CHAPTER 1:

INTRODUCTION

1.0 Introduction

Food is essential and indispensable to human survival and development. This is because it provides elements necessary for man to nourish and develop his physical, as well all intellectual activities. The commercial values of foods with immense health benefit appeared in the main stream market in the 1960's. Highlights during this period include the importance of eating right and that processed foods should retain the natural attributes of their ingredients. Information on the link between a series of medical conditions (such as constipation, cardiovascular diseases, obesity and hypertension) and the intake of excessive amount of certain ingredients become prominent in the 1970's (Cruz *et al,* 2007). These conditions were also shown to be the health consequences of bad eating habits and poor diet, which can be readily aggravated by stress inherent to modern life. This leads to the appreciation of the additional health benefits of eating certain food (e.g. functional foods) to ward off factors that may compromise the body physiological functions.

Herbs have the reputation as health-giving and having curative properties. Their effects on the human body can be generally attributed to the biochemical structure-function relationships of the myriad of chemicals that occur naturally in foods (Labuza, 1994). Herbs are consumed widely as an alternative approaches to enhance health in developed countries and many herbs to have profound therapeutic effects on certain diseases (Marles & Farnsworth, 1994). In fact, most drugs in the markets are derived from active compounds isolated from plants. However, herbal

medicine is increasingly preferred to drugs because the side effects associated with these synthetic drugs are less prevalent when medicinal plants are used.

The present study explored the effects of selected herbs i.e. 'Misai Kucing' (*Orthosiphon stamineus*), 'Pegaga" (*Centella asiatica*) and 'Mengkudu' (*Morinda citrifolia*) when incorporated in yogurt. These plants have high intrinsic medicinal uses in the treatment of patients with diabetes. An important underlying principal of the anticipated changes in the yogurt as a result of the addition of these plants is the modulative effects of the herbal bioactive compounds on the metabolism and growth of starter culture and probiotics.

The objectives of the present study are as follows:

1. To study the effect of *O. stamineus, C. asiatica* and *M. citrifolia* water extract on the changes of yogurt fermentation.
2. To evaluate the viability of *Lactobacillus* spp. and *S. thermophilus* bacteria in the herbal-yogurts.
3. To investigate the *in vitro* inhibition of α-amylase and α-glucosidase, the two key intestinal enzymes linked to diabetes.

CHAPTER 2:

LITERATURE REVIEW

2.1 Yogurt

The word yogurt came from the Turkish *Yogen* which gives the meaning of '*thick'*. Yogurt is a well known dairy product manufactured through the fermentation of milk. Yogurt is traditionally manufactured using *Streptococcus thermophilus* and *L. delbrueckii* ssp. *bulgaricus* as a starter culture. Lactic acid is the main product of the fermentation which enhanced the yogurt texture and flavor. Yogurt is also an important delivery vehicle for friendly bacteria (probiotic organisms) (Shah, 2007) and it is commonly flavored with fruit preserves or other ingredients (Potter & Hotchkiss, 1995) added before or after incubation (Keating & White, 1990). There are many types of yogurt products in the market and these include yogurt mixed with fruits such as strawberry, kiwi, mango and apple.

2.1.1 Health benefits of yogurt

Yogurts or fermented milk products consumption is associated with well being and health benefits (Hughes & Hoover, 1991; Kanbe, 1992; Le, Moulton, Hill, & Kramar, 1986; Mital & Garg, 1992; Nakazawa & Hosono, 1992; Van'tVeer et al., 1989; Yamamoto, Akino, & Takano, 1994). Health benefits that can be obtained from the consumption of yogurt are summarized in the following sections.

a) Anti-diabetic properties

Regular consumption of yogurt was reported to delay the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress in high fructose-induced diabetic rats (Yadav, Shalini, & Sinha, 2007). The slowing down of the onset of these dysfunctions was suggested due to the antioxidative effects in the liver and pancreatic tissues of diabetic animals (Yadav, et al., 2007).

b) Effectiveness against diarrhea

Diarrhea is known as a frequent passing of loose or watery stools. It is usually caused by the disturbances to the natural balance of gut micro flora through infection with pathogenic strains of bacteria (O'Ryan, Prado, & Pickering, 2005). The symptoms of diarrhea are abdominal cramps, abdominal pain, temperature (fever), fatigue, loose (watery stools), bloating and blood in stool (Aranda-Michel & Giannella, 1999).

Certain probiotic strains e.g. *L. acidophilus*, *L. bulgaricus* and *B. longum* are commonly used in the treatment of diarrhea. Rotavirus is one of the most common causes of acute diarrhea in children worldwide. During diarrheal stage of infection the permeability of gut epithelial cells to intact protein is increased and the use of probiotics was found to shorten the duration of rotavirus diarrhea in children (Saavedra, Bauman, Oung, Perman, & Yolken, 1994).

c) Improvement of lactose metabolism

Lactose is a major type of sugar found in milk, milk products and human milk. Lactose consists about up to 8% of the solids in milk and it was not found naturally in any other form of food except from the dairy products. Lactose is a disaccharide composed of monosaccharide glucose and galactose bonded together by glycosidic bond.

Lactose intolerance is caused by the inability of a normal person to digest a significant amount of lactose due to a lack of lactase enzymes production by the small intestine (Casellas, Aparici, Casaus, Rodriguez, & Malagelada, 2010). The function of lactase is to enzymatically cleave the disaccharide sugar into simpler form of sugar i.e. glucose and galactose which are subsequently absorbed into the bloodstream. The failure to digest lactose leads to 'gastric distress' which is caused by the fermentation of lactose in the large intestine that produces hydrogen gas as a byproduct of microbial action (Shah, 1993; Shah, Fedorak, & Jelen, 1992).

Shah (2000) reported that both yogurt and probiotic yogurt are tolerated well by lactose malabsorbers by virtue of the way yogurt is prepared. The culture (i.e., *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) present in yogurt produce substantial quantities of enzyme (*β*-D-galactosidase) that catalyses the hydrolysis of lactose into d-glucose and d-galactose.

d) Antimutagenic properties

Mutagens are agents (physical or environmental) that can induce a genetic mutation or the increase in rate of mutation whereas antimutagens are agents that inhibit the effects of mutagens. The organic acids produced by probiotics were shown using the Ames Salmonella assay to have antimutagenic activities against several mutagens and promutagens (Lankaputhra & Shah, 1998).

e) Protection from carcinogens

Carcinogens are substances that could cause cancer. Lactobacteria has a big potential to bind potential carcinogens and this prevent the host cells from being damaged (Daniel, Roisin, Colette, & Ian, 2005). *L. bulgaricus* can bind with heavy metals and this is associated with anti-tumor properties. Such an action may contribute to the prevention of cancer formation because nitrate can also be a potential carcinogen (Shah, 2007).

Certain strains of *L. acidophilus* and *Bifidobacterium spp.* can decrease the risk of tumor development and this is achieved by a decrease in the levels of enzymes such as β-glucuronidase, azoreductase and nitroreductase responsible for the activation of procarcinogens (Yoon, Benamouzing, Little, Francois-Collange, & Tome, 2000). This was further supported by studies which demonstrated short chain fatty acids largely produced by *L. acidophilus* and *Bifidobacterium*, *L. plantarum* and *L. rhamnosus* are able to inhibit the generation of carcinogen products by reducing enzyme activities (Cenci, Rossi, Throtta, & Caldini, 2002).

f) Inflammatory bowel disease

Inflammatory Bowel Disease (IBD) is a disorder related to the intestinal microflora such as Crohn's disease, ulcerative colitis and pouchitis. The main cause of IBD is abnormal bacteria population in the gut. Probiotics have the potential of effectively reducing the inflammation in inflammatory bowel as shown in studies in rats (enterocolitis) and humans (small bowel bacterial overgrowth in children, pouchitis) (Schultz & Sartor, 2000).

g) *Helicobacter pylori* infection

Helicobacter pylori is a bacterium that cause peptic ulcer disease, chronic gastritis, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma (Adolfsson, Meydani, & Russell, 2004; Personnet, 1993). The S-shaped gram-negative bacterium colonizes the gastric epithelial surface and withstands the stomach's hostile ambience by microaerophilic growth capability and high urease activity (Goodwin & Armstrong, 1990). The treatment of the infection can be obliterated with antibiotics but the use of antibiotics has been associated with side effects and also makes *H. pylori* to be more resistant (Shah, 2007).

Several *in vitro* and animal studies (Aiba, Suzuku, Kabir, Takagi, & Koga, 1998) showed that several strains of *Lactobacillus* are capable to reduce the viability of *H. pylori* and cause less adhesion of the bacteria to human intestine. Examples include *L. johnsonii* La1 and *L. gasseri* OLL2716 which reduced *H. pylori* colonization and inflammation (Felly *et al.,* 2001) and *L. casei* Shirota and *L. acidophilus* which inhibited the growth of *H. pylori* (Cats *et al.,* 2003).

2.2 Probiotic

Probiotic foods are defined as food containing live microorganisms believed to actively enhance health by improving the balance of microflora in the gut (Fuller, 1992). The term ‘‘probiotic’’ refers to cultures of live microorganisms that, when administered to humans or animals (by way of dehydrated cells or fermented foods), benefit the host by improving properties of indigenous microflora (Margoles & Garcia, 2003). The majority probiotics are bacteria with the species of *lactobacillus* and *bifidobacterium* being the most common type of bacteria used. Table 1 shows the list of micro-organism used as probiotics (Anuradha & Rajeshwari, 2005).

Table 1: List of microorganisms used as probiotics.

Bacteria	**Yeast and Moulds**
i. *Lactobacillus: acidophilus, sporogenes, plantarum, rhamnosum, delbrueckii, reuteri, fermentum, lactus, cellobiosus, brevis* ii. *Bifidobacterium: bifidum, infantis, longum, thermophilum, animalis* iii. *Streptococcus: lactis, cremoris, alivarius, intermedius* iv. *Leuconostoc* v. *Pediococcus* vi. *Propionibacterium* vii. *Bacillus* viii. *Enterococcus* ix. *E. faecium*	i. *A.cerevisiae, A. niger, A. oryzae, C. pintolopesii, Sacharomyces boulardii*

Lactic acid and other acids account for over 90% of the organic acids produced by probiotics. The lowering of pH due to lactic acid or acetic acid produced by these bacteria in the gut has bacteriocidal or bacteriostatic effects (Shah, 2007). The benefits of yogurt bacteria (*S. thermophilus* and *L. delbrueckii spp. bulgaricus*) on human health and nutrition (Deeth & Tamime, 1981; IDF, 1984), can be reinforced by the addition of *L. acidophilus* and bifidobacteria (known as probiotic or AB-culture) which are able to tolerate the harsh gastrointestinal effects of acid and bile (Gilliland, 1978; Kanbe, 1992; Lankaputhra & Shah, 1994) and thus improve the microbial balance in the human gastrointestinal tract (GIT) (Schrezenmeir & deVrese, 2001). Probiotics when administered in adequate amounts confer a health benefit on the host (FAO, 2002). Benefits attributed to probiotics include antimutagenic effects, anticarcinogenic properties, improvement in lactose metabolism, reduction in serum cholesterol, and immune system stimulation (Shah, 2007).

The species of the genus *Lactobacillus* with benefits to food and food processing numbered more than 56 (Curry & Crow, 2003). *L. acidophilus* is the most commonly suggested organism for dietary use. *L. acidophilus* can grow as high as at 45^{o}C, but the optimum growth temperature is between 35-40^{o}C. The organisms grow in slightly acidic media at pH 6.4-4.5, with growth ceases to occur when pH of 4.0-3.6 is reached. The acid tolerance of the organisms varies from 0.3% - 1.9% titratable acidity, with the optimum pH at 5.5-6.0 (Curry & Crow, 2003).

L. acidophilus is the most popular bacteria used as a probiotic in food productions. *L. acidophilus* is also known as ‘friendly bacteria’ for human because these bacteria inhibit the proliferation of pathogen bacteria the intestinal tract, vagina

and mouth. *L. acidophilus* is also responsible for producing acetic acid that lowers the natural pH in the intestines which discourages the growth of the other bacteria (Deanne, 1996).

L. acidophilus also utilizes the nutrients that are necessary for unhealthy organisms to survive, thereby depriving them of food. Other health benefits of *L. acidophilus* include boosted immune system (Sander & Klaenhammer, 2001), reduced burden on the liver and reduced levels of serum cholesterol (Ouwehand, Salminen, & Isolauri, 2002). *S. thermophilus* is a gram positive microbe and a facultative anaerobe i.e. organism that can produce ATP by aerobic respiration if oxygen is present. *S. thermophilus* is a lactic acid bacterium that breaks down pyruvate into lactic acid and acetaldehyde. Like *L. acidophilus, S. thermophilus* is also used as a probiotic in producing functional food.

2.3 Medicinal plants

Herbal medicine is an accumulation of therapeutic experiences of generations of practicing physicians on indigenous systems of medicine for over hundreds of years (Kamboj, 2000). In the developing countries, medicinal plants are used traditionally and these plants will continue to play major role in the treatment of various ailments. Herbal medicine is also in increasing demand in the developed world for primary health care because of its efficacy, safety and lesser side effects (Kamboj, 2000).

Bussmann (2002) reported numerous drugs through exploration of ethnopharmacology and traditional medicine have entered the international market. About 25% of the prescription drugs contain active principles derived from the

higher plants (Tiwari & Joshi, 1990). There are more than 800 plant species potentially useful for diabetes mellitus treatment (Perez, Zavala, Perez, & Perez, 1998).

2.3.1 *Orthosiphon stamineus* (Misai Kucing)

O. stamineus, Benth (family *Lamiaceae*) is known locally as 'Misai Kucing'. The plant is used for the treatment of a wide range of diseases in Southeast Asia such as eruptive fever, epilepsy, gallstone, hepatitis, rheumatism, hypertension, syphilis, gonorrhea and renal calculus (Akowuah, Ismail, Norhayati, & Sadikun, 2005). In Malaysia, *O. stamineus* leaves are used to prepare a diuretic tea and reported to be active against kidney and bladder inflammation, gout and diabetes (Wangner, 1982). *O. stamineus* leaf has high antioxidant properties (Chung *et al.*, 1999; Venkatamuru, Patel, & Rao, 1983) and its polyphenol, the most active principle; (Nakasugi & Komai, 1998) has the ability to reduce oxidative stress by inhibiting the formation of lipid peroxidation products in biological systems (Hollman & Katan, 1999).

O. stamineus also contains several chemically active constituents such as terpenoids (diterpenes and triterpenes), polyphenols (lipophilic flavonoids and phenolic acids), and sterols (Tezuka *et al.*, 2000). Sumaryono *et al.*, (1991) reported the identification and quantification of twenty phenolic compounds using HPLC and these include nine lipophilic flavones, two flavonol glycosides, and nine caffeic acid derivatives, such as rosmarinic acid and 2,3-dicaffeoyltartaric acid.

2.3.2 *Centella asiatica* (Pegaga)

Centella asiatica, an ethnomedicinal herbaceous species and originated from India, grows spontaneously in tropical and subtropical countries such as China, Malaysia, Australia, America, South Africa and Madagascar. In Madagascar, the plant is largely used by the local population and is the second medicinal species exported (Pechard, Antona, Aubert, & Badin, 2005). This plant is high in cathechin, rutin and naringin (Zainol, Abdul-Hamid, Yusof, & Muse, 2003) and thus used in porridge for feeding pre-school children in Sri Lanka in combating nutritional deficiencies (Cox, Rajasuriya, Soysa, Gladwin, & Ashworth, 1993).

More specific studies using the extracts of the plant showed *C. asiatica* possess antioxidant activity (Gnanapragasam, Ebenezar, Sathish, Govindaraju, & Devaki, 2004) and antiproliferative effects in tumor cells, improve venous wall alterations in chronic venous hypertension and protect the venous endothelium (Yoshida *et al.,* 2005). *C. asiatica* was also reported to have an anti-rheumatoid effect and wound healing properties (Liu *et al.,* 2008; Lui *et al.,* 2008).

2.3.3 *Morinda citrifolia* (Mengkudu)

M. citrifolia, commonly known as Noni, and locally as ‘Mengkudu’, has a long history of wide use as food and herbal remedies for different disease in tropical regions. The plant is a small evergreen tree. Furusawa & Hirazumi (1994, 1996) reported that the bark, stem, root, leaf and fruit are traditionally as a folk remedy for diabetes, hypertension and cancer and many more.

M. citrifolia is used either in empirical medicine (Chan-Blanco *et al.*, 2006; Mc. Clatchey, 2002; Wang *et al.*, 2002) or as an alternative medicine by virtue of its anti-microbial, anticancer, anti-inflammatory, antioxidant properties (Wang *et al.*, 2002). However, scientific evidence for the benefits of the *M. citrifolia* fruit juice is still limited.

Biological compounds that may be responsible for the medicinal properties of *M. citrifolia* include such as glycosides, polysaccharides, iridoids, alkaloids, lignins, trisaccharide fatty acid esters, antraquinones, scopoletin, morindin, vitamins, and minerals. These compounds are present in *M. citrifolia* fruits, roots, and leaves (Furusawa, Hirazumi, Story, & Jenson, 2003; Hirazumi & Furusawa, 1999; Liu *et al.*, 2001; Sang *et al.*, 2001; Sang *et al.*, 2003; Shotipruk, Kiatsongserm, Pavasant, Goto, & Sasaki, 2004; Su *et al.*, 2005; Wang *et al.*, 2000; Wang *et al.*, 2002). Several classes of compounds have been isolated from *M. citrifolia* include amino acids, anthraquinones, coumarins, fatty acids, flavonoids, iridoids, lignans and polysaccharides (Chan-Blanco *et al.*, 2006). Scopoletin, a coumarin derivative, is one of the representative ingredients in *M. citrifolia*. This compound has potent anti-microbial, antioxidative and anti-inflammatory activities (Deng *et al.*, 2007).

2.4 Diabetes

2.4.1 Introduction

More than 220 million people worldwide (WHO, 2009) and more than 1.2 million people in Malaysia suffer from diabetes (PDM, 2006). These figures are likely to be more than double by 2030 with around 3.2 million deaths occurring every year (i.e. 6 deaths per minute) attributable to complication of diabetes. Almost 80% of diabetes death occurred in low- and middle-income countries where half of diabetes deaths occur in people under the age of 70 years with more than half (i.e. 55%) of the numbers are women (WHO, 2009).

Diabetes is classified into three types i.e. Insulin-dependent Diabetes Mellitus (IDDM) or Type 1 Diabetes (T1D), Noninsulin-dependent Diabetes Mellitus (NIDDM) or Type 2 Diabetes (T2D) and gestational diabetes. Gestational diabetes symptoms are similar to T2D which is most often diagnosed through prenatal screening, rather than reported symptoms (WHO, 2009).

T1D commonly occurs in childhood and adolescence, but it can occur at any age, even late in life. Subject with T1D must administer insulin via injection since insulin cannot be taken orally because the enzymes of the GI tract would digest it (Whitney & Rolfes, 2008).

T2D is the most prevalent form of diabetes and it accounts for 90 to 95% of diabetic cases. The primary defect in T2D is insulin resistance characterized by a reduced sensitivity to insulin stimulation of glucose uptake in muscle, adipose and liver cells (Whitney & Rolfes, 2008).

In Malaysia, patients with T2D make up about 77000 deaths annually due to cardiovascular disease, especially of coronary heart disease and stroke (PDM, 2006). Therefore, the 'epidemic' of T2D is characteristically followed by the 'epidemic' of diabetes-related cardiovascular disease. The serious nature of chronic hyperglycemic is such that the relative risk of coronary death was reported to be higher in individuals with asymptomatic post-prandial glucose rises than in normal glucose tolerant individuals, even after adjustment for other risk factors such as age smoking, blood pressure and cholesterol (Baron, 1998).

T2D is preceded by a long period of asymptomatic hyperglycemia associated with the clustering or cardiovascular risk factor (Wingard & Barrett-Connor, 1995). In this pre-diabetic state, postprandial or post glucose levels are mildly elevated, whereas fasting blood glucose can usually be maintained within the near-normal range. Very often these individuals at risk also have the metabolic syndrome, a multitude of other cardiovascular risk factors, e.g. abdominal obesity, elevated levels of total triglycerides, low levels of high-density lipoprotein (HDL) cholesterol and elevated blood pressure. Thus, these individuals are at a high risk for developing cardiovascular diseases, including stroke (Laakso & Kuusisto, 2007)

Diabetes can damage the heart, blood vessels, eyes, kidneys and nerves (WHO, 2009). People with diabetes have increased chances of getting heart disease and stroke with about 50% of them die of cardiovascular disease (primarily heart disease and stroke).

2.4.2 α-amylase and α-glucosidase inhibition on T2D

T2D is mainly caused by a high digestion rate of refined carbohydrates in the upper part of the small intestine and results in a rapid and high postprandial rise in blood glucose level (Dicarli, Janises, Grunberger, & Ager, 2003). One of therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. Inhibitor of these enzyme delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial glucose rise (Puls, Keup, Krause, Thomas, & Hoffmeister, 1997; Rhabasa-Lhoret & Chiasson, 2004). Examples of such inhibitors which are in clinical use are acarbose, miglitol and voglibose. Inhibitors of α-glucosidase are currently of interest due to their promising therapeutic potential in the treatment of disorders such as diabetes, AIDS, metastatic cancer and lysosomal storage diseases (Melo, Gomes, & Carvalho, 2006).

