are showy with petals whitish up to 2cm long (Stone, 1970).

2.2.1 Traditional medicine usage of *P. guajava*

The essential parts in *P. guajava* are the leaves which are used for medicinal and health care purposes (Khan and Ahmad, 1985). The extract of leaves, bark and roots are used to cure gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, respiratory disease, as well an anti-inflammatory medicine (Doubova *et al.,* 2007; Heinrich *et al.,* 1998 and Morton, 1987). The most important effect of *P. guajava* leaves extract is reduction in level of glucose of blood in diabetics (Aguilar *et al.,* 1994). Decoction of the young shoots is used as a febrifuge and combination of bark and leaf ejected the placenta after childbirth (Burkil, 1994). Hot tea of *P. guajava* leaves was used traditionally to manage, control and treat a plethora of human ailment, including diabetes mellitus and hypertension (Ojewole, 2005 and Oh *et al.,* 2005).

2.2.2 Phytochemistry of *P. guajava* leaves

P. guajava leaves contain essential oil with the main components being α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, cineol, caryophyllene oxide, β-copanene, farnesene,

humulene, selinene, cardinene and curcumene (Zakaria and Mohd, 1994 and Li *et al.*, 1999). Isolated leaves showed existence of flavonoids, and saponins combined with oleanolic acid (Arima and Danno, 2002) whereas triterpenic acids which co-exist with flavonoids, avicularin and its 3-l-4-pyranoside have strong antibacterial action (Oliver-Bever, 1986). The acid components extracted from *P. guajava* leaves include guavanoic acid, guavacoumaric acid, 2α-hydroxyursolic acid, jacoumaric acid, isoneriucoumaric acid, asiatic acid, ilelatifol β-sitosterol-3-*O*-β-d-glucopyranoside, guayavolic acids, ursolic acid, Nerolidiol and crategolic acids (Begum *et al.*, 2002a,b and Iwu, 1993). Proportion of common component in *P. guajava* leaves revealed resin (3.15%), tannin (8.5%), fixed oil (6%), and a number of other fixed substances, tannin, cellulose, fat, mineral salt and chlorophyll (Nadkarni and Nadkarni, 1999). The bark of *P. guajava* contains 12–30% of tannin (Burkill, 1997), resin and crystals of calcium oxalate (Nadkarni and Nadkarni, 1999).

2.2.3 Biological activity of *P. guajava*

2.2.3.1 Anti-diarrheal

Aqueous and ethanol extracts of *P. guajava* at a concentration of 80µg/ml in an organ bath showed more than 70 % inhibition of acetylcholine or KCl solution that induced contraction of isolated guinea pig ileum (Tona *et al.*, 2000). The anti-diarrheal action of the extract may be due, in part, to inhibition of increased watery secretions that occur commonly in all acute diarrheal diseases and cholera (Lutterodt, 1992). Quercetin acts as an anti-diarrheal agent on contraction of Guinea pig ileum *in vitro* and the peristaltic movement of the small intestine mice, and reduced permeability across the capillaries (Heinrich, 1998; Zhang *et al.*, 2003).

2.2.3.2 Anti-bacterial effects

The extracts of *P. guajava* leaves were tested for the major causal agents of intestinal infections in humans and was found to be effective against *Staphylococcus aureus, Streptococcus mutatis, Pseudomonas aeruginosa, Salmonella enteritidis, Bacillus cereus, Proteus* spp. *Shigella* spp. and *Escherichia coli*; (Chah *et al*., 2006; Nair and Chanda, 2007). Aqueous and methanol extracts from *P. guajava* leaves played important roles as inhibitor for spore formation and enterotoxin production by *Clostridium perfringens* type A (Garcia *et al*., 2002). Anti fungal effects of *P. guajava* leaves and bark tincture against six dermatophytes (*Trichophyton tonsurans, Trichophyton rubrum, Trichosporon beigelii, Microsporum fulvum, Microsporum gypseum* and *Candida albicans*) were studied by Dutta and Das (2000). The tincture of bark has shown greater effectiveness in controlling mycelia growth of the dermatophytes than the leaves tincture (Dutta and Das, 2000).

2.2.3.3 Anti-tussive effects

The *P. guajava* leaf extract could be used as a cough remedy. The water infusion from *P. guajava* leaves reduced the frequency of coughing induced by capsaicin aerosol (Jaiarj *et al.,* 1999).

2.2.3.4 Anti-oxidant effects

P. guajava leaf extracts are a potential source of natural antioxidants (Ojan and Nihorimbere, 2004). The phenolic phytochemicals which inhibit peroxidation reaction in the living body (Thaipong *et al.,* 2005) was extracted from decoction *P. guajava* dried leaves (Qjan and Nihorimbere, 2004). The phenolic compounds from *P. guajava* with antioxidant activity contain ferulic acid, protocatechuic acid, quercetin and guavin

B (Thaipong *et al.,* 2005), ascorbic acid, quercetin, gallic acid and caffeic acid (Jimenez *et al.,* 2001).

2.2.3.5 Anti-cancer/anti-tumor effects

An aqueous extract from *P. guajava* leaf inhibited the viability of the cancer cell line (Chen *et al.,* 2007). The essential oil in *P. guajava* leaves also showed effective role to reduce the growth of oral cancer human epidermal (KB) and murine leukemia (P388) cell lines (Manosroi *et al.,* 2006) whereas the monoterpenes present in essential oil act as anti-cancer agent (Cito *et a.l,* 2003). These results suggested that *P. guajava* leaves extracts have the potential effect to prevent growth of tumors and cancer by depressing Tr cells and subsequently shifting to Th1 cells (Salib and Michael, 2004; Seo *et al.,* 2005).

2.2.3.6 Anti-inflammatory/analgesic

A decoction from *P. guajava* leaves extract treated various inflammatory diseases, involving rheumatism. Many polyphenolic compounds, triterpenoids and other chemical compounds present in the leaf extracts can expose the anti-inflammatory and analgesic effects from (Ojewole, 2006). The anti-inflammatory and analgesic effects of 70% ethanol extract from *P. guajava* leaf were studied in rats using the model of carrageenan-induced hind paw edema (Kavimani *et al*., 1997; Olajide *et al*., 1999). The ethanol extracts which showed anti-inflammatory activity showed 58% inhibition anti-inflamatory effects (Muruganandan *et al*., 2001).

2.2.3.7 Cardiovascular, hypotensive effects

The *P. guajava* leaves extract showed cardioprotective effects against myocardial ischemia-reperfusion injury in isolated rat hearts (Ojewole, 2005). High energy phosphates and malondialdehyde in the reperfused heart were significantly reduced in the presence of *P. guajava* leaf water extracts (Conde *et al*., 2003).

2.2.3.8 Anti-diabetic effect

The administration of polar fraction of *P. guajava* extract prepared from 50% ethanol extract reduced the plasma glucose level in the glucose tolerance test and also inhibited increasing in the level of plasma sugar in diabetic rats (Maruyama *et al.*, 1985). In addition, butanol and water soluble fractions were found to suppress the adrenaline- induced lipolysis in fat cells from rat epidimal adipose tissue (Keun *et al.*, 2005 and Maruyama *et al.,* 1985). The butanol-soluble fraction from *P. guajava* leaves extracts prevent hyperglycemia which made it beneficial in treating type 2 diabetes (Keun *et al.,* 2005).

Picture 2.2: leaves and fruit of *P. guajava.*

http://en.wikipedia.org/wiki/File:Psidium_guajava_fruit.jpg

http://www-public.jcu.edu.au/discovernature/weeds/JCUDEV_011784

2.3 *Lycium barbarum*

Lycium barbarum belongs to the Solanaceae plant family. The red fruit of *L. barbarum* was used for thousands of years as traditional Chinese medicinal plant (Gao *et al.,* 2000) with a wide variety of biological activities and pharmacological functions and play an important role in the prevention and treatment of various chronic diseases such as hyperlipidemia, diabetes, cancer, hypo-function immunity, hepatitis, thrombosis, and male infertility (Gao *et al.,* 2000 and Li, 2001).

The *Lycium barbarum* polysaccharide (LBP) known as an active compound in *L. barbarum* water extracts is associated with biological activities which include anti-aging, neuroprotection, anti-fatigue/endurance, increased metabolism, blood sugar control in diabetics, glaucoma, anti-oxidant, immunomodulation, antitumor activity and cytoprotection (Bensky and Gamble, 1993; Zhu, 1998; Chang and But, 2001; Bryan *et al.,* 2008 and Potterat, 2010). Five types of LBP were extracted and separated (LbGp1-LbGp5) from *L. barbarum* (Peng *et al.,* 2001). These LBP's are also implicated having anti-aging and neuroprotective effects against toxins in age-related neurodegenerative diseases (Chang and So, 2008). There are few reports about the effect of plant polysaccharide on male reproductive function that promote fertility (Arlet *et al.,* 1999, Mazaro *et al.,* 2002 and Carro-Juárez *et al.,* 2004). The pro-fertility effect of *L. barbarum* fruit was described by great herbalist Li Shizhen on sixteenth century and also in these days *L. barbarum* fruit is known as a medicinal herb with pro- fertility effect (Wang *et al.,* 2002).

2.3.1 Cardiovascular benefits of *L. barbarum*

LBP reduced vasoconstriction and mediate by production of endothelium-derived relaxation factor (EDRF). *L. barbarum* also increased the maximum

combination capacity of cardiac muscle β receptors in 26-month-old mouse and rats (Liu *et al.*, 1996 and Shi *et al.*, 1998).

2.3.2 Effect of *L. barbarum* on diabetes

L. barbarum showed anti-diabetic effect by reducing oxidation in patient with retinopathy (Li *et al.*, 2000). *L. barbarum* fruit water extract was used to treat diabetic rabbits and this was found to reduce the level of glucose of blood and produced substantial hypoglycemic effects (Qiong Luo *et al.*, 2004).

2.3.3 Eye health benefits

Oral administration of *L. barbarum* reduced the loss of retinal ganglion cells (RGCs), although elevated intraocular pressure (IOP) was not significantly altered (Chan *et al.*, 2007). Thus, the therapeutic function of *L. barbarum* against neurodegeneration in the retina of ocular hypertension rat model suggest that *L. barbarum* may be a potential candidate for development of neuroprotective drugs against loss of retinal ganglion cells in glaucoma (Li, 2007). The lutein and zeaxanthin components of *L. barbarum* acted as restorer visual functions in experimental light-induced phototoxicity and macular degeneration, probably by protecting RGCs from glutamate- and nitric oxide (NO)-induced neuronal apoptosis in the retina (Lam and But, 1999; Sommerburg *et al.*, 1999; Leung *et al.*, 2001). Other studies also indicated that *L. barbarum* protected areas from light damages to the retina pyramid layer of stem cells, the outer nuclear layer, and retinal pigment epithelium in the rat (Liu, Li, and Tso, 1995).

2.3.4 Anti-oxidant activity

L. barbarum and LBP showed antioxidants effect against peroxidation or related conditions (Gong *et al.,* 2005; Luo *et al.,* 2006; Li, Ma and Liu, 2007& Reeve *et al.,* 2010).

2.3.5 Anti-inflammatory

The consumption of *L. barbarum* offered anti-inflammatory benefits in response to a septisemia endotoxin challenge with regard to TNF and IL-6 as seen in male SD rats (Nance *et al.,* 2009). *L. barbarum* also offers skin protection against immune suppression and oxidative stress when consumed for 2 weeks prior to exposure to SSUV radiation (Reeve and Reeve, 2008 & Reeve *et al.,* 2010).

2.3.6 Anti-cancer

LBP has anti-tumor effects because it can increase the number of CD4 (+) and CD (+) T cells in tumor-infiltrating lymphocytes to reduce the immunosuppression and enhance the anti-tumoral immune system (He *et al.,* 2005).

2.3.7 *Lycium barbarum* polysaccharide (LBP)

Polysaccharide protein complex (LBP) was known as the bioactive compound of *L. barbarum* fruit. Several fractions of LBP can be separated by ion exchange chromatography and size exclusion chromatography (Amrani *et al.,* 2009, Gan *et al.,* 2004, Gan *et al.,* 2003 and Huang *et al.,* 1999). LBP contains six monosaccharides (glucose, galactose, arabinose, rhamnose, mannose and xylose) and 18 amino acids (Gan *et al.,* 2004, Gan *et al.,* 2003 and Huang *et al.,* 1998). HPLC analysis showed *L. barbarum* polysaccharide (LBP) composed of two monosaccharides (glucose and fructose) in molar ratios of 1:2 (Shao-Ping Lu and Pin-Ting Zhao**,** 2010). *L. barbarum*

Polysaccharide (LBP) have anti-tumor activity (Sheng *et al.,* 2007), and also can improve immunity through interleukin and antibody (Yang *et al.,* 2008). LBP can improve immune function (Gan *et al.,* 2004 ; Gan *et al.,* 2003), protecting liver damage (Yoon *et al.,* 2005), reduce blood glucose level (Luo *et al.,* 2004), reduce side effect of chemotherapy and radiotherapy (Gong *et al.,* 2005), and act as anti-cancer (Gan *et al.,* 2004 and Gan *et al.,* 2003).

The other polysaccharide compounds in *L. barbarum* are composed of 100 or more monosaccharide. Polysasaccharides can be water-soluble or water insoluble, with the former kinds being glucurono β-glucan and β-glucan, and latter xylo-β-glucan, xylomann-β-glucan, hetero-β-glucan and manno-β-glucan (Huie and Di, 2004 and Sun *et al.,* 2005).

Picture 2.3: *L. barbarum* fruits.

http://4.bp.blogspot.com/_-eXVFD4kwaQ/TCpCHUv-2tI/AAAAAAAAADs/5EV6RnqBilw/s1600/goji.jpg http://1.bp.blogspot.com/-6IJSf-vnCno/TbHHSuuRI/AAAAAAAAAW0/QEgZ_vxTHj0/s1600/goji%2Bberry.jpg

2.4 *Garcinia mangostana*

G. mangostana fruit can be cultivated in tropical countries throughout Asia, e.g. Thailand, India, Malaysia, Vietnam and the Philippines. The white part of *G. mangostana* (aril) is edible portion of fruit that is soft and slightly have sour taste

(Morton, 1987). *G. mangostana* fruits are a rich source of phenolic acids, xanthones, anthocyanins, and condensed tannins (proanthocyanidins) (Mahabusarakam *et al.*, 1987; Pedraza-Chaverri *et al.*, 2008 and Zadernowski *et al.*, 2009).

2.4.1 Health Benefits of *G. mangostana*

G. mangostana has been traditionally used as a medicinal treatment for diarrhea, skin infection and wounds (Pedraza-Chaverri *et al.*, 2008).

2.4.2 Xanthones activity

The xanthones is a major polyphenolic compound in *G. mangostana* (Jung *et al.*, 2006 and Mahabusarakam *et al.*, 1987). It consists of more than fifty xanthones and is present in the pericarp of *G. mangostana* fruit (Jung *et al.*, 2006 and Mahabusarakam *et al.*, 1987). The xanthone nucleus with symmetric forms is known as 9-xanthenone or dibenzo-γ-pyrone (See Figure 2.2) (Vieira and Kijjoa, 2005; Souza and Pinto, 2005 and Gales and Damas, 2005). Xanthones can be classified into five groups: (a) simple oxygenated xanthones, (b) xanthone glycosides, (c) prenylated xanthones, (d) xanthonolignoids and (e) miscellaneous xanthones (Sultanbawa, 1980 and Jiang *et al.*, 2004).

