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Influence of various health beneficial spices on some characteristics of yogurt culture bacteria Lactobacillus acidophilus, and sensor acceptability of spicy probiotic yogurt

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INFLUENCE OF VARIOUS HEALTH BENEFICIAL SPICES ON SOME
CHARACTERISTICS OF YOGURT CULTURE BACTERIA AND
LACTOBACILLUS ACIDOPHILUS, AND SENSORY ACCEPTABILITY
OF SPICY PROBIOTIC YOGURT

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by
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For my parents: René Soto Torres and Margie Vega Braña

I would thank you from the bottom of my heart, but for you my heart has no bottom.

~Author unknown~

For my "baby sister": Karina Alejandra Sánchez Prado

May this work serves as an inspiration to you, so you know that nothing is impossible if

you work hard and set your mind to it.

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ABSTRACT

There is a pronounced public awareness about herbal remedies. Garlic and ginger have antibacterial properties and prevent cardiovascular diseases. Onion and turmeric decrease the risk of diabetes and like garlic, they have anticancer properties. *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* are lactic acid bacteria that produce lactase and reduce the symptoms of malabsorption. Earlier work has shown the influence of spice extracts but the influence of pure spice juice on yogurt culture bacteria is not known. Characteristics of yogurt culture bacteria were measured by suspending freshly thawed cultures in 0.1% peptone water (growth), acidified MRS broth (acid tolerance), MRS-Thio broth with oxgall (bile tolerance) and skim milk (protease activity) with 1% (v/v) of freshly extracted spice juice. Control samples had no spice juice. A probiotic blueberry yogurt was made with 0.05% of individual spice juice. Physico-chemical characteristics of the three bacterial cultures used were determined and a consumer acceptability test was conducted. Results show that these 4 spices did not have an inhibitory effect on the growth of the three culture bacteria. Turmeric improved the protease activity of *L. bulgaricus* and alongside with ginger, it also improved the protease activity of *L. acidophilus*. All four spices decreased the pH of the spicy yogurt. Coliform bacterial growth was significant on turmeric yogurt at day 1 of storage when compared to control and the other spices. *Streptococcus thermophilus* grew better in garlic and ginger yogurt, while *L. bulgaricus* grew better in onion and turmeric yogurt. Color measurements showed a decreased lightness (L*) from all spices, red color space values for the red-green axis (a*), turmeric in the yellow color space and the rest of the spices in the blue color space for the blue-yellow axis (b*).

Apparent viscosity was higher in onion and ginger yogurt. The consumer testing showed a well acceptance of the control and ginger yogurt. Ginger had the highest intent of purchase by consumers. Ginger can be used in yogurts for direct consumption while all 4 spices have potential for a new product line of yogurts for cooking and dips, enabling potential health benefits from both sources.

CHAPTER 1: INTRODUCTION

1.1. Spices

Medicinal plants and herbs are prevalent in the world. Many of these plants are the source of isolation and synthesis of conventional drugs (Lalitha *et. al.* 2009). In the traditional Asian system of medicine, more than 2,000 plant species are known to possess medicinal value. (Agnese *et. al.* 2001).

There is a great interest in the use of herbal remedies. Herbal medicine is based on the principle that plants contain natural substances that can support health and improve diseases (Craig 1999). Spices constitute an important group of herbal goods that have been utilized by people in many countries as tools for alternative, integrated or complementary medicine (Singh *et. al.* 2004). Spices and condiments[1], are utilized by many cultures as traditional methods to improve the nervous system, increase energy, relieve headache due to stress or the common cold, help digestion, and against many other conditions (Purthi 1998, Uhl 2000, Miller and Begona Ruiz-Larea 2002). The healing properties of medicinal plants are caused by the presence of various complex chemical substances of different composition, which occur as secondary metabolites (Karthikeyan *et. al.* 2009, Lozoya and Lozaya 1989). These secondary metabolites are grouped as corticosteroids alkaloids, flavonoids, glycosides, coumarin, and essential oils (Lalitha *et. al.* 2009). Besides these natural products, spices produce certain enzymes that clear carcinogens, block estrogen, prevent cholesterol synthesis, lower blood pressure and prevent blood clotting, among other beneficial effects (Dey and Harborne 1989, Purthi 1998).

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[1] Something used to give a special flavor to food, usually a mixture of spices, salt or other seasonings. (Source: Random House Dictionary)

1.1.1. Garlic

One of the most widely researched medicinal plants and spice that has been used as both medicine and food in many civilizations for more than 400 years is garlic (*Allium sativum*). The *Codex Ebers*, dating to about 1550 B.C is an Egyptian medical papyrus that gives more than 800 therapeutic formulas, of which 22 of them mention garlic as an effective remedy for a variety of disorders including headache, heart problems, tumors, bites and worms (Milner 1996).

Afzal *et. al.* (2000) provides us with a brief background of the use of garlic in ancient times:

> Aristotle, Hippocrates and Aristophanes recommended garlic for its medicinal effects. The Roman naturalist Pliny the Elder cited numerous therapeutic uses for both garlic and onions. Dioscorides, a chief physician to the Roman army in the first century A.D., prescribed garlic as a vermifuge or expeller of intestinal worms. During the first Olympic Games in Greece, garlic was ingested by athletes as a stimulant. The antibacterial properties of garlic were reported as early as 1858 by Louis Pasteur. In India, garlic has been used for centuries as an antiseptic lotion for washing wounds and ulcers.

According to Gupta and Singhvi (2011), in the early 18th century France, gravediggers drank a mixture of crushed garlic in wine that they thought would protect them from the plague that killed many people in Europe. They also mention that during World Wars I and II, soldiers consumed garlic to prevent gangrene.

The uses of garlic with insecticidal, antimicrobial, antiprotozoal and antitumor activities have been researched by many workers with great interest (Moore and Atkins 1977; Dankert *et. al.* 1979; Elnima *et. al.* 1983; Nock and Mazelis 1986). In traditional Chinese, Islamic, folklore medicine and the Ayurvedic system of medicine, several herbs and spices, including garlic, are described as possessing medicinal properties such as antithrombotic, hypolipidaemic and anti-arthritic effects, among other properties

(Makheja and Bailey 1990). In the homeopathic system of medicine, garlic is also an effective remedy for many aliments. In China, garlic and onion tea have long been recommended for fever, headache, cholera and dysentery (Afzal *et. al.* 2000).

Timbo *et. al.* (2006) mention as part of their research that, according to the US Food and Drug Administration survey of 900 people, for the year 2006, garlic stood as the second most utilized supplement (behind Echinacea), with almost 17% of the population using garlic supplements in the preceding 12 months.

1.1.1.1. Components

In accordance to what Castleman (1991) mentions in his book *"Las hierbas que curan"* ("The healing herbs"), the compound with antibiotic properties present in garlic was a mystery until the 1920's, when researchers from the Sandoz Laboratories in Switzerland isolated aliin. He further mentions that this compound by itself does not have any medicinal property, but when garlic is chewed, cut, crushed or mashed, aliin gets combined with an enzyme present in garlic known as alinase, which is transformed in another chemical substance known as allicin, which is then the compound with antibiotic properties.

According with Antlsperger *et. al.* (2003), garlic also contains the sulfur-containing compound ajoene, diallylsulfide, dithiin, S-allylcysteine and enzymes, minerals, B vitamins, proteins, saponins, flavonoids and Maillard reaction products, which are not sulfur-containing compounds. They further mention that it has been shown that ajoene activates signal pathways that may have anti- and pro-apoptotic[2] effects in cells.

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[2] Apoptosis: programmed cell death. (Source: Merriam-Webster Dictionary)

Furthermore, a phytoalexin (allixin) was found, which is a non-sulfur containing compound with antioxidant, antimicrobial, neurotrophic and antitumor promoting effects and that also inhibits aflatoxin B2 DNA binding (Kodera *et. al.* 1989, Yamasaki *et. al.* 1991).

1.1.1.2. Medicinal Properties and common uses

The known medicinal benefits of garlic and its constituents include inhibition of platelet aggregation, the lowering of serum cholesterol level and increased fibrinolysis (Agarwal 1996, Rahman 2001), stimulation of immune function through activation of macrophages and induction of T-cell proliferation (Lau *et. al.* 1991, Lamm and Riggs 2000). Other attributes include reduction of blood glucose levels (Sheela *et. al.* 1995, Augusti and Sheela 1996), radioprotection (Singh *et. al.* 1995), improvement of memory and learning deficit (Moriguchi *et. al.* 1996, Nishiyama *et. al.* 1997), protection against microbial, viral and fungal infections (Cellini *et. al.* 1996, Avato *et. al.* 2000, Guo *et. al.* 1993), and anticancer effects (Milner[a] 2001, Thompson and Ali 2003).

While the science is not definite, research shows promise for garlic in the areas of cancer protection and heart-related risk factors for patients (Phalke and Ravindra 2010). According to the University of Maryland-Medical Center, (UM-MC[a] 2011), garlic is rich in antioxidants, which help destroy free radicals. The antioxidant agents in garlic are reported to be its sulfur-containing compounds (Kourounakis and Rekka 1991, Prasad *et. al.* 1995).

When garlic is used to prevent cardiovascular diseases, this one may help decrease LDL (bad cholesterol) and total cholesterol levels while raising the HDL (good cholesterol). It

also lowers the aggregation of platelets to help the blood flow more easily, and it decreases the blood pressure as well (Rivlin 2006).

Garlic is also thought to be beneficial against the common cold. The base for this belief is traditional use and some laboratory findings that serve as evidence that garlic has antiviral and antibacterial properties. A study of 146 participants done by Lissiman *et. al.* (2009) found that people who took garlic every day for three months (instead of a placebo) had fewer colds. When these participants suffered from a cold, the length of the illness was similar in both groups (4.63 versus 5.63 days). While this study was encouraging, there is a need for randomized controlled trials to support these findings. Skin rash and odor were included in the possible side effects in this small trial.