CHAPTER 3:

MATERIALS AND METHODS

3.1 Materials

3.1.1 Herbs

a) *Orthosiphon stamineus* (Misai Kucing)

O. stamineus leaves were collected in the late afternoon, when the leaves, white flowers and stems were less turgid and therefore less likely to be damaged. The whole samples were rinsed under running tap water and left to dry in the oven (Memmert) for 3 days at 55^{o}C. These were then ground (Waring Commercial) into powder form and were kept in sealed airtight container and stored away from direct sunlight.

b) *Centella asiatica* (Pegaga)

C. asiatica was purchased from local night market. The whole plants were washed under running tap water and the roots were removed. The leaves and petiole were dried in the oven for 3 days at 55^{o}C before being ground (Waring Commercial) to powder form. The powdered *C. asiatica* was then kept in sealed airtight containers and stored away from direct sunlight.

c) *Morinda citrifolia* (Mengkudu)

Mature pale white but firm *M. citrifolia* fruits were obtained from Noni trees in Klang, Selangor. The fruits were washed with tap water before being cut into pieces of about 20-30g. These were then blended (Waring Commercial) and then transferred into sealed airtight containers and stored at 4^{o}C and used within 3 days.

3.1.2 Yogurt making

a) Milk

Pasteurized Dutch Lady Full cream milk was purchased from local grocery stores. Milk with minimum 7 days from the date of expiry was used to ensure similar milk freshness.

b) Yogurt bacteria and probiotics mixture

Mixture of *L. acidophilus LA-5, Bifidobacterium* Bb-12, *L. casei LC-10,* and *Streptococcus thermophilus Th-4* in sachets (Nn Yogurt Mix, Malaysia) and mixture of *Lactobacillus acidophilus NCFM, L. bulgaricus, L. casei, L. rhamnosus, Bifidobacterium bifidum, B. infantis* and *B. longum* in capsules (Bio-life Sdn. Bhd., Malaysia) were used to prepare starter culture.

c) Chemicals

All chemicals used, unless stated otherwise, were of analytical grade purchased either from Sigma Chemical Aldrich Co., Merck or Oxoid through local suppliers.

3.2 Methods

3.2.1 Water extraction of herbs

a) Extraction method for *O. stamineus* and *C. asiatica*

The water extraction of *O. stamineus* and *C. asiatica* was carried out as described by Tabak *et al.* (1996). The herb water ratio used was 1:10 (10g herb powder in 100ml distilled water). Herbs were placed in separate Scott bottle (200ml) followed by the addition of 100ml distilled water. The bottle was capped and the mixtures were incubated in a water bath (Memmert, 70^{o}C) overnight followed by filtration using filter paper (Whatman No.1). The filtrates were then centrifuged (2000rpm, 15min, at 4^{o}C) and the supernatants harvested were used as herbal water extract in herbal yogurt preparation.

b) Extraction method for *M. citrifolia*

The water extraction of *M. citrifolia* fruits was carried out as described by Maskat & Tan (2011). The blended *M. citrifolia* fruit (see section 3.1.1c) was mixed with water at 1:9 ratio (1 part mashed *M. citrifolia* in 9 parts of distilled water) in a 500ml beaker. The mixture was mixed thoroughly in the beaker followed by heating (80^{o}C) under continuous stirring for approximately 2 hours. The water extract was filtered using tea strainer to remove the seeds and large particles. The filtrate was then used as *M. citrifolia* water extract in herbal yogurt preparation.

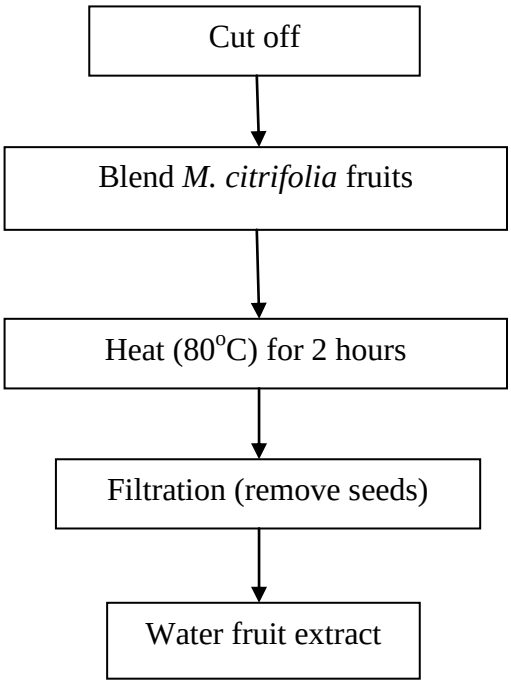

Figure 3.1: *M. citrifolia* fruit water extract procedure.

3.2.2 Yogurt preparation

a) Starter culture preparation

Pasteurized full cream milk (1000 ml) was poured into a sterilized 2L beaker and the content was covered with a sterile aluminum foil. The milk was heated in the water bath (Memmert) until it reached 41^{o}C. The heated milk was mixed (by using sterile glass rod) with a sachet and a capsule of yogurt bacteria starter culture (see section 3.1.2). Incubation was carried out for 24 hours at 41^{o}C and the yogurt formed was stored in a fridge (4^{o}C) and used as starter culture within 7 days.

b) Herbal-yogurt preparation

Herbal-yogurts were prepared by adding herbal water extracts at three different concentrations i.e. 1, 5 and 10% v/v. Starter culture was added at 5% v/v. Pasteurized full cream milk was added to make up the volume to 100%. The mixtures were stirred well using sterile glass rod followed by incubation in the water bath (41^{o}C) until the pH reached to 4.5, which normally took approximately 5-6 hours. Plain-yogurts were prepared essentially in the same manner as described for herbal-yogurt with the exception that distilled water was used in place of herbal water extract. Yogurts formed were stored in the refrigerator (4^{o}C) for predetermined periods.

3.2.3 Effects of herbal extracts on yogurt fermentation

a) Measurement of pH

pH of yogurts was determined by using a digital pH meter (Cyper Scan 510). The pH meter was routinely calibrated at pH 4.0 and 7.0 before and after the pH measurement (Olson & Aryana, 2008). Samples of yogurts (1ml) were thoroughly mixed with 3 ml of dH_2O in a test tube. The changes of pH were monitored on days 0, 7, 14 and 21 of refrigeration.

b) Measurement of titratable acidity (TA)

Yogurt was titrated using 0.1N NaOH to determine titratable acidity (TA). Yogurt samples (1.0ml) were transferred into a beaker (50ml) containing 9ml of dH_2O and a few drops of 0.1% phenolphthalein were added. The mixtures were thoroughly mixed and NaOH was titrated into the solutions drop by drop under continuous stirring by a magnetic stirrer until the color of the solution changed from clear to a constant pink.

The content of lactic acid was calculated as follows:

Percentage of lactic acid = df x V_{NaOH} x 0.009g x 0.1N x 100%

df = dilution factor (in this case = 10)

V_{NaOH} = Volume of NaOH to neutralized lactic acid

0.1 = Normality of NaOH

3.2.4 Preparation of yogurt water extracts

Yogurt was mixed with water in the ratio of 1.0: 0.25 i.e. 10g of yogurt with 2.5ml of dH_2O. The mixture was homogenized (Eppendorf 5804R, 10000rpm, 30 seconds) followed by incubation in water bath (45^oC) for 10 minutes. The homogenate was centrifuged (10000 rpm, 15 min, 4^oC) followed by the neutralization to pH 7.0 by the addition of 0.5M NaOH. The supernatant was centrifuged again (10000 rpm, 15min and 4^oC) to remove residual precipitates. The clear supernatant obtained, now referred to as yogurt water extract, was harvested and stored at -20^oC until required for analysis.

3.2.5 Antioxidative activity by 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical inhibition (DRI) assay

The method of estimating free radical-scavenging activity of the extract of herbal yogurt was adapted from Shetty *et al.* (2007). 60µM DPPH (3 ml) in ethanol was pipetted into 4.5ml cuvette and added with 250µl of neat, or diluted (2 or 4 times diluted in water) herbal yogurt water extract. The decrease in absorbance was monitored at 517 nm until a constant reading was obtained. Negative controls consist of 250µl of dH_2O instead of the herbal yogurt water extract.

The free radical-scavenging activity (FRSA) was calculated by using the following formula:

FRSA (%) = $[(A_c - A_s) / A_c] \times 100$,

A_c: the absorbance of the control

A_s: the absorbance of the tested sample after the reading is constant.

3.2.6 Determination of total phenolic content

The total phenolic content (TPC) in each herbal yogurt was determined using Folin-Ciocalteu reagent method according to Shetty *et al.* (1995). Herbal yogurt water extract (1.0ml) was pipetted into a test tube followed with the addition of 1ml of 95% ethanol and 5ml of dH_2O. After a thorough mixing, 0.5ml of 50% (v/v) Folin-Ciocalteu reagent was added to each sample. After 5 minutes, 1ml of 5% Na_2CO_3 (Merck) was added to the mixture. The solution was allowed to stand at room temperature for 1 hour after which the absorbance at 725nm was measured by a spectrophotometer (Shimadzu UV Mini 1240). Gallic acids in the range of 10-200mg/L were prepared and the total phenolic content determined in the same manner as described for the yogurt water extract. TPC in yogurt water extract was read against garlic acid standard curve and expressed as gallic acid equivalent (GAE; mg/L).

3.2.7 Microbial count

a) Enumeration of *Lactobacillus* spp.

Lactobacillus spp. was cultured on deMan, Rogosa and Sharpe (MRS) agar, the preparation of which was adapted from the manufacturer (Ovoid Product Detail Code: CM0361). MRS agars (62g/l) was suspended in 1 liter of distilled water and boiled to dissolve the medium completely. It was then autoclaved at 121^{o}C for 15 minutes and cooled to 45-50^{o}C. Enumeration of lactobacilli species was carried out

using the pour plate method. A series of prepared decimal dilution (dilution from 10^{-3} to 10^{-7}) of yogurts were used by mixing yogurt sample (1ml) and 9ml of 1.5% sterile buffered peptone. Samples were mixed thoroughly and each mixture was serially repeated (1:10 dilution) 6 times using peptone water (Oxoid, UK) as the diluents. Diluted yogurts (1ml) in triplicate were then placed on empty petri dishes and 15ml of cooled (45^{o}C) autoclaved MRS agar was poured in. The mixtures were mixed thoroughly by swirling the petri dishes. The agar was placed on the flat surface for about 15 minutes to allow the agar to solidify. The lid was sealed by using parafilm and the petri dishes were incubated in an inverted position in the incubator at 37^{o}C for 24 hours followed by colony counting the next day.

b) Enumeration of *S. thermophilus*

M17 agar was used for the selective enumeration of *S. thermophlius* and the agar was prepared according to Oxoid (Code: CM0785). M17 agar powder (48.25g) was rehydrated in 950ml distilled water followed by autoclaving in 121^{o}C for 15 minutes. The agar was cooled down to 50^{o}C and 10% solution (w/v) of lactose monohydrate (50ml) was added and the mixture was mixed thoroughly immediately prior to pouring (15ml) onto petri dishes. The petri dishes were left cool to room temperature to allow the agar to solidify. Diluted yogurt samples (0.1ml) were delivered by sterile pipette onto the surface of the agar. Sterile spreaders were used to spread inoculums evenly over the surface of the agar. The agar plates were incubated (37^{o}C) in inverted position and the colonies formed after 28-48 hours incubation were then counted.

c) Viable cell count calculation

Bacteria in each diluted yogurt sample were enumerated by counting the colony forming units (CFU) on the agar. The bacterial population in each ml of yogurt was then calculated using the following formula:

$$\text{CFU/ml} = \frac{\text{Number of colonies in a plate}}{\text{Inoculum (ml)}} \times (\text{Dilution factor})$$

3.2.8 Sensory evaluation on herbal-yogurts

The sensory evaluation of herbal-yogurt and plain-yogurt was carried out after an overnight storage at 4°C. A panel of 16 people (mean age = 24 years old) assessed six descriptors i.e. visual appearance, body texture, aroma, sweetness, sourness and overall taste using a sensory rating scale of 1-10 (1 for 'extremely dislike' to 10 for 'extremely like') (Larmond, 1987). The panels recognized the yogurts only by codes. Each panel was requested to rinse their mouth by drinking mineral water after assessing each yogurt.

3.2.9 Inhibition of α-glucosidase activity

The assay for α-glucosidase activity was performed according to the method described by Shetty *et al.* (2007). A volume of 500µl of homogenized herbal yogurt water extract and 1000µl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase (Sigma Chemical Co.) solution (1.0 U/ml) were incubated in water bath at 25°C for 10 min. Then, 250µl of 5mM p-nitrophenyl-α-D-glucopyranoside

solution in 0.1M phosphate buffer (pH 6.9) was added to each well. The reaction mixtures were incubated at 25°C for 5 min. Absorbance readings were recorded at 405nm before and after incubation and compared to a control which had 500µl of buffer solution in place of the extract.

The inhibition of α-glucosidase (%) by the yogurt extract was calculated as follows:

$$\% \text{ inhibition} = [(\Delta \text{ control}_{A450} - \Delta \text{ sample extract}_{A450}) / \Delta \text{ control }_{A450}] \times 100\%$$

3.2.10 Inhibition of α-amylase activity

The inhibition of α-amylase by herbal yogurt water extract was assayed according to Shetty *et al.* (2007). Porcine pancreatic α-amylase (EC 3.2.1.1, 0.5mg/ml, Sigma Chemicals Co.) in 0.02M sodium phosphate buffer (500µl, pH 6.9 with 0.006 M sodium chloride) was mixed with 500µl yogurt water extracts in a test tube followed by incubation at 25°C for 10 min. Starch solution (1%, 500µl) in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) was then added to each tube and the reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped by the addition of 1.0 ml of dinitrosalicylic acid (DNSA) color reagent. The test tubes were then incubated in a boiling water bath for 7 minutes followed by the addition of 1ml of tartarate (18.2%). After cooling to room temperature, the reaction mixture was then diluted by adding 10 ml of distilled water prior to absorbance reading at 540 nm (OD). The inhibition of α-amylase (%) yogurt water extract was calculated as follows:

$$\text{Inhibitory activity (\%)} = (OD_{control} - OD_{sample}) / OD_{control} \times 100$$

3.2.11 Calculation of IC_{50} for α-amylase and α-glucosidase inhibition activity

Enzyme inhibition was expressed as the concentration of inhibitory compound that inhibit 50% of α-amylase or α-glucosidase activity (IC_{50}), assuming that the activity of the blank was 100%. To enable the determination of IC_{50}, the inhibition of enzymes at 3 different volume of yogurt extracts (500µl, 250µl and 125µl; with dH_2O used when necessary to make up the volume to 500µl) were measured. IC_{50} values were calculated using ED50plus vol.1 software, developed by Mario H. Vargas, MD (Shetty *et al.,* 2007).

3.2.12 Statistical analysis

All experiments were carried out using three independent batches of yogurts and each sample, unless otherwise stated was analysed in duplicate. Data were subjected to one-way ANOVA by SPSS®, version 17.0. Duncan's multiple range tests was used to compare the means among treatments and significant effect was established by ANOVA at $p \leq 0.05$.

CHAPTER 4

RESULTS

4.1 pH and titratable acidity (TA) of yogurt after fermentation and during refrigerated storage

The pH of yogurts was recorded on day 0, 7, 14 and 21 of refrigerated storage (Figure 4.1). The initial pH of yogurts (day 0; 4.52) reduced ($p<0.05$) to 4.09-4.14 during refrigerated storage. The initial TA of plain-yogurt on day 0 (1.0% lactic acid equivalent) increased to 1.4% ($p<0.05$) after one week but this was later reduced to about 1.3% ($p<0.05$) during extended (day 7, 14 and 21) refrigerated storage.

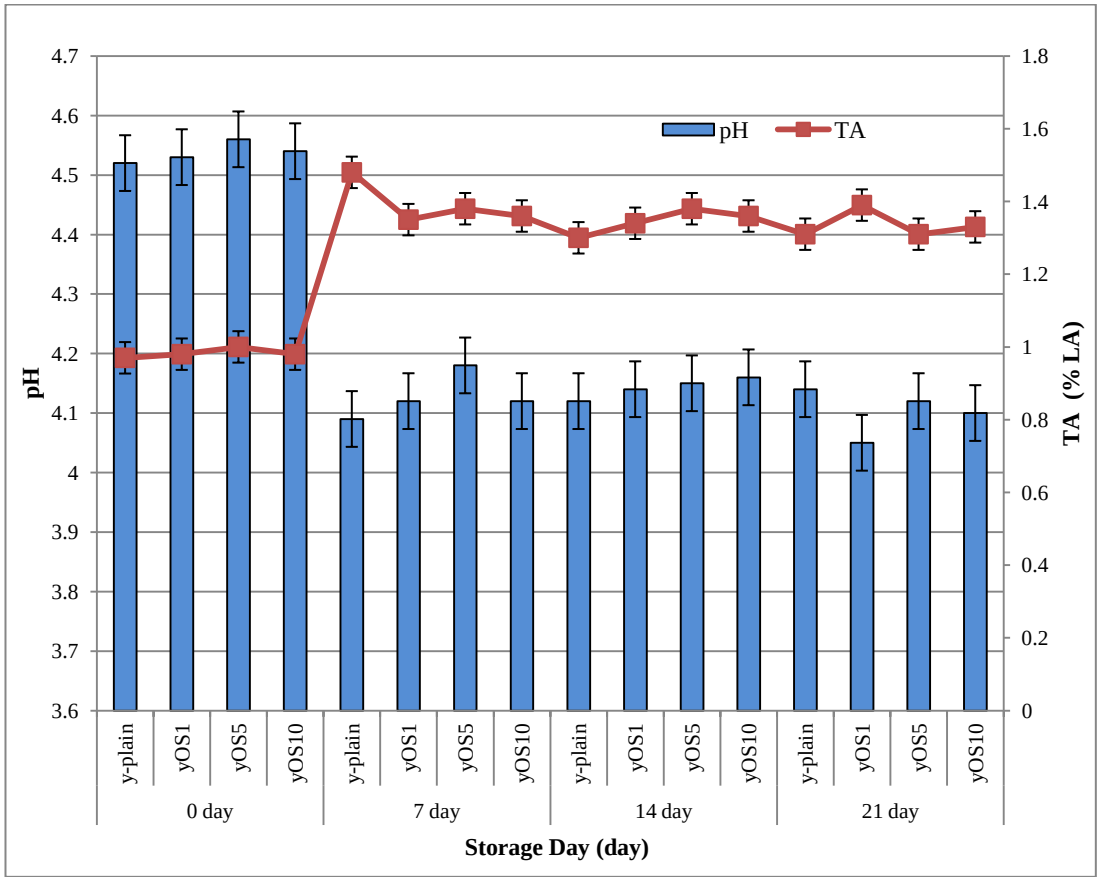

Figure 4.1: Effects of *O. stamineus* on the changes of pH and TA in yogurt at the 0, 7, 14 and 21 days of storage at 4^{o}C.

Table 4.1: The percentage (%) of titratable acidity (TA) and pH of *O. stamineus*-yogurts and plain-yogurt during 21 days of storage (4℃) (*n*=3).

	0 day		**7 day**		**14 day**		**21 day**	
	pH	TA	pH	TA	pH	TA	pH	TA
y plain	4.52±0.02	4.14±0.01	4.09±0.02	1.48±0.01	4.12±0.02	1.30±0.01	4.14±0.01	1.31±0.02
yOS1	4.53±0.08	4.05±0.01	4.12±0.01	1.35±0.00	4.14±0.03	1.34±0.01	4.05±0.01	1.39±0.01
yOS5	4.56±0.03	4.12±0.03	4.18±0.02	1.38±0.00	4.15±0.03	1.38±0.01	4.12±0.03	1.31±0.01
yOS10	4.54±0.05	4.10±0.00	4.12±0.01	1.36±0.01	4.16±0.03	1.36±0.01	4.10±0.00	1.33±0.01

4.1.1 Effects of *O. stamineus* on pH and TA of yogurt

The presence of *O. stamineus* water extract at different concentrations (1%, 5% and 10% for yOS1, yOS5 and yOS10 respectively) showed no differences ($p>0.05$) on pH of the yogurt compared to plain-yogurt (control) during storage (Figure 4.1). The TA of *O. stamineus*-yogurts was lower than plain-yogurt after 7 days of storage (1.35 and 1.48% respectively; $p<0.05$). However the differences seen on day 7 were absent after extended refrigerated storage.

4.1.2 Effects of *C. asiatica* on pH and TA of yogurt

Refrigerated storage reduced ($p<0.05$) pH of all yogurts to between 4.04 and 4.20 (Figure 4.2).