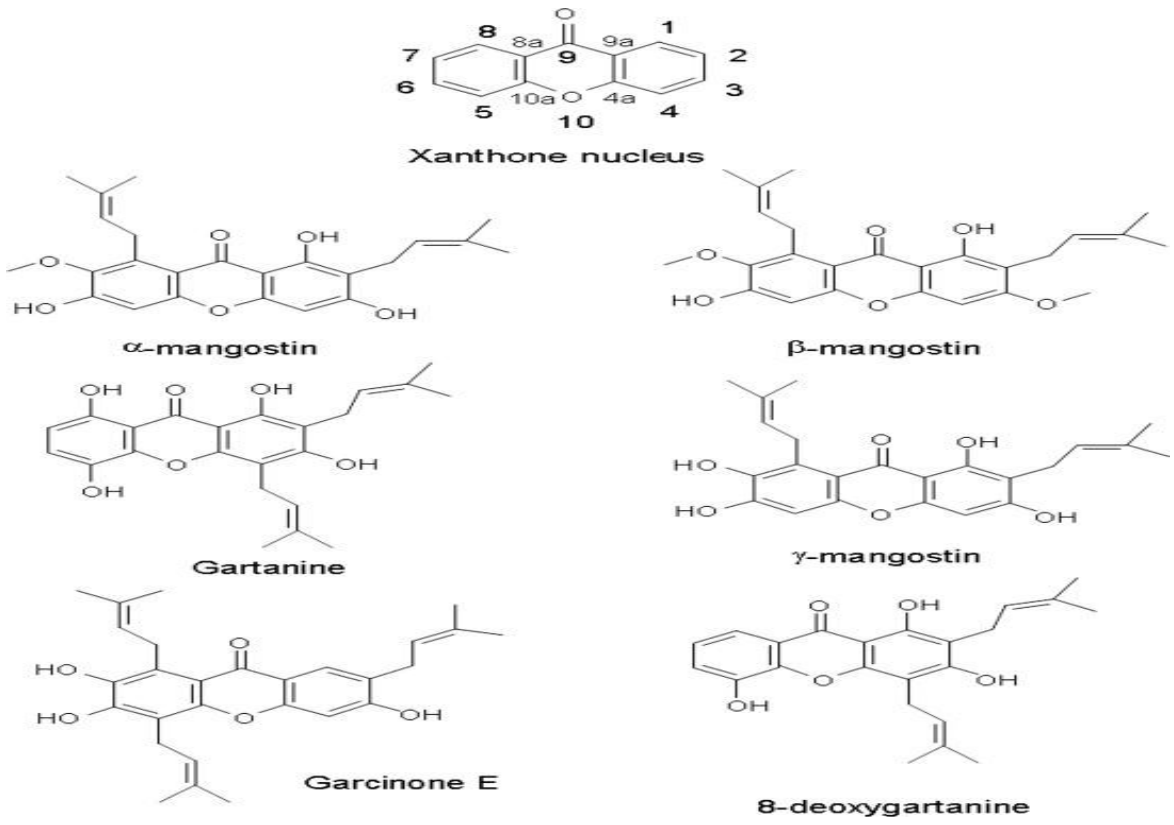


Figure 2.2: Xanthone nucleus with IUPAC numbers of carbons and chemical structure of the most studied xanthones.

Plant materials, such as vegetables, fruits, roots, spices, leaves, and barks, are potential sources of natural antioxidants (Chyau *et al.,* 2002; Ellnain-Wojtaszek *et al.,* 2002; Naczk *et al.,* 2003 and Picerno *et al.,* 2003). There are several xanthones with antioxidant activities which can be isolated from *G. mangostana* pericarp. 8-hydroxycudraxanthone G, gartanin, γ-mangostin, α-mangostin, and smeathxanthone secreted from *G. mangostana* exhibited the strongest antioxidant activities (Jung *et al.,* 2006). Air-dried *G. mangostana* exhibited 1,3,6,7-tetrahydroxy-2,8-(3-methyl-2-butenyl) and this xanthone displayed a strong free radical-scavenging activity (Yu, Zhao, Yang, Zhao, and Jiang 2007). The phenolics compounds from unripe *G. mangostana* fruit rind produce stronger free radical-scavenging activity from than mature fruit rind (Pothitirat, Chomnawang, Supabhol, and Gritsanapan 2009).

2.4.3 Anticancer activity

Several anticancer activities of xanthones from *G. mangostana* fruit pericarp are shown in Table 2.1.

Table 2.1: Antitumoral properties of xanthones isolated from *Garcinia mangostana*

Effects	References
Cytotoxic effect on hepatoma cells lines as well as on the gastric and lung cancer cell lines by Garcinone E.	Ho *et al.,* (2002)
Six xanthones from the pericarp of GML showed antiproliferative activity against human leukemia HL60 cells. In addition, α-mangostin induced caspase 3-dependent apoptosis in HL60.	Matsumoto *et al.,* (2003)
The treatment with dietary α-mangostin inhibits cells proliferation in the colon lesions in rats injected with DMH.	Nabandith *et al.,* (2004)
Aqueous extract of the fruit rind GML showed antileukemic activity in four cells lines.	Chiang *et al.,* (2004)
Ethanolic and methanolic extracts of GML showed antiproliferative effect on human breast cancer SKBR3 cells.	(Moongkarndi *et al.,* 2004a, 2004b)
The antiproliferative effect of α- and γ-mangostins, was associated with apoptosis in human colon cancer DLD-1 cells.	Matsumoto *et al.,* (2005)
α-Mangostin inhibited DMBA-induced preneoplastic lesions in a mouse mammary organ culture.	Jung *et al.,* (2006)
On the three human cancer cell lines mangostenone C, mangostenone D, demethylcalabaxanthone, β-mangostin, gartanin, garcinone E, α-mangostin, mangostinone, γ-mangostin, garcinone D, and garcinone C have cytotoxic effects.	Suksamrarn *et al.,* (2006)
Antitumoral activity against DLD-1 cells has been showed by α-Mangostin.	Nakagawa *et al.,* (2007)

2.4.4 Anti-inflammatory activity

The anti-allergy and anti-inflammatory properties of *G. Mangostana* in different *in vitro* models, such as RBL-2H3 cells (Nakatani *et al.,* 2002), C6 glioma cells from rat (Nakatani *et al.,* 2002a,b, 2004;. Yamakuni *et al.,* 2006), the rabbit thoracic aorta, Guinea-pig trachea (Chairungsrilerd *et al.,* 1996a,b) and several in vivo models in rats (Shankaranarayan *et al.,* 1979; Nakatani *et al.,* 2004) are indicated in Table 2.2.

Table 2.2: Anti-inflammatory effects of GML.

Effects	References
Antiiflamatory activity have been showed by a-Mangostin, 1-isomangostin, and mangostin triacetate in several experimental models in rats	Shankaranaray an *et al.,* 1979
In several experimental models of inflammation in rats and guinea pigs showed anti-inflammatory effects by a-Mangostin, i.p	Gopalakrishna n *et al.,* 1980
a-Mangostin ameliorates the histamine-induced contraction of aorta and trachea from male guinea pigs	Chairungsriler d *et al.,* 1996
The crude methanol extract from GM legume blocked the histaminergic and serotonergic response in isolated rabbit aorta strips. The histaminergic response was blocked by a-mangostin and c-mangostin blocked the serotonergic response	Chairungsilerd *et al.,* 1996,b.
c-Mangostin is 5HT2A receptor antagonist in vascular smooth muscles and platelets	Chairungsriler d *et al.,* 1998,a.
c-Mangostin inhibits 5-FMT-induced head-twitch response in mice by blocking 5-HT2A receptors	Chairungsriler d *et al.,* 1998,b.

Table 2.2: Anti-inflammatory effects of GML.

Effects	References
c-Mangostin inhibited A2318 induced PGE2 release in C6 cells and arachidonic acid conversion to PGE2 in isolated microsomes as well as the activities of both constitutive COX-1 and inducible COX-2	Nakatani *et al.*, 2002,b.
Extracts of *mangosteen* pod inhibited histamine release in RBL-2H3 cells and decreased A23187 induced PGE2 synthesis in C6 rat glioma cells	Nakatani *et al.*, 2002,b.
inhibited COX-1 and -2 activity and PGE2 synthesis in C6 rat glioma cells was inhibired by c-Mangostin (a), LPS-induced expression of COX-2 protein and its mRNA was inhibited by c-Mangostin (b) , c-Mangostin (c) reduced the LPS-inducible activation of NF-kB, and c-Mangostin (d) inhibited rat carrageenan-induced paw edema	Nakatani *et al.*, 2004
PGE2 was induced by A23187 and LPS-induced transcription of NF-kB-mediated in C6 rat glioma cells that A23187 and LPS was reduced by garcinone B	Yamakuni *et al.*, 2006
LPS-stimulated citotoxicity was inhibited by a- and c-mangostins. a-Mangostin showed a potent inhibition on paw oedema in mice	Chen *et al.*, 2008
a-Mangostin inhibits human 12-LOX	Deschamps *et al.*, 2007

2.4.5 Antibacterial activity

Xanthones from *G. mangostana* have antibacterial, antiviral and antifungal properties. Sundaram *et al.* (1983) inspected the antibacterial and antifungal properties of a-mangostin and its derivatives. Their findings showed *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium* and *Bacillus subtilis* were highly sensitive to xanthones, whereas *Proteus sp., Klebsiella sp.* and *Escherichia coli* were only quite sensitive to them (Sundaram *et al.*, 1983).

Picture 2.4: *G. mangostana* fruit.
http://en.wikipedia.org/wiki/File:Mangosteen.jpeg

2.5 Exopolysaccharide (EPS)

Exopolysaccharide is used widely in food industry and as thickening, gelling and stabilizing agents (Sutherland, 1998). In yogurt the presence of EPS produced by LAB improve their physical properties and slow down syneresis by binding water (Hess, Roberts, & Ziegler, 1997; Hassan, Corredig, & Frank, 2001; Hassan, Corredig, & Frank (2002) and Ruas-Madiedo, Tuinier, Kanning, & Zoon, 2005).

Many bacterial strains produce EPS and they include *Lactobacillus acidophilus* (Robijn *et al.*, 1996), *L. delbrueckii* subsp. *bulgaricus* strain RR (Gruter *et al.*, 1993), *L.*

helveticus (Staaf *et al.,* 1996, Stingele *et al.,* 1997), *L. paracasei* (Robijn *et al.,* 1996), *L. reuteri* (van Geel-Schutten *et al.,* 1999), *L. rhamnosus* (Vanhaverbeke *et al.,* 1998), *L. sake* (Robijn *et al.,* 1995), *Lactococcus lactis subsp. Cremoris* (Nakajima *et al.,* 1992, Gruter *et al.,* 1992), and *Streptococcus thermophilus* (Lemoine *et al.,* 1997, Faber *et al.,* 1998).

This is reflected in the many dairy products in the Europe market which contain *S. thermophilus* and exopolysaccharide (EPS)-producing capacity in milk and enriched milk medium (Vaningelgem *et al.*, 2004).

2.5.1 EPS structure

Glucose, galactose and rhamnose are the major monosaccharide composition of polysaccharide substances produced by lactic acid bacteria (LAB) (Doco, Wieruszeski, & Fournet, 1990; Stingele, Nesser, & Mollet, 1996). The relative amount of each monosaccharide in EPS however appear to depend on the type of LAB.

The exopolysaccharide of *S. thermophilus* S3, produced in skimmed milk, is composed of D-galactose and L-rhamnose in a molar ratio of 2:1 (Elisabeth Faber *et al.*, 2001; see Figure 2.3).

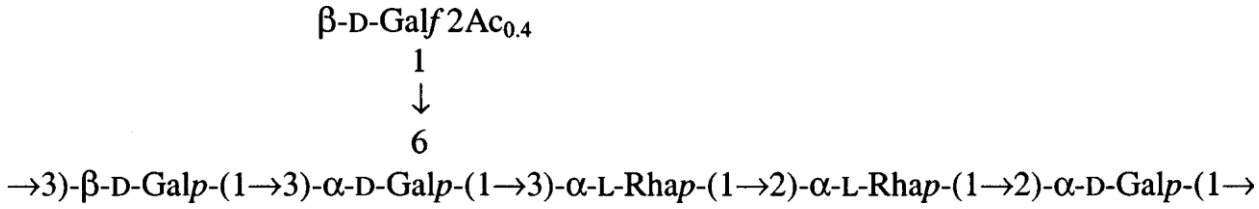


Figure 2.3: Exopolysaccharide of *S. thermophilus*.

On the other hand the neutral exopolysaccharide (EPS) produced by *Lactobacillus delbrueckii* ssp. *bulgaricus* LBB.B26 in skimmed milk compose of d-glucose and d-galactose in a molar ratio of 2:3 (Gerwig**,** Urshev and Kamerling, 2007; See Figure 2.4).

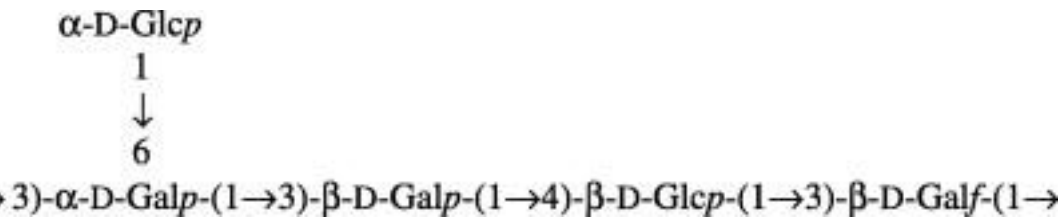


Figure 2.4: Exopolysaccharide of *Lb. delbereuckii bulgaricus.*

The exopolysaccharide (EPS) produced by *L. acidophilus* LMG9433 in a semi-defined medium was found to be more heterogenous, the EPS produced is more heteropolymer with a composition of D-glucose, D-galactose. D-glucuronic acid, and 2-acetamido-2-deoxy-D-glucose in the molar ratios of 2:1:1:1 (Robijn *et al.,* 1996; see Figure 2.5).

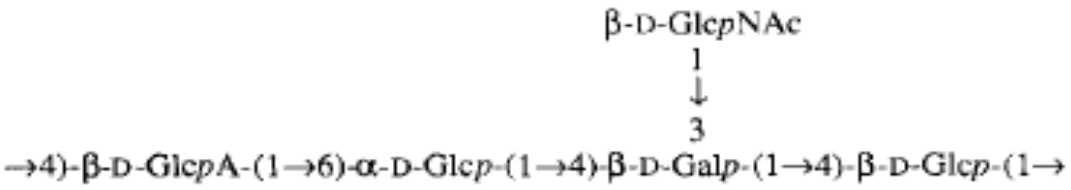


Figure 2.5: Structure of exopolysaccharide was produced by *L. acidophilus.*

Because of the unique differences in monosaccharide composition in EPS produced by different LAB *L. delbrueckii* ssp. *Bulgaricus* strains are commonly applied with *S. thermophilus* strains as commercial starters.

The structure and molecular mass of exopolysaccharides influence on their rheological and physical properties (Faber *et al.,* 1998).

2.5.2 Effect of carbohydrate and protein on EPS

The carbohydrate and protein content of the fermentation media are known to affect the production of EPS by LAB (Grobben *et al.,* 1997; Marshall *et al.,* 1995 and Petry *et al., 2003*). The EPS is increased when protein content increases in the medium

(Grobben *et al.,* 1998). Other medium components such as carbohydrates, specific amino acids and minerals can also affect the structure and yield of EPS (De Vuyst and Degeest, 1999 & Grobben *et al.,* 1998). Since EPS production depends on the LAB counts, the growth of bacteria which is supported by the balance between the nitrogen and carbon content is also essential to obtain high EPS yields (De Vuyst and Degeest, 1999 and De Vuyst *et al.,* 1998).