Animal and *in vitro* studies have provided evidence of an anticarcinogenic potential of several bioactive compounds in garlic (Wargovich *et. al.* 1996). Garlic is rich in flavanols, mainly kaempferol, which aids in the detoxification of carcinogenic compounds (Bilyk and Sapers 1985, Hertog *et. al.* 1992). Preclinical studies with cancer models appear to provide some of the most compelling evidence that garlic and related sulfur constituents can suppress cancer risk and alter the biological behavior of tumors (Milner[b] 2001). Diallyl, which is a sulfur-containing compound, have anticarcinogenic effects that have been demonstrated in animals (Reddy *et. al.* 1993). According to Kaschula *et. al.* (2010), ajoene, one of the components of garlic, possesses a broad spectrum of biological activities that include anticancer activity. In accordance with their findings, it's cytotoxicity towards cancer cells is believed to occur via an apoptotic

mechanism involving activation of the mitochondrial-dependent caspase[3] cascade. Kaschula *et. al.* (2010) have also found that the substitution of the terminal end allyl groups in ajoene for alkyl, aromatic, or heteroaromatic groups produces some analogs with superior *in vitro* anticancer action to ajoene, opening up the way to developing ajoene-based anticancer agents. Thus, the chemoprotective action of garlic is well recognized, and it has been observed that individuals who regularly consume large amounts of garlic (~20 grams or more per day) are less susceptible to cancer than those with a low intake, particularly in the case of gastric or intestinal cancers (Ernst 1997, Iscovich *et. al.* 1992, Dausch and Nixon 1990, Steinmetz *et. al.* 1994, You *et. al.* 1989, Dorant *et. al.* 1996).

Garlic also possesses neuroprotective abilities that have been tested *in vitro* (Peng *et. al.* 2002). According to these studies, aged garlic has been looked to for multiple benefits that some researchers believe may address a number of underlying mechanisms that contribute to the classic Alzheimer syndrome. According to Chauhan (2006), "garlic is expected to produce cumulative benefits and enhanced neuroprotection by virtue of being a natural statin, natural NSAID, natural antioxidant, natural anti-apoptotic agent and a memory enhancer", a combination used often in synthetic pharmaceutical drugs used currently for Alzheimer's therapy, but with less adverse effects. In accordance with Bongiorno *et. al.* (2008), "given the multiple-mechanistic possibilities and minimal risk associated with its use, garlic seems a prudent recommendation for prevention and treatment of Alzheimer's and since aged garlic is best studied in relation to this syndrome, it may be the best form to employ it".

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[3] Caspases are a family of cysteine proteases that play essential roles in apoptosis (programmed cell death), necrosis (tissue death), and inflammation. (Alnemri *et. al.* 1996)

Garlic can also be used to treat parasites. The antiparasitic effects of freshly crushed garlic were known by many ancient Asian cultures. Freshly crushed garlic has been used to treat people suffering from dysentery or intestinal worms (Ankri and Mirelman 1999). Mirelman *et. al.* (1987) found that the human intestinal protozoan parasite *Entamoeba histolytica*, is very sensitive to allicin, one of the components of garlic. According to his unpublished results, Mirelman also found that allicin also efficiently inhibited the growth of other protozoan parasites such as *Leishmania major, Giardia lamblia*, *Crithidia fasciculate* and *Leptomonas colosoma*.

According to Castleman (1991) and Gupta *et. al.* (2010), garlic is capable of killing the bacteria responsible for tuberculosis (*Mycobacterium tuberculosis*), and can also prevent an infection with the influenza virus. Furthermore, in his book "*Las hierbas que curan*" ("The healing herbs"), Castleman (1991) mentions that garlic has been used successfully to treat criptococcal meningitis, athlete's foot infections by means of killing the *Trichophyton mentagrophytes* fungi and vaginal yeast infections by means of killing the *Candida albicans* yeast. Also, during *in vitro* testing, garlic extracts were found to be effective against a broad spectrum of Gram-negative and Gram-positive bacteria (Fenwick and Hanley 1985). The inhibitory effects of garlic extract have been observed against *Helicobacter pyroli* and *Pseudomonas aeruginosa* (O'Gara *et. al.* 2000, Tsao and Yin 2001, Sivam *et. al.* 1997).

Other less common properties of garlic include anti-allergic properties (Usui and Suzuki 1996) and effectiveness against memory loss (Moriguchi *et. al.* 1994).

1.1.2. Ginger

A trend toward use of non-chemical drugs and complementary therapies has developed in the last few decades (O'Brien and Zhou 1995). One of the proposed herbal remedies is ginger provided in capsule form (Ozgoli *et. al.* 2009). Ginger (*Zingiber officinale*) is a nutritional complement and is on the US Food and Drug Administration (FDA) list of safe herbal preparations (FCPG 2001). It is also on the list of herbal drugs in the WHO monograph (WHO 1999).

Shogaol and Gingerol are the effective substances in ginger that have local effects on the digestive system (Ozgoli *et. al.* 2009). Ginger has been used for thousands of years in several countries, including China (Ozgoli *et. al.* 2009), as well as a remedy in Asian, Indian and Arabic herbal traditions since long-ago (UM-MC[b] 2011). As a matter of fact, in China, ginger is believed to be an antidote for seafood intoxication and that is the main reason why Chinese seafood dishes are often seasoned and accompanied with this spice (Castleman 1991).

Castleman (1991) in his book "*Las hierbas que curan*" ("The healing herbs") gives a brief background on the evolutionary use of ginger:

> The ancient Greek adopted ginger as a digestive aid, eating it wrapped in bread after their meals. As time went by, ginger was incorporated into the bread, giving rise to the famous gingerbread. The Romans also used ginger as a digestive aid, but after the fall of Rome, this spice became scarce in Europe and became very expensive. Once the market with Asia was renewed and ginger was available again, the demand was very high. In England and their American colonies, ginger was incorporated into a beverage to calm the stomach known as ginger beer, the precursor of what is known today as ginger ale, which is still a popular house remedy for nausea, vomiting and diarrhea.

Ginger is also listed in the pharmacopoeias of the United Kingdom, Thailand, and China as an effective herb in treatment of nausea and vomiting during pregnancy (Blumenta 1998; Blumenta 2000).

1.1.2.1. Components

Ginger contains approximately 1.0% - 3.0% volatile oils and a number of pungent compounds (Chrubasik *et. al.* 2005). Gingerols are the most abundant pungent compounds in the fresh rhizome, and several gingerols of several chain lengths (n6 to n10) are present in ginger, with 6-gingerol being the most abundant (Zick *et. al.* 2008). Shogaols, the dehydrated form of gingerols, are only found in small quantities in the fresh rhizome and are mainly found in the dried and thermally treated rhizome, with 6-shogaol being the most abundant (Jolad *et. al.* 2004).

1.1.2.2. Medicinal Properties and common uses

In China, ginger has been used for headaches and common cold (Grant and Lutz 2000), to aid digestion and treat upset stomach, diarrhea (Gilani and Ghayur 2005) and nausea (Grant and Lutz 2000) for more than 2,500 years. This spice is universally reputed for its use in gastrointestinal disorders as a stomachic[4], laxative and prokinetic[5], and at the same time as an antidysenteric, antispasmodic and anticolic aid (Gilani and Ghayur 2005). In the Mediterranean (Sharma and Clark 1998) and Western parts, ginger has been used in herbal medicine practice for the treatment of rheumatological conditions, arthritis and muscular soreness (Bordia *et. al.* 1997; Langner *et. al.* 1998). Due to these properties, it has gained considerable attention as a botanical dietary supplement in the United States

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[4] A stomachic medicine is one that serves to tone the stomach, improving its function and increasing appetite. (Farlex Partner Medical Dictionary 2012)
[5] Stimulating movement or motility, such as a drug that promotes GI motility. (Mosby's Medical Dictionary, 8th edition, 2009)

and Europe through the years, and especially for its use in the treatment of chronic inflammatory conditions (Srivastava and Mustafa 1992; Kiuchi *et. al.* 1992; Srivastava 1984; Tjendraputra *et. al.* 2001; Park and Pizzuto 2002; Aggarwal and Shishodia 2004). According to Liang (1992), ginger has also been used to help treat arthritis, colic and heart conditions. Besides to these therapeutic uses, ginger continues to be valued around the world as an important cooking spice and is believed to help treat headaches, flu-like symptoms, the common cold, and even painful menstrual cramps. Liang (1992) also mentions that ginger has also been suggested for the treatment of various other conditions, including atherosclerosis, high cholesterol, ulcers, rheumatoid arthritis, migraines, depression, and impotence.

Studies in animal models have shown that ginger and its phenolic constituents (i.e., 6-gingerol) suppress carcinogenesis in the skin (Katiyar *et. al.* 1996), gastrointestinal tract (Yoshimi *et. al.* 1992), colon (Bode 2003; Manju and Nalini 2005), and breasts (Nagasawa *et. al.* 2002).

Health care professionals today commonly recommend ginger to help prevent or treat nausea and vomiting associated with motion sickness, pregnancy related nausea and vomiting, and cancer chemotherapy. Successful use of ginger in treating the nausea and vomiting of pregnancy has been demonstrated in several modern reports (Fischer-Rasmussen *et. al.* 1991; Mazzota and Magee 2000; Vutyvanich *et. al.* 2001; Keating and Chez 2002). However, there is no consistent evidence to show safe dosage and form of ginger administration during pregnancy. Although, Fischer-Rasmusen *et. al.* (1991) and Vutyavanich *et. al.* (2001) demonstrated significant improvement in pregnancy nausea and vomiting in women using capsules containing 250 mg of ginger, 4 times a day for 4

days. Keating and Chez (2002) showed a significant decrease in first-trimester nausea and vomiting in women who consumed ginger syrup that contained 250 mg of ginger in honey and water in 1 tablespoon 4 times per day for 14 days. As for the treatment of motion sickness, according to a study by Mowrey and Clayson (1982), the action of ginger against nausea also alleviates motion sickness and vertigo better than the commonly used Dramamine™[6].

Ginger also represents a potentially effective novel treatment for chemotherapy induced nausea (Levine *et. al.* 2008). Lien *et. al.* (2003) showed that ginger reduces nausea, gastric dysrhythmia, and plasma vasopressin, a hormone that has been shown to increase along with nausea. Likewise, ginger prolonged latency before nausea onset and shortened recovery time after exposure to a rotating optokinetic drum, a stimulus that often induces symptoms of motion sickness. Gonlachanvit *et. al.* (2003) reported that one gram of ginger reduced the gastric dysrhythmia and nausea resulting from the infusion of dextrose to produce hyperglycemia in healthy humans. In addition, it has been shown that ginger reduces postoperative nausea and vomiting (Chaiyakunapruk *et. al.* 2006).

According to the Medical Center of the University of Maryland (UM-MC[b] 2011), "ginger is also used as a digestive aid for mild upset stomach, as support in inflammatory conditions such as arthritis, and may even be used in heart disease or cancer. Although it is too early to tell if ginger will benefit those with heart disease, their preliminary studies suggest that ginger may lower cholesterol and help prevent the blood from clotting. Each of these effects may protect the blood vessels from blockage and the damaging effects of blockage such as atherosclerosis, which can lead to a heart attack or stroke. Their

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[6] Active ingredient in each tablet: 50mg of Dimenhydrinate; Purpose: Antiemetic. (http://dramamine.com/)

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laboratory studies have also found that components in ginger may have anticancer activity".