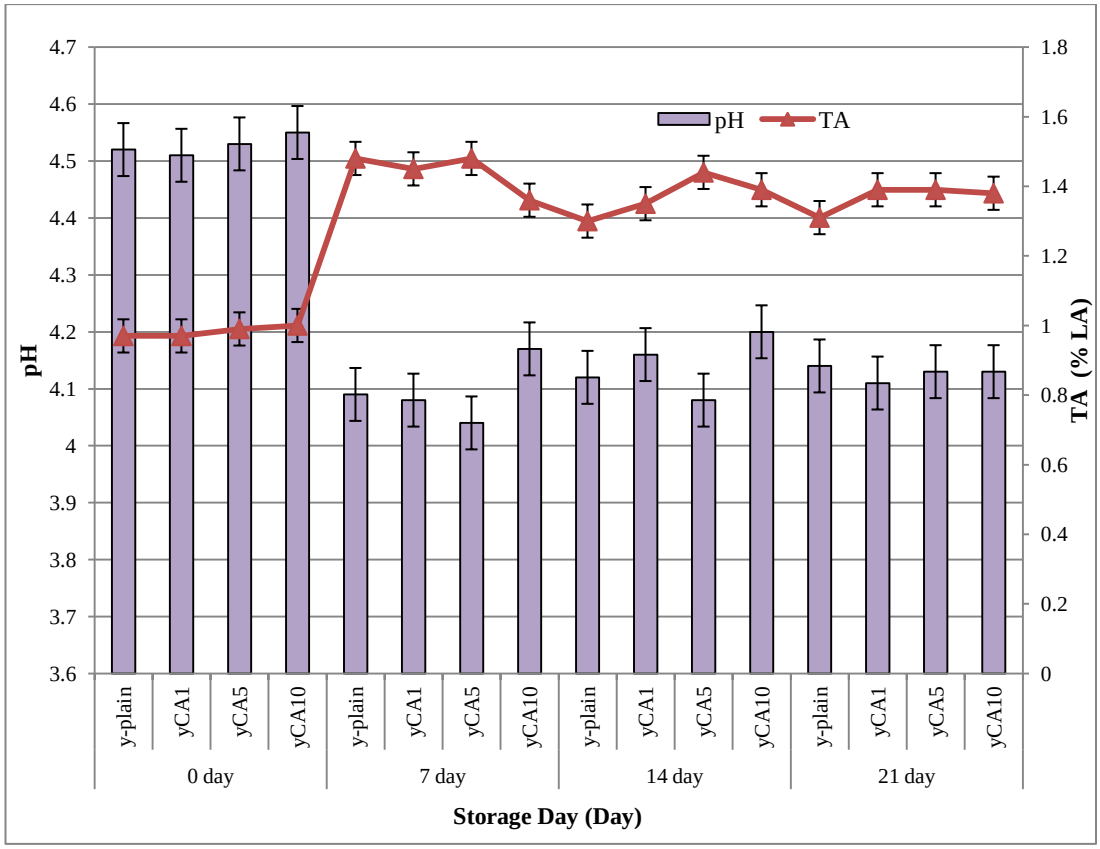

Figure 4.2: Effects of *C. asiatica* on the changes of pH and TA in yogurt at the 0, 7, 14 and 21 days of storage at 4°C.

The initial TA (1.0% LA equivalent) of yogurt increased ($p<0.05$) to between 1.36 and 1.48% during refrigerated storage. No effects of treatments were seen on the differences in pH or TA.

Table 4.2: The percentage (%) of titratable acidity (TA) and pH of *C. asiatica*-yogurts and plain-yogurt during 21 days of storage (4℃) (*n* =3).

	0 day		**7 day**		**14 day**		**21 day**	
	pH	TA	pH	TA	pH	TA	pH	TA
y plain	4.52±0.02	0.97±0.01	4.09±0.02	1.48±0.01	4.12±0.02	1.30±0.01	4.14±0.01	1.31±0.02
yCA1	4.51±0.03	0.97±0.01	4.08±0.01	1.45±0.00	4.16±0.03	1.35±0.01	4.11±0.01	1.39±0.01
yCA5	4.53±0.04	0.99±0.01	4.04±0.01	1.48±0.01	4.08±0.01	1.44±0.01	4.13±0.01	1.39±0.01
yCA10	4.55±0.05	1.00±0.01	4.17±0.01	1.36±0.01	4.20±0.03	1.39±0.01	4.13±0.01	1.38±0.02

4.1.3 Effects of *M. citrifolia* on pH and TA of yogurt

The initial TA (1.0% LA equivalent) on day 0 increased (1.23-1.4% LA equivalent) during refrigerated storage for all yogurt treatments (Figure 4.3).

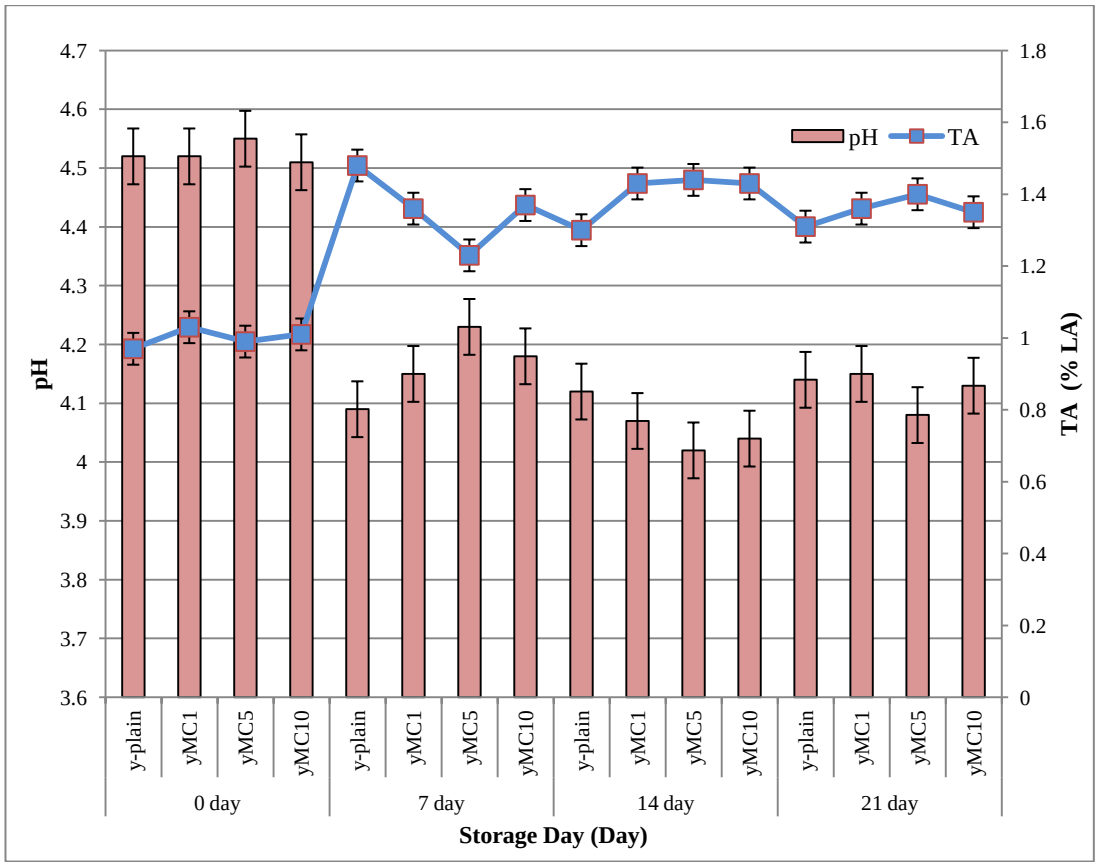

Figure 4.3: Effects of *M. citrifolia* on the changes of pH and TA in yogurt at the 0, 7, 14 and 21 days of storage at 4^{o}C.

pH reduced ($p<0.05$) to between 4.02 and 4.23 during storage. The presence of *M. citrifolia* water extract tended to lower pH of yogurt to the lowest values on day 14 compared to day 7 and 21 and this was reflected in TA of *M. citrifolia*-yogurts being lower ($p<0.05$) and then higher ($p<0.05$) than plain-yogurt on day 7 and 14 respectively.

Table 4.3: The percentage (%) of titratable acidity (TA) and pH of *C. asiatica*-yogurts and plain-yogurt during 21 days of storage (4℃) (*n* =3).

	0 day		**7 day**		**14 day**		**21 day**	
	pH	TA	pH	TA	pH	TA	pH	TA
y plain	4.52±0.02	0.97±0.01	4.09±0.02	1.48±0.01	4.12±0.02	1.30±0.01	4.14±0.01	1.31±0.02
yMC1	4.52±0.04	1.03±0.01	4.15±0.01	1.36±0.03	4.07±0.03	1.43±0.02	4.15±0.02	1.36±0.02
yMC5	4.55±0.02	0.99±0.01	4.23±0.01	1.23±0.01	4.02±0.01	1.44±0.01	4.08±0.02	1.40±0.01
yMC10	4.51±0.05	1.01±0.01	4.18±0.01	1.37±0.02	4.04±0.00	1.43±0.01	4.13±0.03	1.35±0.02

4.2 Total phenolic content (TPC) and antioxidant capacity in yogurt

The TPC of plain-yogurt after fermentation (day 0) was 10.9±0.2µg/ml and this value was gradually increased to 14.9±1.6µg/ml by day 14 of storage (Figure 4.4). Plain-yogurt on day 21 of storage had the lowest TPC (11.1±2.2µg/ml). DPPH inhibition by plain-yogurt on day 0 (18.65±0.49%) and day 7 (22.98±7.78%; $p>0.05$) reduced when storage was extended to 14 and 21 days (4.75±1.26% and 0.36±0.03% respectively).

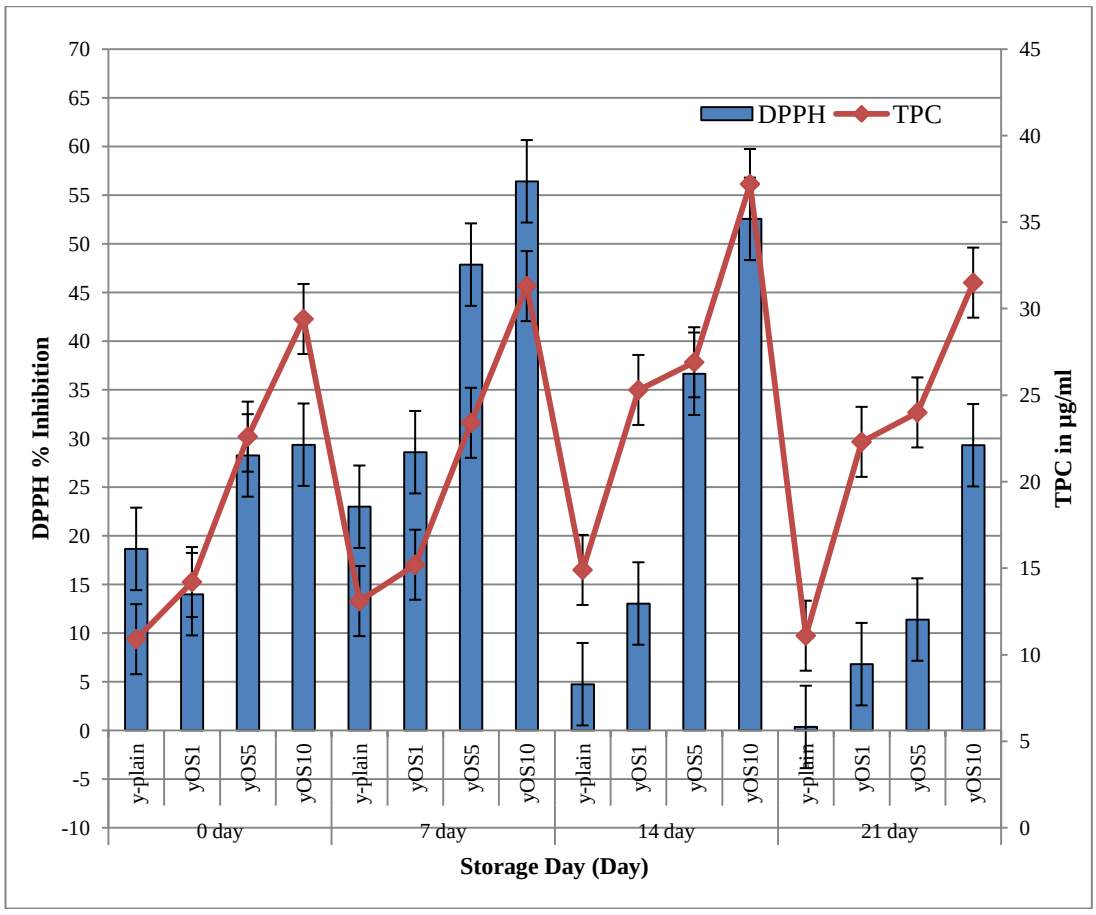

Figure 4.4: Effects of *O. stamineus* on the total phenolic content and antioxidant activity at the 0, 7, 14 and 21 days of storage at 4°C.

Table 4.4: The percentage (%) of DPPH inhibition and total phenolic compound (TPC) in *O. stamineus*-yogurts and plain-yogurt during 21 days of storage (4℃) (n=3).

	0 day		**7 day**		**14 day**		**21 day**	
	DPPH	TPC	DPPH	TPC	DPPH	TPC	DPPH	TPC
y plain	18.65±0.49	10.9±0.2	22.98±7.8	13.1±2.9	4.75±1.26	14.9±1.6	0.36±0.03	11.1±2.2
yOS1	14.00±0.18	14.2±0.9	28.58 ± 6.0	15.2±0.2	13.04±1.10	25.3±1.6	6.81±0.06	22.3±1.7
yOS5	28.25±1.45	22.6±0.4	47.85±4.8	23.4±0.3	36.64±3.90	26.9±1.0	11.39±0.27	24.0±1.0
yOS10	29.35±1.24	29.4±0.4	56.41±4.9	31.3±0.7	52.55±4.10	37.2±2.7	29.30±1.49	31.5±0.7

4.2.1 Effects of *O. stamineus* on total phenolic content and antioxidant capacity in yogurt

The addition of *O. stamineus* increased yogurt TPC values in dose dependent manner (14.2±0.9µg/ml to 15.2±0.2 µg/ml, 22.6±0.4µg/ml to 23.4±0.3 µg/ml, and 29.4±0.4µg/ml to 31.3±0.7µg/ml for yOS1, yOS5 and yOS10 respectively) on day 0 and 7 of refrigerated storage (Figure 4.4). Maximum TPC in *O. stamineus*-yogurt was seen in yOS10 on day 14 (37.2±2.7µg/ml) but this was reduced to 31.5±0.7µg/ml by day 21 of storage.

The DPPH inhibition capacity of yOS1 (14.00± 0.18%) was not different from plain-yogurt on day 0 but higher DPPH inhibition (28.58±6.00%) than plain-yogurt (22.98±7.78%) was recorded on day 7 of storage (Figure 4.4). An increased inclusion of *O. staminues* in yogurt resulted in significantly higher DPPH inhibition than plain-yogurt on day 7 (47.85±4.80% and 56.41± 4.90% for yOS5 and yOS10 respectively). YOS10 showed reduction in DPPH inhibition during extended storage (52.55±4.10% and 29.30± 1.49% on day 14 and 21 respectively) compared to yOS5 (36.64±3.90% and 11.39±0.27% on day 14 and 21 respectively).

4.2.2 Effects of *C. asiatica* on total phenolic content and antioxidant capacity in yogurt

C. asiatica inclusion at 1% had small (14.8±0.3µg/ml) but significant ($p<0.05$) effect on the increase of TPC of yogurt (Figure 4.5). Higher level of *C. asiatica* inclusion (5 and 10%) did not increase TPC tremendously (15.0±0.9 and 16.7±1.8µg/ml respectively). Refrigerated storage increased TPC values with highest increase ($p<0.05$) compared to day 0 yogurt seen on day 14 of storage.

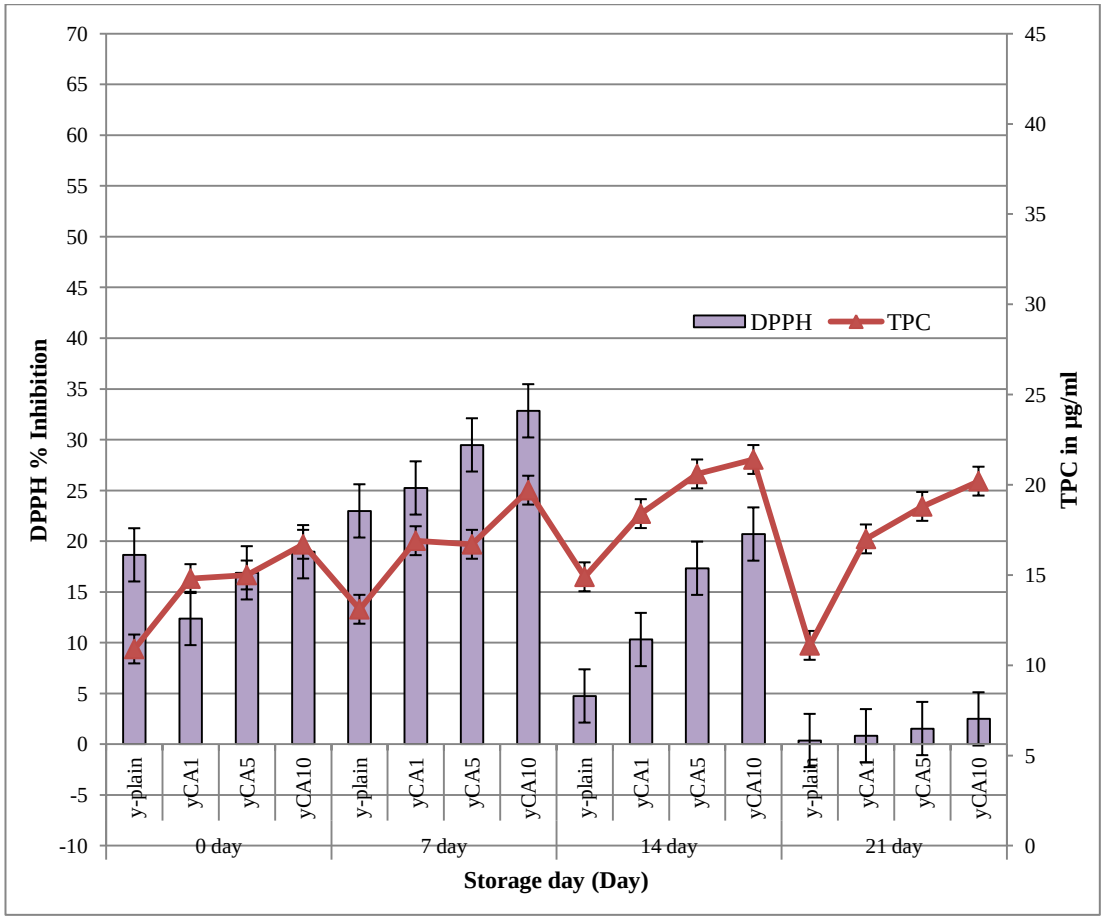

Figure 4.5: Effects of *C. asiatica* on the total phenolic content and antioxidant activity at the 0, 7, 14 and 21 days of storage at 4°C.

The presence of *C. asiatica* did not increase the ability to inhibit DPPH oxidation by fresh (day 0) yogurt. However, refrigeration for 7 days had positive

effect on the enhancement of *C. asiatica*–yogurts to inhibit DPPH oxidation with significant effect ($p<0.05$) seen by yCA1, yCA5 and yCA10 (25.24±0.36%, 29.48±3.49% and 32.84±7.18% respectively) compared to plain-yogurt (22.98±7.78%).

Refrigerated storage for 14 days reduced yogurts inhibition of DPPH oxidation. Increasing *C. asiatica* content in yogurt had no effect to minimize the loss of antioxidant activities of yogurt due to storage. All *C. asiatica*–yogurts on day 14 had higher antioxidant activities than plain-yogurt (4.75±1.26%) with significant effects seen in yCA1, yCA5 and yCA10 (10.31±1.43%, 17.33±1.58% and 20.7±0.41% respectively; $p<0.05$). Antioxidant activities were at the minimum (<3%) for all yogurts refrigerated for 21 days.

Table 4.5: The percentage (%) of DPPH inhibition and total phenolic compound (TPC) in *C. asiatica*-yogurts and plain-yogurt during 21 days of storage (4℃) (n=3).

	0 day		**7 day**		**14 day**		**21 day**	
	DPPH	TPC	DPPH	TPC	DPPH	TPC	DPPH	TPC
y plain	18.65±0.49	10.9±0.2	22.98±7.78	13.1±3.0	4.75±1.26	14.9±1.6	0.36±0.03	11.1±2.2
yCA1	12.38±0.47	14.8±0.3	25.24±0.36	16.9±1.3	10.31±1.43	18.4±2.8	0.83±0.06	17.0±2.1
yCA5	16.88±0.45	15.0±0.9	29.48±3.49	16.7±0.4	17.33±1.58	20.6±1.3	1.54±0.20	18.8±2.1
yCA10	18.96±0.67	16.7±1.8	32.84±7.18	19.7±0.5	20.7±0.41	21.4±1.4	2.49±0.28	20.2±1.5

4.2.3 Effects of *M. citrifolia* on total phenolic content and antioxidant capacity in yogurt

Refrigerated storage tended to increase TPC of yogurt with yMC1 increased ($p<0.05$) from 14.1±1.3µg/ml on day 0 to 16.9±1.3µg/ml on day 14 (Figure 4.6). YMC5 and yMC10 showed an increase ($p<0.05$) of TPC value (from 14.9±1.3 µg/ml to 16.3±0.36µg/ml and from 15.6±0.5µg/ml to 17.0±0.28µg/ml respectively) on day 0 to day 7.