2.5.3 Ropy and capsular EPS

There are two type of exopolysaccharide produced by LAB in yogurt or dairy products, like ropy and capsular type of EPS. Each EPS has its own physical attributes and thus the final textural characteristics of yogurt are dependent on the relative amount of ropy and capsular in the EPS (Bouzar, Cerning, & Desmazeaud, 1997; Hess, Roberts, & Ziegler, 1997). Capsular EPS and ropy EPS have different behavior in relation to the interaction with milk proteins during the manufacture of yogurt. Their differences in structure and in the protein network (Folkenberg *et al.*, 2005; Hassan, Ipsen, Janzen, & Qvist, 2003) resulted in pronounced effect on the viscosity and consistency of yogurt (Hassan, Corredig, & Frank, 2002).

2.5.4 EPS effects and health benefits

The EPS molecules affect on the rheology, texture and mouth feel of food products and have been used as thickeners, stabilizers, gelling or water-binding agent and emulsifiers (Banik *et al.,* 2000 and Sutherland, 1998). Furthermore, claims have been made associating bacterial EPS with health-promoting characteristics such as antitumor effects, reduced blood cholesterol, immunostimulatory activity, and enhancing colonization of probiotic bacteria in the gastrointestinal tract (Welman and Maddox, 2003).

2.6 *Streptococcus thermophilus*

Streptococcus thermophilus is commonly isolated from dairy products, and even from plant samples (Michaylova *et al.,* 2002). *S. thermophilus* is identified as anaerobic, aerotolerant, catalase-negative and gram-positive. It grows as linear chains of ovoid cells and unable to grow at 10°C, pH 9.6 or in 6.5% NaCl broth (Moschetti *et al.,* 1998). Species identification of *S. thermophilus* is based on the hydrolysis of arginine and esculin, cellobiose, inulin, maltose, mannitol, raffinose broths and N-acetylglucosamine and the ability to grow at 45°C (Facklam, 2002 and Moschetti *et al.,* 1998).

S.thermophilus includes different properties although there is difference in the species level by DNA-DNA hybridization (Kılıç 2001).

2.6.1 Role of *S. thermophilus* in dairy products

S. thermophilus is a thermophilic lactic acid bacteria (LAB) widely used as starter culture in the manufacture of dairy products and can be considered the second most important industrial dairy starter after *L. lactis* (Hols *et al.,* 2005). It is traditionally used in making cheese and yogurt, for example, hard-cooked cheese (Emmental, Gruyere, Parmesan and Grana-type, etc.), mozzarella and cheddar. *S. thermophilus* is able to grow or survive at high temperatures (45°C) required in several production processes. To make cheese, *S. thermophilus* is used alone or in combination with several mesophilic and lactobacilli starter, for yogurt, it is always used with *Lactobacillus delbrueckii* ssp. *bulgaricus* (Auclair and Accolas, 1983).

2.6.2 EPS production by *S. thermophilus*

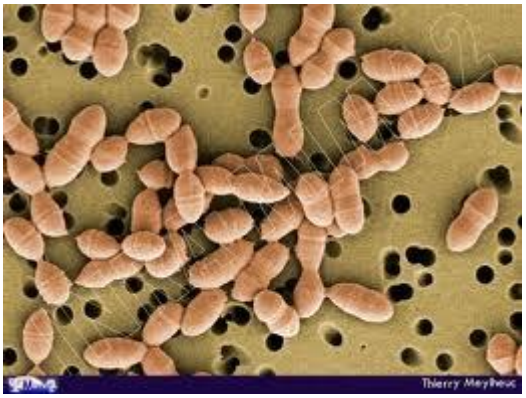
Several strains of *S. thermophilus* produce exopolysaccharides (EPS) which contribute to the desirable viscous texture and rheological properties of fermented milk products, especially yogurt. The physiological role of the EPS of *S. thermophilus* is not well understood but it was confer considerate neither to advantage for growth nor survival of the bacteria in milk (Broadbent *et al.,* 2003). EPS from strains of *S. thermophilus* are heteropolysaccharides which display a great diversity in terms of their monomer composition, molecular weight (MW) and structure of the repeat unit. The first *S. thermophilus* EPS structure to be identified is those from strains CNCM 733, 734, and 735, which EPS produced consist of the same repeating unit structure →3)-β-d-Gal*p*-(1→3)-[α-d-Gal*p*-(1→6)]-β-d-Glc*p*-(1→3)-α-d-Gal*p*NAc-(1→ (Doco *et al.,* 1990).

The production of EPS by *S. thermophilus* is dependent on the growth rate and influence of environmental factors such as pH, temperature, carbon source, carbon to nitrogen ratio (C:N) and concentration of carbon (De Vuyst *et al.,* 1998; Degeest and De Vuyst, 1999; Zisu and Shah, 2003).

2.6.3 Probiotic strains of *S. thermophilus*

Probiotics combination of *S. thermophilus* have been identified as having positive effects on diarrhea in young children, inflammatory gut disease and enterocolitis in premature neonates (Bibiloni *et al.,* 2005; Bin-Nun *et al.,* 2005 and Shamir *et al.,* 2005). In probiotic mixture the efficacy of *Bifidobacteria* was shown improved by *S. thermophilus* prevented rotaviral diarrhea (Bin-Nun *et al.,* 2005 and Saavedra *et al.,* 1994). Specific strains of *S. thermophilus* can also interfere with the

adhesion of either oral cariogenic bacteria to dental plaque, or *Candida* spp. to silicone voice prostheses, the increasing the lifetime (Busscher *et al.*, 1997 and Comelli *et al.*, 2002).



Picture 2.5: form of *S. thermophilus* in yogurt.

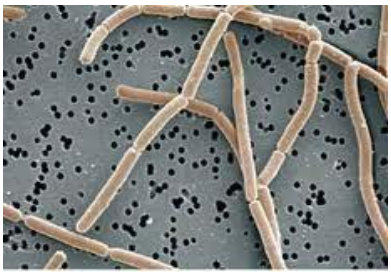
http://www.probioticsnews.creativetesting.co.uk/_resources/media/documents/libraryimages/hires/streptococcus_thermophilus_4.jpg

2.7 *Lactobacillus delbrueckii*

Lactobacillus delbrueckii are non-motile, Gram-positive, facultative anaerobic, and non spore-forming rod-shaped (the cell size: 0.5-0.8 x 2.0 to 9.0 mm) lactic acid bacteria. *L. delbrueckii* has homofermentetive metabolism and its optimum temperature for growth is 42-45 °C (Robinson 1999). *L. delbrueckii*, like other lactic acid bacteria, is tolerant to acid, cannot synthesize porphyrins, and possess a strictly fermentative metabolism with lactic acid as major metabolic end production (Axelsson, 1998; Hammes and Vogel, 1995; Kandler and Weiss, 1986). The *L. delbrueckii* species includes three subspecies, *L. delbrueckii* subsp. *delbrueckii, L. delbrueckii* subsp. *lactis*, and *L. delbrueckii* subsp. *bulgaricus*. *L. delbrueckii* subsp. *bulgaricus* grows on a

relatively small number of carbohydrates and generally requires pantothenic acid and niacin (Hammes and Vogel, 1995).

Lactic acid bacteria are widely used in fermentative food processes. *L. delbrueckii* in corporation with *S. thermophilus* are universally used as starters for milk fermentation in yogurt production. While fermenting milk *L. delbrueckii* subsp. *bulgaricus* produces acetaldehyde, which imports unique yogurt aroma (Zourari, Accolas and Desmazeaud, 1992), as well as flavor and texture properties (Curry & Crow, 2003). *L. delbruckii* subsp. *bulgaricus* also produce bacteriocins which kill undesired bacteria (Simova *et al.*, 2008).



Picture 2.6: *Lactobacillus spp.* forms in yogurt.

http://microbewiki.kenyon.edu/images/5/5f/Lactobacillus_bulgaricus.jpg

2.8 Colony forming unit (CFU)

CFU is a measure of viable bacteria or fungal numbers and the results are given as CFU/ ml. The digestive tract is colonized by beneficial bacteria, several of these microbes are introduced from environment, mostly through food and they colonize the digestive tract. These microbes called probiotics because of their potentials benefits to the host, play important role in human metabolism. The probiotics are capable of producing different molecules as a result of fermentation of unabsorbed feed

components in the large intestine. The organic acids produced from the probiotic fermentation of undigested carbohydrate have several healthy benefits including modulation of host immune system, inhibition *Helicobacter pylori*, decreasing lactose intolerance, assimilate cholesterol, preventing autoimmunity, and exhibiting antimutagenic properties (Parvez *et al.*, 2006).

Yogurt is one type of food which contains huge amount of probiotics; *L. bulgaricus* and *S. thermophilus* formed the most part of probiotics in yogurt that have important role in digestive tract and producing exopolysaccharide. The survival and viability of probiotic bacteria is often low in yogurt and results in less than 10^7–10^8 cfu/g which is the daily recommended intake to confer health benefits (Lourens-Hattingh & Viljoen, 2001). The viability of yogurt bacteria in yogurts depends on a number of factors such as the strain of probiotic bacteria incorporated, the yogurt starter cultures used and any interaction between the species present, culture conditions, fermentation time and storage conditions, pH of the yogurt (post-acidification during storage), sugar concentration (osmotic pressure), milk solids content, availability of nutrients, the presence of hydrogen peroxide, dissolved oxygen content (especially for *Bifidobacterium* spp.), buffering capacity and β-galactosidase concentration in the yogurt (Dave & Shah, 1998).

2.9 Syneresis

Appearance and physical characteristics are important parameters of quality of yogurt. Good quality yogurt should be thick and smooth, without signs of syneresis. Set yoghurt with a high level of syneresis on the surface can be considered a poor quality product. Syneresis is spontaneous whey separation on the surface of set yogurt and is an undesirable occurrence. This problem can be reduced or eliminated if the total solid of set yogurt be increased to 14% (w/ w) (Shah, 2003 and Tamime & Deeth, 1980) or by

using stabilizers. However, the uses of stabilizers such as gelatin, modified starches or gums can affect consumer perception of yogurt are prohibited in some European countries (De Vuyst & Degeest, 1999). Thus the use of dairy ingredients such as dried skim milk powder (SMP), whey protein isolate (WPI), whey protein concentrate (WPC), sodium (Na) caseinate or calcium (Ca)-caseinate are commonly used to increase the content solids yogurt mix thus reducing syneresis. Nevertheless, the enrichment of these ingredients affects production costs.

Casein-based ingredients (SMP, Na-caseinate or Ca-caseinate) fortifiy yogurt, resulting in an increase in firmness (or viscosity) and reduction in syneresis compared with unfortified yogurt (Modler *et al.,* 1983; Guzmán-González *et al.,* 2000 and Remuef *et al.,* 2003). The addition of whey protein (WP)-based ingredients (WPI or WPC) on the other hand, showed no consistent trends to improve firmness and syneresis of yogurt. For instance yogurt supplement with WPC had lower apparent viscosity and firmness than control yogurt made without fortification (Modler *et al.,* 1983 and Guzmán-González *et al.,* 1999). Baig and Prasad (1996) on the other hand found that supplementation of milk with WPC improved the apparent viscosity and textural properties of the resultant yogurt. These differences were suggested to stem from the variations in the composition of whey protein-based ingredients (Baig and Prasad, 1996 and Bhullar, Uddin, and Shah, 2002). Extending the heating time of milk supplemented with WPC from 1 to 5 min at 90 ºC was shown to increase the apparent viscosity of stirred yogurt. On the other hand, the apparent viscosity of stirred yogurt fortified with Na- and Ca-caseinate was not affected by the increase in the heating time. Nonetheless, fortification with WPC reduced syneresis dramatically (Remuef *et al.,* 2003).

Yogurt production using of EPS – producing starter culture can replace and reduce the use of stabilizers as well as added dairy ingredients (Puvanenthiran,

Williams, and Augustin, 2002). Whey protein and EPS are known to have high water binding capacity (Walzem, Dillard & German, 2002; Cerning, 1990 and De Vuyst & Degeest, 1999) and both may act cooperatively in preserving water in the gel structure. In fact yogurts made using EPS-producing starter cultures (both capsular and ropy) had a lower level of syneresis than yogurt made by non-EPS-producing starter cultures (Shah, 2003; Marshall & Rawson, 1999; Wacher-Rodarte *et al.,* 1993). Ropy bacteria strain producing ropy starch reduce syneresis and increase viscosity better than non-ropy bacteria strain in yogurt containing 12% total solids (Wacher-Rodarte *et al.,* 1993).

2.9.1 Methods to determine whey syneresis

There are 2 methods commonly used to determine whey syneresis i.e. by, drainage and centrifugation (Bhullar *et al*., 2002 and Jaros *et al*., 2002).

2.9.1.1 Drainage method

Susceptibility to syneresis can also be measured in laboratory conditions using a drainage method. This method employs a sieve which separates the whey under gravitational force from a certain quantity of set yogurt (disturbed or undisturbed gel) at a fixed temperature. The whey separation is calculated as the percent weight of the separated whey over the initial weight of the gel (Harwalkar and Kalab 1986 and Guzmán-González *et al*., 1999, 2000).

2.9.1.2 Centrifugation method

The water-holding capacity of a product is determined by centrifugation method. A certain quantity of set yogurt (disturbed or undisturbed gel) is centrifuged at a specified speed over a certain period at a fixed temperature. The whey separation is

calculated as the percent weight of the separated whey over the initial weight of the gel (Bhullar *et al*., 2002 and Jaros *et al*., 2002).

Although these methods give high-precision results, they do not represent the actual value of spontaneous whey separation in set yogurt. The breakage of the yogurt gel as well as the presence of EPS may influence the separation of whey (Lucey *et al*., 1998).

2.10 Total solid

Total solids and fat content make up important chemical composition of the milk and has important effects on the activity of starter cultures. Concentrated yogurt produced by different methods was shown to influence the growth and activity of starter cultures (Ozer and Robinson, 1999). This type of yogurt has superior features with its protein (2.5x) and mineral (1.5x) contents being higher whereas, lactose content and fat content being lower than regular yogurt. Because of these compositional features concentrated yogurt is regarded to command significant market potential (Salji, 1991).

The growth of starter culture bacteria is influenced by many factors such as the chemical composition of milk, milk temperature, and the amount of inoculums, incubation time and cooling time of the milk (Mirnezami, 1999).

The supplementation of milk with whey, casein hydrolysate and milk protein can improve the acidification of yogurt and this has the benefit of reducing the fermentation time to about 55% (Oliveria, Sodini, Remeuf and Corrieu, 2001).

The addition of these supplements has little impact on fermentation because the total solids content had no deleterious effect on starter activity or coagulation time (Abd-El-Salam and El-Alamy, 1982).

In fact the increase in milk total solids from 16 to 23% had an important beneficial effect on reducing the rate of pH reduction during fermentation (Tamime, Kalab and Davies, 1989; Ozer, Bell, Grandison and Robinson, 1998).

An increase in milk fat content increased the initial pH of the samples with the rate of reducing pH during incubation of high fat samples was lower than others (Shaker et al., 2000).

Several methods for milk fortification are used by the dairy industry. Skim milk powder (SMP) is traditionally added to enrich the milk before fermentation. Whey protein concentrates (WPCs) may also be used as effective additions when compared to SMP as partial such as evaporation of milk is another method to increase total solids content to a desired level. The removal of water from milk by membrane filtration or under vacuum also reduced syneresis and improves the stability of the coagulum during storage (Tamime *et al.,* 2001). Using this method the evaporation of goat's milk was found did not only improve the consistency, but also reduce the goaty flavor of the end-product (Tamime and Robinson, 1985).

The uses of whey protein concentrate (WPCs) and caseinates in milk supplementation for yogurt production (Lucey, Munro, & Singh, 1999; Patocka, Cervenkova, Narine, & Jelen, 2006; Remeuf, Mohammed, Sodini, & Tissier, 2003 and Sodini, Montella, & Tong, 2005) which improves yogurt's rheological properties (Fox, 2001 and Tamime *et al.,* 2001) increases protein content and this enhances the development of chains and aggregates of casein micelles (Prentice, 1992).