1.1.3. Onion

Onion (*Allium cepa*) is one of the most widely and largely consumed vegetables (Park *et. al.* 2009). From epidemiologic studies, Hertog *et. al.* (1993) state that onion consumption is known to be related to low rates of coronary heart disease and it has a long history of healing.

According to Goulart (1995), the Egyptian medical papyrus Codex Ebers listed over 8,000 onion-alleviated ailments. Also, in ancient Greece, Hippocrates prescribed onions as diuretics, wound healers and pneumonia fighters. And finally in the Far East, onions were used to treat infections, hypertension and more.

1.1.3.1. Components

Bioactive components of onion are quercetin and dipropyl disulfides (Saulis *et. al.* 2002). Onion is one of the major sources of various biologically active phytomolecules, e.g., flavonoids, phenolic acids, thiosulfinates, cepaenes, and anthocyanins (Singh *et. al.* 2009). The major flavonoids found in the dry peel of the onion, which has usually been discarded, contain large amounts of quercetin, quercetin glycoside and their oxidative products, which are effective antioxidants against the lethal effect of oxidative stress (Gulsen *et. al.* 2007; Prakash *et. al.* 2007).

1.1.3.2. Medicinal Properties and common uses

Medicinal properties of onion include diuretic, expectorant, and useful for bleeding piles or hemorrhoids (Meena *et. al.* 2010). Onion is also used for the treatment of skin keloids and scars. The most recent addition to the collection of available treatments for the

management of scars is Mederma™ (Contractubex, Merz, Frankfurt, Germany), which is a topical gel containing *Allium cepa* (onion extract) as the active ingredient (Jackson and Shelton 1999). Onion has been found to contain both antibacterial and fibrinolytic activity (Augusti 1996; Danker *et. al.* 1979). The regular consumption of onions in food is also associated with a reduced risk of neurodegenerative disorders, cancer, cataract, ulcer, osteoporosis, vascular and heart disease (Kaneko and Baba 1999; Kawaii *et. al.* 1999; Sanderson *et. al.* 1999; Shutenko *et. al.* 1999).

In accordance with a research by Srinivasan (2005), onion (*Allium cepa*) and the closely related garlic (*Allium sativum*) are two spices that have been widely examined for an antidiabetic potential. The research also found that both these spices contain hypoglycemic agents and a review to ancient literature indicates their use in the treatment of diabetes.

1.1.4. Turmeric

Turmeric (*Curcuma longa*) is an herbaceous perennial plant belonging to the botanical family of *Zingiberaceae*, or the ginger family (Mathew and Pushpanath 2005), and is native to tropical South Asia (Norman 1991). Ammon and Wahl (1991) mention that this vibrant yellow spice derived from the rhizome of the plant, has a long history of use in traditional medicines of China and India, where the rhizome of turmeric has been crushed into a powder and used in Asian cuisine, cosmetics, remedies, and fabric dying for more than 2,000 years. Early European explorers to the Asian continent introduced this important spice to the Western world in the 14th century (Aggarwal *et. al.* 2007). Other uses of turmeric include food preservative and coloring material commonly used in the Indian subcontinent (Aggarwal *et. al.* 2007, Chattopadhyay *et. al.* 2004). Many

medicinal properties are attributed to this spice. Since the time of Ayurveda (1900 BC) numerous therapeutic activities have been assigned to turmeric for a wide variety of illnesses and conditions, including those of the skin, gastrointestinal and pulmonary systems, aches, pains, sprains, wounds, and liver disorders (Aggarwal *et. al.* 2007).

In food and manufacturing, turmeric is currently used in perfumes and as a natural yellow coloring agent, as well as an approved food additive to flavor various types of curries and mustards (Tilak *et. al.* 2004; Shishodia *et. al.* 2005).

1.1.4.1. Components

Curcumin was first isolated by Vogel in 1842 and structurally characterized by Lampe and Milobedezka in 1910 (Milobedzka *et. al.* 1910). It was synthesized and confirmed in 1913 (Lampe and Milobedezka 1913). Typical extracts of *Curcuma longa* contain the structures I–III, of which I is the most common (Ramsewak *et. al.* 2000). Reports conflict as to whether I or III is the most potent as an antioxidant and anti-tumor agent (Ruby *et. al.* 1995; Ramsewak *et. al.* 2000).

According to Chattopadhyay *et. al.* (2004), "turmeric contains a wide variety of phytochemicals, including but not limited to curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols". Extensive research within the last half century has proven that most of the therapeutic activities once associated with turmeric are due to curcumin, which is the most important fraction of this plant (Naz *et. al.* 2010). Turmeric contains three different analogues of curcumin (i.e., diferuloylmethane, demethoxycurcumin, and bisdemothycurcumin). It is not clear whether all the three analogues exhibit equal activity (Balaji and Chempakam

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2010). The melting point of curcumin, $C_2H_2OO_6$, is 184°C. It is soluble in ethanol and acetone, but insoluble in water (Joe *et. al.* 2004).

1.1.4.2.   Medicinal Properties and common uses

The extract of turmeric has many medicinal properties including antioxidant (Das and Das 2002, Ruby *et. al.* 1995), anti-inflammatory (Ammon *et. al.* 1993; Literat *et. al.* 2001, Lukita-Atmadja *et. al.* 2002), antiviral, antibacterial (Negi *et. al.* 1999), antifungal (Apisariyakul *et. al.* 1995; Roth *et. al.* 1998), and cancer chemo-preventive actions (Bush *et. al.* 2001; Gescher *et. al.* 2001; Shao *et. al.* 2002). As part of the ancient Ayurveda Indian medical system, a compress of turmeric paste is used to treat eye infections, and to dress wounds, treat bites, acne, burns and various skin diseases (Thakur *et. al.* 1989).

According to Pandeya (2005), in the north part of India, women are given a tonic of fresh turmeric paste with powder of dried ginger roots and honey in a glass of hot milk to drink twice daily after childbirth. Also, a poultice of turmeric is applied to the perineum to aid in the healing of any lacerations in the birth canal.

Turmeric is widely used in traditional Indian medicine to cure and/or treat several conditions, with biliary disorders, hepatic disorders, cough, diabetic wounds, anorexia, rheumatism, and sinusitis among them (Shishodia *et. al.* 2005). In fact, powdered turmeric is taken with boiled milk to treat cough and related respiratory ailments (Thakur *et. al.* 1989). This ancient remedy is also used to treat dental diseases, digestive disorders such as indigestion, dyspepsia, acidity, flatulence, ulcers, as well to alleviate the hallucinatory effects of hashish and other psychotropic drugs (Tilak *et. al.* 2004).

Laboratory studies have shown that turmeric have the capability of fighting protozoa, which relates to the original use of this spice for the treatment of dysentery (Castleman 1991).

Furthermore, local application of turmeric is a common household remedy in India for several conditions such as skin diseases, insect bites and chicken pox (Nadkarni 1976). Actually, the American pharmaceutical company Johnson & Johnson even makes turmeric Band-Aids for the Indian market (MacGregor 2006).

According to Maheshwari *et. al.* (2006), based on the ancient use of turmeric in wound healing, earlier studies evaluated the effect of curcumin on enhancement of wound healing. In accordance to these studies, curcumin-treated wound biopsies showed a large number of infiltrating cells such as macrophage, neutrophils and fibroblasts as compared to untreated wounds. The presence of myofibroblast in curcumin-treated wound demonstrated faster wound contraction (Sidhu *et. al.* 1998). According to Castleman (1991), to help prevent bacterial infections in wounds, it is recommended to sprinkle a small amount of turmeric on previously cleaned cuts and scrapes.

Curcumin is also a powerful antioxidant and, in addition, ancient texts of Indian medicine describe the use of curcumin for a wide variety of inflammatory diseases including sprains and swellings caused by injury, wound healing, and abdominal problems (Ammon and Wahl 1991). Turmeric contains several anti-inflammatory compounds, including six different cyclooxygenase 2 (COX-2) inhibitors[7].

In addition, some research from the Medical Center of the University of Maryland (UM-MC[c] 2011) also suggests that turmeric may be helpful for several conditions, including

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[7] A form of non-steroidal anti-inflammatory drug (NSAID) that directly targets COX-2, an enzyme responsible for inflammation and pain. (Marnett and Kalgutkar 1998)

indigestion or dyspepsia, by stimulating the gallbladder to produce bile. Besides this, other uses include maintenance of remission of ulcerative colitis, ability to reduce inflammation and relieve of the symptoms of osteoarthritis, prevention of atherosclerosis by lowering cholesterol and preventing blood clots from building up along the walls of the arteries.

Evidence from *in vitro* and animal studies suggests that curcumin may also help avoid, regulate, or destroy several types of cancers (Gescher *et. al.* 2001), including prostate (Dorai *et. al.* 2001), breast (Kim *et. al.* 2001), skin (Phan *et. al.* 2001), and colon (Johnson and Mukhtar 2007). The effects of curcumin may be due to its ability to stop the growth of the blood vessels that supply cancerous tumors, and its preventive effects may come from its strength as an antioxidant, protecting cells from damage (Gescher *et. al.* 2001).

Some recent research shows that curcumin may have the potential to improve chronic inflammatory conditions in obesity (Woo *et. al.* 2007). Also, curcumin has now been used to treat cardiovascular diseases, diabetes, osteoporosis, Crohn's disease, Alzheimer's disease, psoriasis, and other conditions (Shishodia *et. al.* 2005).

To understand how turmeric may help treat Alzheimer's disease, we need to know how this disease forms in the first place. Thomas-Eapen (2009) explains that "Beta-amyloid is responsible for forming the plaques in the brain causing the cognitive decline in Alzheimer's disease. Also, turmeric extracts contain a number of natural agents that block the beta-amyloid formation". A low dose of dietary curcumin at 160 ppm reduced the insoluble and soluble beta-amyloid plaque 43% to 50% more than a higher dose of 5,000 ppm (Lim *et. al.* 2001). The incidence of Alzheimer's disease in rural India is low

compared to the United States (Chandra *et. al.* 2001). Although this may be due to multifactorial causes, extensive dietetic use of turmeric may have an important role in this finding (Thomas-Eapen 2009).