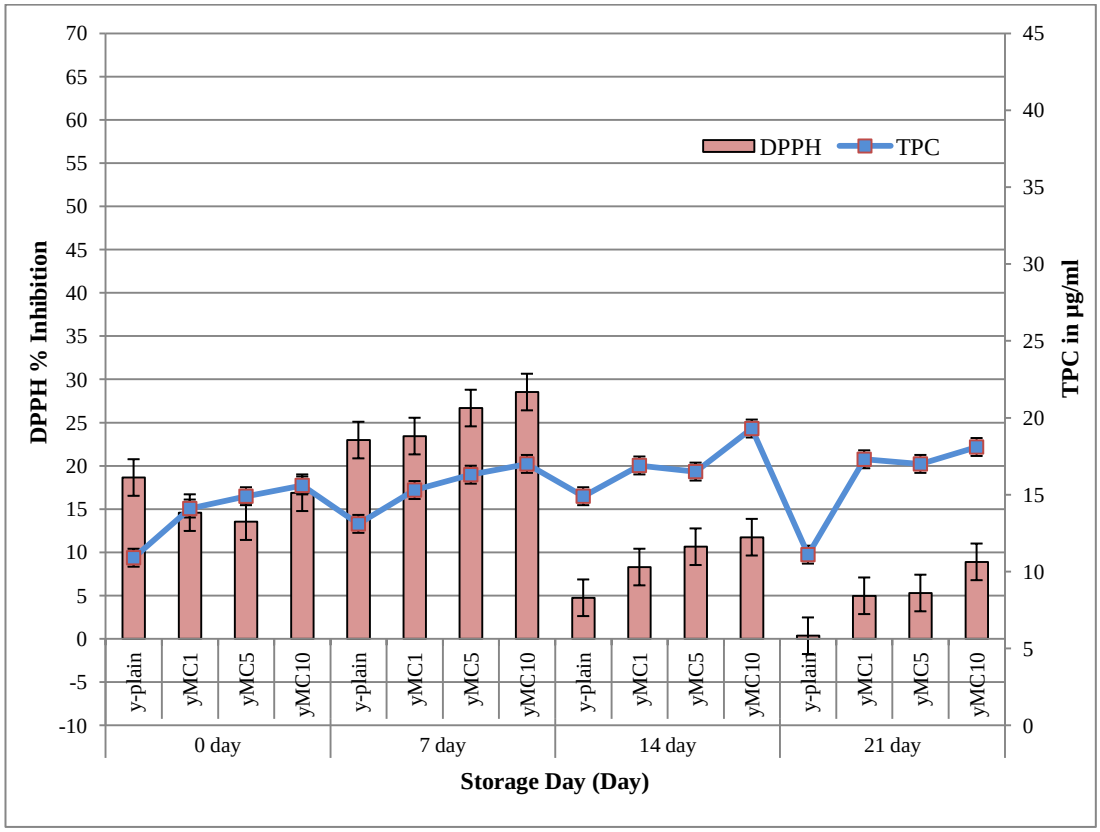

Figure 4.6: Effects of *M. citrifolia* on the total phenolic content and antioxidant activity at the 0, 7, 14 and 21 days of storage at 4^{o}C.

DPPH inhibition by plain-yogurt on day 0 (18.65±0.49%) increased slightly ($p>0.05$) to (22.98±7.78%) on day 7 of storage. Extended storage to 14 and 21 days reduced DPPH inhibition to 4.75±1.26% and 0.36±0.03% respectively.

YMC1 had showed lower DPPH inhibition (14.59±0.50%) compared to y-plain (18.65±0.49%) during day 0 of storage but higher inhibition during extended during at day 7 (23.44±0.92%; yMC1 and 22.98±7.78%). YMC5 had lower DPPH inhibition capacity (13.55±0.52%) than plain-yogurt on day 0 but it showed higher DPPH inhibition (26.68±1.12%) than plain-yogurt (22.98±7.78%) on day 7 of storage. YMC10 showed the highest (28.53±1.52%) DPPH inhibition and this occurred on day 7 of storage. Extended storage of *M. citrifolia*-yogurt to 14 and 21 days reduced DPPH inhibition ($p<0.05$) compared to day 0 to between 4 and 9%.

Table 4.6: The percentage (%) of DPPH inhibition and total phenolic compound (TPC) in *M. citrifolia*-yogurts and plain-yogurt during 21 days of storage (4℃) (n=3).

	0 day		**7 day**		**14 day**		**21 day**	
	DPPH	TPC	DPPH	TPC	DPPH	TPC	DPPH	TPC
y plain	18.65±0.49	10.9±0.2	22.98±7.78	13.1±3.0	4.75±1.26	14.9±1.6	0.36±0.03	11.1±2.2
yMC1	14.59±0.50	14.1±1.3	23.44±0.92	15.3±0.3	8.30±0.49	16.9±1.3	4.98±0.28	17.3±1.3
yMC5	13.55±0.52	14.9±0.4	26.68±1.12	16.3±0.4	10.65±1.38	16.5±0.8	5.30±0.79	17.0±0.5
yMC10	16.89±0.89	15.6±0.5	28.53±1.52	17.0±0.3	11.75±0.57	19.3±2.0	8.90±0.55	18.1±0.5

4.3 Viability of *Lactobacillus* spp. in yogurt

Lactobacillus spp. in plain-yogurt was 5.60×10^8cfu/ml in fresh (day 0) yogurt (Figure 4.7a). Refrigerated storage of yogurt resulted in small increase in viable bacteria count with mean values being significantly higher on days 7 and 14 yogurt compared to day 0 yogurts.

4.3.1 Effects of herbs extract on *Lactobacillus* spp. counts in yogurt

O. stamineus did not affect the growth of *Lactobacillus* spp. during fermentation (i.e. no differences in bacterial counts in day 0 yogurt) but it increased ($p<0.05$) the growth of *Lactobacillus* spp. after 7 days storage with highest effects seen in yOS1 (1.62×10^9cfu/ml) and yOS5 (1.60×10^9cfu/ml) followed by yOS10 (1.20×10^9cfu/ml) compared to plain-yogurt (7.00×10^8cfu/ml, $p<0.05$) (Figure 4.7a).

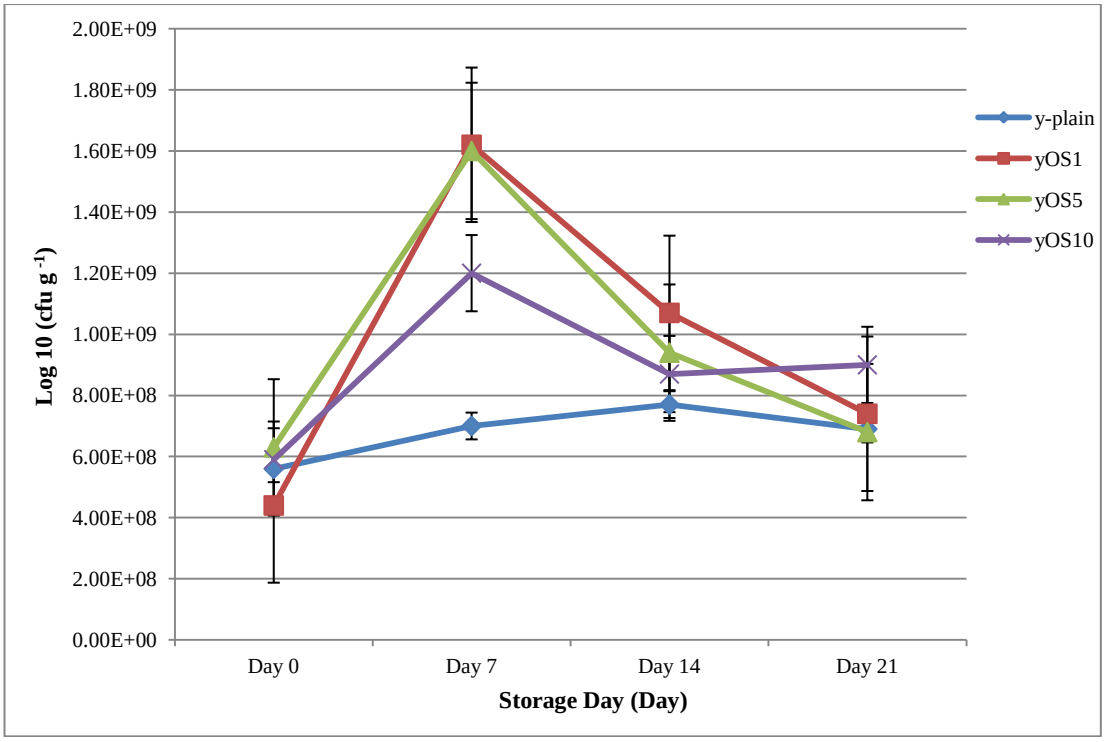

Figure 4.7a: Effects of *O. stamineus* on the viability of *Lactobacillus* spp. during storage at 4°C. Each data represents the means
\of triplicate determination.

Extended refrigerated storage resulted in reduction in viable *Lactobacillus* spp. counts by day 14 ($8.70x10^8$ to $1.07x10^9$cfu/ml) and day 21 ($6.80x10^8$ to $9.00x10^8$cfu/ml) and these were not different from plain-yogurt at respective storage days.

Table 4.7: Enumeration (Log_{10}: cfu g^{-1}) of *Lactobacillus* spp. growth in *O. stamineus*-yogurts and plain-yogurt during 21 days of storage (4℃) (n=3).

	Day 0	Day 7	Day 14	Day 21
y-plain	5.60E+08	7.00E+08	7.70E+08	6.90E+08
yOS1	4.40E+08	1.62E+09	1.07E+09	7.40E+08
yOS5	6.30E+08	1.60E+09	9.40E+08	6.80E+08
yOS10	5.90E+08	1.20E+09	8.70E+08	9.00E+08

C. asiatica also did not affect *Lactobacillus* spp. growth during yogurt fermentation. *Lactobacillus* spp. counts in fresh *C. asiatica*-yogurts ranged between $5.30x10^8$cfu/ml and $6.70x10^8$cfu/ml (Figure 4.7b).

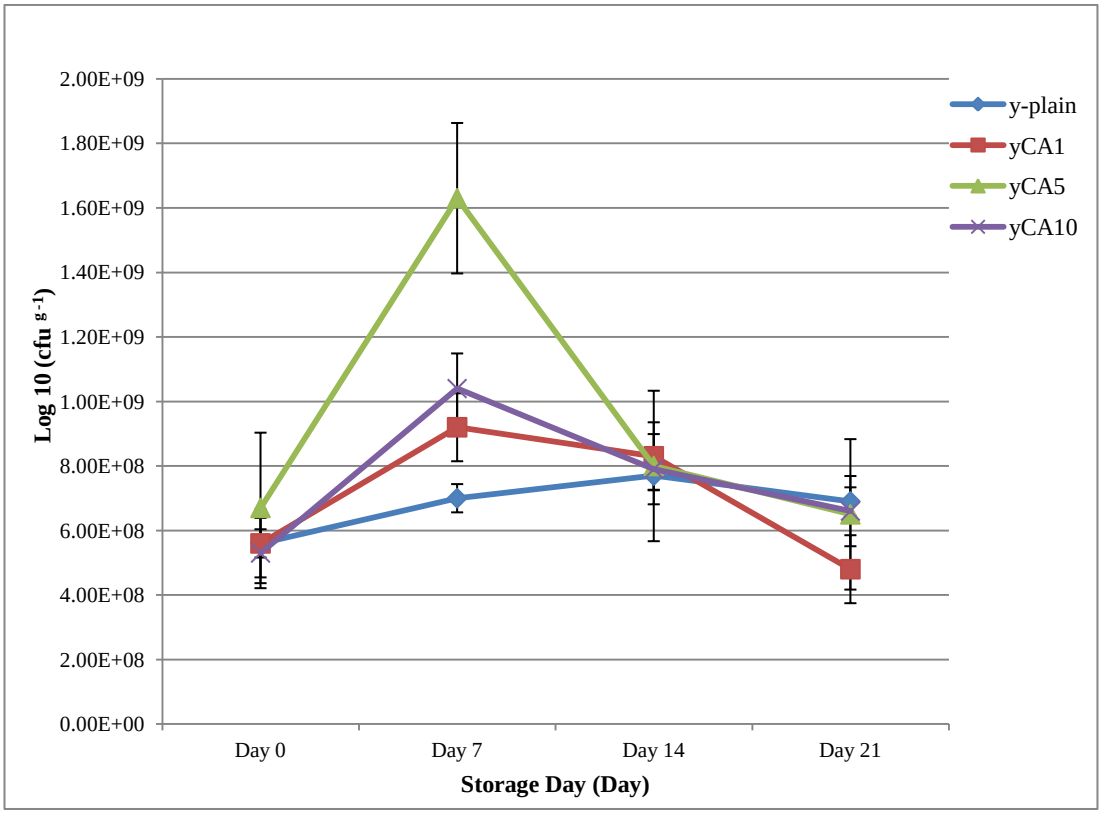

Figure 4.7b: Effects of *C. asiatica* on the viability of *Lactobacillus* spp. during storage at 4°C. Each data represents the means of triplicate determination.

Lactobacillus spp. count after 7 days of refrigerated storage increased with yCA5 containing highest number ($1.63x10^9$cfu/ml) followed by yCA10 ($1.04x10^9$cfu/ml) and yCA1 ($9.20x10^8$cfu/ml). Extended storage to 14 and 21 days resulted in a decreased viable *Lactobacillus* spp. counts in *C. asiatica*-yogurts to values similar to those in plain-yogurt.

Table 4.8: Enumeration (Log_{10}: cfu g^{-1}) of *Lactobacillus* spp. growth in *C. asiatica*-yogurts and plain-yogurt during 21 days of storage day (4℃) (*n*=3).

	Day 0	Day 7	Day 14	Day 21
y-plain	5.60E+08	7.00E+08	7.70E+08	6.90E+08
yCA1	5.60E+08	9.20E+08	8.30E+08	4.80E+08
yCA5	6.70E+08	1.63E+09	8.00E+08	6.50E+08
yCA10	5.30E+08	1.04E+09	7.90E+08	6.60E+08

M. citrifolia-yogurts did not affect the growth of *Lactobacillus* spp. during yogurt fermentation. *Lactobacillus* spp. counts in fresh (day 0) yogurt was between $4.20x10^8$cfu/ml to $5.40x10^8$cfu/ml (Figure 4.7c). The growth of *Lactobacillus* spp. after 7 days of refrigerated storage was highest in yMC5 ($9.50x10^8$cfu/ml) followed by yMC10 ($8.10x10^8$cfu/ml) and yMC1 ($7.50x10^8$cfu/ml).

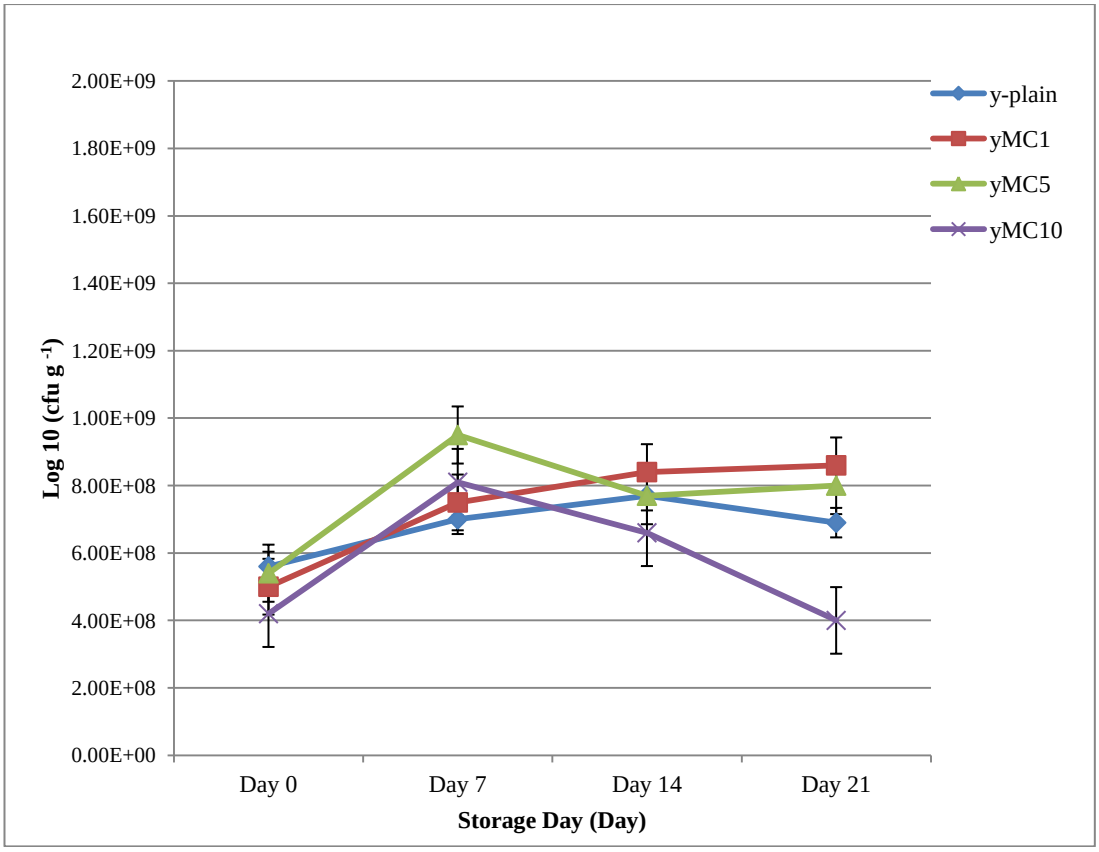

Figure 4.7c: Effects of *M. citrifolia* on the viability of *Lactobacillus* spp. during storage at 4^{o}C. Each data represents the means of triplicate determination.

Extended refrigerated storage of *M. citrifolia*-yogurts resulted in reduction in viable *Lactobacillus* spp. counts by day 14, (7.70x10^{8}cfu/ml and 6.60x10^{8}cfu/ml for yMC5 and yMC10 respectively). The highest concentration of *M. citrifolia* (10%v/v) caused a profound effect on the viability of *Lactobacillus* spp. during storage. YMC10 showed the lowest *Lactobacillus* spp. counts (4.00x10^{8}cfu/ml) on day 21 of storage. YMC1 on the other hand showed consistent increase in viable *Lactobacillus* spp. during storage.

Table 4.9: Enumeration (Log_{10}: cfu g^{-1}) of *Lactobacillus* spp. growth in *M. citrifolia*-yogurts and plain-yogurt during 21 storage day (4℃) (*n*=3).

	Day 0	Day 7	Day 14	Day 21
y-plain	5.60E+08	7.00E+08	7.70E+08	6.90E+08
yMC1	5.00E+08	7.50E+08	8.40E+08	8.60E+08
yMC5	5.40E+08	9.50E+08	7.70E+08	8.00E+08
yMC10	4.20E+08	8.10E+08	6.60E+08	4.00E+08

4.4 Viability of *S. thermophilus* in yogurt

The viable count of *S. thermophilus* in plain-yogurt on day 0 was $1.37x10^9$cfu/ml (Figure 4.8a) and was not changed on day 7 of storage. Extended storage to day 14 decreased ($p<0.05$) *S. thermophilus* count to $8.40x10^8$cfu/ml. The count decreased further to $7.80x10^8$cfu/ml after 21 days of storage.

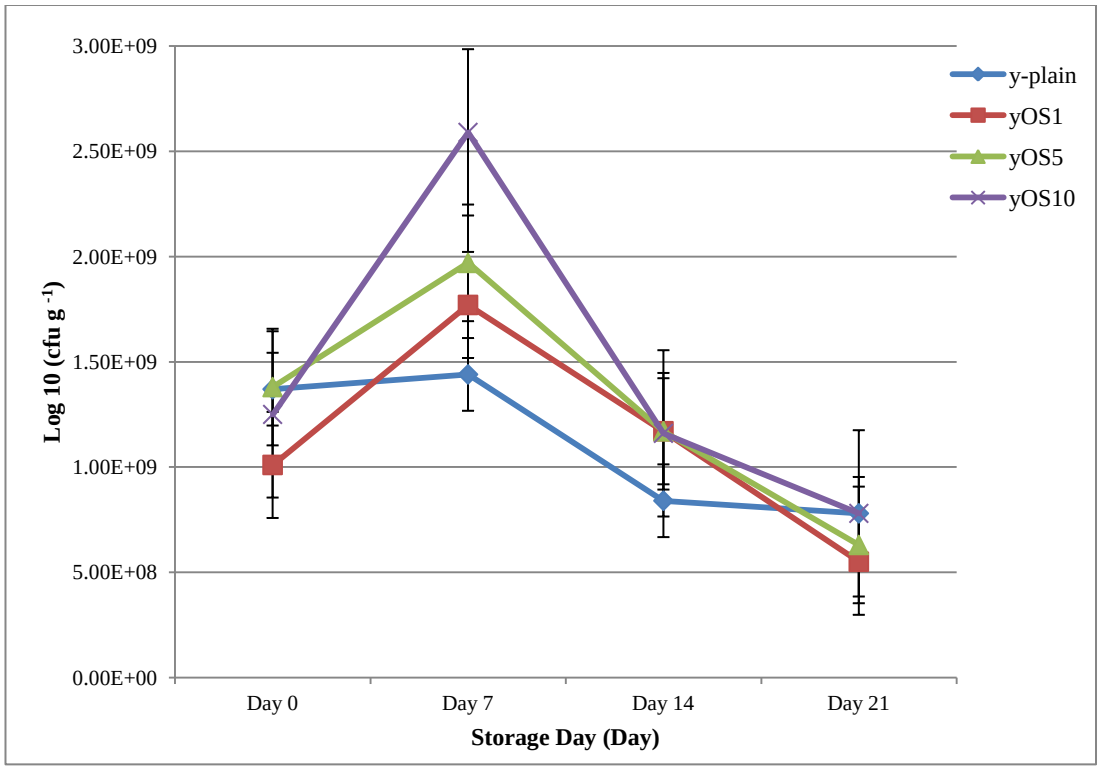

Figure 4.8a: Effects of *O. stamineus* on the viability of *S. thermophilus* during storage at 4°C. Each data represents the means of triplicate determination.