The nature and relative proportions of the different proteins in the dry matter significantly influence the texture of the final product (Almeida, Tamime, & Oliveira, 2008; Penna, Converti, & Oliveira, 2006; Puvanenthiran, Williams, & Augustin, 2002 and Sodini *et al.*, 2004). Penetrometry is used to perform texture profile analyses, which complement instrumental and sensory evaluation of texture. This instrument measures

the force required to push a probe into yogurt to a fixed depth of penetration which reflects the hardness or firmness of yogurt (Pons & Fiszman, 1996).

2.11 Yogurt

Yogurt is a dairy product produced by fermenting milk, with or without added non-fat dry milk (NFDM), by *L. bulgaricus* and *S. thermophilus* bacteria (Lee *et al.*, 1990). Any type of milk can be consumed to produce yogurt but modern production is dominated by cow's milk. Milk is initially homogenized with NFDM and other stabilizers to obtain 12-14% total milk solids followed by heating. Heating of milk prior to fermentation achieves the following: 1) to destroy pathogens or undesirable organisms; 2) to either stimulate or inhibit activity of lactic starter cultures; and 3) to denature milk proteins which provide the proper viscosity and gelation, and limit syneresis in the final product (Tamime and Robinson, 1999).

There should be at least 2 million lactic acid bacteria (mono-cellular) per gram live in starter cultures (Viljoen *et al.,* 2001). The lipidic part of fermented products e.g. yogurt, almost remains same as original milk while the proteins (milk casein) hydrolyzed partially and became more digestible (Viljoen *et al.,* 2001). The serum protein of yogurt (lacto-albumin and lacto-globulin) in comparison with cheeses remain within the product and simultaneous presence of lactose and lactic acid allows micro-components such as calcium and phosphorus which can be found in abundance in both milk and yogurt to become more widely and immediately available (Haertlle *et al.*, 2002).

Some changes in milk component during yogurt production refer to fermentation and the ingredients added during manufacturing. Induced changes in the fermentation involved the action of fermentative inoculated starter cultures and the

secretion of nutrients and chemicals by microorganisms as well as the presence of microorganisms and their associated enzymes (Gurr, 1987).

The consumption of yogurt continues to follow an upward trend because this dairy product is ready to be consumed, relatively low in fat, rich in nutrients and by virtue of LAB has several functional properties. The demand for functional foods includes yogurt has been strengthened in recent years due to growing awareness among consumers of the link between diet and health (FitzGerald, 2004).

2.12 Bio-yogurt

The commercial value of yogurt increased with the addition of new live cultures and phyto-nutrients for instance bio-yogurt contains live strains of *L. acidophilus* and species of *Bifidobacterium* (known as AB-cultures), in addition to the conventional yogurt organisms, *S. thermophilus* and *L. bulgaricus* (Akın, 2006).

CHAPTER 3: MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Starter culture

The starter culture used in the present studies contains the following mixture of bacteria (1: *Lactobacillus acidophilus LA-5,* 2: *Bifidobacterium spp.,* 3: *Lactobacillus casei LC-01,* 4: *Streptococcus thermophilus Th-4* and 5: *L. delbruckii ssp bulgaricus*), *L. rhamnosus, Bifidobacterium bifidum,B. infantis, B. longum.* (Nn Yogurt Mix, cosway, Malaysia).

3.1.2 Milk

Both pasteurized and powdered full cream milk were purchased from local hypermarket. The pasteurised milks used were at least 7 days prior to the expiry date.

3.2 METHODS

3.2.1 PLANT MATERIALS

Four types of plant materials were used in the present studies. These were 1) *Momordica grosvenori* (fruit), 2) *Psidium guajava* (leaf), 3) *Lycium barbarum* (fruit) and 4) *Garcinia mangostana* fruit. Dried fruits of *M. grosvenori* and *L. barbarum* were purchased from local Chinese medicinal shop. Partially dried *M. grosvenori* was subjected to further drying in the oven (50°C) for 72 hrs. *Psidium guajava* leaves were harvested from a fruit orchard in Port Dickson, Negri Sembilan and these were initially washed clean of visible impurities followed by drying in the oven (50°C) for 72 hrs. The dried *M. grosvenori* fruit and *P. guajava* leaves were ground using coffee grinder to powder form. These were placed in airtight containers and stored at room temperature away from direct sunlight. *L. barbarum* (approximately 30g) was

meshed using mortar and pestle in the presence of double amount of distilled water in g to assist the formation of thick paste. The *L. barbarum* paste was then stored in the refrigerator (4°C) and used within 3 days. Fresh *G. mangostana* fruits were purchased from local fruit market. The fresh white pulp and the soft purple inner-skin were spooned outweighed (g) and subsequently ground using blender in the presence of distilled water in 1g: 1ml ratio.

3.2.2 Preparation of plant water extract.

Plant water extract for optical density (OD) studies (Section 3.2.5) was prepared by the extraction of water from water-herbal mixture. The materials from plants (leaves or fruits) prepared in Section 3.2.1 were mixed in appropriate amount of water to yield plant: water ratio of 6: 100. Plant water extract for co-incubation with milk and starter culture for yogurt making (see Section 3.2.3.2) was prepared for *L. barbarum* (LB), *M. grosvenori* (MG) and *P. guajava* (PG) by separately mixing the dried plant materials (see Section 3.2.1) in distilled water (dH_2O) in a ratio of 2: 10. The mixtures were incubated in water bath (70°C) overnight (18 hrs). A coffee sieve was used to separate coarse materials from the resultant solution. The filtrates were centrifuged (2000 rpm, 15 minutes, 4°C) and the clear supernatant obtained was used as water herbal extract in the making of herbal-yogurt.

3.2.3 YOGURT

3.2.3.1 Starter culture preparation

Pasteurized full cream milk (1 liter) was pre-heated to 41°C. Small volume of pre-heated milk (100ml) was placed into a sterilized beaker and the content from a

sachet of yogurt-mix powder was added. The mixture was stirred thoroughly and more warm milk was added to make up the volume to 1 liter. Incubation was carried out overnight (18 hrs) in a water bath (41°C). The yogurt formed was stored in the refrigerator (4°C) and used as starter culture within 3 days.

3.2.3.2 Yogurt preparation

Herbal-yogurts were prepared as follows: 30ml of plant water extract was added into 540ml of pre-heated full cream milk followed by the addition of 4.2g of full cream milk powder to correct the milk solid content. Starter culture (30g) was then added into the beaker and the contents were thoroughly mixed. Control was prepared essentially in the same manner as for herbal-yogurt except that 30ml of dH_2O was used place of plant water extract. The milk-starter culture-plant water extract mix was aliquoted (100ml) into pre-weighed (correct to 4 decimal places) disposable plastic containers. Fermentation of milk was carried out by placing all containers in the same water bath (41°C). A designated container for each treatment (tracer container) was used to provide samples to track the status of yogurt acidification (see Section 3.2.4). Yogurts were incubated until the pH reached 4.5 upon which were then stored in the refrigerator (4°C) until required for further analysis.

3.2.4 pH measurement and Total Titratable Acid (TA %)

The changes in pH during the fermentation of yogurt was measured every one hour (or 30 minutes when necessary) until the pH reached 4.5. Known volume (1ml) of the incubated milk-starter culture mixture was taken out from the incubator at predetermined periods and placed in a test tube containing 1ml of ice-cold distilled water. The mixture was homogenized and the pH was measured using a digital pH meter (Mettler-Toledo 320) correct to 2 decimal places. The titratable acid (TA) was

determined by titration using NaOH. Samples of yogurt (1ml) taken at the same time for pH determination were placed in Erlenmeyer flask containing 9 ml dH_2O followed by the addition of three drops of 0.1% phenolphthalein which was prepared by dissolving 1g of phenolphthalein in 100ml of ethanol. NaOH (0.1N) was titrated into the solution and the mixture was properly mixed by swirling the flask. The titration was continued until the colour of the solution changed to slightly pink which was stable for at least 15-20 seconds. The amount of acid produced during fermentation was calculated as follows:

$$TTA\% = \text{Dilution factor (10)} \times V\ NaOH \times 0.1\ N \times 0.009 \times 100\ \%$$

where V is the volume of NaOH required to neutralize the acid and 0.009 represent the weight of lactic acid (g) neutralized by 1ml 0.1N NaOH.

3.2.5 OPTICAL DENSITY (OD)

Optical density (OD), measured in a spectrophotometer, can be used as a measure of bacteria mass in a suspension. As visible light passes through a cell suspension the light is scattered. Greater scattering indicates that more bacteria or other material is present. The amount of light scattered can be measured in a spectrophotometer. Typically mid log-phase of bacteria growth is measured by measuring absorbance at 600nm (OD_{600}), but time course measurement of OD may also be used to estimate the rate of microbial growth in a medium suspension (de Oliveira *et al.,* 2010).

3.2.5.1 Preparation of yogurt bacteria suspension

Starter culture (1.0ml) was initially diluted 10X by mixing in 9 ml sterile peptone water buffer. The yogurt bacteria was then cultivated on MRS and M17 medium as described on sections 3.2.6.4 and 3.2.6.5 to separately

cultivate *Lactobacillus spp.* and *S. thermophilus*. respectively. Samples were poured into cuvette and then transferred to spectrophotometer for measuring OD_{600} control sample (0%) was used as blank. OD was recorded every 5 minutes to monitor the growth rate of yogurt bacteria. After 2 nights of incubation, samples from several colonies on MRS agar (*Lactobacillus spp.* Colonies) were placed into MRS broth media and re-incubated at 37°C for 24-48 hours to prepare *Lactobacillus spp.* suspension culture. Samples from several *S. thermophilus* colonies from M17 agar were cultivated in sterile mixture of peptone water buffer and lactose solution prior to incubation at 37°C for 48 hours to prepare *S. thermophilus* suspension culture.

3.2.5.2 Assessment of yogurt bacteria growth in suspension by measuring OD_{600}

The optical density (OD) of increasing microbial mass of yogurt bacteria (*Lactobacillus spp.* and *S. thermophilus*) during incubation at 37°C was measured in the presence of four types of herbs at different concentration (0.75%, 1.5% and 3.0% w/v). Different strengths of plant water extracts (1.5% and 3%) were made by adding bacteria culture, sterile peptone water buffer and 6% herbal solution (see Section 3.2.2) at different ratio. Plant water extracts at different strengths were initially prepared. Plant water extract (6.0%; see Section 3.2.2) was mixed in equal volume of sterile peptone water buffer to yield 3% herbal concentration. This solution was further diluted in peptone water buffer to yield 1.5% solution. Peptone water buffer was used as 0% plant water extract concentration. Bacterial suspension for OD measurement was prepared by mixing known volume of prepared yogurt bacteria (see Section 3.2.5.1) with equal volume of 6.0, 3.0 and 1.5% plant water extracts to yield

bacterial suspensions in the presence of 3.0, 1.5 and 0.75% plant water extracts.

3.2.6 Enumeration of probiotic bacteria (CFU)

Yogurt bacteria were enumerated using spread plate method and pour plate method for *S. thermophillus* and *Lactobacillus* spp. respectively. There are two methods for the culture of LAB bacteria: 1) pour plate method using MRS agar medium to support the growth of *Lactobacillus* spp. under anaerobic condition and 2) Spread plate method using M17 agar medium to support the growth of *S. thermophilus.* Samples of yogurts were diluted to 10^{-4}, 10^{-5} and 10^{-6} in sterile peptone water (see Section 3.2.6.3) and 1.0ml or 0.1ml aliquots of the diluted yogurt were plated on individual MRS or M17 plates respectively. Procedures to prepare M17 and MRS medium are explained in sections 3.2.6.1 and 3.2.6.2 respectively.

3.2.6.1 MRS agar preparation

MRS agar (62g) was properly suspended in 1L distilled water. The mixture was dissolved by mild heating (45°C) with frequent agitation followed by boiling for one minute. The agar was sterilized by autoclaving (121ºC for 15 min) followed by cooling to 45-50ºC prior to pouring (15ml) into Petri dishes.

3.2.6.2 M17 agar preparation

M17 agar is a nutritionally rich medium used for the cultivation and enumeration of fastidious *lactic streptococci*. M17 agar (55g) was resuspended in 1L dH_2O with the assistance of mild heating (45°C) and with frequent agitation. The solution was then boiled for one minute and then sterilized by autoclaving (120°C, 15 min) followed by cooling to 45-50ºC. The molten M17

agar (15ml) was then transferred into Petri dishes. Lactose (10g) was dissolved in 100ml of dH_2O to prepare 10% (w/v) lactose solution and sterilized by autoclaving. The sterilized lactose solution was later added into the M17 medium and the mixture was mixed thoroughly.

3.2.6.3 Preparation of Peptone Water Buffer

Buffered peptone water (20g) was dissolved in 1L dH_2O and mixed thoroughly. The solution was distributed into different 15ml-centrifuged tubes and were then sterilized by autoclaving at 121°C for 15 minutes.

3.2.6.4 Pour plate method using MRS media

The *Lactobacillus* spp. counts were determined as described by Kailasapathy *et al.,* (2008). Samples were initially diluted serially to the desired dilution factor using sterilized peptone water buffer. Diluted yogurt sample (1ml) was initially transferred onto sterile Petri dishes followed by gentle pouring of 15ml of sterile MRS culture. The contents in the Petri dishes were evenly mixed by gently tilting and swirling the dishes. The Petri dishes were left for 10 minutes at room temperature to allow MRS agar to solidify. Parafilm was used for sealing the Petri dishes to prevent the entry of air because *Lactobacillus* spp. are anaerobic bacteria. The Petri dishes were incubated at 37°C for 24-48 hours and the colonies formed were counted and the viable cell count in the sample was expressed as colony forming units per milliliter sample (CFU/ ml) using the following formula:

$$\text{CFU*/ ml} = \frac{\text{Number of colonies} \times \text{dilution factor of sample}}{\text{Volume of culture on plate}}$$

*CFU= colony forming unit

3.2.6.5 Spread plate method using M17 media

The spread plate count method as described by Dave and Shah, (1996) was used. Samples were serially diluted as described in Section 3.2.6.4. Sterilized M17 media was transferred into Petri dishes and the agar was allowed to solidify for 10 minutes. Then 0.1ml serially diluted samples was transferred onto M17 agar surface and was spread using bent glass rod (hockey stick shape) in three different directions. The Petri dishes were then incubated at 37°C for 48 hours and the colonies formed after incubation was expressed as CFU/ ml (See Section 3.2.6.4).

3.2.7 Exopolysaccharide (EPS) isolation

EPS was isolated according to Amatayakul *et al.,* (2005). Yogurt was initially diluted with dH_2O in 1: 1 ratio. Proteins in the diluted samples were precipitated by adding 4ml of 20% (w/v) trichloroacetic acid (TCA) and these were separated by centrifugation (3500g for 30 minutes at 4°C). The supernatant was harvested and the pH was adjusted to 6.80 using 40% (w/v) NaOH, followed by boiling at 100°C for 30 minutes to denature whey proteins. After boiling, the solution was subjected to another centrifugation (3500g, 30 minutes, 4°C) to separate denatured whey proteins. The supernatant was transferred into test tubes and was mixed with equal volume of cold absolute ethanol (99% ethanol) to precipitate the carbohydrate from supernatant. The precipitation was carried out overnight at 4°C. The suspension formed was separated by centrifugation (3500 xg, 30 minutes, 4°C). The carbohydrate pellet was completely dissolved in 10ml of dH_2O or Milli-Q water and resultant suspension was subjected to sonication for 1 hour at room temperature. The suspensions were individually dialyzed at 4°C in dialysis membrane tubes with molecular weight cut-off 13,000 Da against tap water over 2 weeks period. Water was changed once in two days. The EPS

concentration was quantified by using the phenol-sulfuric method of Dubois *et al.* (1956) and was expressed as glucose equivalent.