Likewise, turmeric is used for skin health. Traditionally, turmeric paste is applied on the face and skin as a mask to improve skin appearance and aid in the fading of blemishes (Thomas-Eapen 2009). Also a curcumin treatment has been shown to correct defects associated with cystic fibrosis (Egan *et. al.* 2004).

Finally, a preliminary study suggests that curcumin may help treat uveitis, which is an inflammation of the eye. A study of 32 people with uveitis revealed that curcumin appeared to be as effective as corticosteroids, which is the type of treatment generally prescribed for this eye disorder (UM-MC[c] 2011).

1.1.5. Spices and Autoclaving

Spices contain sulfur containing volatiles that get reduced when they are submitted to autoclaving temperatures (121°C) (Azu *et. al.* 2007). In fact, the spices lose their antibacterial activities within 20 minutes at 100°C (Chen *et. al.* 1985). Also, autoclaving causes Maillard (browning) reaction, and formation of other compounds that alter the beneficial components of the spices (Kim *et. al.* 2011).

1.2. Probiotics

Probiotics are microorganisms introduced orally in the gastrointestinal tract that are able to contribute positively to the activity of intestinal microflora and, therefore, to the host health (Saarela *et. al.* 2002). The term probiotic is a relatively new word meaning "for life" and it is currently used to name bacteria associated with beneficial effects for humans and animals (FAO/WHO 2001). Probiotics have been with us for as long as

people have consumed fermented milks, but their association with health benefits dates only from the turn of the century (Fuller 1991). The original observation of the positive role played by some selected bacteria is attributed to Elie Metchnikoff (FAO/WHO 2001). Metchnikoff (1907) drew attention to the adverse effects of the gut microflora on the host and suggested that ingestion of fermented milks improved this so called "autointoxication". This Nobel Prize winner further suggested that "The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes". Fuller (1989), in order to indicate the microbial nature of probiotics, redefined the word as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". A similar definition was proposed by Havenaar and Huis in't Veld (1992): "A viable mono or mixed culture of bacteria which, when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora". A more recent, but most likely not the last definition states that a probiotic is a "live microorganism, which when consumed in adequate amounts, confer a health effect on the host" (Guarner and Schaafsma 1998).

There are certain guidelines to be used in order to assess the properties of probiotics. According to the established ones from FAO/WHO (2001), for use in foods, probiotic microorganisms should not only be capable of surviving passage through the digestive tract but also have the capability to multiply in the gut. This means they must show resistance to gastric juices and ability to grow in the presence of bile under intestinal conditions, or be consumed in a food that allows them to survive passage through the stomach and exposure to bile. They also have to be Gram positive bacteria.

According to Fuller (1991), the probiotic preparations that are currently available on the market are mainly based on lactic acid bacteria (*Bifidobacteria*, *Lactobacilli* and *Streptococci*). These three genera have been shown to be important components of the gastrointestinal microflora and are all relatively harmless. Fuller (1991) also mentions that yogurt starter bacteria (*L. bulgaricus and S. thermophilus*) are also considered as probiotics because yogurt has been associated with health benefits in the past. He finally mentions that a probiotic preparation may contain one or several different strains of bacteria.

According to Gorbach (1990), it is known that certain *Lactobacilli* species adhere to the gut mucosal surface and in a certain way inhibit the attachment of gram-negative bacteria. He also mentions that *Lactobacilli* species has been reported to increase the availability of minerals. Figure 1 show other health benefits associated with the consumption of probiotics.

1.2.1.   *Streptococcus thermophilus*

In a study conducted with infants (Saavedra *et. al.* 1994), it was observed that the lactic acid bacteria *S. thermophilus* may be able to improve gastrointestinal function in infants. This bacterium (*S. thermophilus*) multiplies and proliferates within the human gastrointestinal tract and produces lactase activity, which helps with the digestion of lactose in milk and can decrease the symptoms of malabsorption, which often accompanies acute infectious diarrhea (Marteau *et. al.* 1990). Besides acute infectious diarrhea, strains of *S. thermophilus* have also been proven to reduce the risks of antibiotic associated diarrhea.
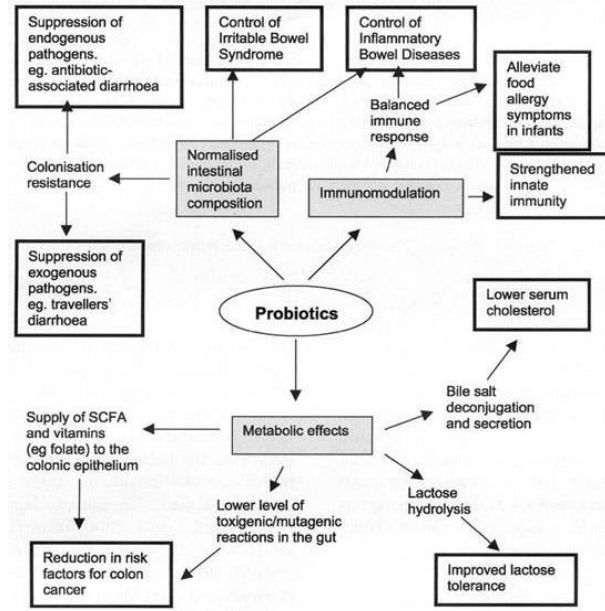
Figure 1. Proposed health benefits associated with the consumption of probiotics (Source: Saarela *et. al.* 2002)

According to Beauchamp (2004), antibiotics can have the adverse effect of destroying beneficial bacteria and causing harmful bacteria to multiply, which invokes diarrhea. According to her study, Dr. Beauchamp (2004) states that adults who ate yogurt that contains *S. thermophilus* while on treatment with antibiotics had lower rates of diarrhea than a control group (12.4% vs. 23.7%). As for infants, a study by Corrêa *et. al.* (2005) revealed that 16% of those given *S. thermophilus* supplements acquired antibiotic associated diarrhea, compared to 31% of infants who acquired the antibiotic associated diarrhea but did not receive the supplement with *S. thermophilus*.

It has been demonstrated that live cultures of *Streptococcus thermophilus* make it easier for people who are lactose-intolerant to digest dairy products because the bacteria break down lactose, the sugar in milk that lactose-intolerants find difficult to digest (Taylor and Mitchell 2007).

Chemotherapy often causes mucositis, which is a severe inflammation of primarily the small intestines (Ridge *et. al.* 2008). According to Whitford *et. al.* (2009), currently there is no treatment to alleviate the symptoms of mucositis caused by chemotherapy. In their study, Whitford *et. al.* (2009) found that when rats were inflicted with mucositis by chemotherapy medications, cells in the infected areas functioned more healthily and the tissue was less distressed after the intake of *S. thermophilus*.

According to Carper (1998), consuming yogurt with *S. thermophilus* stimulates enough immunity to block lung cancer in mice. Her book, "*Food: your miracle medicine*" mentions a study in which lab mice were fed with a yogurt that contained *Lactobacillus lactis* and *S. thermophilus*, and then they were injected with cancer cells. According to the results of this study, the consumption of the yogurt decreased the expected number of cancer cells by one-third.

1.2.2. *Lactobacilli* species

Most probiotic bacteria are lactic acid bacteria (Lee & Salminen 1995) and, among them, *Lactobacilli* represent certainly one of the fundamental microbial groups, because of their significant contribution to the maintenance of the intestinal ecosystem and in the immune stimulation of the host (Saarela *et. al.* 2002). *Lactobacilli* have been isolated from all portions of the human gastrointestinal tract, where they are considered normal inhabitants (Greene & Klaenhammer 1994). Most of them are able to survive the low pH of the stomach, which is normally destructive for most microbes (Holzapfel *et. al.* 1998). Moreover, *Lactobacilli* are among the dominant bacteria in the small intestine, although at much lower levels than in the colon (Simon & Gorbach 1995).

1.2.2.1. *Lactobacillus bulgaricus*

Lactobacillus bulgaricus is identified in the dairy industry as a "starter culture" that encourages the growth of other probiotic microbes during the production of cheese and yogurt; this functions as an early adapter in harsh environments may offer a glimpse into the prominence of *L. bulgaricus* in the role of a beneficial probiotic (Probiotic.org 2011).

According to Courtin and Rul (2003), *L. bulgaricus* is commonly used alongside *S. thermophilus* as a starter for making yogurt. They further explain that the two species have a synergistic relationship, with *L. bulgaricus* producing amino acids from proteins in milk, which are then utilized by *S. thermophilus*, and that both species are lactic acid producers, which provides yogurt with its characteristic tart flavor and acts as a preservative. The resulting decrease in pH also coagulates the milk proteins partially, which results in the characteristic thickness of yogurt (Kaláb 1997; Zourari *et. al.* 1992). While fermenting milk, *L. bulgaricus* produces acetaldehyde, one of the main yogurt aroma/flavor components (Zourari *et. al.* 1992). Some strains of *L. bulgaricus* also produce bacteriocins, which kill undesired bacteria (Simova *et. al.* 2008).

Lactobacillus bulgaricus, in similarity with *S. thermophilus*, is also often helpful to people who suffer of lactose intolerance (Taylor and Mitchell 2007). In addition, this probiotic strain may also be used to lower the cholesterol in the blood. According to a study by Tok and Aslim (2010), *L. bulgaricus* has the capacity to remove cholesterol from MRS broth by means of exopolysaccharides[8], with and without the presence of Oxgall.

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[8] High molecular weight polymers that are composed of sugar residues and are secreted by a microorganism into the surrounding environment (Kumar and Mody 2009).

1.2.2.2. *Lactobacillus acidophilus*

L. acidophilus occurs naturally in the human and animal gastrointestinal tract (European Bioinformatics Institute 2011), and some strains of *L. acidophilus* may be considered to have probiotic characteristics (Ljungh and Wadström 2006). These strains are commercially used in many dairy products, sometimes together with *S. thermophilus* and *L. bulgaricus* in the production of yogurt.

Some strains of *L. acidophilus* have been studied for potential health effects. Among these health effects, it has been determined that adequate daily intake of *L. acidophilus* may facilitate lactose digestion in lactose-intolerant subjects (Sanders and Klaenhammer 2001). Also, according to some research, (Anderson and Gilliland 1999), it has been shown that, likewise *L. bulgaricus*, *L. acidophilus* may also be helpful for reducing serum cholesterol levels. Additionally, *L. acidophilus* has shown to inhibit growth of breast cancer cells, and has a positive effect on chemotherapy patients by relieving the symptoms of chemotherapy induced diarrhea (Lee and Salminen 2008).