4.4.1 Effects of herbs extract on *S. thermophilus* counts in yogurt

The presence of *O. stamineus* had no effect on *S. thermophilus* counts in fresh yogurt (Figure 4.8a). The viable counts of *S. thermophilus* in *O. stamineus*-yogurts however increased after 7 days of refrigerated storage ($1.77x10^9$, $1.97x10^9$, $2.59 x10^9$cfu/ml for yOS1, yOS5 and yOS10 respectively; $p<0.05$ compared to day 0).

Extended storage resulted in reduction of viable of *S. thermophilus* counts towards day 0 values by day 14 of storage.

Table 4.10: Enumeration (Log_{10}: cfu g^{-1}) of *S. thermophilus* growth in *O. stamineus*-yogurts and plain-yogurt during 21 days of storage (4℃) (*n*=3).

	Day 0	Day 7	Day 14	Day 21
y-plain	1.37E+09	1.44E+09	8.40E+08	7.80E+08
yOS1	1.01E+09	1.77E+09	1.17E+09	5.50E+08
yOS5	1.38E+09	1.97E+09	1.17E+09	6.30E+08
yOS10	1.25E+09	2.59E+09	1.16E+09	7.80E+08

S. thermophilus counts in *C. asiatica*-yogurts on day 0 ranged $8.90x10^8$cfu/ml to $1.62x10^9$cfu/ml. The viable cell counts increased to between 1.83 and $2.01x10^9$cfu/ml ($p<0.05$ for yCA5 in comparison to plain-yogurt $1.44x10^9$cfu/ml) after 7 days refrigerated storage (Figure 4.8b). Extended storage to 21 days resulted in a decrease in viable cell counts for *S. thermophilus* for all yogurts to values below their respective day 0 values.

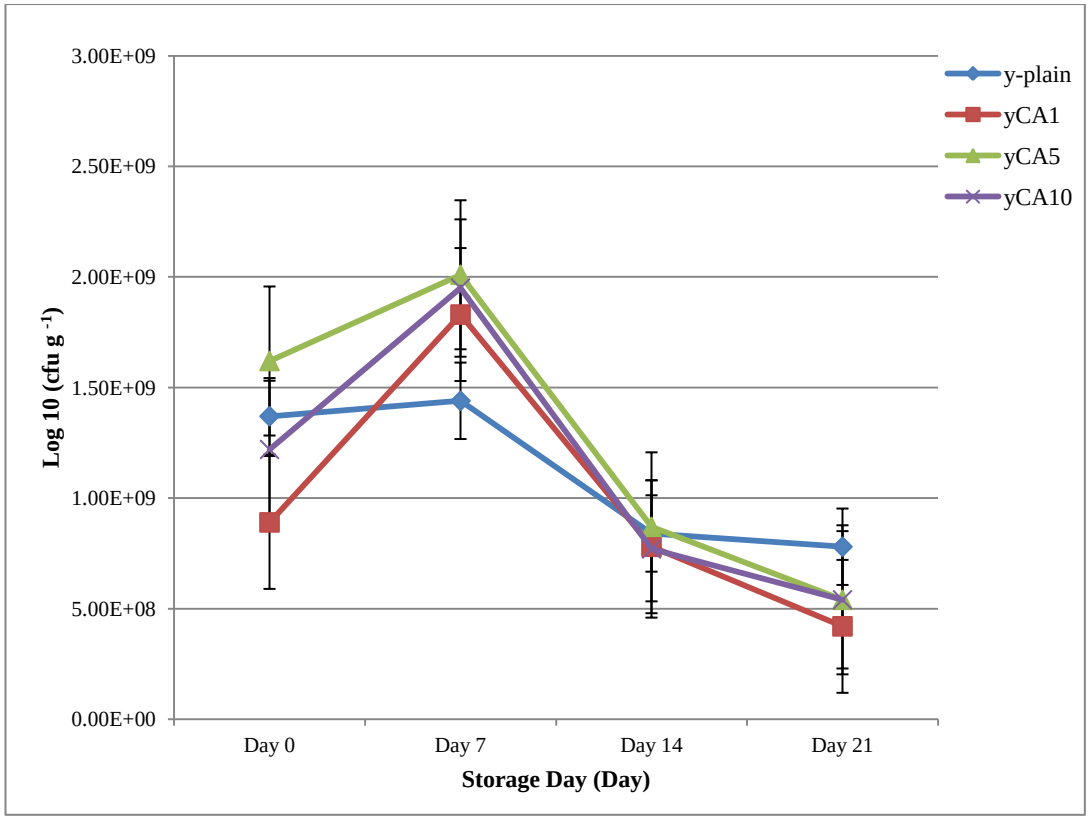

Figure 4.8b: Effects of *C. asiatica* on the viability of *S. thermophilus* during storage at 4°C. Each data represents the means of triplicate determination.

Table 4.11: Enumeration (Log_{10}: cfu g^{-1}) of *S. thermophilus* growth in *C. asiatica*-yogurts and plain-yogurt during 21 days of storage (4℃) (*n*=3).

	Day 0	Day 7	Day 14	Day 21
y-plain	1.37E+09	1.44E+09	8.40E+08	7.80E+08
yCA1	8.90E+08	1.83E+09	7.80E+08	4.20E+08
yCA5	1.62E+09	2.01E+09	8.70E+08	5.40E+08
yCA10	1.22E+09	1.95E+09	7.70E+08	5.40E+08

S. thermophilus counts in *M. citrifolia* yogurts on day 0 ranged from $9.70x10^8$cfu/ml to $1.87x10^9$cfu/ml (Figure 4.8c). The viable cell counts increased to 1.63 to $2.38x10^9$cfu/ml ($p<0.05$ for yMC5; $2.38x10^9$cfu/ml) in comparison to plain-yogurt ($1.44x10^9$cfu/ml) after 7 days refrigerated storage. Extended storage to 14 and

21 days resulted in reduction of viable of *S. thermophilus* counts towards day 0 values.

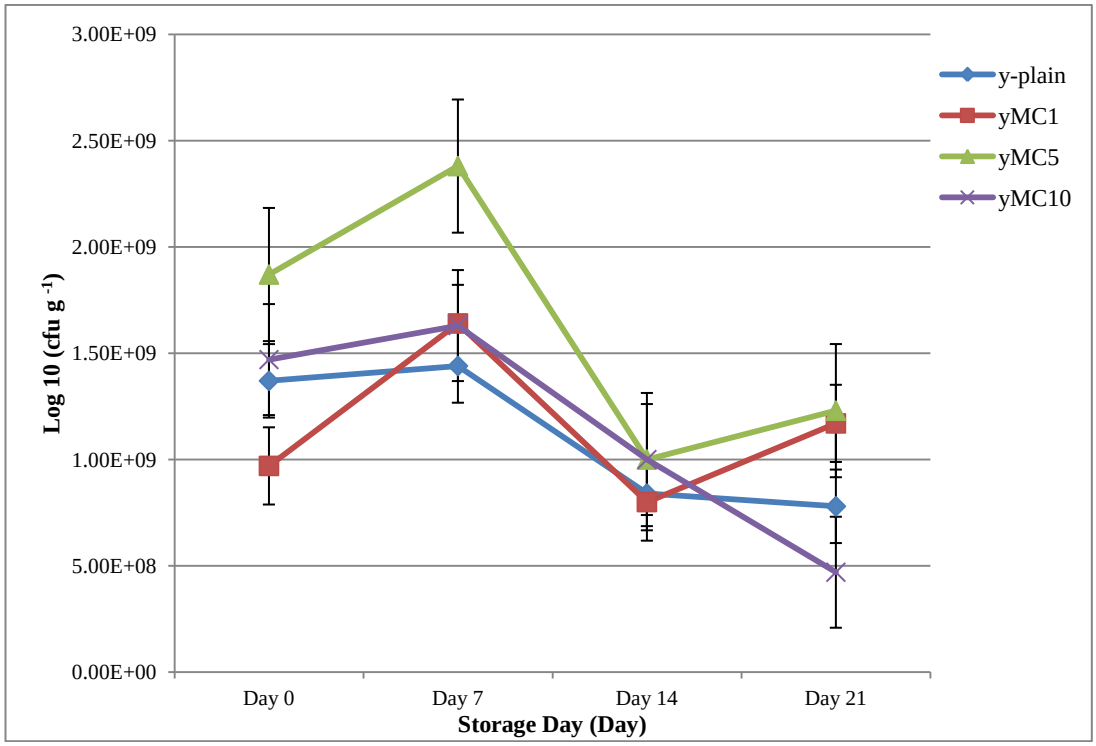

Figure 4.8c: Effects of *M. citrifolia* on the viability of *S. thermophilus* during storage at 4^{o}C. Each data represents the means of triplicate determination.

Table 4.12: Enumeration (Log_{10}: cfu g^{-1}) of *S. thermophilus* growth in *M. citrifolia*-yogurts and plain-yogurt during 21 days of storage (4℃) (*n*=3).

	Day 0	Day 7	Day 14	Day 21
y-plain	1.37E+09	1.44E+09	8.40E+08	7.80E+08
yMC1	9.70E+08	1.64E+09	8.00E+08	1.17E+09
yMC5	1.87E+09	2.38E+09	1.00E+09	1.23E+09
yMC10	1.47E+09	1.63E+09	1.00E+09	4.70E+08

4.5 α-amylase inhibition by yogurt

Fresh plain-yogurt inhibited 37.1±2.7% of α-amylase activity (Figure 4.9). Refrigerated storage of plain-yogurt resulted in gradual linear increase in the inhibition on α-amylase activity with time maximum values obtained on the 21 days of storage. Difference in α-amylase inhibition compared to day 0 of storage was significant ($p<0.05$) on day 7 (47.6±3.9%), day 14 (51.0±0.5%) and day 21 (54.8±5.8%).

4.5.1 α-amylase inhibition by *O. stamineus*-yogurts

All *O. stamineus*-yogurts had higher inhibition on α-amylase activity, both in fresh and in stored yogurts. α-amylase inhibition by fresh *O. stamineus*-yogurts ranged between 46 to 48% (Figure 4.9).

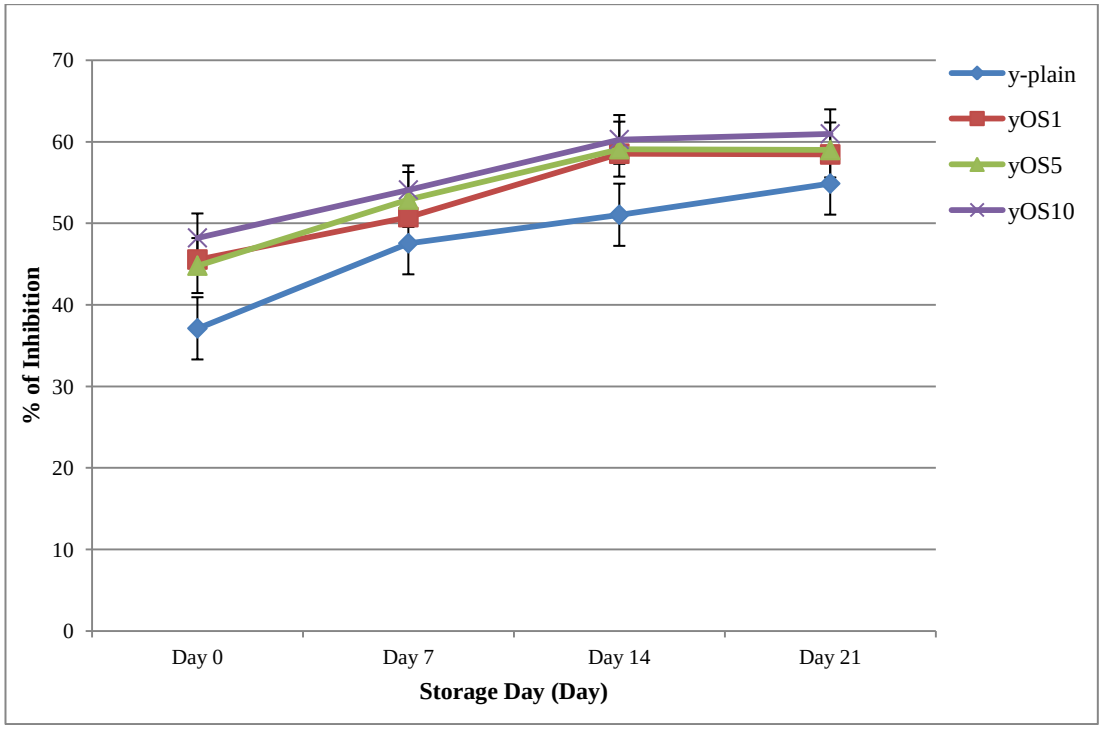

Figure 4.9: α-amylase inhibition by *O. stamineus*-yogurts extract prepared from yogurt refrigerated for 0, 7, 14 and 21 days.

Maximal inhibition of α-amylase by *O. stamineus*-yogurts was shown after 14 days of storage, whereby all *O. stamineus*-yogurts preparation showed higher inhibition (58 to 60%) on α-amylase than plain-yogurt (51.0±0.5%; $p<0.05$). Yogurt with the highest inclusion of *O. stamineus* (60.9±0.4%; yOS10) showed higher ($p<0.05$) inhibition on α-amylase at all storage days.

Table 4.13 : Percentage of α-amylase inhibition by *O. staminues*-yogurts and plain-yogurt during 21 days of storage.

	Day 0	Day 7	Day 14	Day 21
y-plain	37.1±2.7	47.6±3.9	51.0±0.5	54.8±5.8
yOS1	45.5±6.5	50.7±0.7	58.5±1.5	58.4±1.2
yOS5	44.8±4.5	52.9±1.3	59.0±2.3	58.9±0.1
yOS10	48.2±2.2	54.1±0.5	60.2±0.4	60.9±0.4

The changes in IC_{50} of α-amylase inhibition by yogurts as a result of the addition of *O. stamineus* and refrigerated storage are presented in Figure 4.10. Higher inclusion percentage of *O. stamineus* in yogurt resulted in increasingly higher potency (reduction in IC_{50} values) of yogurts to inhibit α-amylase.

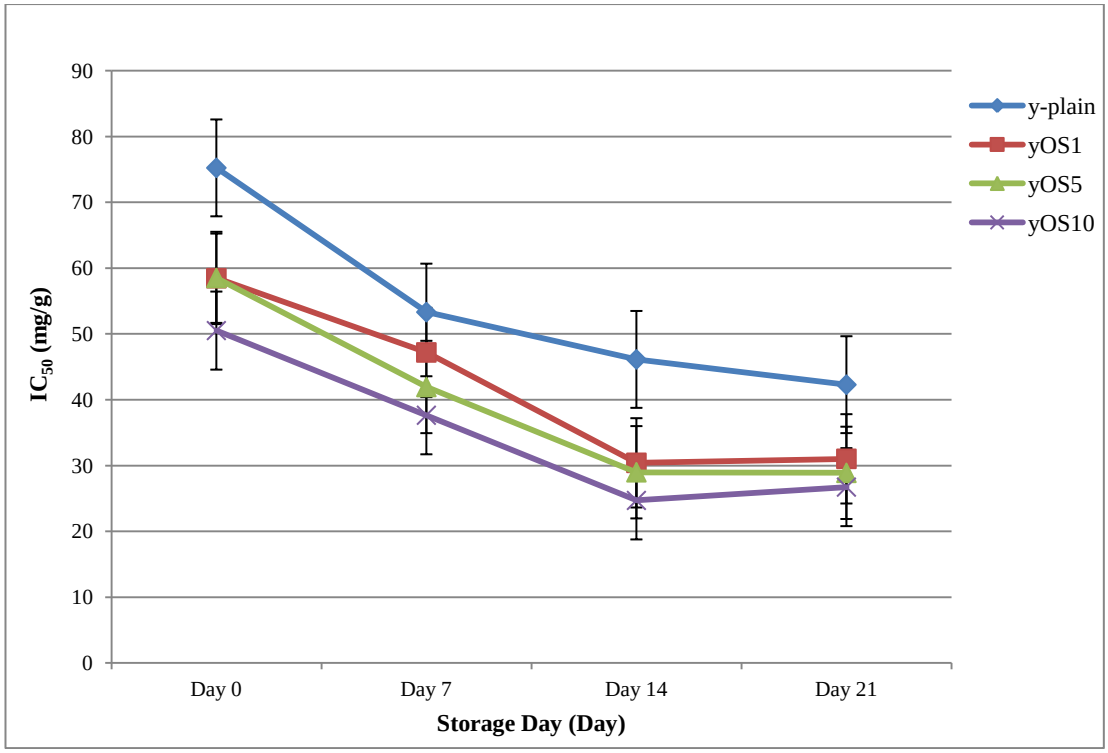

Figure 4.10: IC_{50} values for α-amylase inhibition by *O. stamineus*-yogurts at different refrigerated ($4^{o}C$) storage periods.

The increase in the potency of *O. stamineus*-yogurts to inhibit α-amylase occurred until day 14 of storage whereas for plain-yogurt the IC_{50} value continued to decrease until day 21 of storage. Highest potency to inhibit α-amylase was shown by day 14 for *O. stamineus*-yogurts (30.41±3.11, 28.98±1.75, 24.70±1.21mg/g for yOS1, yOS5 and yOS10 respectively) but only by day 21 for plain-yogurt (IC_{50} 42.29±1.45mg/g).

Table 4.14: IC_{50} (mg/g) values for α-amylase inhibition by *O. stamineus*-yogurts and plain-yogurt during 21 days of storage.

	Day 0	Day 7	Day 14	Day 21
y-plain	75.24±5.63	53.32±1.26	46.13±2.10	42.29±1.45
yOS1	58.47±3.72	47.19±4.21	30.41±3.11	31.02±1.25
yOS5	58.51±2.86	41.94±2.30	28.98±1.75	28.89±3.21
yOS10	50.51±3.20	37.64±1.20	24.70±1.21	26.72±2.68

4.5.2 α-amylase inhibition by *C. asiatica*-yogurts

The presence of *C. asiatica* increased the fresh yogurt inhibition of α-amylase to 44.7±2.8%, 50.5±1.4% and 51.9±0.6% (for yCA1, yCA5 and yCA10 respectively) compared to plain-yogurt (37.1±2.7%; Figure 4.11).

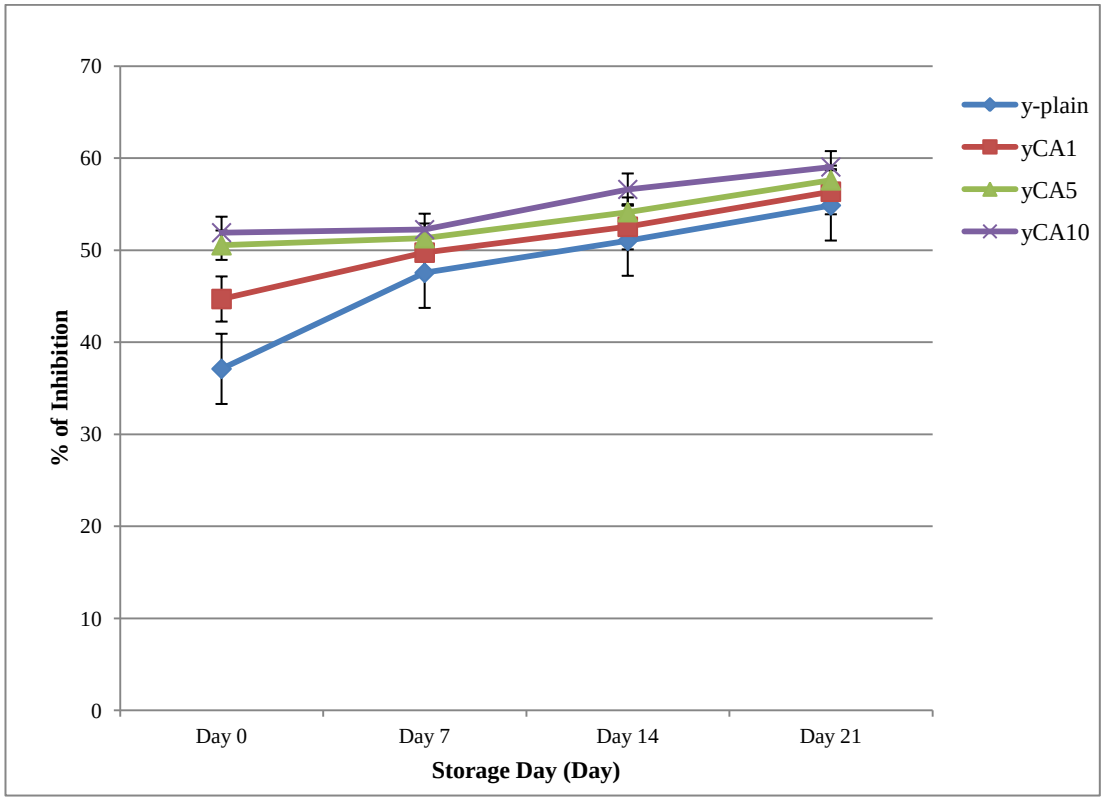

Figure 4.11: α-amylase inhibition by *C. asiatica*-yogurts extract prepared from yogurt refrigerated for 0, 7, 14 and 21 days.