3.2.7.1 Phenol-sulfuric method

The phenol-sulfuric acid assay was based on the absorbance of 490nm of a colored aromatic complex formed between phenol and the carbohydrate which determined reducing and non-reducing sugars and carbohydrate. This assay was used to prepare the glucose standard curve and the unknown amount carbohydrate present in the isolated EPS was determined by comparison with a calibration curve using a spectrophotometer.

Glucose (1.0g) was dissolved in 10ml of dH_2O to produce 100mg/ml stock solutions which were the aliquoted (500µl) and stored (-20°C) for later use. Thawed stock solution (10µl) was further diluted by mixing in 990µl dH_2O to yield glucose concentration of 1mg/ml. Known volumes from this solution were transferred into different test tubes and diluted so that the following final glucose concentrations were obtained: 0 (blank), 10, 20, 30, 40, 50, 80 and 100µg/ml. Each glucose standard (1.0ml) solution was thoroughly mixed with 500µl of 4% (v/v) phenol solution. Concentrated sulfuric acid (98%, 2.5ml) was carefully added drop by drop using Pasteur pipette into glucose-phenol solution and the mixture was then thoroughly vortexed. The glucose-phenol-sulfuric acid mixtures were allowed to cool down to room temperature and the absorbance at 490nm was read after 15 minutes.

3.2.8 Syneresis

Syneresis was carried out using siphon method as described by Amatayakul, Sherkat, and Shah (2006). A cup of yogurt (prepared in Section 3.2.3.2) was weighed correct to 4 decimal places and the container was then positioned at an angle of 45° for 2 hours at 5°C. The whey accumulated was removed from the yogurt surface by using a syringe and the cups were then re-weighed. Syneresis was reported in terms of the percentage of whey lost using the following formula:

$$\text{Syneresis (\%)} = (\text{whey lost} / \text{sample weight}) \times 100\%$$

3.2.9 WATER HOLDING CAPACITY (WHC)

Water hold capacity was performed as described by Minto, Michael, Randall and Karen (2010). The making of yogurt was essentially as described in section 3.2.3.2 but aliquotes of 30ml were directly fermented into pre-weighed 50ml centrifuge tubes ($Wt_{empty\ tubes}$; Oak Ridge Centrifuge Tubes). The tubes were weighed (Wt_{sample}) and subsequently placed in the refrigerator (4°C; when the pH of yogurt in the tracer container (section 3.2.3.2) has reached 4.5 and these were centrifuged (9500 rpm, 40 minutes, 10°C) the next day. Separated supernatant was discarded and tubes were reweighed (Wt_{pellet}). The weight of the pellet was determined by weight differences. Water holding capacity was reported in terms of percentage of pellet weight by using the following formula:

$$\text{WHC (\%)} = [\ (Wt_{pellet} - Wt_{empty\ tubes}\ )/\ (Wt_{sample} - Wt_{empty\ tubes})\ ] \times 100\%$$

3.2.10 TOTAL SOLIDS

The total solid was determined as described by Hooi *et al.* (2004). Approximately 3g of yogurt samples were placed in pre-weighed aluminum pans ($Wt_{empty\ pan}$) and these were weighed ($Wt_{before\ drying}$) and subsequently placed into

atmospheric oven at 100°C for 5 hours drying. The dried samples were cooled down to room temperature in a desiccator containing cobalt (II) chloride anhydrous. The aluminum pans containing dried yogurt samples were re-weighed ($Wt_{after\ drying}$) and the total solid was reported in terms of percentage of yogurt solids using the following formula:

Total solids (%) = [($Wt_{after\ drying}$ - $Wt_{empty\ pan}$)/ ($Wt_{before\ drying}$ – $Wt_{empty\ pan}$)] x100%

3.2.11 STATISTICAL ANALYSIS

The experiments were carried out in three different batches of yogurt (n=3). All assays were performed in triplicates and the results were expressed as mean ± S.E.M (standard mean error) values of the 3 batches. The statistical analysis was performed using one way analysis of variance (ANOVA, SPSS 19.0), followed by Duncan's post hoc test for mean comparison. The criterion for statistical significance was $p<0.05$.

CHAPTER 4: RESULTS

4.0 RESULTS

4.1 Effects of *M. grosvenori*-, *P. guajava*-, *L. barbarum*- and *G.mangostana*- yogurts on changes of pH and TTA in yogurt

Typical changes in pH and TTA in yogurt during the first 4 hours of yogurts incubation are shown in Figures 4.1 and 4.2 respectively.

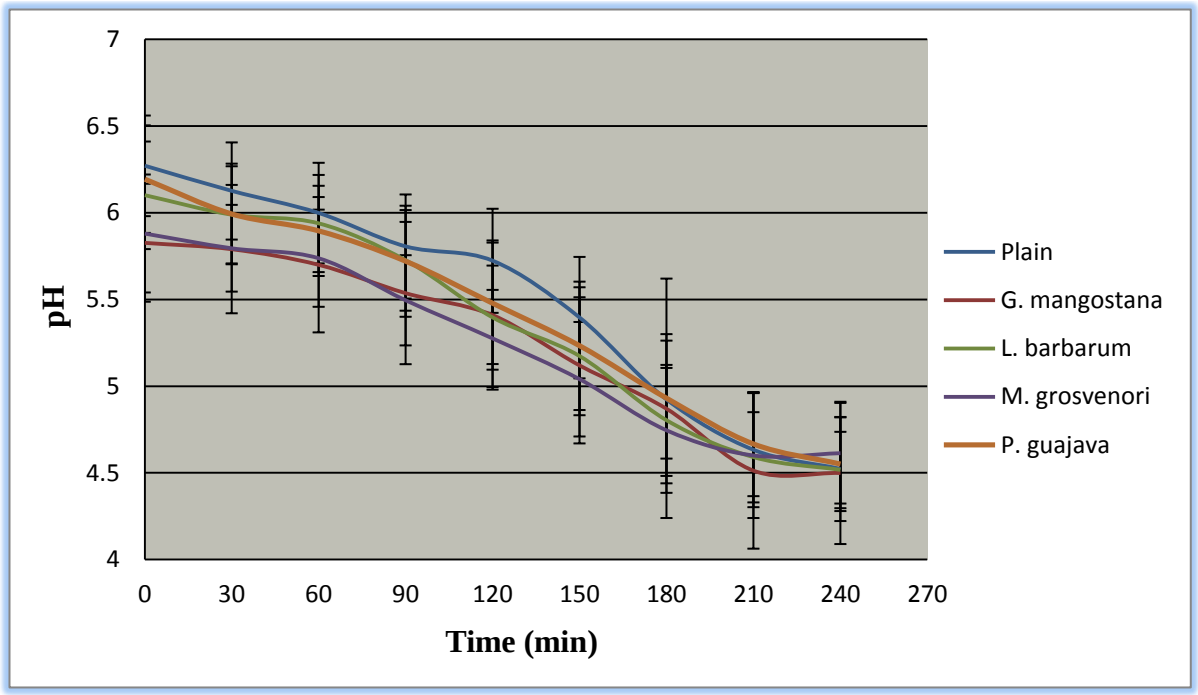


Figure 4.1: pH changes in yogurts during the first 4 hours of yogurt fermentation at 41°C.

The initial pH of milk ranged 5.83-6.27 as a result of mixing with starter culture and plant/ fruit extract. The fermentation of milk for all treatments underwent similar pH changes during the first 120 minutes after which *L. barbarum*-, *M. grosvenori*-, *P. guajava*- and plain- yogurts experienced faster fermentation (approximately -0.33 pH/hr) than *G. mangostana*- yogurts (-0.205 pH/hr). Time taken to reach pH=4.5 for *L. barbarum*-, *M. grosvenori*-, *P. guajava*-, *G. magostana*- and plain- yogurts was 240 min.

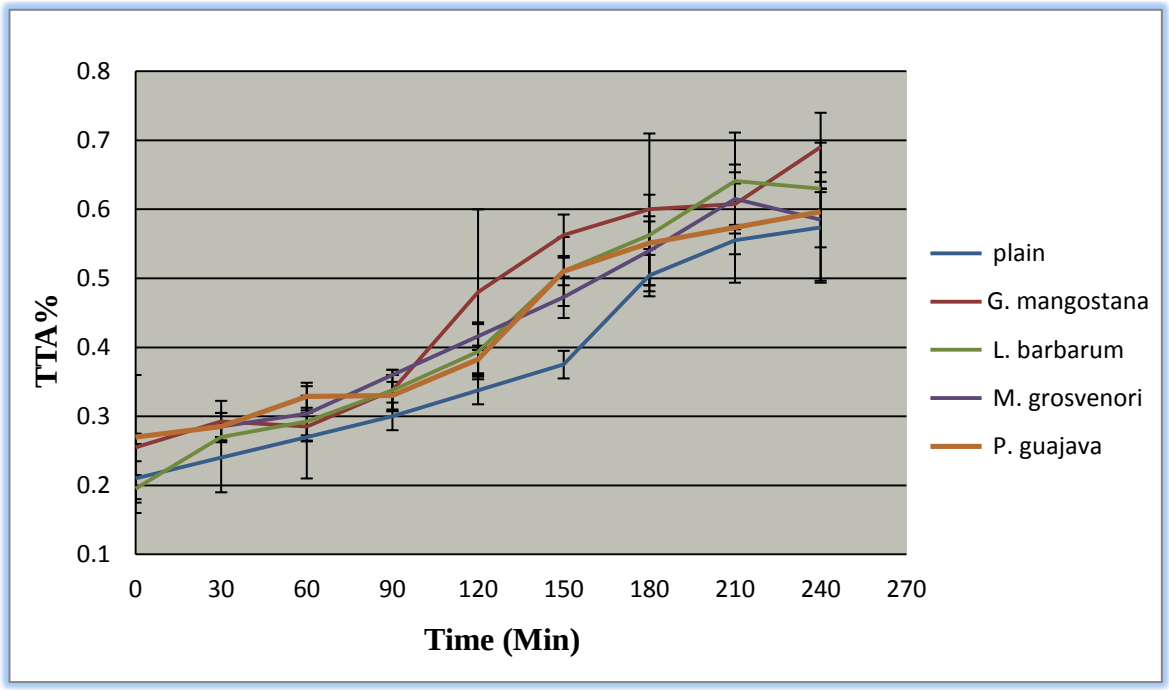


Figure 4.2: TA% changes in yogurts during the first 4 hours of fermentation at 41°C.

The TA of milk-starter culture-plant/ fruit extract ranged 0.19-0.27% whereas the TA values ranged 0.57 – 0.69% at the end of yogurt fermentation (pH = 4.5). The acidification during the first 90 min of fermentation was similar for plain and all herbal yogurts showed slow increasing in TA, after which log phase were reached for plain-, *P. guajava*-, *M. grosvenori*- and *L. barbarum*-yogurts at t = 90-210 min, followed by *G. mangostana*-yogurt at t= 90-180 min. Plateau phase were reached after 210 min incubation in Plain-, *P. guajaya*-, *M. grosvenori*- and *L. barbarum*- yogurts. On the other hand *G. mangostana*- yogurt showed plateau at t= 180-210 min and sudden increasing in acidification at t=210-240. On average *G. mangostana*- and *L. barbarum*-yogurts experienced faster acidification during fermentation (approximately 0.11 TTA/ hr) whereas the acidification rate for *M. grosvenori, P. guajava*- and plain- yogurts was approximately 0.08 TTA/ hr.

4.2 Growth of bacteria in medium suspension

The growth of bacteria as measured by the increase in turbidity of MRS growth medium containing *Lactobacillus* spp. and peptone buffer containing *S. thermophillus* are shown in Figures 4.3 to 4.6 and Figures 4.7 to 4.10 respectively.

4.2.1 Effects of plant water extract on growth of *Lactobacillus* spp. in MRS growth medium

The growth of *Lactobacillus* spp. in the presence of plant water extracts increase with incubation time and generally began to plateau after about 20-25 minutes. The presence of plant water extracts at 0%, 0.75% and 1.5% had similar effects on growth of *Lactobacillus* spp. whereas *L. barbarum* water extract at 3.0% increased growth of *Lactobacillus* spp. around 3 fold higher ($OD_{plateau}$= 0.113) compared to control ($OD_{plateau}$= 0.043, Figure 4.3). The growth of bacteria also began to plateau at much later time (i.e. after 30 mins) at 3.0% *L. barbarum* than that for control ($t_{plateau}$= 20 minutes; 0% *L. barbarum*).

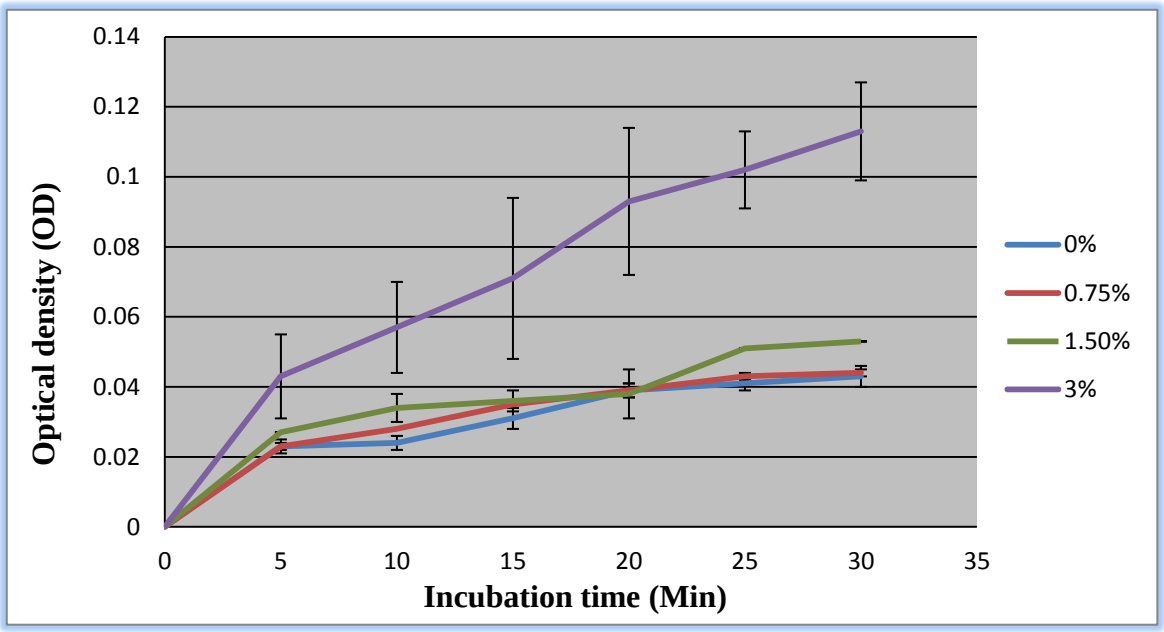


Figure 4.3: Effects of different concentration of *L. barbarum* on the changes in optical density (OD) of *Lactobacillus* spp. growing in MRS broth.

The growth of *Lactobacillus* spp. in the presence of plant water extracts increase with incubation time and generally began to plateau after 25 minutes. When *P. guajava* was added at 0.75% it stimulated the growth of *Lactobacillus* spp. (OD at plateau = 0.025, Figure 4.4). The stimulatory effects on bacterial growth was enhanced by 1.5% *P. guajava* inclusion (OD at plateau=0.053 and $t_{plateau}$= 25 mins). The addition of higher amount of *P.guajava* water extract (3.0%) also increased bacterial growth further (OD=0.124) but plateau was reached at much later time (i.e. after 30 mins).