Lactobacillus acidophilus produces the bacteriocin CH5, which is both antibacterial and inhibitory against certain yeasts and molds and is effective against both *Salmonella typhimurium* and *Campylobacter jejuni* (Lee and Salminen 2008). These authors also claim that this microorganism has been shown to "improve bowel regularity and also to have a preventative effect against traveller's diarrhea, as well as antibiotic related bowel issues", (can help re-colonize the gut of normal intestinal microflora after antibiotic treatment).

1.3.   Spices and probiotics: potential effects and influences

The importance of a balanced diet and the presence of probiotic bacteria in the gut are well recognized in maintaining general gut health (Sutherland *et. al.* 2009). The "hygiene hypothesis" holds that foods, bacteria and health are indistinguishably linked, due to the fact that early life exposure to whole foods and a range of bacteria via breastfeeding and environmental sources contributes to the development of a normal inflammatory response (Isolauri *et. al.* 2001; Elston 2006). According to Sutherland *et. al.* (2009), in the developed world, people live in an increasingly sanitized environment and eat an increasingly over processed diet and it has been suggested that these two factors may contribute to a lack of tolerance to non-harmful antigens.

In a study by Sutherland *et. al.* (2009), a research was focused to address the interactions between food extracts and human gut bacteria and such study reported the new evidence of how certain foods can impact directly on the growth of nutritionally important bacteria as well as inhibiting the growth of health damaging bacteria. The bacteria used in the study mentioned above included probiotic bacteria (*Lactobacillus reuteri, Lactobacillus rhamnosus* and *Bifidobacteria lactis*) and pathogenic bacteria (*Escherichia coli* 0157:H7 and LF82). A total of 37 food products were used in the study, but the ones of interest are onion (*Allium cepa*), ginger (*Zingiber officinale*), garlic (*Allium sativum*), and turmeric (*Curcuma longa*). Table 1 summarizes the major findings of this study. The findings of the previous research open a possibility of the utilization of various spices to promote the growth and development of probiotic strains, which are beneficial to consumers and at the same time act as a bactericide for harmful microorganisms.

Table 1. Major findings of interest from "*In vitro* effects of food extracts on selected probiotic and pathogenic bacteria" (Sutherland *et. al.* 2009)

Spice	Predominant Effects
Garlic	• Probiotic (*L. reuteri, L. rhamnosus and B. lactis*) growth enhancement • Pathogen (*E. coli* 0157:H7 and LF82) growth inhibition
Ginger	• Significant inhibition of pathogens (*E. coli* 0157:H7 and LF82) • Minimal enhancement of probiotic (*L. reuteri, L. rhamnosus and B. lactis*)
Onion	• Significant inhibition of pathogens (*E. coli* 0157:H7 and LF82) • Minimal enhancement of probiotic (*L. reuteri, L. rhamnosus and B. lactis*)
Turmeric	• Significantly antibacterial against the probiotics (*L. reuteri, L. rhamnosus and B. lactis*) and pathogens (*E. coli* 0157:H7 and LF82)

Billing and Sherman (1998) indicated that the main reason that spices are used is to improve palatability of foods. However, it is most likely that the main reason spices are used is because they help keep the foods free of unwanted microorganisms and thus contribute to health (Brul and Coote 1999). In a study by Leuschner and Zamparini (2002), the growth and endurance of *Escherichia coli* 0157 and *Salmonella enterica* serovar *enteritidis* were researched in the presence of garlic, ginger, mustard and cloves, with garlic and clove showing bacteriostatic and bactericidal activities towards both microorganisms. On the other hand, Licón *et. al.* (2012) reported that the addition of the saffron spice slowed down slightly the viable counts of lactic acid bacteria, such as *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris Lactococcus lactis ssp. lactis biovar diacetylactis, and Streptococcus thermophilus* on pressed ewe milk cheese.

Pianpumepong and Noomhorm (2010) studied the possibility of obtaining lactic acid bacteria from fresh turmeric rhizomes with the potential of being used as starter cultures.

The probiotic characterization was determined by acid and bile tolerance, among other tests. According to their results, all lactic acid bacteria showed a high acid and bile tolerance (> 89% survival). Mater *et. al.* (2005) reported that *S. thermophilus* by itself resisted a pH as low as 2 and maintained its viability. Furthermore, Lick *et. al.* (2001) found that *S. thermophilus* is able to survive gastrointestinal passage in vivo and detected viable counts in human duodenal samples after fresh yogurt ingestion.

Spices can have a direct impact in the digestive process. For an example, the amount of gastric secretion in the stomach is around 1.5 liters per day and one of the many things that stimulate this secretion is bitters[9], like those present in ginger (Schwabl *et. al.* 2004). As for the bile and pancreatic duct, these two open in the duodenal section of the small intestine, and is here where spices like turmeric and ginger can be helpful by aiding the necessary enzymes to pass into the intestine in order to break down the food into smaller components (Schwabl *et. al.* 2004).

Proteases constitute one of the biggest functional group of proteins that are involved in many normal and pathological processes (Tripathi *et. al.* 2011). The protease inhibition by pathogenic organisms could help in the control of several diseases (Supuran *et. al.* 2002). Plants and herbs are known to synthesize self-protective compounds that can directly affect microbes (Huynh and other 1992). Spices like turmeric are known to contain proteases and to have proteolytic activity (Nagarathnam *et. al.* 2010). In China, an oriental-style type of cheese, called "Jiangzhinai", is made with ginger proteases, and according to a study by Huang *et. al.* (2011), it is suggested that ginger has the potential to be a rennet substitute in the manufacture of cheeses.

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[9] A liquid containing a bitter-tasting substance, used as a tonic to stimulate the appetite or improve digestion (Collins English Dictionary 2003)

Spices can play an important role in the color of foods. Licón *et. al.* (2012) reported the effects that the addition of saffron spice had in hard cheese, where there was a significant change in color as the concentration of saffron spice increased, imparting a characteristic red color to the product. On the other hand, Tarakci *et. al.* (2011) studied the influence of garlic in a traditional Turkish herby-pickled cheese. This cheese is made with raw milk and a mixture of around 25 kinds of herbs, including *Allium* sp, *Tymus* sp, *Mentha* sp and *Ferula* sp., among others. Their study reported that increasing the level of garlic in the herbed pickled cheese decreased the L* (lightness) and the b* (blue-yellow axis), but the values of a* (red-green axis) increased. This study also reported that the pH values for herby cheeses were lower when compared to control (Tarakci *et. al.* 2011).

Amirdivani and Baba (2011), measured the pH on yogurts that were exposed to peppermint, dill and basil. According to their study, the pH of the herbal-yogurts decreased 0.2 pH units/hour faster than plain-yogurt. Behrad *et. al.* (2012) measured the pH on yogurts that were exposed to cinnamon and licorice and they reported that there were no significant differences in pH between herbal-yogurts and plain-yogurt during fermentation and storage.

Yang *et. al.* (2012), studies the effects of the addition of ginger juice to milk and they reported that there was a significant ($P < 0.05$) influence on the viscosity of yogurt exposed to concentrations of 2 to 10% of ginger juice. Hassan *et. al.* (2010) manufactured a yogurt using concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% of aqueous garlic extract. According to their results, after 15 days of storage, the resultant yogurt in all different concentrations of garlic had higher values than control. Foda *et. al.* (2007) prepared a set yogurt using buffalo milk and supplemented with turmeric powder at

concentrations of 0.1, 0.25, 0.50, 0.75 and 1%. According to their study, there was an increase in the firmness and viscosity of the yogurt as the concentration of turmeric increased.

Yogurt is known for having high content of nutrients, and contrary to regular fluid milk, yogurt can be digested more easily (Yang *et. al.* 2012). According to László (2006), "there has been an increasing interest in the use of natural food additives and the incorporation of health-promoting substances into the diet". Spices like ginger and garlic has been incorporated into yogurts (Yang *et. al.* 2012; Hassan *et. al.* 2010). Most of the researches done with spices include a range of different concentrations, while the ideal situation would be to follow the recommended daily dosages. Table 2 shows the recommended daily dosages of some spices.

Taking into consideration the values from Table 2, the most appropriate percentage of spice juice to be incorporated into a yogurt product would be between 1-4%.

Table 2. Recommended daily dosages of some spices

Form	Garlic	Ginger	Onion	Turmeric
	Amount	Amount	Amount	Amount
Fresh raw	~2-4g[b]	~2-4g[b]	~50g[c]	~1.5-3g[b]
Dried powder	~900mg[a]	~1g[b]	~20g[c]	~1-3g[b]
Essential oil	~0.09-0.36mL[b]	(no info found)	(no info found)	(no info found)
Extract (solid)	~600-1,200mg[b]	(no info found)	(no info found)	~0.4g[b]
Juice	~4mL[b]	~1.5-3mL[b]	~50mL[c]	~4mL[b]

Source: [a]Amagase *et. al.* 2001, [b]University of Maryland-Medical Center 2011, [c]Blumental *et. al.* 1998

Another matter to consider when incorporating fresh spice juice to a yogurt product is the strength in flavor and aroma from the spice, which in the case of garlic, for example, can

be pretty strong. A yogurt product with spice juice should serve to provide the combined health benefits from the spice plus those benefits from the gut-healthy bacteria present in the yogurt. This yogurt product should not have the intention of providing the full recommended daily dosage of a certain additive, because a formulation with this intention could compromise the overall likeness and acceptance from the consumers.

1.4. Justification

The functional foods market has been increasing in the last few years. In 2009, United States was the second largest market for functional foods, whereas Japan was the first. The functional foods market is expected to increase from $7.1 billion that it was worth in 2009 to an estimated $8.6 billion in 2015 (Starling 2010). The yogurt market increased from $9.7 billion in 2005 to $15.4 billion in 2010 (Heller 2006).

Probiotics have been valued for their potential benefits to overall health. The normal microflora of the human gut is known to limit the ability of potential pathogens to attach to the intestinal mucosa and thus infect. By boosting the consumption of probiotics, the defense mechanism of the body is also boosted and this could prevent illnesses, it could decrease the use of antibiotics and furthermore avoid the spread of pathogens that are antibiotic-resistant.

On the other hand, there is a growing market and an influx of spicy foods from other countries. Mexico, China, Thailand and India are some examples of countries that have spices as a central ingredient in their cuisine. Spices have already being used in the dairy food industry. Products like jalapeño cheese, Jack cheese, garlic cheese and Cajun spice cheese among others are among consumer's favorites and all of them have something in common: spices. An increase in the tendency of consumption of spicy foods can have an

influence on the microbial flora in the human gut. Spices have been studied for their medicinal properties with antimicrobial, antiviral, antifungal and antiparasitic properties among them. We have to take into consideration the effects that certain spices can have on the good bacteria that naturally inhabits the human gut when people consume them. Earlier work has shown the influence of spice extracts but the influence that the pure spice juice can have on culture bacterial strains and a probiotic bacterium commonly used in the manufacture of yogurt is not known.