Refrigerated storage yogurt increased the α-amylase inhibition ($p<0.05$ for yogurts after 14 days storage) with highest inhibition achieved by day 21 for yCA10 (59.0±2.5%). Maximal difference between plain-yogurt and *C. asiatica*-yogurts was seen on day 0 but these differences were diminished after 7 days of refrigeration.

Table 4.15: Percentage of α-amylase inhibition by *C. asiatica*-yogurts and plain-yogurt during 21 days of storage.

	Day 0	Day 7	Day 14	Day 21
y-plain	37.1±2.7	47.6±3.9	51.0±0.5	54.8±5.8
yCA1	44.7±2.8	49.7±2.1	52.5±0.3	56.3±1.0
yCA5	50.5±1.4	51.3±3.3	54.1±0.5	57.6±0.3
yCA10	51.9±0.6	52.2±2.0	56.6±1.2	59.0±2.5

The changes in IC_{50} of α-amylase inhibition by yogurt as a result of the addition of *C. asiatica* and refrigerated storage are presented in Figure 4.12. Higher inclusion percentage of *C. asiatica* in yogurt resulted in increasingly higher potency (reduction in IC_{50} values) of yogurts to inhibit α-amylase.

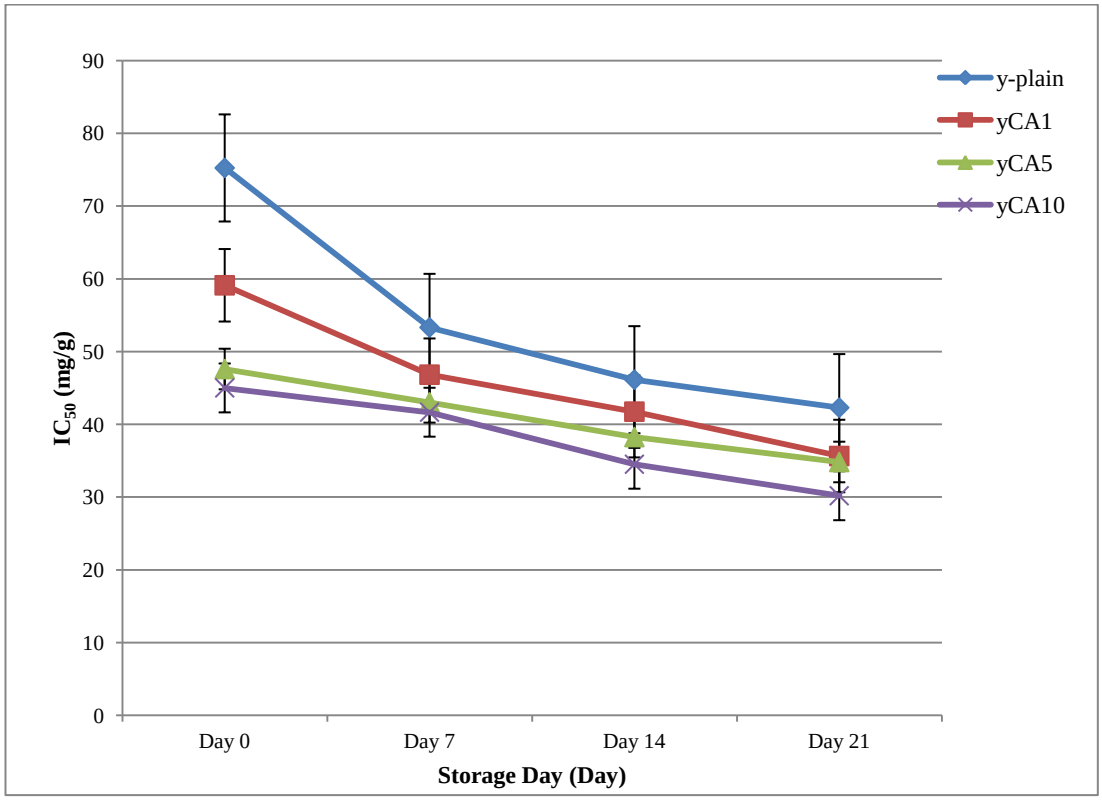

Figure 4.12: IC_{50} values for α-amylase inhibition by *C. asiatica*-yogurts at different refrigerated ($4^{o}C$) storage periods.

The increase in the potency of all yogurts to inhibit α-amylase occurred until day 21 of storage. The highest potency to inhibit α-amylase at this storage day was 42.29±1.45, 35.64±1.35, 34.82±1.24 and 30.18±0.65mg/g for plain-yogurt, yCA1, yCA5 and yCA10 respectively.

Table 4.16: IC_{50} (mg/g) values for α-amylase inhibition by *C. asiatica*-yogurts and plain-yogurt during 21 days of storage.

	Day 0	Day 7	Day 14	Day 21
y-plain	75.24±5.63	53.32±1.26	46.13±2.10	42.29±1.45
yCA1	59.11±1.33	46.82±2.15	41.74±1.22	35.64±1.35
yCA5	47.60±1.56	43.02±3.11	38.24±1.65	34.82±1.24
yCA10	45.00±0.98	41.66±2.56	34.51±2.01	30.18±0.65

4.5.3 α-amylase inhibition by *M. citrifolia*-yogurts

The inhibition of α-amylase by fresh *M. citrifolia*-yogurts (45-52%) was higher than plain-yogurt (37.1±2.7%; $p<0.05$) (Figure 4.13). There was very small increase in *M. citrifolia*–yogurts inhibition on α-amylase after 7 days of refrigerated storage 49.9±4.3%, 50.0±2.8% and 52.2±1.4% for yCA1, yCA5 and yCA10 respectively and this diminished the significant difference from plain-yogurt.

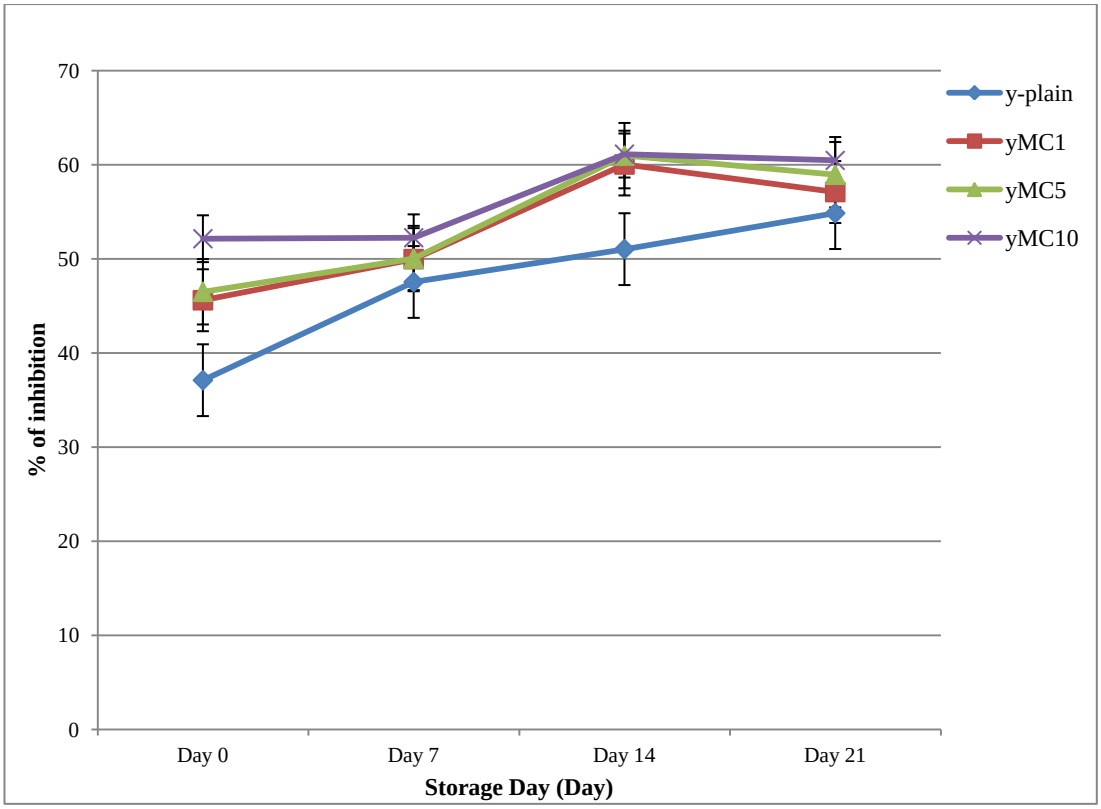

Figure 4.13: α-amylase inhibition by *M. citrifolia*–yogurts extract prepared from yogurt refrigerated for 0, 7, 14 and 21 days.

Refrigerated storage to 14 days resulted in the highest ($p<0.05$) inhibition of α-amylase (60.0±1.2%, 60.9±1.4% and 61.1±2.3% for yMC1, yMC5 and yMC10 respectively). Extended storage to 21 days resulted in no further increase in *M. citrifolia*–yogurts inhibition of α-amylase.

Table 4.17: Percentage of α-amylase inhibition by *M. citrifolia*-yogurts and plain-yogurt during 21 days of storage.

	Day 0	Day 7	Day 14	Day 21
y-plain	37.1±2.7	47.6±3.9	51.0±0.5	54.8±5.8
yMC1	45.6±5.9	49.9±4.3	60.0±1.2	57.1±0.1
yMC5	46.5±1.5	50.0±2.8	60.9±1.4	58.9±1.0
yMC10	52.1±2.2	52.2±1.4	61.1±2.3	60.4±0.3

The changes in IC_{50} of α-amylase inhibition by yogurt as a result of the addition of *M. citrifolia* and refrigerated storage are presented in Figure 4.14. Higher inclusion percentage of *M. citrifolia* in yogurt resulted in increasingly higher potency (reduction in IC_{50} values) of yogurts to inhibit α-amylase.

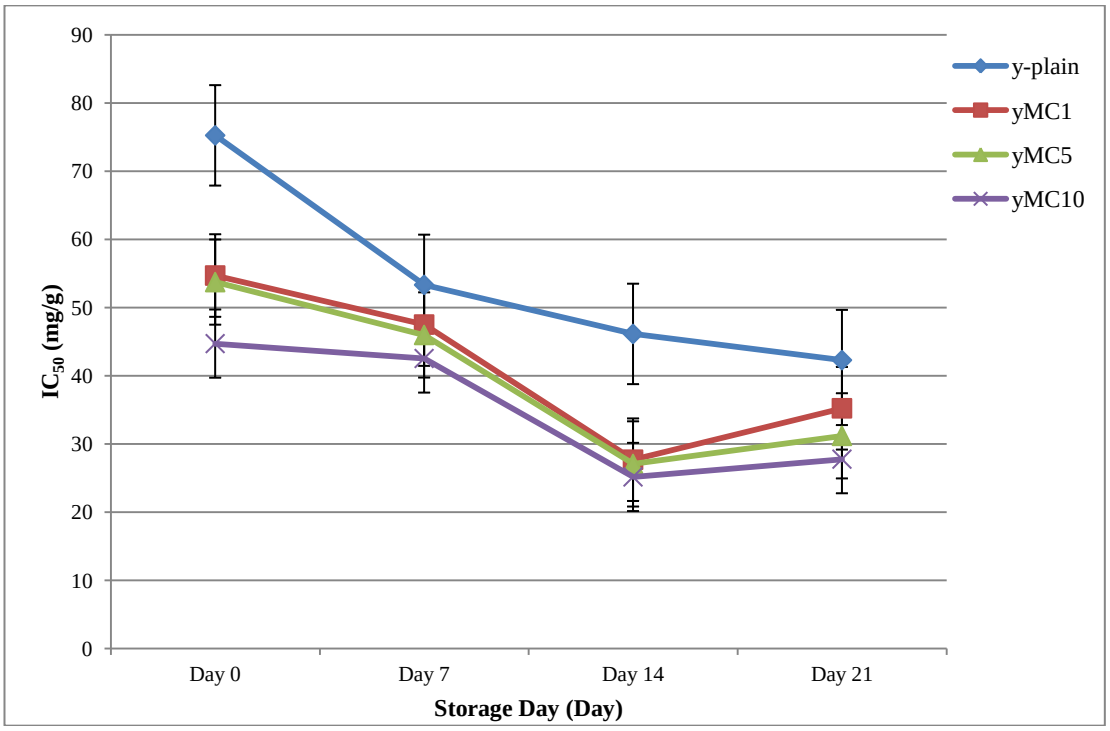

Figure 4.14: IC_{50} values for α-amylase inhibition by *M. citrifolia*–yogurts at different refrigerated (4^{o}C) storage periods.

Table 4.18: IC_{50} (mg/g) values for α-amylase inhibition by *M. citrifolia*-yogurts and plain-yogurt during 21 days of storage.

	Day 0	Day 7	Day 14	Day 21
y-plain	75.24±5.63	53.32±1.26	46.13±2.10	42.29±1.45
yMC1	54.69±3.74	47.50±3.12	27.69±1.02	35.23±2.51
yMC5	53.73±2.61	45.98±1.57	27.07±1.08	31.19±2.41
yMC10	44.71±1.57	42.53±2.10	25.16±1.68	27.77±1.33

The highest potency of *M. citrifolia*–yogurts to inhibit α-amylase occurred on 14 day of storage for *M. citrifolia*–yogurts (35.23±2.51, 31.19±2.41 and 27.77±1.33mg/g for yMC1, yMC5 and yMC10 respectively) and on day 21 of storage for plain-yogurt (42.29±1.45mg/g).

4.6 α-glucosidase inhibition by yogurt

The inhibition of α-glucosidase by plain-yogurt on day 0 (5.8±0.36%) increased with time to the highest value (15.87±0.71%) by day 21 of refrigerated storage (Figure 4.15). IC_{50} for plain-yogurt decreased from 506.85 mg/g (day 0) to 247.43 mg/g (day 7) (see Figure 4.16).

4.6.1 α-glucosidase inhibition by *O. stamineus*-yogurts

The addition of *O. stamineus* increased ($p<0.05$) the fresh yogurt inhibition of α-glucosidase (18.05±0.47%, 19.27±0.36%, and 21.22±0.44% for yOS1, yOS5 and yOS10 respectively) (Figure 4.15). Refrigerated storage to 21 days resulted in minimal changes in *O. stamineus*-yogurts inhibition of α-glucosidase.

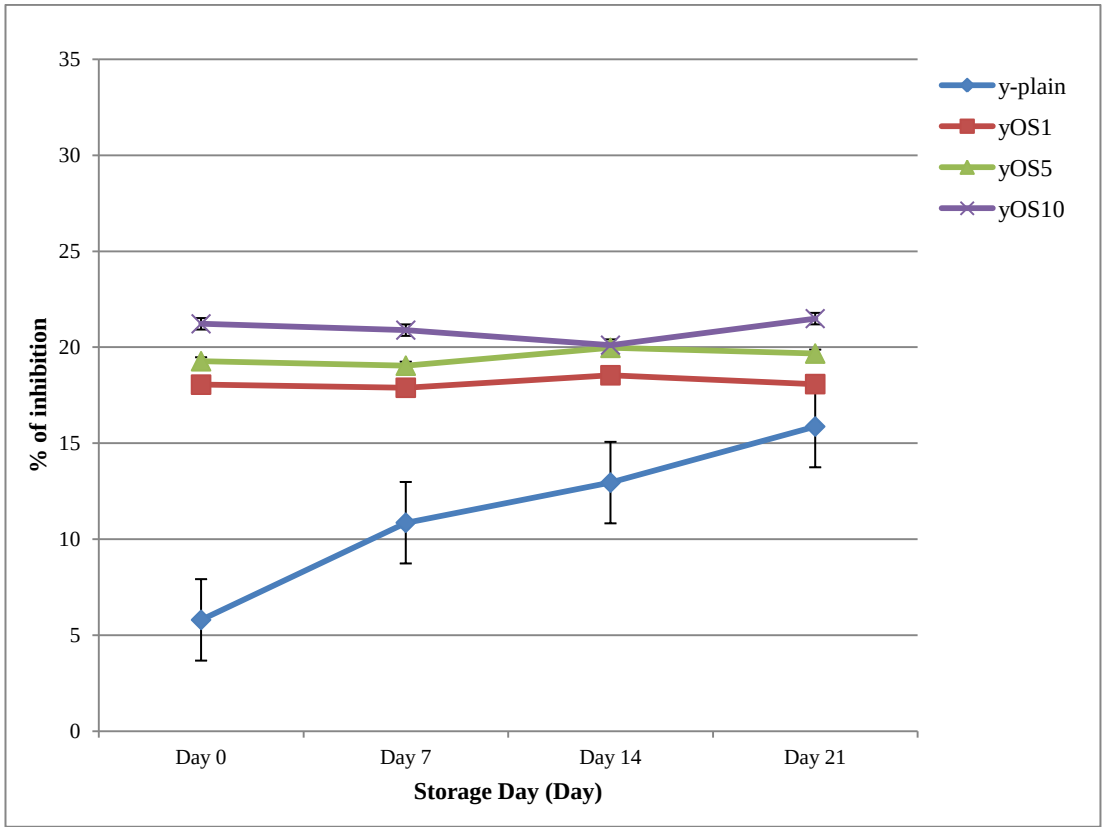

Figure 4.15: α-glucosidase inhibition by *O. stamineus*-yogurts extract prepared from yogurt refrigerated for 0, 7, 14 and 21 days.

Table 4.19 : Percentage of α-glucosidase inhibition by *O. stamineus*-yogurts and plain-yogurt during 21 days of storage.

	Day 0	Day 7	Day 14	Day 21
y-plain	5.80±0.36	10.86±0.23	12.95±0.31	15.87±0.71
yOS1	18.05±0.47	17.89±0.30	18.54±0.38	18.08±0.45
yOS5	19.27±0.36	19.04±3.09	19.97±0.39	19.67±0.39
yOS10	21.22±0.44	20.89±0.66	20.11±0.98	21.49±0.63

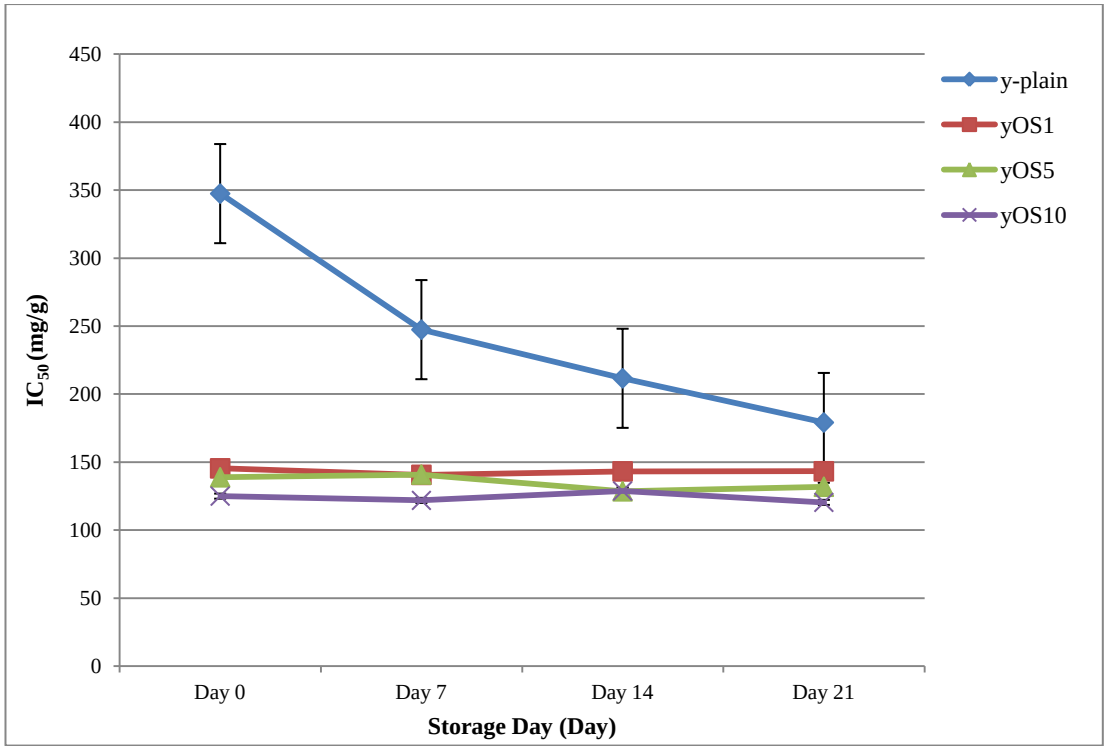

Figure 4.16: IC_{50} values for α-glucosidase inhibition by *O. stamineus*-yogurts at different refrigerated ($4^{o}C$) storage periods.