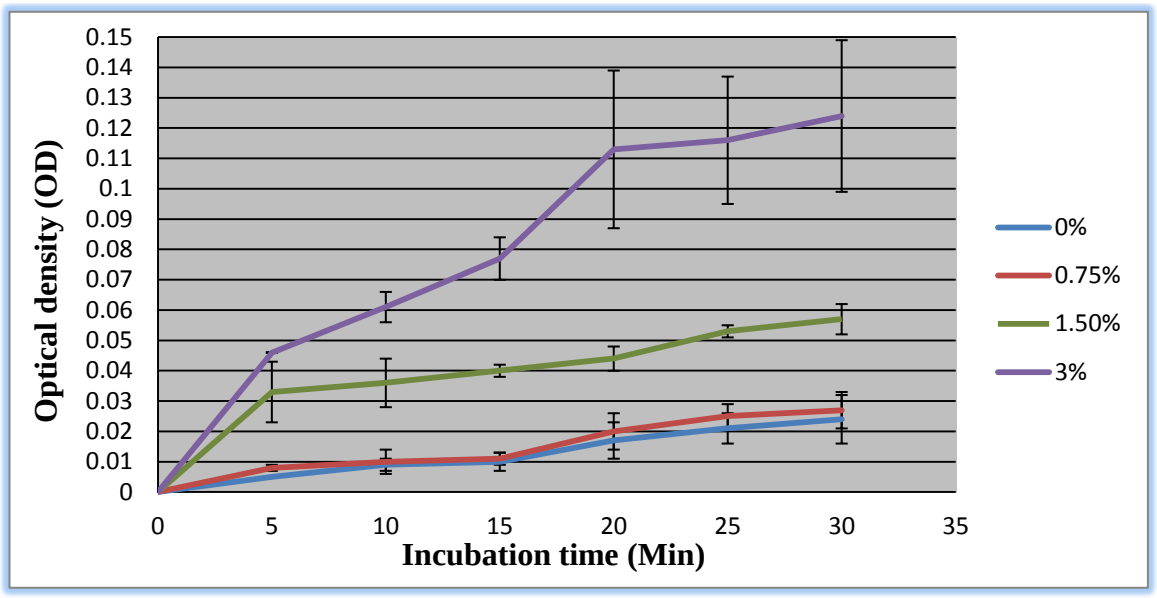


Figure 4.4: Effects of different concentration of *P. guajava* on the changes in optical density (OD) of *Lactobacillus* spp. growing in MRS broth.

M. grosvenori water extract at 0.75% and 1.5% had little effect in stimulating the growth of *Lactobacillus* spp. ($OD_{plateau}$ = 0.07 and 0.10 respectively compared to control $OD_{plateau}$ = 0.06, Figure 4.5). *M. grosvenori* water extract at 3% increased bacterial growth 2 fold higher ($OD_{plateau}$ = 0.22).

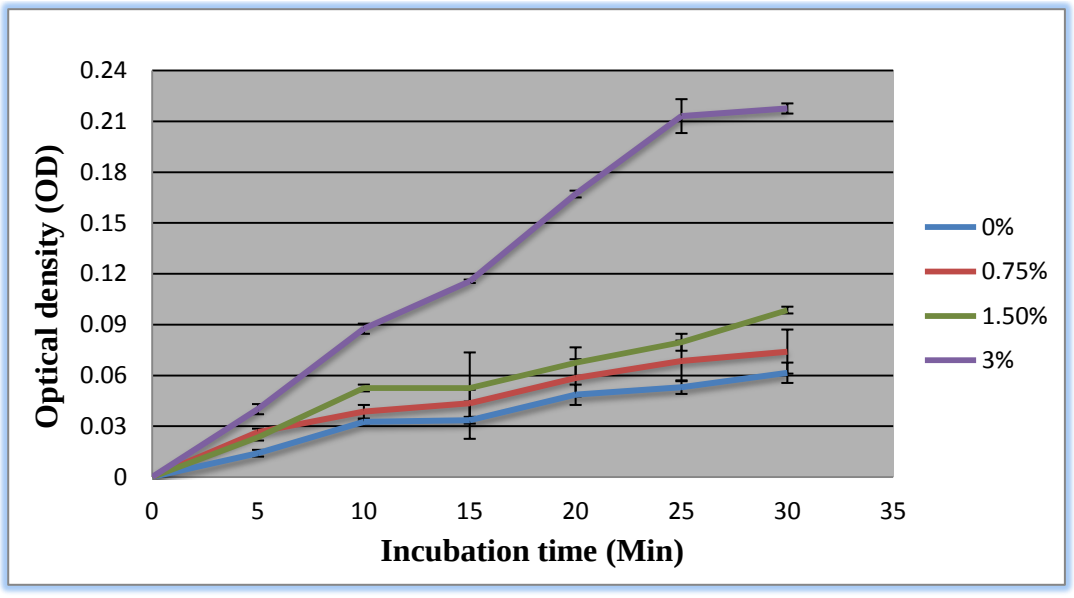


Figure 4.5: Effects of different concentration of *M. grosvenori* on the changes in optical density (OD) of *Lactobacillus* spp. growing in MRS broth.

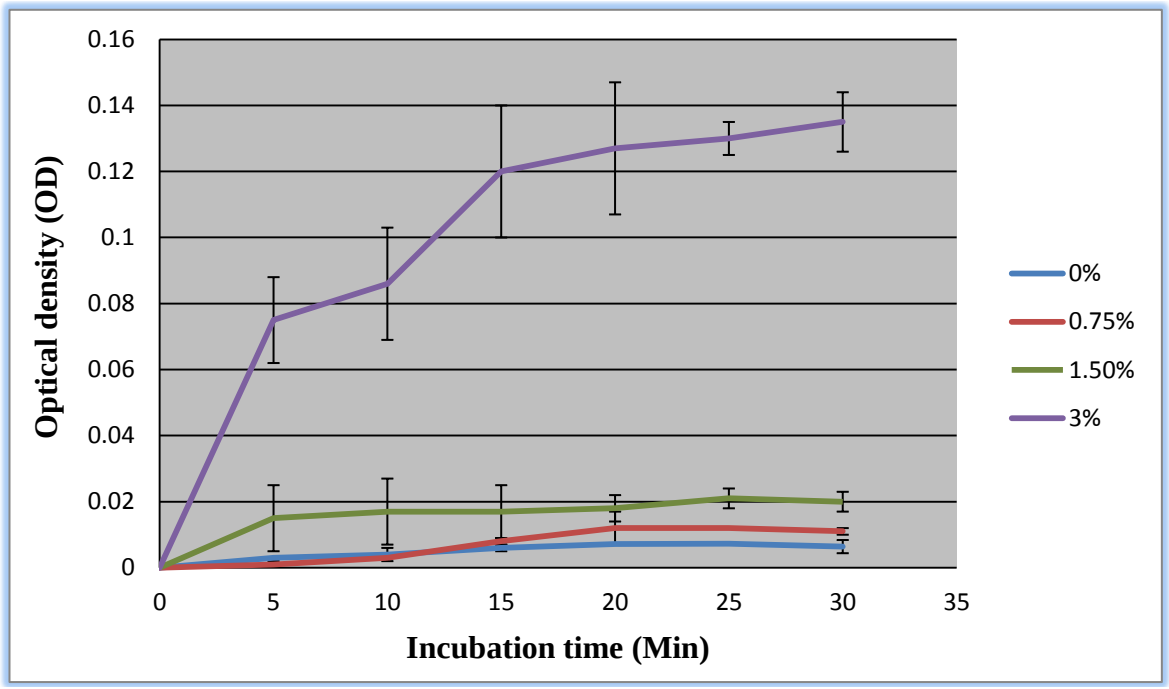


Figure 4.6: Effects of different concentration of *G. mangostana* on the changes in optical density (OD) of *Lactobacillus* spp. growing in MRS broth.

G. mangostana water extract stimulated *Lactobacillus* spp. growth in the same manner as *M. grosvenori.* Inclusion of *G. mangostana* water extract at 0.75% had no effect on bacterial growth compared to control ($OD_{plateau}$ = 0.01, Figure 4.6). The addition of *G. mangostana* 1.5% increased the growth of *Lactobacillus* spp. by about 100% ($OD_{plateau}$ = 0.02). The inclusion of *G. mangostana* water extract at 3.0% increased bacterial growth 14 fold higher ($OD_{plateau}$ = 0.14) compared to control.

4.2.2 Effects of plant water extract on *S. thermophillus* growth in peptone buffer

The growth of *S. thermophillus* in the absence of plant water extracts increase with incubation time but plateau was not reached by the end of incubation (t=30 mins). There was a dose response effects of plant water extracts for all plant studied on the growth of *S. thermophilus.* Inclusion of *L. barbarum* at 0.75% and 1.5% stimulated the growth of *S. thermophilus* ($OD_{plateau}$ = 0.04 and 0.05 respectively compared to control $OD_{plateau}$ = 0.017 at the 30^{th} minutes of incubation, Figure 4.7). The inclusion of *L. barbarum* water extract at 3% did not only increase *S. thermophilus* growth by 3 fold

higher compared to control ($OD_{plateau}$= 0.058, Figure 4.7) but also resulted in plateau in bacterial growth at t=20 mins.

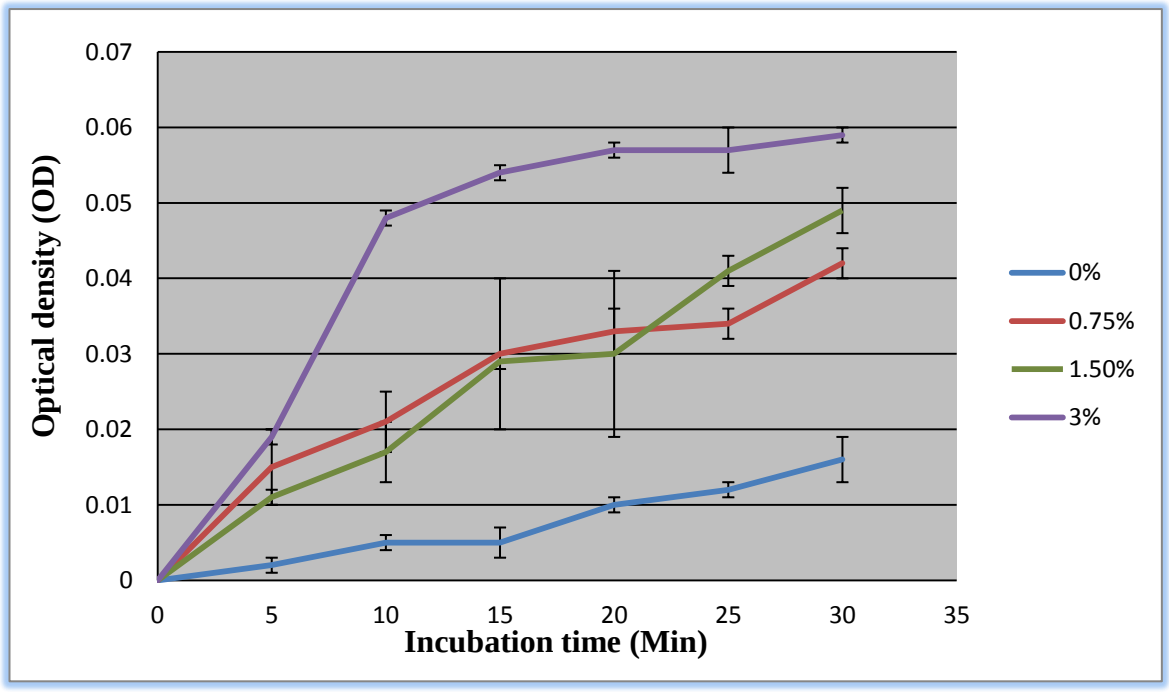


Figure 4.7: Effects of different concentration of *L. barbarum* on the changes in optical density (OD) of *S. thermophilus* growing in peptone buffer.

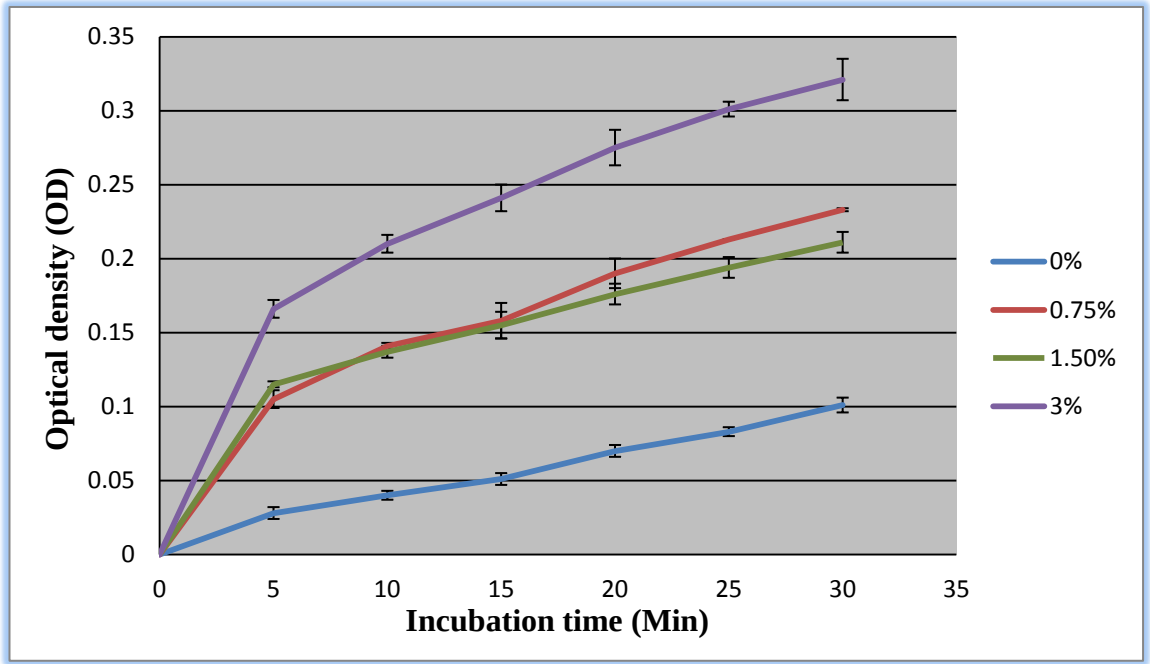


Figure 4.8: Effects of different concentration of *P. guajava* water extract on the changes in optical density (OD) of *S. thermophilus* growing in peptone buffer.

P. guajava water extract at 0.75% and 1.5% stimulated the growth of *S. thermophilus* to similar extent ($OD_{plateau}$ = 0.24 and 0.21 respectively compared to control $OD_{plateau}$ = 0.10, Figure 4.8) after 30 min of incubation. Increasing the water extract to 3.0% resulted in the highest OD achieved ($OD_{plateau}$ = 0.32) by the end of incubation.

M. grosvenori water extract at 0.75% and 1.5% water extract inclusion stimulated the growth of *S. thermophilus* to similar extent ($OD_{plateau}$= 0.111 and 0.115 respectively compared to control $OD_{plateau}$= 0.093, Figure 4.9). The 3.0% water extract tested also had little effect in stimulating the growth of *S. thermophillus* but during the first 15 min, after which showed significant stimulation in *S. thermophillus* growth ($OD_{plateau}$= 0.130, Figure 4.9).