1.5. Hypothesis

The hypothesis for this research was different spice juices have different influences on probiotic characteristics and yogurt attributes.

1.6. Research objectives

- To determine the influence of 1% (v/v) of garlic, ginger, onion and turmeric juice on the growth of *Streptococcus thermophilus* ST-M5, *Lactobacillus bulgaricus* LB-12 and *Lactobacillus acidophilus* LA-K.

- To elucidate the influence of 1% (v/v) of garlic, ginger, onion and turmeric juice on acid tolerance, bile tolerance and protease activity of *S. thermophilus*, *L. bulgaricus* and *L. acidophilus*.

- To manufacture a spicy probiotic yogurt and to elucidate the influence of 0.05% (v/v) of garlic, ginger, onion and turmeric juice on the growth of *S. thermophilus*, *L. bulgaricus* and *L. acidophilus* in yogurt.

- To elucidate the influence of 0.05% (v/v) of garlic, ginger, onion and turmeric juice on the physico-chemical characteristics (pH, titratable acidity, coliform bacterial growth, color and apparent viscosity) of spicy probiotic yogurt.

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- To study the influence of 0.05% (v/v) of garlic, ginger, onion and turmeric juice on sensory characteristics of spicy probiotic yogurt and to determine the consumer acceptability of spicy probiotic yogurt.

CHAPTER 2: MATERIALS AND METHODS

2.1.   Experimental design

Freshly thawed *Streptococcus thermophilus* ST-M5, *Lactobacillus bulgaricus* LB-12 and *Lactobacillus acidophilus* LA-K culture were suspended in sterilized 0.1% peptone water and 1% (v/v) of freshly extracted spice juice individually of garlic, ginger, onion or turmeric. The four spice juice treatments were performed in a random manner. The control was the sample in which no spice juice was added. Growth was determined by plating the control and the treated samples every 12 hours for 60 hours of incubation for *S. thermophilus* and every two hours for 8 hours of incubation for the *Lactobacilli*. Bile tolerance was determined by plating the control and the treated samples every hour for 5 hours of incubation. Acid tolerance was determined by plating the control and the treated samples every hour for 2 hours of incubation. Protease activity was determined by measuring the optical density (absorbance) at 0, 12 and 24 hours of incubation. A spicy probiotic yogurt was manufactured with 0.05% of the juice, individually of the 4 spices. Physico-chemical characteristics, namely pH, titratable acidity, color, apparent viscosity and coliform bacterial growth, as well as growth of the three organisms in yogurt were measured weekly for a period of 35 days. Three replications were conducted for each experimental condition. The experimental design for the yogurt culture bacteria characteristics and the physico-chemical characteristics, (except for apparent viscosity) of the spicy yogurt was Repeated Measures. For the apparent viscosity and the yogurt consumer test, the experimental design was a randomized block design (RBD) with the individual containers and the participants, respectively, as blocks.

2.2. Sample preparation

The fresh spices were obtained from local vendors and they were peeled, washed with steaming hot water and dried with paper towels. Using a Juiceman 2-in-1 juice extractor (Model JM8000S, Applica Consumer Products, Inc, Miramar, FL), the juice of each spice was obtained just before it was needed. This step was done in this manner to avoid oxidation reactions, which included "greening" of garlic, "pinking" of onion, and "darkening" of turmeric (Imai *et. al.* 2006). Control and spice treated samples for growth analysis were prepared by inoculating 11mL of freshly thawed culture of *Streptococcus thermophilus* ST-M5, *Lactobacillus bulgaricus* LB-12 and *Lactobacillus acidophilus* LA-K (F-DVS, Chr. Hansen's Laboratory, Milwaukee, WI,) into 99mL of sterile peptone water (growth), sterile acidified MRS broth at pH 2 (acid tolerance), sterile skim milk (protease activity) or sterile MRS broth with sodium thioglycolate, which acted as an oxygen scavenger for the *Lactobacilli* (bile tolerance), containing 1mL juice of each spice (garlic, ginger, onion or turmeric). Sodium thioglycolate was not used for the *Streptococci*. Control samples were prepared in the same manner, but no spice juice was added.

2.3. Treatments

The effects of 1% (v/v) of garlic, ginger, onion and turmeric juice were determined by means of serial dilutions. One (1) milliliter of spice juice (garlic, ginger, onion or turmeric) was added to a dilution bottle containing 99mL of sterile peptone water (growth) or sterile MRS broth (for acid or bile tolerance) and was shaken vigorously for the purpose of mixing the contents. From this first dilution (10^1), 11mL of the contents were transferred to the remaining 7 peptone water containing dilution bottles (10^2 to 10^8)

and the respective serial dilutions were made. For protease activity, skim milk was used as a medium. The control samples did not contained any spice juice at all.

2.4. Preparation of diluents and media
2.4.1. Peptone water

Peptone water (0.1%) was prepared by dissolving 1g of peptone medium (Bacto™ Peptone, Difco, Dickinson and company, Sparks, MD) in 1L of distilled water, and autoclaved in 99mL portions at 121°C for 15 minutes.

2.4.2. MRS broth for bile tolerance

The MRS broth for the test of bile tolerance was prepared according to the method proposed by Pereira and Gibson (2002) in the following manner: 100 mL of sterile distilled water was mixed with the aid of a magnetic stirring rod in a volumetric flask with 5.5 grams of MRS broth (Difco™, Dickinson and company, Sparks, MD), 0.3 grams of Oxgall (bovine bile) (USBiological, Swampscott, MA) and 0.2 grams of sodium thioglycolate (Acros Organic, Fair Lawn, NJ). After the mixture was homogenized, 99 mL of this broth was transferred into a graduated cylinder and from there it was transferred to a dilution bottle and autoclaved to sterilize.

2.4.3. MRS broth for acid tolerance

The MRS broth for the test of acid tolerance was prepared according to the method proposed by Pereira and Gibson (2002) with slight modifications. The three bacterial cultures were inoculated in acidified MRS broth (Difco™, Dickinson and company, Sparks, MD) previously adjusted to pH 2. The broth was prepared by mixing 5.5g of MRS broth powder in 100mL of sterile distilled water and the pH was adjusted with 1M HCl and mixed thoroughly. After the mixture was homogenized, 90 mL of this broth was

transferred into a graduated cylinder and from there it was transferred to a dilution bottle and autoclaved to sterilize.

2.4.4. *Streptococcus thermophilus* agar

The *Streptococcus thermophilus* agar was prepared in the following way: 10g of Bacto Tryptone (Becton, Dickinson and Co., Sparks, MD), 10g of RNAse and DNAse free sucrose (Amresco, Solon, OH), 5g of Bacto yeast extract (Becton, Dickinson and Co., Sparks, MD) and 2g of K_2HPO_4 (Fisher Scientific, Fair Lawn, NJ) was dissolved in 1L of distilled water. The pH of the mixture was adjusted to 6.8 ± 0.1; after this 6mL of 0.5% bromocresol purple (Fisher Scientific, Fair Lawn, NJ) and 12g of agar powder (Fisher Scientific, Fair Lawn, NJ) were added to the mixture. The medium was then autoclaved at 121°C for 15 minutes (Dave and Shah 1996).

2.4.5. *Lactobacilli* MRS agar

The MRS agar was prepared according to the instructions given by the manufacturer (Difco™, Dickinson and company, Sparks, MD): 55g of MRS broth powder (Difco™, Dickinson and company, Sparks, MD) was dissolved in 1 L of distilled water with the aid of a magnetic stirrer. The pH was adjusted to 5.2 ± 0.1 and 15 grams of agar powder (Fisher Scientific, Fair Lawn, NJ) was added and mixed. The medium was then autoclaved at 121°C for 15 minutes (Tharmaraj and Shah 2003).

2.4.6. *Lactobacilli* MRS agar without dextrose

The MRS agar was prepared according to the instructions given by the manufacturer (Difco™, Dickinson and company, Sparks, MD) following a modification of Dave and Shah (1996) and Tharmaraj and Shah (2003): 1 liter of distilled water was added to a graduated cylinder. MRS base medium without dextrose was prepared by weighing the

appropriate proportion of 10g of proteose peptone #3 (USBiological, Swampscott, MA), 10g of beef extract (Becton, Dickinson and Co., Sparks, MD), 5g of yeast extract (Becton, Dickinson and Co., Sparks, MD), 1g of polysorbate 80 (Sigma-Aldrich, St. Louis, MO), 2g of ammonium citrate (Fisher Scientific, Fair Lawn, NJ), 5g of sodium acetate, anhydrous (Fisher Scientific, Fair Lawn, NJ), 0.1g of magnesium sulfate, anhydrous (EMD Chemicals Inc, Gibbstown, NJ), 0.05g of manganese sulfate, monohydrate (Sigma-Aldrich, St. Louis, MO), 2g of dipotassium phosphate (Fisher Scientific, Fair Lawn, NJ), and 15g of agar powder (Fisher Scientific, Fair Lawn, NJ). The medium was then autoclaved at 121°C for 15 minutes. A 10% (w/v) sorbitol (EMD Chemicals Inc, Gibbstown, NJ) solution was prepared and filter sterilized with a Nalgene® membrane filter units (Nalge Co., Rochester, NY), and the appropriate amount of this solution was aseptically added to the MRS base medium to form a 10% sorbitol solution and 90% MRS base medium mixture immediately before pouring the plates.

2.4.7. *o*-phthaldialdehyde (OPA) solution for protease activity

The OPA solution was prepared following the method proposed by Oberg *et. al.* (1991) by combining the following reagents and diluting to a final volume of 50mL with distilled water: 25 mL of 100 m*M* sodium borate (Fisher Scientific, Fair Lawn, NJ), 2.5 mL 20% (wt/wt) Sodium Dodecyl Sulfate (Fisher Scientific, Fair Lawn, NJ), 40 mg of OPA reagent (Alfa Aesar, Ward Mill, MA) dissolved in 1 mL methanol (Sigma-Aldrich, St. Louis, MO) and 100 µL of β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO).