IC_{50} α-glucosidase for all *O. stamineus*-yogurts on day 0 was similar (125 to 145 mg/g) and these remained unchanged throughout refrigerated storage up to 21 days (Figure 4.16).

Table 4.20: IC_{50} (mg/g) values for α-glucosidase inhibition by *O. stamineus*-yogurts and plain-yogurt.

	Day 0	Day 7	Day 14	Day 21
y-plain	347.45±6.52	247.43±4.65	211.64±2.67	179.14±2.54
yOS1	145.41±5.84	140.64±4.72	143.18±3.41	143.26±2.31
yOS5	138.86±5.21	140.86±4.11	128.55±5.44	131.92±4.41
yOS10	124.99±3.11	121.85±3.95	128.80±6.21	120.40±3.11

4.6.2 α-glucosidase inhibition by *C. asiatica*-yogurts

The inhibition of α-glucosidase by fresh *C. asiatica*-yogurts (17.21±0.36%, 22.53±0.28%, and 25.45±0.12% for yCA1, yCA5 and yCA10 respectively) were higher compared to plain-yogurt (5.8±0.36%; $p<0.05$) (Figure 4.17).

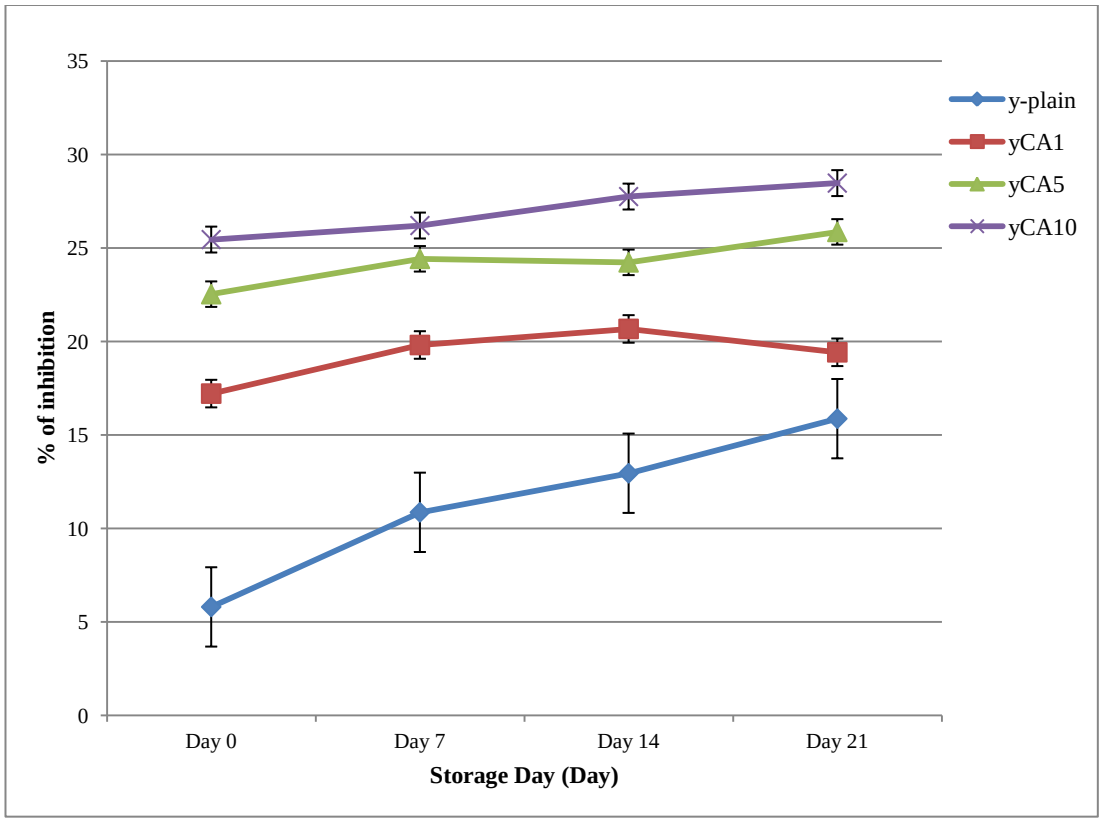

Figure 4.17: α-glucosidase inhibition by *C. asiatica*-yogurts extract prepared from yogurt refrigerated for 0, 7, 14 and 21 days.

All yogurts showed a gradual increase in the inhibition of α-glucosidase during storage with yCA10 recorded the highest inhibition followed by yCA5 and yCA1.

Table 4.21 : Percentage of α-glucosidase inhibition by *C. asiatica*-yogurts and plain-yogurt during 21 days of storage.

	Day 0	Day 7	Day 14	Day 21
y-plain	5.80±0.36	10.86±0.23	12.95±0.31	15.87±0.71
yCA1	17.21±0.36	19.81±0.78	20.67±0.33	19.42±0.26
yCA5	22.53±0.28	24.42±0.45	24.23±0.60	25.86±4.76
yCA10	25.45±0.12	26.20±0.42	27.75±4.82	28.47±0.51

IC_{50} of α-glucosidase by *C. asiatica*-yogurts are as shown in Figure 4.18. Fresh *C. asiatica*-yogurts (101 to 163mg/g) showed higher potency than plain-yogurt (347.45±6.52mg/g) with yCA10 showing the most potent effects on α-glucosidase inhibition at all storage duration tested.

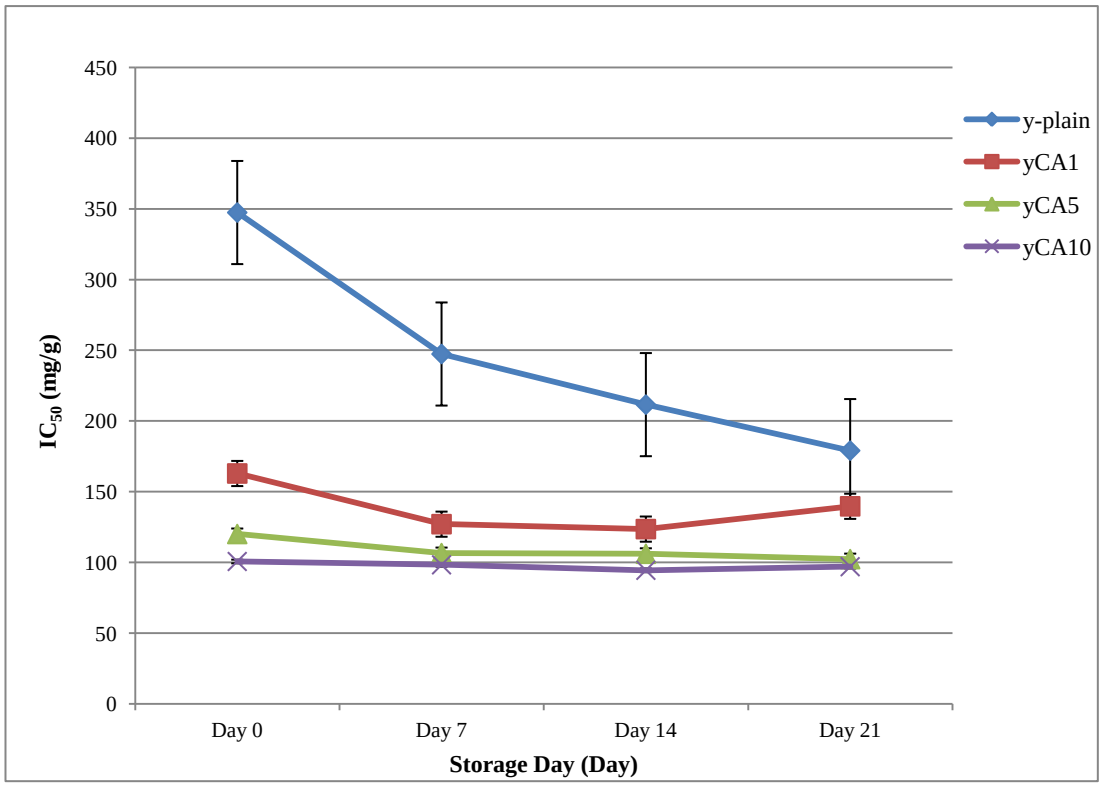

Figure 4.18: IC_{50} values for α-glucosidase inhibition by *C. asiatica*-yogurts at different refrigerated (4^{o}C) storage periods.

Refrigerated storage caused reduction IC_{50} values for plain-yogurt ($p<0.05$) but not for *C. asiatica*-yogurts. Refrigerated storage for 21 days resulted in small or no increase (yCA5 and yCA10) on potency, which were in contrast to marked increase in the potency of plain-yogurt to inhibit α-glucosidase during the same storage period.

Table 4.22 : IC_{50} (mg/g) values of α-glucosidase inhibition by *C. asiatica*-yogurts and plain-yogurt.

	Day 0	Day 7	Day 14	Day 21
y-plain	347.45±6.52	247.43±4.65	211.64±2.67	179.14±2.54
yCA1	162.97±3.15	127.14±3.56	123.66±4.11	139.77±2.88
yCA5	120.19±4.12	106.72±4.23	106.22±3.62	102.45±2.51
yCA10	100.77±3.85	98.40±3.12	94.52±3.81	97.10±2.81

4.6.3 α-glucosidase inhibition by *M. citrifolia*-yogurts

The inhibition of α-glucosidase by fresh *M. citrifolia*-yogurts increased with increasing *M. citrifolia* content in the yogurt (15.08±5.24%, 18.25±0.74%, and 20.68±4.96% for yMC1, yMC5 and yMC10 respectively) which were higher than plain-yogurt (5.8±0.36%; $p<0.05$) (Figure 4.19).

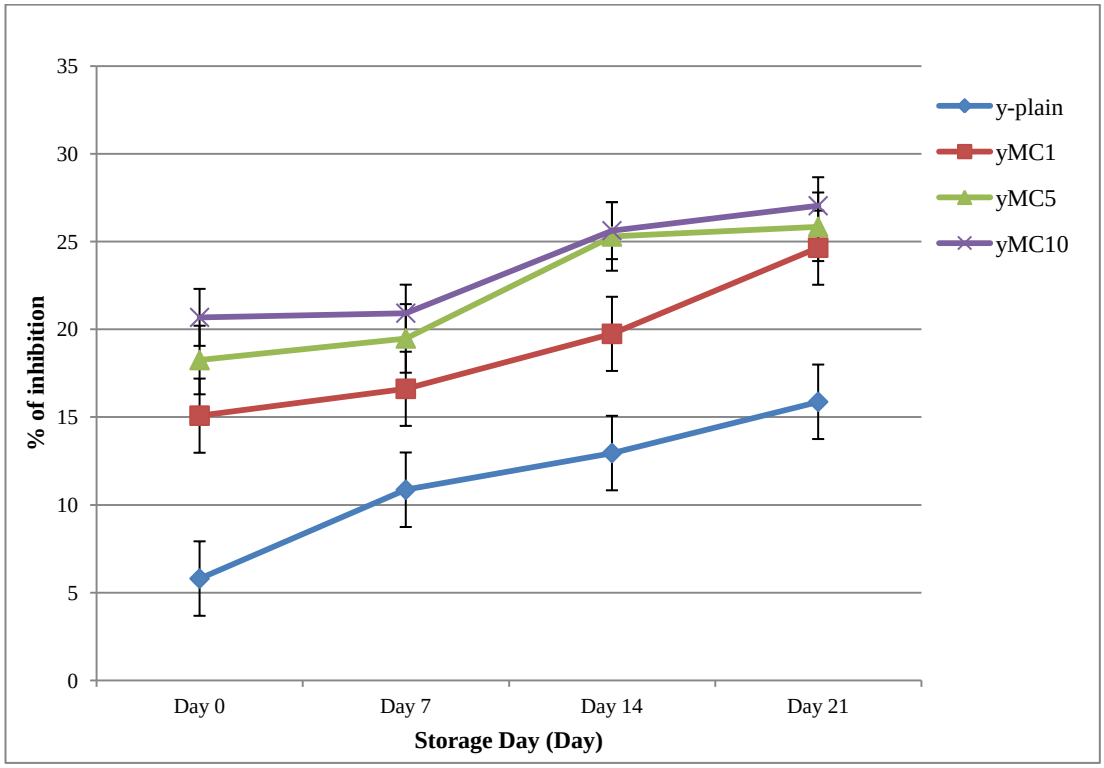

Figure 4.19: α-glucosidase inhibition by *M. citrifolia*-yogurts extract prepared from yogurt refrigerated for 0, 7, 14 and 21 days.

Refrigerated storage of yogurts resulted in a gradual increase in α-glucosidase inhibition with yMC10 recorded the highest inhibition of α-glucosidase activity followed by yMC5 and yMC1. Differences in α-glucosidase inhibition by yogurt between *M. citrifolia*-yogurts and plain-yogurt were significant ($p<0.05$) at all storage days.

Table 4.23 : Percentage of α-glucosidase inhibition by *M. citrifolia*-yogurts and plain-yogurt during 21 days of storage.

	Day 0	Day 7	Day 14	Day 21
y-plain	5.80±0.36	10.86±0.23	12.95±0.31	15.87±0.71
yMC1	15.08±5.24	16.61±1.54	19.74±2.05	24.65±3.99
yMC5	18.25±0.74	19.48±0.42	25.29±0.62	25.84±5.24
yMC10	20.68±4.96	20.92±0.23	25.62±0.35	27.04±8.99

IC_{50} values for day 0 for all *M. citrifolia*-yogurts (192.37±5.11, 156.47±3.54 and 124.17±4.15mg/g for yMC1, yMC5 and yMC10 respectively) were lower ($p<0.05$) than plain-yogurt (347.45±6.52mg/g) (Figure 4.20).

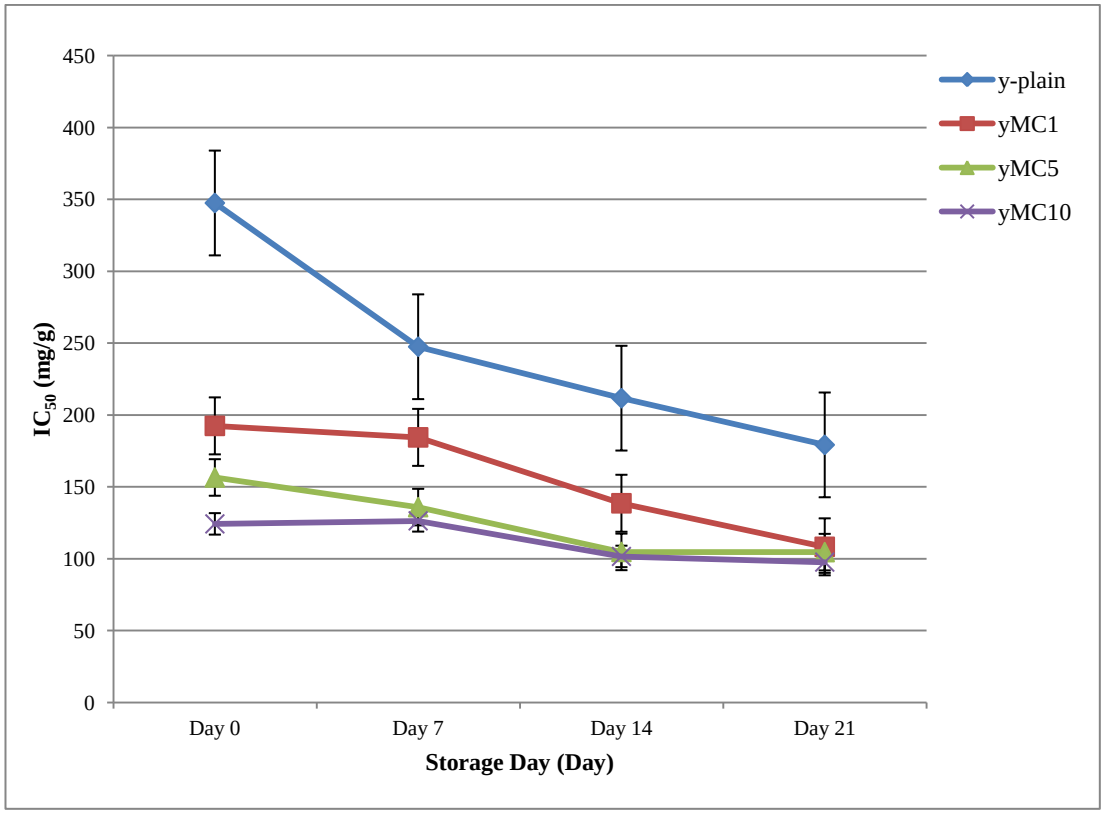

Figure 4.20: IC_{50} values for α-glucosidase inhibition by *M. citrifolia*–yogurts at different refrigerated (4^{o}C) storage periods.

Extended refrigerated storage resulted only in minimal decrease in IC_{50} for all *M. citrifolia*-yogurts. The lowest IC_{50} on day 21 were similar for all *M. citrifolia*–yogurts (97 to 108mg/g) which were lower than plain-yogurt (179.14±2.54mg/g; $p<0.05$).

Table 4.24 : IC_{50} (mg/g) values of α-glucosidase inhibition by *M. citrifolia*-yogurts and plain-yogurt.

	Day 0	Day 7	Day 14	Day 21
y-plain	347.45±6.52	247.43±4.65	211.64±2.67	179.14±2.54
yMC1	192.37±5.11	184.36±2.25	138.52±2.61	108.21±3.14
yMC5	156.47±3.54	135.84±3.58	104.68±2.55	104.52±3.18
yMC10	124.17±4.15	126.28±1.38	101.53±2.33	97.56±3.17

4.7 Correlation between TPC and plant biological activities

Table 4.25: Regression values of correlation between TPC and antioxidant activity, α-amylase and α-glucosidase.

	Plain-yogurt	*O. stamineus*-yogurt	*C. asiatica*-yogurt	*M. citrifolia*-yogurt
TPC vs Antioxidant	0.00	0.33	0.00	0.09
TPC vs α-amylase	0.20	0.37	0.60	0.72
TPC vs α-glucosidase	0.23	0.61	0.49	0.68

Linear regression is used to describe the relationship between two quantitative variables (x and y). Correlative studies were carried out to compare between TPC and three biological activities i.e. antioxidant activities and inhibition of α-amylase and α-glucosidase. Good correlations were found to exist between TPC and inhibition of enzymes but not between TPC and antioxidant (Table 4.1). Amongst the herbal yogurts, *M. citrifolia*-yogurt showed the strongest correlation between TPC and α-amylase (r^2 = 0.72) followed by *C. asiatica*-yogurt (r^2 = 0.60) and *O. stamineus*-yogurt (r^2 = 0.37). For TPC and α-glucosidase, both *M. citrifolia*-yogurt (r^2 = 0.68) and *O. stamineus*-yogurt (r^2 = 0.61) showed stronger correlation than *C. asiatica*-yogurt (r^2 = 0.49).

4.8 Sensory assessment

Table 4.26 : Organoleptic properties score for fresh (0 day) *O. stamineus*-yogurt, *C. asiatica*-yogurt, *M. citrifolia*-yogurt and plain-yogurt on the visual appearance, body & texture, aroma, sweetness, sourness and overall taste scores (mean±SE). All scores were evaluated on a 1 to 10 scale.

	Visual Apppearance	Body & Texture	Aroma	Sweetness	Sourness	Overall Taste
yOS5	5.40±0.05^{a}	5.04±0.39^{a}	3.77±0.42^{a}	2.36±0.30^{a}	4.36±0.66^{a}	3.22±0.37^{a}
yCA5	5.18±0.46^{a}	5.63±0.47^{ab}	4.31±0.36^{a}	2.50±0.31^{ab}	6.63±0.54^{a}	4.45±0.41^{ab}
yMC5	6.00±0.54^{ab}	4.90±0.47^{a}	3.86±0.39^{a}	2.60±0.30^{ab}	4.86±0.63^{a}	3.59±0.44^{a}
y plain	6.90±0.32^{b}	6.68±0.31^{b}	5.59±0.43^{b}	3.45±0.40^{b}	7.18±0.44^{b}	5.45±0.46^{b}

*****Data are presented as means of n= 16. Means with different superscripts within a column were significantly different (P<0.05). Herbal-yogurt was made by incorporating 5% v/v herbal water extract.