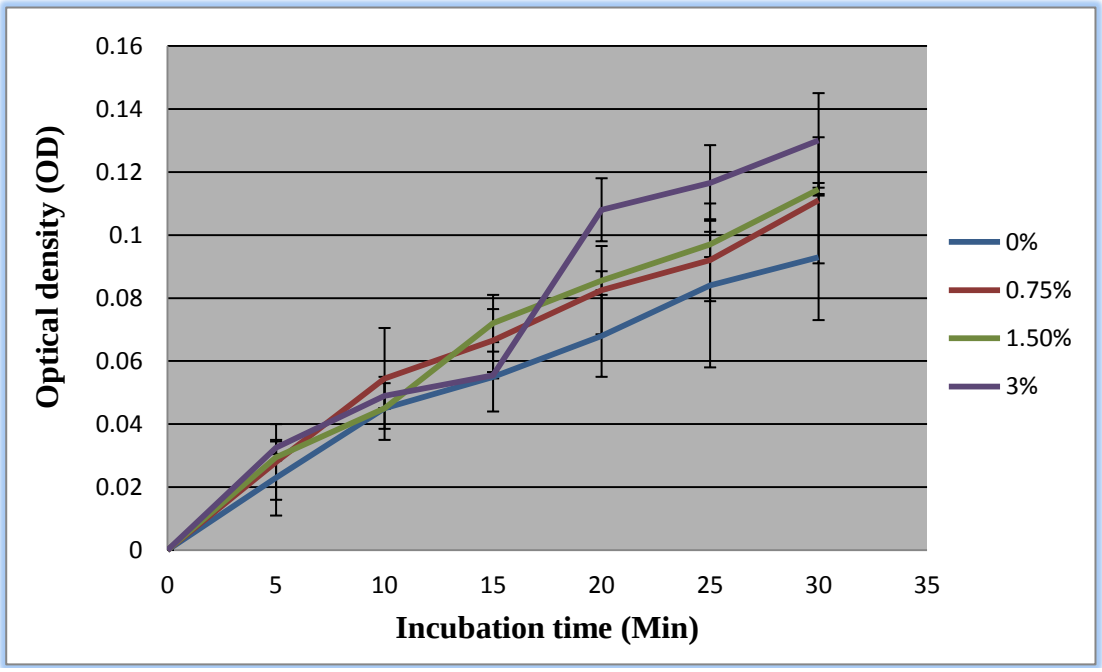


Figure 4.9: Effects of different concentration of *M. grosvenori* on the changes in optical density (OD) of *S. thermophillus* growing in peptone buffer.

G. mangostana water extract stimulated *S. thermophilus* growth (Figure 4.10) in the same manner as *P. guajava.* Inclusion of *G. mangostana* water extract at 0.75% had a little effect of stimulating bacterial growth ($OD_{plateau}$ = 0.02) compared to control ($OD_{plateau}$ = 0.01). The addition of *G. mangostana* at 1.5% increased the growth of to $OD_{plateau}$ = 0.056. The inclusion of *G. mangostana* water extract at 3.0% increased *S. thermophilus* growth 10 fold higher ($OD_{plateau}$ = 0.10) compared to control.

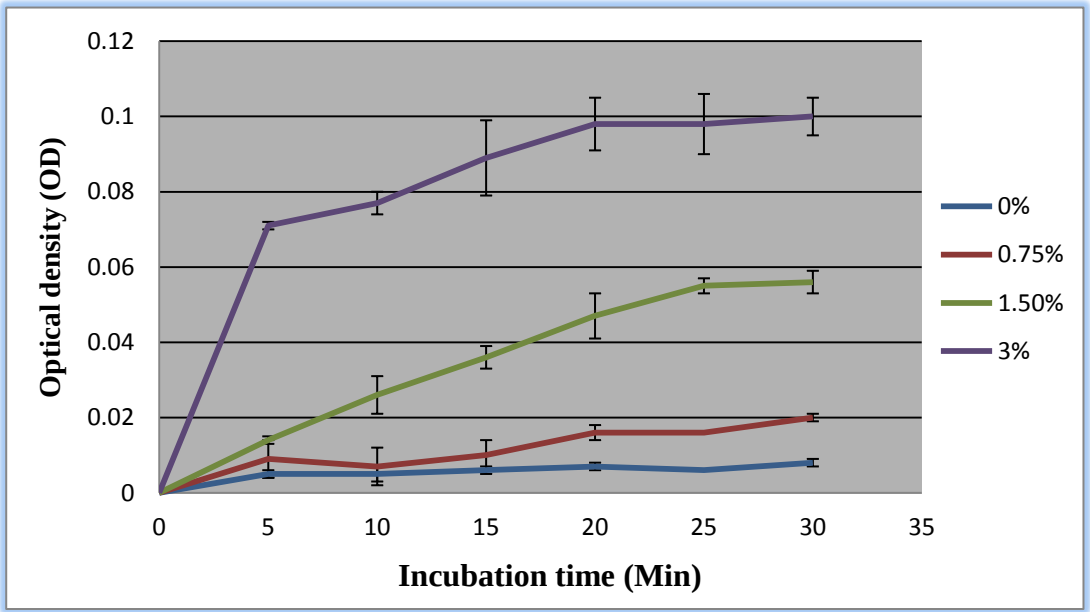


Figure 4.10: Effects of different concentration of *G. mangostana* on the changes in optical density (OD) of *S. thermophilus* growing in peptone buffer.

4.3 Comparison of *Lactobacillus* spp. and *S. thermophilus* density at 3% plant water extract

The ratios of the effects of plant water extracts at 3.0% on OD of *Lactobacillus* spp. in relation to that of their respective controls with incubation time are presented in Figure 4.11. Both *G. mangostana* and *P. guajava* showed high OD ratios at t = 5 mins with a period of low OD ratios at t = 10-30 mins. *M. grosvenori* and *L. barbarum* on the other hand showed consistent lower effects on *Lactobacillus* spp. growth with incubation time.

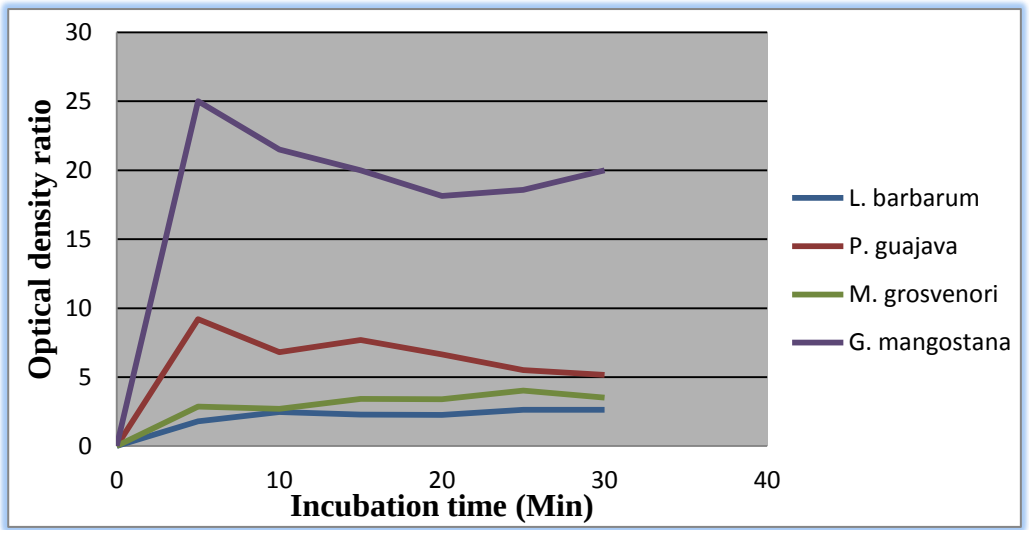


Figure 4.11: Changes in the optical density (OD) ratio with time for *Lactobacillus* spp. grown in 3% plant water extracts in relation to their respective control.

The OD ratios of the effects of 3.0% plant water extracts on *S. thermophillus* in relation to that of their respective controls with incubation time are presented in Figure 4.12. *G. mangostana* had highest OD ratio for *S. thermophilus* growth and this occurred at t = 10 and 25 mins of incubation (figure 4.12). *P. guajava* and *L. barbarum* had maximal OD ratio at t = 5 and t = 10 mins respectively but the ratio decreased with incubation time. *M. grosvenori* water extract showed the lowest OD ratio but it showed consistent increase in OD ratio with incubation time which reached its plateau at t = 20 mins.

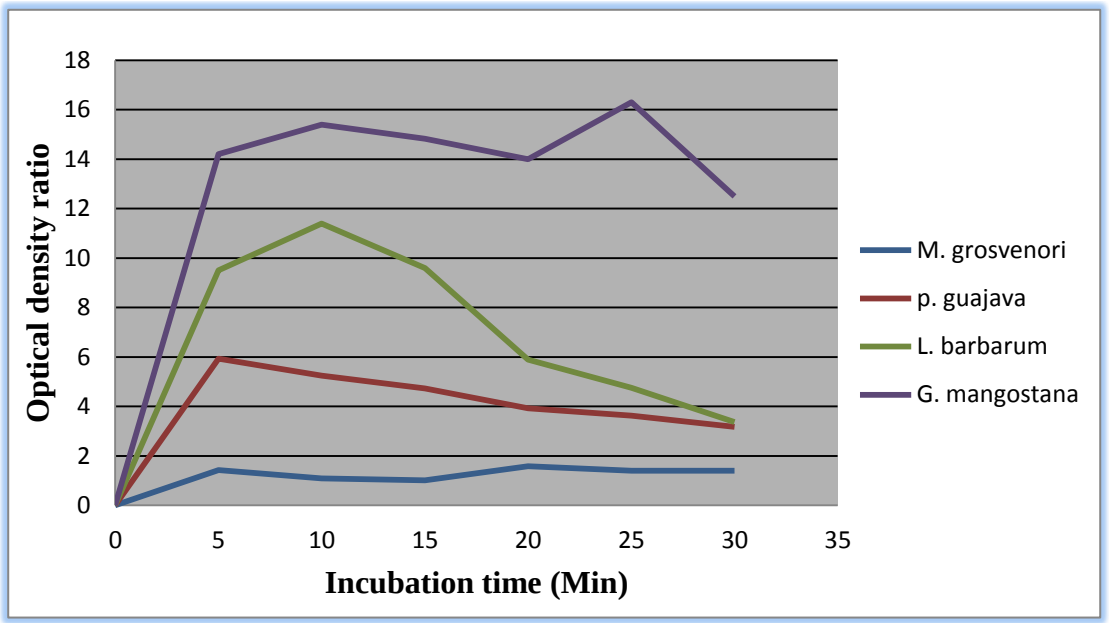


Figure 4.12: Changes in the density (OD) ratio with time for *S. thermophilus* grown in 3% plant water extracts in relation to their respective control.

4.4 Enumeration of yogurt bacteria in yogurts

Yogurts were diluted (See section 3.2.6) and subjected to clonal plating (section 3.2.6.4-5) when the pH of yogurt during fermentation reached 4.5. *S. thermophilus* (615 ± 92 × 10^6 cells/ml; $p<0.05$) was higher than *Lactobacillus* spp. (1.01 ± 0.25 ×10^6 cells/ml) in plain- yogurt (see Table 4.1). The addition of plant water extract increased the growth of both yogurt bacteria in *L.barbarum-, P. guajava- and M. Grosvenori-* yogurts with the counts for *Lactobacillus* spp. being lower ($p<0.05$) than *S. thermophilus*. There was a reduction in *Lactobacillus* spp. And *S. thermophilus* counts (0.69 ± 0.43 × 10^6 cells/ml and 610 ± 14 ×10^6 cells/ml respectively; $p>0.05$) due to the presence of *G. mangostana*-water extract compared to control.

Table 4.1: Colony forming unit (CFU) of *Lactobacillus* spp. and *S. thermophilus* from plain- and herbal- yogurts.

Yogurt samples	**CFU (10^6 cells/ml)**	
	Lactobacillus spp.	*S. thermophilus*
Plain	1.01 ± 0.25	615 ± 92
M. grosvenori	1.23 ± 0.09	805 ± 21
P. guajava	1.32 ± 0.03	1045 ± 49
L. barbarum	1.52 ± 0.16	785 ± 261
G. mangostana	0.69 ± 0.43	610 ± 14

4.5 Exopolysaccharide content in yogurts

Table 4.2: Purified exoplysaccharide from plain- and herbal-yogurts.

Samples	**EPS concentration µg/ ml**
Plain	132.0 ± 39.6
M. grosvenori	183.9 ± 18.2
P. guajava	258.7 ± 22.0
L. barbarum	181.4 ± 57.0
G. mangostana	113.1 ± 56.2

The EPS in plain yogurt was 132± 39.6 µg/ml (see Table 4.2). The addition of plant water extracts increased EPS production in *M. grosvenori*- ($p>0.05$), *P. guajava*- ($p<0.05$) and *L. barbarum*- ($p>0.05$) yogurts but not in *G. mangostana*-yogurt ($p>0.05$). *P. guajava*- and *G. mangostana*- yogurts had produced the higher and lower EPS concentration respectively (EPS= 258.7 and 113.1 µg/ ml respectively).

4.6 Physicochemical properties of plain- and herbal-yogurts

Syneresis in plain-yogurt was 3.23 ± 0.3% (see Table 4.3). The addition of plant water extract tended to result in higher syneresis in *P. guajava*-yogurt ($p < 0.05$) but lower syneresis in *G. mangostana*-yogurts (2.90 ± 0.3%; $p > 0.05$). The WHC and total solid for herbal-yogurts were found to be similar with those in plain-yogurt.

Table 4.3: Syneresis, water holding capacity (WHC) and total solids of plain- and herbal-yogurts.

Samples	Syneresis (%)	WHC (%)	Total Solid (%)
Plain	3.23 ± 0.3	24.1 ± 1. 4	13.5 ± 0.51
M. grosvenori	3.01 ± 1.5	25.0 ± 2.6	13.6 ± 0.36
P. guajava	3.61 ± 0.7	22.8 ± 1.4	13.3 ± 0.08
L. barbarum	3.14 ± 0.7	26.5 ± 0.6	14.6 ± 0.46
G. mangostana	2.90 ± 0.3	28.1 ± 2.0	14.9 ± 0.50

4.7 Effects of plant water extracts on the physicochemical properties and EPS production

The present results showed higher titratable acid (TA) in herbal- yogurt than control can be assigned to the changes in syneresis% and WHC%. There are positive correlation between TA with syneresis% and TA with WHC%. Titratable acids were increased in the presence of plant water extracts which impose reduction in syneresis% and expose increasing in WHC%. The changes in EPS production showed no relation with changes in physicochemical properties in herbal- yogurts.

Table 4.4: Effects of plant water extracts on the physicochemical properties and EPS production.

Samples	EPS	TA at pH=4.5	Syneresis (%)	WHC (%)	Total Solid (%)
Plain	132	0.57	3.23	24.1	13.5
M. grosvenori	184	0.58	3.01	25.0	13.6
P. guajava	259	0.60	3.61	22.8	13.3
L. barbarum	181	0.63	3.14	26.5	14.6
G. mangostana	113	0.69	2.90	28.1	14.9

CHAPTER 5: DISCUSSION

5.0 Discussion

5.1 Effects of *M. grosvenori, P. guajava, L. barbarum* and *G. mangostana* on the acidification of yogurt

Acidification occurs during yogurt fermentation and it is an important process of milk gelation. This process can be easily monitored during fermentation by determining the pH and titratable acid (TA) which unveiled with time principally as a result of lactic acid formation from lactose catabolism by yogurt bacteria (Afonso & Maia, 1999; Beal *et al.,* 1999). The fermentation dynamics for different herbal yogurts may be compared by the differences in the 1) lag phase (slow pH decline), 2) logarithmic phase (rapid pH decrease) and 3) plateau phase (slowing down of acidification). The shape of fermentation curve is affected by many parameters such as the milk base, the type and concentration of supplemented ingredients, starter culture, incubation temperature, and milk heat treatment (Soukoulis *et al.,* 2007).