2.5.   Analytical procedures
2.5.1. Growth

Growth was determined by plating the control and the spice-treated samples along with the three probiotic strains of interest every 2 hours for 8 hours for the *Lactobacilli* and

every 12 hours for 60 hours for *S. thermophilus*. The incubation for *S. thermophilus* and *L. acidophilus* was at 37°C and the incubation for the *L. bulgaricus* was at 43°C. *S. thermophilus* was incubated aerobically for 48 hours and the *Lactobacilli* were incubated anaerobically for 72 hours. After incubation, the plates were observed and any colony growth was counted using a Darkfield Quebec colony counter (American Optical, Buffalo, NY).

2.5.2. Acid tolerance

The acid tolerance was determined according to the method proposed by Pereira and Gibson (2002) with slight modifications.   The bacterial cultures of *Streptococcus thermophilus* ST-M5, *Lactobacillus bulgaricus* LB-12 and *Lactobacillus acidophilus* LAK were inoculated in acidified MRS broth previously adjusted to pH 2 using 10M HCl.   The acidified MRS broth inoculated with *S. thermophilus* was incubated aerobically and the acidified MRS broth inoculated with *L. acidophilus* was incubated anaerobically, both at 37°C.   The acidified MRS broth inoculated with *L. bulgaricus* was incubated anaerobically at 43°C.   At 0, 1 and 2 hours of incubation, 11mL of the inoculated broths were serially diluted in 99mL of 0.1% peptone water and 1mL of each dilution was pour plated using *Streptococcus thermophilus* agar for *S. thermophilus,* *Lactobacilli* MRS agar for *L. bulgaricus* and *Lactobacilli* MRS agar without dextrose for *L. acidophilus*. The petri dishes were incubated aerobically at 37°C for 48 hours for *S. thermophilus*, while the petri dishes with *L. bulgaricus* and *L. acidophilus* were incubated anaerobically for 72 hours at 43°C and 37°C, respectively. After incubation, the plates were observed and any colony growth was counted using a Darkfield Quebec colony counter (American Optical, Buffalo, NY).

2.5.3. Bile tolerance

The bile tolerance was determined according to the method proposed by Pereira and Gibson (2002) with slight modifications. The bile tolerance of the cultures were analyzed in MRS-THIO broth supplemented with 0.3% (wt/vol) oxgall (bovine bile) and 0.2% (vol/vol) sodium thioglycolate for *L. bulgaricus* and *L. acidophilus*, but no sodium thioglycolate for *S. thermophilus*. Oxgall was added to test bile tolerance of the bacteria and sodium thioglycolate was used in the broth as an oxygen scavenger. For this test, 11 mL of freshly thawed cultures were inoculated in MRS-THIO broth, 1mL of spice juice was added and the mixture was incubated at 43°C for *L. bulgaricus* and 37°C for *S. thermophilus* and *L. acidophilus* for 5 hours. At 0 hours (immediately after inoculation) and hourly up to 5 hours after inoculation, the inoculated broths were serially diluted in 99mL of 0.1% peptone water and 1mL of each dilution was pour plated using *Streptococcus thermophilus agar* for *S. thermophilus, Lactobacilli* MRS agar for *L. bulgaricus* and *Lactobacilli* MRS agar without dextrose for *L. acidophilus*. The petri dishes were incubated aerobically at 37°C for 48 hours for *S. thermophilus*, while the petri dishes with *L. bulgaricus* and *L. acidophilus* were incubated anaerobically for 72 hours at 43°C and 37°C, respectively. After incubation, the plates were observed and any colony growth was counted using a Darkfield Quebec colony counter (American Optical, Buffalo, NY).

2.5.4. Protease activity

The protease activity of the spice treated and control samples were determined by *o*-phthaldialdehyde (OPA) electrophotometric method proposed by Oberg *et. al.* (1991) with a slight modification. The samples were incubated in sterile skim milk at 40°C for

0, 12 and 24 hours. After incubation, 2.5 mL from each sample was mixed with 1mL of distilled water individually and was transferred into each of the test tubes containing 5mL of 0.75N trichloroacetic acid (TCA) (Fisher Scientific, Fair Lawn, NJ) and the test tubes were vortexed. After setting at room temperature for 10 minutes, the acidified samples were filtered through a Whatman number 2 filter paper (Clifton, NJ). The filtrate obtained from each test tube were filtered a second time using a 13mm 0.22µm nylon syringe filter (Advantec, Dublin CA). Aliquots from each TCA filtrate were analyzed by OPA testing and the protease activity was determined by measuring the optical density (absorbance) of both the control and the spice-treated samples at 0, 12 and 24 hours of incubation by means of a spectrophotometer at 340nm.

2.5.5. Yogurt manufacture

For the manufacturing of a high quality probiotic yogurt, previously cleaned and sanitized stainless steel pails with a 3-gallon capacity were used. The nonfat dry milk (250g per gallon) was dissolved with the skim milk (2 gallons per pail). The pails were placed inside a vat with hot water and this mixture was heated to 140°F. Once this temperature was reached, the mixture was homogenized at 1,500psi for the first stage and at 500psi for the second stage in the homogenizer (type 300 DJP4 2PS, Manton-Gaulin MFG Co Inc., Everett, MA, USA). After homogenization, the pails with the mix were returned to the steaming vat and were vat pasteurized at 185°F for 30 minutes and were stirred constantly. The mix was then lowered to 104°F and it was inoculated with *S. thermophilus*, *L. bulgaricus* and *L. acidophilus* (0.75mL per gallon). Immediately after inoculation, the pails with the product were placed inside the incubator at 40°C for 3 hours. After the first 3 hours of incubation elapsed, a sample of the yogurt was taken and

the pH was measured. When the pH reached a reading of 4.6, the pails with the yogurt were transferred to a cooler at 40°F for purpose of product setting. The spice juice (0.05%) and 20% (v/v) of blueberry puree were added and mixed in at the following day.

2.5.6. Physico-chemical characteristics of yogurt
2.5.6.1. Growth of *S. thermophilus* ST-M5, *L. bulgaricus* LB-12 and *L. acidophilus* LAK in spicy blueberry yogurt as influenced by 0.05% spice juice

Growth of *S. thermophilus*, *L. bulgaricus* and *L. acidophilus* was measured weekly. The same procedure discussed on section 2.5.1 was used for this testing with the major difference that yogurt was used to supply the bacteria, instead of freshly thawed culture. The procedure was repeated weekly for a period of 5 weeks. Dilution samples were plated in three separate petri dishes and each had a specific agar for each bacterium, (see sections 2.4.4 thru 2.4.6). The concentration used was 0.05% of spice juice to avoid compromising the overall flavor and aroma of the final product.

2.5.6.2. Measurement of pH of spicy blueberry yogurt as influenced by 0.05% spice juice

The pH was measured weekly from a sample of yogurt for a period of 5 weeks using an Oyster series pH meter (Extech Instruments, Waltham, MA). The pH meter was calibrated using commercial buffers (Fischer Scientific, Fair Lawn, NJ) at pH 4 and pH 7 and the instrument's temperature was adjusted to that of the sample ($10°C \pm 2$) before reading.

2.5.6.3. Measurement of titratable acidity of spicy blueberry yogurt as influenced by 0.05% spice juice

The titratable acidity was measured weekly for a period of 5 weeks. The titratable acidity was determined by weighing 9 grams of yogurt and around 5 drops of phenolphthalein

were added to the yogurt sample. This mixture was titrated with1M NaOH until a slight pink color was observed and the final volume of NaOH used was recorded.

2.5.6.4. Measurement of coliform bacterial growth of spicy blueberry yogurt as influenced by 0.05% spice juice

The spicy blueberry yogurt was tested for coliforms in a weekly basis for 5 weeks using coliform petrifilm (3M®, St. Paul, MN) containing violet red bile agar. The procedure was done by weighing 11g of yogurt samples, and adding it to a dilution bottle containing 99mL of sterile 0.1% of peptone water (Difco, Detroit, MI). The contents were agitated and 1mL of the dilution was plated on a previously labeled petrifilm and incubated at 32°C for 24 hours. After incubation, the petrifilm were observed and any growth was counted using a Darkfield Quebec colony counter (American Optical, Buffalo, NY).

2.5.6.5. Measurement of color of spicy blueberry yogurt as influenced by 0.05% spice juice

The L*, a*, b*, C* and h* values on the spicy blueberry yogurt were determined by measuring a yogurt sample weekly for a period of 5 weeks using a colorimeter (Miniscan XE plus model 45/0-L, Hunter associates laboratory, Inc. Reston, VA) and the Universal software (v4.10). The instrument was calibrated by using the white and black standard tiles included with the instrument. The operating conditions were 10° observer, D65 illuminant and 45/0 sensor. The averages of the L*, a* and b* values at each time point measured were used to calculate the magnitude of the total color difference (ΔE) using the following equation (HunterLab 2001): $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$

2.5.6.6. Measurement of apparent viscosity of spicy blueberry yogurt as influenced by 0.05% spice juice

The apparent viscosity was measured weekly for a period of 5 weeks using a viscometer (Brookfield model DV-II and Brookfield helipath stand, Brookfield engineering laboratories, Inc. Stoughton, MA) and the Windgather 32 software (Brookfield engineering laboratories, Inc. Stoughton, MA). A T-C spindle was used at 20 RPMs and 100 data points were taken. All measurements were done from different cups, but from the same batch. The spindle was inserted in the sample at a constant depth of 2 cm from the top level of the sample. The helipath was fixed in downward motion so the spindle could cut circular layers at increasing depths of the sample. The container used to store the sample had a top diameter of 4.55", a bottom diameter of 3.25" and it was 2.45" tall.

2.5.7. Consumer acceptance test

The Institutional Review Board (IRB) exempted this study from continued oversight, with the exemption number HE12-17 (See appendix A). A consumer acceptance test was conducted using 100 random participants. The participants consisted in students and faculty at Louisiana State University (Baton Rouge, LA) that wished to participate willingly in the study. The yogurt was 2.5 weeks old when it was served to the participants of the acceptance test. Each participant received and signed an informed consent that was previously approved by the Institutional Review Board (IRB), in which the potential risks of the study were explained. Each participant received 5 samples in three digits-random number coded 3.25oz. plastic cups. Water and non-salted saltine crackers were provided to the participants, so they could clean their palates in between each sample and 5 single use spoons were also provided, one for each sample. Participants were instructed not to talk or discuss their samples with other participants

during the evaluation. A 9-point hedonic scale (Peryam and Pilgrim 1957), where 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely, was used to evaluate overall appearance, color, aroma, taste, texture (thickness and graininess) and overall like of the product (See appendix B). The form also provided for acceptability, intent of purchase if the product were commercially available and intent of purchase knowing the product contained a spice with health benefits.