Herbal-yogurts showed lower ($p<0.05$) score than plain-yogurt for all characteristics assessed (Table 4.2). The presence of *O. stamineus* and *C. asiatica* reduced yogurt visual appearance (5.40±0.05 and 5.18±0.46 respectively) compared to plain-yogurt (6.90±0.32; $p<0.05$). Body and texture scores were lower for *O. stamineus*-yogurt and *M. citrifolia*-yogurt (5.04±0.39 and 4.90±0.47 respectively) than in y-plain (6.68±0.31; $p<0.05$). *O. stamineus*-yogurt scored lower values ($p<0.05$) than plain-yogurt for all organoleptic properties. *C. asiatica*-yogurt was different ($p<0.05$) from plain-yogurt only for visual appearance, aroma and sourness (5.18±0.46, 4.31±0.36 and 6.63±0.54 respectively). *M. citrifolia*-yogurt on the other hand, was different ($p<0.05$) from plain-yogurt for body and texture, aroma and sourness (4.90±0.47, 3.86±0.39 and 4.86±0.63 respectively).

CHAPTER 5: DISCUSSION

The presence of herbal water extract in yogurt has tremendous effects on the growth and metabolism of yogurt bacteria and yogurt quality. This can be ascertained from the changes in acidification of yogurt, yogurt bacteria counts as well as eating and functional values as a result of the addition of herbs (*O. stamineus, C. asiatica* and *M. citrifolia*). This chapter attempts to discuss the changes taken place in yogurt due to herbal water extracts and subsequently consider the potential benefits of herbal-yogurts for consumers with special health concern.

5.1 The effects of herbal water extract on the changes of yogurt pysiochemical properties.

5.1.1 The changes of pH and TA in herbal-yogurts

Acidification is the main metabolic process that takes place during milk fermentation and this can be monitored by measuring the pH and titratable acidity (TA). Lactic acid, citric acid, pyruvic acid, succinic acid, formic acid, acetic acid, propionic acid and butyric acid are several organic acids produced by the lactic acid bacteria (LAB) as a result of degradation of glucose and galactose (Thomas & Crow, 1984) during energy generation for microbial growth (Donkor, Henriksson, Vasiljevic, & Shah, 2006; Ostile, Treimo, & Narvhus, 2003). In addition, hydrolysis of milk protein by the proteinases produced by the LAB is an important source of amino acids to support yogurt bacteria proliferation. The pH and the organic acid content in yogurt are inversely associated to each other i.e. when the level lactic acid content increases, pH levels correspondingly decrease during the fermentation (Lourens-Hattingh & Viljoen, 2001). Therefore any changes in the rate of pH

reduction and elevation of TA during fermentation or storage would implicate effects herbs on microbial growth and metabolism (Prajapati and Dave, 1994).

Fermentation of yogurt was terminated at pH 4.5 as this is the best pH value for commercial yogurt (Hamann & Marth, 1983) and to prevent the growth of pathogenic organism (Micanel, Haynes, & Playne, 1997). The presence of *O. stamineus*, *C. asiatica* and *M. citrifolia* extracts reduced the pH further to between 4.04 and 4.23 by day 7 of storage. This was associated with the increased of TA during storage. Different phytochemical contents in the herbs may have influenced post-acidification to varying degree during storage in the same manner as the effects of fruits on yogurt during storage (Kailsapathy, Harmstorf, & Philips, 2007). This could occur by the phytochemical modulation of growth and activity of *S. thermophilus* (Kailasapathy *et al.,* 2007) and other LAB (Prajapati & Dave, 1994) which are responsible of pH decline during refrigerated storage.

5.1.2 The addition of herbal extract on the total phenolic content in yogurt

Phenolic compounds are secondary metabolites in plants, fruits and vegetables several of which are essential in human and animal diets (Bravo, 1998; Crozier *et al.,* 2000; Vattem, Ghaedian, & Shetty, 2005). The phenolic compounds may consist of flavonoids, cinnamic acid derivatives, coumarins, tocopherols and polyfunctional organic acid groups (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993). The inclusion of herbal extract in yogurt resulted in higher TPC with *O. stamineus*-yogurt (day 14, yOS10, 37.2±2.71µ/ml) showing the highest value followed by *C. asiatica*-yogurt (day 14, yCA10, 21.4±1.41 µ/ml), *M. citrifolia*-yogurt (day 14, yMC10, 19.3±2.0 µ/ml) and plain-yogurt (day 14, 14.9±1.55 µ/ml).

The TPC values in plain-yogurt, despite an absence of plant extracts, reflect the phenolic nature of proteins and amino acid with benzene rings.

Plants have a potential in protecting themselves from microbial attack (pathogen) and resist to biotic and abiotic stresses with the presence of phenolic compounds (Karaaslan, Ozden, Vardin, & Turkoglu, 2011). In herbal-yogurts, phenolic compounds may protect the probiotic growth and may sustain its viability during the refrigerated storage. These compounds are commonly used in the food industry including dairy sector by the addition of fruit juices (Coisson, Travaglia, Piana, Capasso, & Arlorio, 2005) rich in phenolics. Phenolic compounds are favored due to their antioxidant, anti-inflammatory, anti-mutagen, and anti-clotting powers which have been correlated with a declined risk of cardiovascular diseases and cancer development (Fresco *et al.,* 2010). The higher phenolic compound found in the herbal-yogurts may also contribute in the same manner and future studies need to be carried out to identify the specific phenolic compounds responsible for these properties.

5.1.3 Effects of herbal extract on the antioxidant capacity in yogurt

As addressed in previous section, an immediate positive attribute of the addition of herbs into yogurt is the fortification of yogurt with phenolic compounds. This potentially increases the value of yogurt because most phenolic compounds have antioxidant activities. Apart from flavoring, this is one of the reasons why herbs are added to other milk products such as cheese to make it more “functional” (Coisson *et al.,* 2005) and also possibly to extend the product’s shelf life (Coppen, 1994). Antioxidants can neutralize the oxidizing effects of free radicals by donating

electrons (Sherwin, 1978) and thus the wide use of antioxidants in the food industry to delay the oxidation process (Berset, Brand-Williams, & Cuvelier, 1994). Antioxidant activity in the yogurt is not only limited to phenolic compounds because the presence of different antioxidant components in the extracts such as sugar, organic acid and milk protein proteolysis (Lourens-Hattingh & Viljoen, 2001) may also function as hydrogen donor.

In the present study *O. stamineus*-yogurt, *C. asiatica*-yogurt and *M. citrifolia*–yogurt showed higher capacity to inhibit DPPH oxidation than plain-yogurt (Figure 4.4, 4.5 and 4.6). The phytochemicals in the herbal water extract used *O. stamineus* (Akowuah, Ismail, Norhayati, & Sadikun, 2005), *C asiatica* (Zainol, Abdul-Hamid, Yusof, & Muse, 2003) and *M. citrifolia* (Yang, Paulino, Janke-Stedronsky, & Abawi, 2007), have by themselves, the antioxidant activities. DPPH inhibition attribute to yogurt is derived from milk protein proteolysis (Lourens-Hattingh & Viljoen, 2001) and organic acids (Correia *et al.,* 2004) resulted from fermentation and post-acidification during storage. Thus, the higher inhibition of antioxidant by herbal-yogurts than by plain-yogurt could either be due to herbal water extract or increased production of antioxidants from fermentations or both.

Despite phenolic compounds in herbs having potential antioxidant properties, both were shown not to be well correlated with each other (Table 4.25; Cai, Luo, Sun, & Corke, 2004; Akowuah *et al.,* 2004). The DPPH radical-scavenging assay determined free antioxidants in *M. citrifolia* product, whereas the assay of total phenolics with Folin-Ciocalteu reagents determined both free phenolics and bound phenolics in *M. citrifolia* product (Singleton, 1999). Therefore, the bound of antioxidant in *M. citrifolia* may not contribute in the free-radical-scavenging activity (RSA) in the DPPH assay (Yang *et al.,* 2007).

5.2 The viability of *Lactobacillus* ssp. and *S. thermophilus* in herbal-yogurts

The growths of microorganism are influenced by several factors including moisture, oxygen concentration, temperature, nutrients, pH and inhibitors (Mountney & Gould, 1988). The viability of specific bacteria in nutrient rich food, for example probiotic bacteria in yogurt, is affected by more defined factors such as acidity, pH, hydrogen peroxide (Dave & Shah, 1996), oxygen content (Ishibashi & Shimamura, 1993), concentrations of lactic and acetic acids and temperature of storage during both the fermentation and storage of yogurt (Lankaputhra & Shah, 1995; Lankaputhra, Shah & Britz, 1996). Probiotic should have three characters i.e. able to inhabit the gastrointestinal tract, survive through the stomach and maintain its viability and metabolic activity in the intestine (Hyun, & Shin, 1998). Extended storage period can affect the viability of *L. acidophilus* and the best shelf-life for yogurt storage at 4^{o}C is considered to be between 20-40 days (Dave & Shah, 1997; Nighswonger, Brashears, & Gililand, 1996; and Gilliland & Speck, 1977).

In the present study, the viability of probiotic in herbal-yogurts during storage was taken into consideration because they should remained high at the acceptable level (Heller, 2001) despite being exposed to herbal extracts known to possess antibacterial activities. Most strains of *L. acidophilus* do not survive well in fermented milk during storage because post-acidification lowers the pH further (Shah, 2007), and it is difficult to maintain high viable cell numbers in relatively more acidic environment.

The viability of *Lactobacillus* spp. and *S. thermophilus* in herbal-yogurts in the present studies remained high (10^6 to 10^7) throughout the storage period which qualifies the products as 'live yogurt' (Dave & Shah, 1997). There is no agreement on the minimum concentration of probiotics to achieve therapeutic benefit (Donkor *et al.*, 2006). While some researchers suggest concentration levels above 10^6cfu mL^{-1} (Kurmann & Rasic, 1991) others stipulate $>10^7$ and 10^8cfu mL^{-1} as satisfactory levels (Davis, Ashton, & McCaskill, 1971; Kailasapathy & Rybka, 1997; Lourens-Hattingh & Viljoen, 2001). It can be concluded from the present studies that the presence of herbs did not significantly reduce the viability of yogurt bacteria.

Several ways to improve the survival of probiotics presence in yogurt have been suggested. The addition of inulin is able to improve the probiotic viability (Capela, Hay & Shah, 2006 & Ozer, *et al.*, 2005). The inclusion of exopolysaccharide (EPS) showed some improvement in the survival of *L. acidophilus* until day 21 of storage (Ramchandran & Shah, 2010). EPS is very sufficient to protect the microbial cells against phagocytosis, phage attacks, antibiotics, toxic compound, osmotic stress and bacteriocins which effectively reduce microbial death during yogurt fermentation (Durlu-Ozkaya, Aslim, & Ozkaya, 2007; Ruas-Madiedo & de los Reyes-Gavilan, 2005). The production of EPS by LAB in herbal-yogurts was not investigated in the present studies but it is possible that LAB produced more EPS in the presence of more 'challenging' environment produced by the herbal extracts.

5.3 *In vitro* inhibition of α-amylase and α-glucosidase.

Type 2 Diabetes (T2D) is a metabolic disease characterized by hyperglycemia resulting from a defect in insulin secretion or insulin action or both. The craving for food rich in carbohydrate and simple sugars is one of the causes for diabetes. The therapeutic approach of treating T2D is to decrease the postprandial hyperglycemia by inhibiting carbohydrate-hydrolysing enzymes (α-amylase and α-glucosidase) thus reducing the rate of glucose absorption from intestine into blood (Bhandari, Nilubon, Gao, & Kawabata, 2008).

Acarbose and miglitol, synthetic diabetes drugs, are used conventionally as glucosidase inhibitors but these medicine are responsible for side effects such as abdominal pain, flatulence and diarrhea in the patients (Fujisawa, Ikegami, Inoue, Kawabata, & Ogihara, 2005). Thus more effective and safe inhibitor of α-amylase and α-glucosidase are required (Bhandari, Nilubon, Gao, & Kawabata, 2008). In the present studies it was found that the inhibition of α-amylase and α-glucosidase by yogurt can be enhanced by three different plant water extracts. Manipulation of food capacity to inhibit α-amylase and α-glucosidase opens up additional possibilities for the control of gastrointestinal carbohydrate digestion and thus the rate of glucose absorption into the systemic circulation.

5.3.1 *In vitro* inhibition of α-amylase by herbal-yogurts

Amylase is present in both salivary and pancreatic secretion (Ramasubbu *et al.,* 2004) and is responsible for cleaving large malto-oligosaccharides to maltose, a substrate for intestinal α-glucosidase (Loizzo *et al.,* 2008). The consumption of yogurt was shown beneficial to those with diabetes by virtue of the anti-amylase activities by this milk product (Salminen, Gueimonde, & Isolauri, 2005). *O. stamineus*-yogurt, *C. asiatica*-yogurt and *M. citrifolia*-yogurt showed more pronounced inhibition on α-amylase activity than could plain-yogurt (Figures 4.9, 4.11 and 4.13). This finding indicates that the therapeutic benefits gain from yogurt (Chowdhury, Chakraborty, & Raychaudhuri, 2008) may be further enhanced by the addition of certain herbal extracts.

The inhibitory effect of herbal-yogurts on α-amylase could be explained by the presence of phytochemicals in the plant water extracts used. The total phenolic content showed good correlations with α-amylase inhibition, with the strongest for *M. citrifolia*-yogurt followed by *C. asiatica*-yogurt, *O. stamineus*-yogurt and plain-yogurt (0.72, 0.60, 0.37 and 0.20 respectively). Phytochemicals may consist of hundreds of compound which may categorized as natural monophenols, flavonoids, phenolic acids, hydroxycinnamic acids, lignans, tyrosol esters and stilbenoids. *O. stamineus* (Adisakwattana *et al.,* 2010), *C. asiatica* (Adisakwattana, et al., 2010; Odhav, Kandasamy, Khumalo, & Baijnath, 2010) and *M. citrifolia* (Martirosyan, 2009) extracts by themselves can inhibit α-amylase activity and these plants are widely used to help diabetic patients manage their blood glucose. The phenolic compounds were reported capable of playing a role in mediating amylase inhibition

(McCue & Shetty, 2004). Further analysis need to be carried out to identify the specific phenolic compound responsible for the inhibition of α-amylase.

5.3.2 *In vitro* inhibition of α-glucosidase by herbal-yogurts

All herbal-yogurts had higher inhibitory effects on α-glucosidase compared to plain-yogurt. The inhibitory effects of herbs on α-glucosidase were found to be greatest in *C. asiatica*-yogurt followed by *M. citrifolia*-yogurt and *O. stamineus*-yogurt. The inhibition of α-glucosidase activity may be related to the presence of the plant phenolic compounds since there is some correlation between α-glucosidase inhibition (IC_{50}) and total phenolic content of herbal-yogurts (r^2 = 0.61, 0.49, 0.68 for *O. stamineus*-yogurt, *C. asiatica*-yogurt and *M. citrifolia*-yogurt respectively) as opposed to plain-yogurt (r^2 =0.23).

As previously discussed for α-amylase, this highlights the potential use of selected herbs to enhance the therapeutic properties of yogurt for patients with diabetes. This is because α-glucosidase, an enzyme release by the small intestine, cleaves di- and oligosaccharides into monosaccharide glucose prior to its absorption into the blood circulation (Kawabata, Gao, Huang, & Xu, 2007). α-glucosidase inhibitors such as acarbose and miglitol, being modified saccharides, exert their effects through competitive and reversible inhibition of intestinal α-glucosidase enzyme (Vadivel & Biesalski, 2011). The delay of glucose absorption by α-glucosidase inhibition may thus reduce the hyperglycemia impact of an otherwise fast carbohydrate digestion. The apparent presence of α-glucosidase inhibitors in the plants used in the

present studies offer safe and potentially fewer side effects commonly attributed to inhibitory effects of natural plant compounds (McCue & Shetty, 2004).

5.4 Organoleptic properties of herbal-yogurts

The evaluation of sensory properties of herbal-yogurts is important to know consumer preference and for marketing purposes. The organoleptic properties of yogurt are correlated to several factor including TA content (Guler & Multu, 2005) rheology, viscosity and firmness. During fermentation, *S. thermophilus* main products metabolism are lactic acid, acetic acid, acetaldehyde, ethanol and diacetyl (Ozer, Kirmaci, Oztekin, Hayaloglu, & Atamer, 2007) which all contributed to the sourness of fermented yogurt (Hugenholtz and Kleerebezem, 1999). The presence of acetaldehyde contributed to yogurt aroma (Pette & Lolkema, 1950). The concentration of these metabolites were not measured in the present studies but the formation of these compounds may be altered by the added herbal extract during in yogurt fermentation as demonstrated for lemon yogurt (Boeneke & Aryana, 2008).

Plain-yogurt scored better for all sensory parameters compared to herbal-yogurts (Table 4.26). The phytochemicals in the herbs used can be considered to play important role in causing poor organoleptic properties of herbal-yogurts. Herbs contain a diverse range of metabolites which may responsible for their unique taste and flavor (Rivasseau *et al*, 2006). For example, ripe *M. citrifolia* fruit emanates a strong byturic-acid-like rancid smell (Morton, 1992; Dixon *et al*. 1999) which originates from hexanoic and octanoic acid (Matsuo, Sugaya, Yasukawa, Aigaki, & Fuyama, 2007). The presence of tannin in *O. stamineus* extract (Chew *et al.,* 2011) can affect the astringency and bitterness (Takeda, 1994). In addition, *O. stamineus*

also contains lipophilic flavones, flavonol glycosides and caffeic acid derivatives such as rosmarinic acid and 2,3-dicaffeoyltartaric acid (Sumaryono *et al.,* 1991). In previous studies, *C. asiatica* was shown to contain γ-terpinene and *β*-pinene. The presence of γ-terpinene contributes bitter flavor (Wongfhun, Gordon, & Apichartsrangkoon, 2010) whereas *β*-pinene gives rise to plastic and pine-like aroma (Wongfhun, Gordon, & Apichartsrangkoon, 2010). Other compounds contain in *C. asiatica* are β-caryophyllene, humulene, *E*-β-farnesene, α-copaene, β-elemene, and alloaromadendrene (Wongfhun, Gordon, & Apichartsrangkoon, 2010) but the effects of these compounds on the taste flavored have not been studied. It is not possible from the studies to associate which metabolites are responsible for the low organoleptic score of herbal yogurt. Therefore, future studies are required to establish possible relationship between these compounds and organoleptic properties of herbal-yogurts.

5.5 Implication of findings

The presence *O. stamineus, C. asiatica* and *M. citrifolia* extracts were shown to stimulate the acidification process during yogurt fermentation, enhanced bacterial growth and resulted in higher yogurt *in vitro* inhibition of α-amylase and α-glucosidase activities. These changes may be regarded as highly desirable because the proportionately more milk lactose catabolized, higher bacterial counts and enhanced inhibition of diabetic enzymes make herbal-yogurts better than plain-yogurt with respect to nutritional and functional properties.

Functional food products are relatively new to Malaysian consumers. The eating of yogurt as functional food is now seen as very trendy largely because of the

association of milk consumption with affluence and wellness. Most yogurt products in the market are currently made to appeal to the healthy bacteria it contains and other essential nutrients such as calcium, folate and vitamin D. The proposition of herbal-yogurt as a functional food should therefore be unique and relate to its herbal taste, health benefits, probiotic survival and stability of the product during storage.

One of the challenges in making herbal-yogurt more appealing to consumers is to improve its organoleptic properties. All herbal-yogurts in the present studies showed less organoleptic scores than plain-yogurt, probably due to the high plant phenolic and flavonoid compounds. Future studies to enhance the taste and texture of herbal-yogurt should be carried out. This is despite the fact that the higher antioxidant contents, bacterial counts and higher inhibition of α-amylase and α-glucosidase in these yogurts make organoleptic properties relatively of small issues. The addition of natural additives such as non-caloric sweeteners (e.g. stevia) (Savita, Sheela, Sharan, Shankar, & Parama, 2004) and EPS-producing LAB to prevent whey separation thus enhancing the rheology, texture and mouth feel (Jolly, Sebastien, Duboc, & Neeser, 2002; Welman & Maddox, 2003) are examples of approaches to improve herbal-yogurt organoleptic properties.

Efforts in making herbal-yogurt more acceptable for the organoleptic points of view stem from three encouraging health and wellness developments: an ever increasing trend in the consumption of a) yogurts and b) herbal products and c) the continuing search for natural, and safe with little side effects treatment for diabetes. The trend for yogurt and yogurt drink consumption in Malaysia is on the rise. This phenomenon is in line with those in the developed countries (Jones & Jew, 2007). Yogurt market in Malaysia for the year 2010 generated revenues of $79.2 million with yogurt drinks sales proved as the most favourite for Malaysian yogurt market

because the sale for this sector alone was $70.9 million (Datamonitor, 2010). Malaysian spent about RM91/person/year on herbal product compared to RM45/person/year for the American (Hussin, 2001). In fact there is an increasing shift away from the use of conventional medicines to natural substances like herbs is associated with the belief and accumulated evidence that many herbal products are safer than synthetic substances (Hussin, 2001; Han, Abas, & Sabariah, 2008).

From the abovementioned deliberations, it is anticipated that herbal-yogurts have the potential in the near future to be accepted as main-stream yogurt products, especially after the issues related to current apparently low organoleptic properties have been addressed.

5.6 Conclusion

The presence of *O. stamineus, C. asiatica* and *M. citrifolia* changed yogurt fermentation. The herbal extracts sustained the viability of probiotic, *Lactobacillus* spp. throughout refrigerated storage. *O. stamineus*-yogurt, *C. asiatica*-yogurt and *M. citrifolia*-yogurt formed enhanced the inhibition of α-amylase and α-glucosidase activity *in vitro.*