No obvious lag phase occurred for *P. guajava*-yogurt but it took 4 hours of fermentation to achieve desired yogurt pH of 4.5 (see Figure 4.1). In contrast *M. grosvenori-, L. barbarum*- and plain- yogurts had lag phase of about 1 hr prior to reaching pH 4.5 for a total period of 4 hrs. *G. mangostana*-yogurt also had discernibly longer lag phase (nearly 2 hrs) than plain-yogurt and required a total of 4 hrs to reach pH 4.5 (see Figure 4.1). The modification of pH changes during the entire fermentation period suggests the alteration of metabolic activity or growth rate of bacteria as a result of the addition of plant water extract. This is further elaborated in Section 5.3 which showed different viable yogurt bacteria counts at the end of incubation (i.e. at pH 4.5). Acidification in particular plays important role in the gelation of milk (Girard and

Schafferlequart, 2007) thus the remarkable differences in syneresis and water holding capacity of different herbal-yogurts (see Section 5.5).

In contrast to changes in pH, there were two phases for TA, the first phase is from 0 until 180 minutes which showed higher rate of increase in TA than the second phase which occurred from 180 until 240 minutes (see Figure 4.2). TA reflects total acid regardless of strong (readily dissociable H^+) or weak (organic acids) acids which measures acidic compounds developed as a result of lactic acid bacteria metabolic activity (Kurman and Rasic, 1991). The rates of fermentation in the first phase in general represent both *Lactobacillus* spp. and *S. thermophilus* activities whereas the second reflects *Lactobacillus* spp. activities. This is because the growth of *S. thermophilus* was reduced by the low pH (<5.0) due to the accumulation of acids by the time fermentation entered the second phase (Faergemand *et al.,* 1999).

5.2 Effects of plants water extract on the growth of *Lactobacillus* spp. and *S. thermophilus*

5.2.1 Measurement of yogurt bacteria cell mass by turbidity measurement

Optical density (OD), also known as turbidity or absorbance, can be used as a measure of the concentration of bacteria in a suspension because the amount of light absorbed by a suspension of cells, within limits, is proportional to biomass (Koch, 1981). Although most of the effects of plant water extracts on bacterial growth were dose-dependent for *Lactobacillus* spp. several showed stimulation of bacterial growth when higher amount of plant water extracts were added into the growth medium (Figure 4.3 to 4.6). This was seen for the growth of *S. thermophilus* which increased during 30 minutes incubation at 3.0% for all plant water extract (Figure 4.7 to 4.10). However this method, which compares dose-response effects on cell mass, allows quick appreciation

of the growth of yogurt bacteria in the presence of potentially complex growth modulators present in plant water extracts.

Several factors contributed to the changes in OD during incubation and these include pH and temperature (Aslim *et al.,* 2005), glucose (Kabanova *et al.,* 2009), and metabolic stress factors (lactic acid, acetic acid and hydrogen peroxide; Kong and Davidson, 1980; Miyoshi *et al.,* 2003). Apart from natural plant colourings which directly affect the colorimetric absorbance (Aminot and Rey 2000) the plant water extracts also contribute varying amount of phytochemicals which may directly affect the growth and metabolism of yogurt bacteria. These are further discussed in section 5.2.2.

5.2.2 Measurement of yogurt bacteria cell numbers by indirect viable cell counts

This method involved plating diluted cell suspension on a suitable medium which allow a viable unit to grow and form a colony. This method is related to the viable number of bacteria in the sample, its sensitivity (theoretically, a single cell can be detected), and it allows for inspection and positive identification of the organism counted. In general the bacterial counts for *S. thermophilus* were more than *Lactobacillus* spp. by $10^2 – 10^3$ cells (see section 4.4). The presence of plant extracts in general has small stimulatory effects on both yogurt bacteria ($p>0.05$ for *Lactobacillus* spp., $p<0.05$ for *S. thermophillus*), except for a clear inhibitory effects ($p>0.05$) of *G. mangostana* water extract on *Lactobacillus* spp and *S. thermophillus* (Table 4.1).

Lactic acid bacteria (LAB) play important role in yogurt making which is typically produced by fermenting pasteurized milk using *S. thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*. Milk lactose is converted to lactic acid during fermentation. Thus it is important to inspect the differences in the TA accumulation and cell counts as a result of the presence of different plant water extracts

during yogurt fermentation. It is interesting to note that the TA in different yogurts varied despite the fact that yogurt fermentation was stopped at the same pH 4.5 (Table 5.1).

Table 5.1. The acidification of yogurt and the yogurt bacteria counts in herbal- and plain- yogurts at pH=4.5.

Yogurt treatments	Incubation Time (Min) pH=4.5	TA at pH=4.5	*Lactobacillus* spp. CFU ($x10^6$ cells/ml)	*S. thermophilus* CFU ($x10^6$ cells/ml)
Plain	240	0.47	1.01 ± 0.25	615 ± 92
M. grosvenori	240	0.58	1.23 ± 0.09	805 ± 21
P. guajava	240	0.59	1.32 ± 0.03	1045 ± 49
L. barbarum	240	0.63	1.52 ± 0.16	785 ± 261
G. mangostana	270	0.69	0.69 ± 0.43	610 ± 14

The starter culture used in making the yogurt consist of both homofermentative and heterofermentative lactic acid bacteria and as such they produce acetic and propionic acid as well as lactic acid. These acids are known to cause intracellular acidification and protein denaturation (Huang *et al.,* 1986) because their higher pK_a values and higher percent of undissociated forms made them more active antimicrobial agents than lactic acid.

The relatively longer time taken to reach pH 4.5 for *G. mangostana* may be partially explained by possible negative effects of this fruit extract on *Lactobacillus* spp. and *S. thermophilus* growth in the yogurt. The number of *Lactobacillus* spp. and *S. thermophilus* was lower than other types of yogurts sample (see Table 5.1). This explains the slow rate of pH reduction in *G. mangostana*-yogurt whereas *S. thermophilus* population, known to proteolytically liberate amino acids and peptides

from the milk protein (Miller and Kandler, 1967) and making these materials available for the growth of *Lactobacillus* spp. (Faergemand *et al.,* 1999).

The mechanism responsible for the inhibitory or negative effects of *G. mangostana* on *Lactobacillus* spp. and *S. thermophillus* is not known. However, xanthones, the most important content of *G. mangostana* with antibacterial property are known to inhibit growth of gram positive (e.g. *S. aureus*) and negative (*S. enteritidis*) bacteria (Iinuma *et al.,* 1996; Yasunaka *et al.,* 2005; Chanarat *et al.,* 1997). This inhibitory property of xanthones could have affected *Lactobacillus* spp. And *S. thermophillus* growth and metabolism in similar manner. *L. barbarum* yogurt had higher *Lactobacillus* spp. count than plain-yogurt and this could be attributed to higher saccharide contents in *Lycium barbarum* polysaccharide (LBP). The glucose, galactose, arabinose, rhamnose, mannose and xylose in LBP (Gan *et al.,* 2004, Zhang & Gan *et al.,* 2003 and Huang *et al.,* 1998) was reported capable of stimulating *Lactobacillus* spp. growth in similar manner with oligosaccharides (Du *et al.,* 2011). Findings from the present studies was also in agreement with Lin *et al.* (2004) which showed the addition of 5% *L. barbarum* to soymilk increased *L. paracasei* subsp. *paracasei* growth (Lin, Chiu and Pan, 2004).

5.3 Effects of plant water extracts on EPS production by yogurt bacteria

Exopolysaccharides are produced by lactic acid bacteria (LAB) during fermentation (Cerning, 1990) can act as natural biothickeners. The *in situ* production of EPS have significant role in food industry because they can improve product consistency and texture of fermented food (Ricciardi and Clement, 2000; De Vuyst and Degeest, 1999; De Vuyst *et al.* 2001) and reduce syneresis in yogurt (Majumder and Goyal, 2009; Robitaille *et al.,* 2009). The method used to isolate EPS in the present

studies did not differentiate polysaccharide from those either produced by lactic acid bacteria or those originating from the plants used in making herbal-yogurts. For instance *L. barabrum*-polysaccharide (LBP) isolated by ion exchange consist of 6 types of monosaccharide (Gan *et al.,* 2004) and thus the LBP in *L. barbarum* may have overestimated the quantification of EPS isolated from the *L. barbarum*-yogurt. Thus care has to be taken to interpret the differential effects caused by plant water extracts on LAB production of EPS. All herbal-yogurts except *P. guajava* with highest EPS concentration (258.6 µg/ ml, $p<0.05$) produced EPS in the range of 113.1-183.9 µg/ ml ($p>0.05$) which were not much different from plain-yogurt (132.0 µg/ ml) (Table 4.2).

EPS production is strictly coupled to bacteria growth (Grobben *et al.,* 1995; Grobben *et al.,* 1996) and simple carbohydrate addition can support EPS-production by *S. thermophilus* and *Lactobacillus spp.* (Grobben *et al.,* 1997; Aslim and Yuksekdag, 2008). *G. mangostana*-yogurt contained the least amount of EPS and this was despite the fact that *G. mangostana* contains relatively high amount of simple carbohydrate for microbial metabolism (Teh *et al.,* 2009). Thus, it is highly likely that the phytochemicals present in the added *G. mangostana* may have contributed to a reduced EPS content in *G. mangostana*- yogurt. One possible explaination is the direct effects of *G. mangostana* phytochemicals on the growth of yogurt bacteria during yogurt fermentation. It was previously reported that EPS production by *Lactobacillus* spp. can be higher than by *S. thermophilus* (Aslim *et al.*, 2005). The lower bacterial count for *Lactobacillus* spp. and *S. thermophillus* in the presence of *G. mangostana* water extracts may collectively contribute to the lowered EPS production by *G. mangostana*-yogurt (113.1 µg/ ml) compared to by control (132.0 µg/ ml; see Table 5.1). The present studies illustrate a potentially natural way of manipulating the production of EPS by the addition of plant water extracts. The effects of plant water extracts on yogurt may not be exclusively evaluated based on EPS production alone because a good and acceptable

yogurt need also have excellent physicochemical properties, particularly the firmness and texture which are largely determined by to a large extent the syneresis and water holding capacity of yogurt (Lee and Lucey, 2006).

5.4 Effects of plant extracts on physicochemical properties of yogurt

Three physicochemical properties, namely i.e. syneresis (S), water holding capacity (WHC) and total solids (TS) were measured because they play major role in determining firmness and texture of yogurt (Lee and Lucey 2004; Lee and Lucey, 2010). Syneresis of yogurt in the presence of plant water extracts was either reduced (*G. mangostana*, *L. barbarum* and *M. grosvenori*) or increased (*P. guajava*) compared to plain-yogurt (Table 4.3 and 4.4). Syneresis is the separation of the liquid phase in gel caused by the weakening of the yogurt gel and reduced yogurt quality (Singh, 2009). The acidification and decreasing pH are suggested to increase the formation of protein network and gel formation which collectively decrease syneresis (Alting *et al,* 2003). Therefore prominent changes to the physicochemical properties of yogurt as the result of the addition of plant water extracts must be considered in relation to changes in the acidification of yogurt.

The addition of plant water extracts introduced phytochemicals and simple and complex carbohydrates that may directly influenced yogurt physicochemical properties. Phenolic compounds, the most abundant phytochemicals in plants had no effects in syneresis (Harbourne *et al.*, 2011) and thus may not also be causing the differences in syneresis in herbal yogurts. Sucrose (3.8-11.3%) on the other hand can reduce syneresis up to 10 times in yogurt (Braga and Cunha 2005) principally by acting as substrate for energy production and thus the accumulation of pH reducing organic acids. The potentially sweet property brought about by *G. mangostana* and *M. grosvenori* is not

expected to be responsible for the syneresis-reducing effects seen in the herbal-yogurts. This is because the sugar in *G. mangostana* (17% sugar w/v; Verheij, 1992), by calculation contribute only 1.02 % sugar (sucrose, glucose and fructose) to the yogurt mixture, whereas *M.grosvenori* despite the sweetness properties of mogroside V (300x sweeter than sucrose) has low caloric value (Takemoto *et al.,* 1983 and Matsumoto *et al.*, 1990) and is expected not to be catabolyzed by yogurt bacteria to form organic acids (Suzuki *et al,* 2005) responsible for reduction in pH.

Water holding capacity (WHC) measures the amount of water absorbed in the protein structure (Parnell-Clunies *et al.* 1986). The mobility of water molecules in yogurt, as reflected in WHC values, can affect yield, sensory evaluation, stability (in physical terms) and texture. In fact WHC is an essential quality parameter such that the viscosity can be increased, gel-structures can be created or the physical stability can be lengthened by changing the WHC (Mao *et al.*, 2001). Thus changes in WHC as a result of functional additive such as plant water extracts may modify the properties of yogurt in a predetermined manner. In the present studies plant water extracts caused increased (*G. mangostana*- 28.1 %, *M. grosvenori* 25.0% and *L. barbarum*- yogurts 26.5%) or reduced (*P. guajava*- yogurt 22.8%) WHC compared to control yogurt (24.1%) (Table 4.3 and 4.4). Phenolic compounds, at the very low concentration used in the present studies (typically <26 ug galic acid equivalent/ml,; Amirdivani and Baba, 2011) also have no significant effect on water holding capacity although it is important to take note that at much higher amount they may slightly decrease water mobility in yogurt which cause increase WHC (Harbourne *et al,.* 2011). *β*-glucan composite a type of polysaccharide in plants, had no influence on gel firmness and WHC whereas the addition of *β*-glucan increased viscosity values in yogurt (Sahan *et al.*, 2008). Thus *L. barbarum* polysaccharide may not be causing the increase in WHC.

The total solids (TS) in many commercial yogurt ranged 14-15% (Tamime and Robinson, 1999). The addition of plant water extracts has no effect for *P. guajava*- (13.3%) and *M. grosvenori*- (13.6%) yogurts with slightly higher TS (~15%) were recorded in *G. mangostana*- and *L. barbarum*-yogurts (14.9-14.6%, respectively; $p<0.05$) compared to control (13.5%) (Table 4.3 and 4.4). Since the differences in TS were not significantly different ($p<0.05$), TS is considered not affected by the addition of plant water extracts. Thus potential differences to the physicochemical properties of herbal-yogurts are contributed by either the syneresis or WHC or both, especially in the light of previous reports whereby reduced whey separation of set yogurt occurred only when the total solids were increased (Harwalkar and Kalab, 1986; Jaros *et al*., 2002; Amatayakul *et al.*, 2006; Mahdian and Tehrani, 2007).

Attempts were made to compare the relationship between changes in fermentation products and two important factors which determine the firmness and texture of yogurt i.e. syneresis and WHC (see Table 5.2).

Table 5.2. Relationship between acidification and EPS content on syneresis and water holding capacity of yogurt.

	Syneresis	WHC
EPS	$y = 0.008x + 3.035$ $R^2 = 0.019$	$y = -0.062x + 26.35$; $R^2 = 0.018$
Acidity (TA)	$y = -1.520x + 4.050$ $R^2 = 0.672$	$y= 12.00x + 18.40$; $R^2 = 0.727$
EPS vs Acidity	$y = -0.010x + 0.750$ $R^2 = 0.101$	

The correlation with syneresis and WHC were very weak with EPS (r^2 = 0.019 and 0.018 respectively), in contrast to when comparisons were made with the acidification of yogurt (i.e. TAr^2 = 0.672 and 0.727 respectively). This suggests strongly that the modification yogurt acidification had occurred via the modulation of growth (Section 5.2.2) and metabolism of yogurt bacteria by different components of phytochemicals in the added plant water extracts.

CONCLUSION

The addition of plant water extracts (*L. barbarum, P. guajava, M. grosvenori* and *G. mangostana*) has altered yogurt fermentation. This is reflected in the higher titratable acidity (i.e. byproducts of fermentation) for all herbal-yogurts than in control yogurt. All plant water extracts stimulated *Lactobacillus* spp. and *S. thermophilus* growth except *G. mangostana* which inhibited *Lactobacillus* spp. and *S. thermophilus* growth. The changed microbial growth can be assigned to the changes in EPS production because all herbal-yogurts, except *G. mangostana*, had higher EPS than in control. However acidification is more important than EPS production in determining the water separation from yogurt.