2.6.   Statistical analysis

Differences of least square means were used to determine significant differences at $P <$ 0.05 for main effects (spice and time), and two way interaction effects (spice * time). Data is presented as mean ± standard error of the means. Significant differences were determined at $\alpha = 0.05$. Significant differences ($P < 0.05$) among the main effects were analyzed using Tukey's adjustment. Data was analyzed using Proc Mixed model of Statistical Analysis System (SAS[®]). Data from yogurt consumer testing was also analyzed using Statistical Analysis System (SAS[®]). For the yogurt consumer testing, ANOVA was done to analyze the questions with the 9-point hedonic scale (Peryam and Pilgrim 1957). A frequency count was used to analyze the Yes/No questions. Finally, a McNemar's test (McNemar 1947) was used to analyze for changes in positive purchase intent after potential health benefit of the product had been given.

CHAPTER 3: RESULTS AND DISCUSSION

3.1.   Growth
3.1.1. *Streptococcus thermophilus* ST-M5

The growth of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice is shown in Figure 2. There was a significant ($P < 0.05$) effect for treatment, time and treatment * time (Table 3).
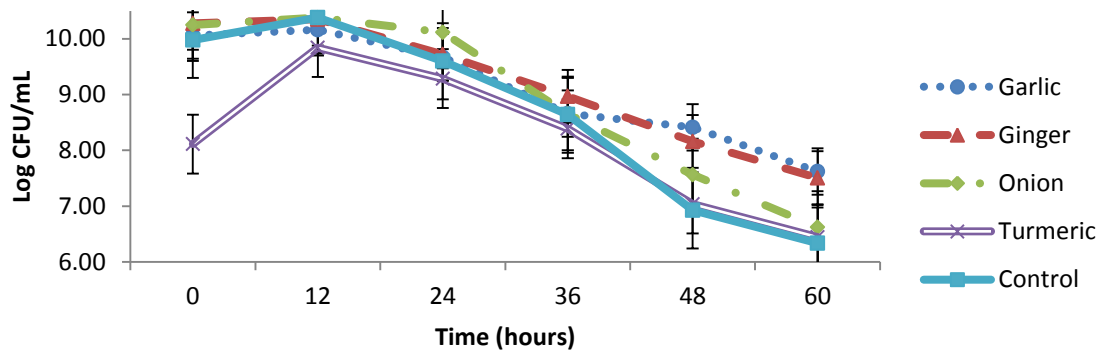
Figure 2. Growth of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice

Table 3. Probability > F (Pr > F) of fixed effects for the growth of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice

EFFECT	Pr > F
TREATMENT	<0.001
TIME	<0.001
TREATMENT * TIME	<0.001

Time = incubation period of 60 hours

Turmeric was significantly ($P < 0.05$) different with lower viable counts at 0 and 12 hours of incubation when compared to control while the other spices were not (Table 4, and Figure 2). At 12 hours of incubation, the control and all spices, except for turmeric (9.84 Log CFU/mL) showed similar numbers in viable counts, which ranged in between 10.16 to 10.38 Log CFU/mL (Table 4 and Figure 2).

Table 4. Least Square Means for growth of *Streptococcus thermophilus* ST-M5 as influenced by spice juice

Treatment	0 hrs	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs
Control	9.97 [ABCDE]	10.38 [A]	9.59 [EF]	8.64 [HIJ]	6.92 [NO]	6.33 [P]
Garlic	10.05 [ABCDE]	10.16 [ABCD]	9.67 [DEF]	8.66 [HI]	**8.41** [IJ]	**7.62** [KL]
Ginger	10.27 [AB]	10.34 [AB]	9.72 [CDEF]	8.96 [GH]	**8.16** [IJ]	**7.50** [LM]
Onion	10.24 [ABC]	10.38 [A]	10.11 [ABCDE]	8.65 [HI]	**7.56** [LM]	6.62 [NOP]
Turmeric	**8.11** [JK]	**9.84** [BCDE]	9.28 [FG]	8.38 [IJ]	**7.03** [MN]	6.44 [OP]

[ABC] LSMeans with different letter within the table are significantly different

At 24 hours of incubation none of the spices were significantly different when compared to control (Table 4) and all spices, except for onion (10.11 Log CFU/mL) showed a decrease of 1 Log in their viable counts (Table 4 and Figure 2). At 36 hours of incubation, none of the spices were significantly different when compared to control (Table 4) and all spices showed a decrease of 1 Log, except for onion which decreased 2 Logs (Table 4 and Figure 2). At 48 hours of incubation, all spices, except for turmeric, were significantly ($P < 0.05$) different when compared to control (Table 4), which showed a decrease by 2 Logs in viable counts (6.92 Log CFU/mL), while onion (7.56 Log CFU/mL) and turmeric (7.03 Log CFU/mL) decreased by 1 Log (Table 4 and Figure 2). At 60 hours of incubation, garlic and ginger were significantly ($P < 0.05$) different when compared to control, while onion and turmeric did not show any significant difference when compared to control (Table 4 and Figure 2). In addition, all spices had higher values in viable counts than control (6.33 Log CFU/mL), and garlic (7.62 Log CFU/mL) and ginger (7.50 Log CFU/mL) did not show any significant difference when compared to the rest of the spices and control (Table 4 and Figure 2). Mean log

reductions of the viable counts of *S. thermophilus* subjected to different spice juices were obtained by subtracting counts at 60 hours of incubation from counts at 0 hours of incubation and are shown in Table 5.

Table 5. Mean Log reduction of the viable counts of *Streptococcus thermophilus* ST-M5 treated with 1% of spice juice

Treatment	Log CFU/mL
Control	**3.64**
Garlic	2.43
Ginger	2.77
Onion	**3.62**
Turmeric	**1.67**

In Table 5, a high number indicates high bacterial death and a lower number indicates low bacterial death. The log reduction of turmeric showed the lowest bacterial death when compared with the rest of the spices, while onion and control showed the highest bacterial death.

3.1.2. *Lactobacillus bulgaricus* LB-12

The growth of *Lactobacillus bulgaricus* LB-12 as influenced by 1% of spice juice is shown in Figure 3. There was a significant effect for treatment, time and treatment * time (Table 6). None of the spices were significantly different at 0 and 2 hours of incubation when compared to control (Table 7 and Figure 3). At 4 hours of incubation, all spices, except for ginger, were significantly ($P < 0.05$) different when compared to control (Table 7). In addition, ginger (9.74 Log CFU/mL) and control (9.72 Log CFU/mL) decreased by 1 Log (Table 7 and Figure 3). Turmeric was significantly ($P < 0.05$) different when compared to control after 6 hours of incubation while the other spices were not (Table 7), while all spices and control had a decrease by 1 Log in their viable counts, showing a range of 9.45 to 9.65 Log CFU/mL (Table 7 and Figure 3).
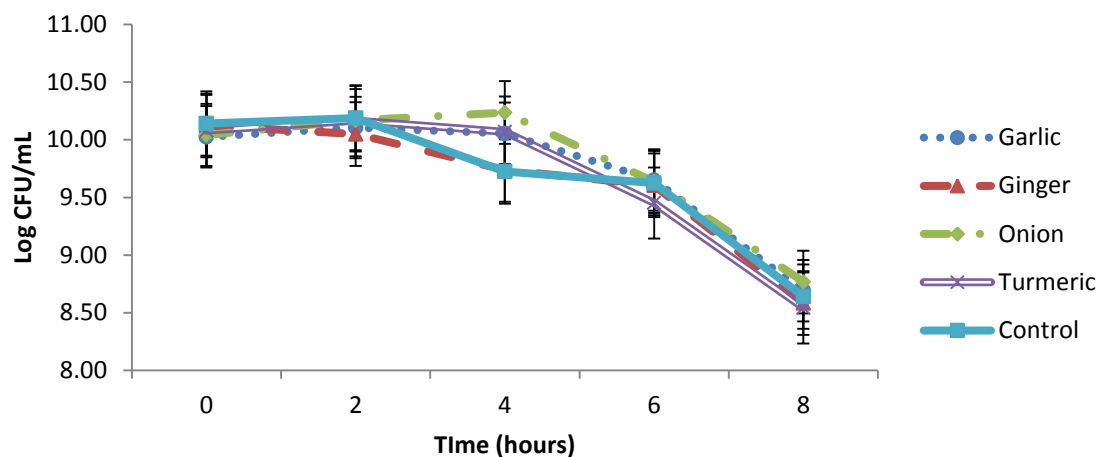
Figure 3. Growth of *Lactobacillus bulgaricus* LB-12 as influenced by 1% of spice juice

Table 6. Probability > F (Pr > F) of fixed effects for the growth of *Lactobacillus bulgaricus* LB-12 as influenced by 1% of spice juice

EFFECT	Pr > F
TREATMENT	**<0.001**
TIME	**<0.001**
TREATMENT * TIME	**<0.001**

Time = incubation period of 8 hours

Table 7. Least Square Means for the growth of *Lactobacillus bulgaricus* LB-12 as influenced by spice juice

Treatment	0 hrs	2 hrs	4 hrs	6 hrs	8 hrs
Control	10.14 [ABC]	10.19 [AB]	9.72 [D]	9.62 [D]	8.64 [FG]
Garlic	10.02 [C]	10.10 [ABC]	**10.05** [BC]	9.65 [D]	8.69 [FG]
Ginger	10.12 [ABC]	10.05 [BC]	9.74 [D]	9.60 [DE]	8.58 [G]
Onion	10.04 [BC]	10.16 [ABC]	**10.24** [A]	9.63 [D]	8.77 [F]
Turmeric	10.08 [ABC]	10.16 [ABC]	**10.07** [BC]	**9.45** [E]	8.54 [G]

[ABC] LSMeans with different letter within the table are significantly different

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At 8 hours of incubation, none of the spices were significantly ($P < 0.05$) different when compared to control (Table 7) and all spices and control showed a decrease of 1 Log in their viable counts, showing a range of 8.54 to 8.77 Log CFU/mL (Table 7 and Figure 3). Mean log reduction of the viable counts of *L. bulgaricus* subjected to different spice juices were obtained by subtracting counts at 8 hours of incubation from 0 hours of incubation and are shown in Table 8.

Table 8. Mean Log reduction of the viable counts of *Lactobacillus bulgaricus* LB-12 treated with 1% of spice juice

Treatment	Log CFU/mL
Control	1.50
Garlic	1.33
Ginger	**1.54**
Onion	**1.27**
Turmeric	**1.54**

In Table 8, a high number indicates high bacterial death and a lower number indicates low bacterial death. The log reduction of onion showed the lowest bacterial death when compared with the rest of the spices, while ginger and turmeric showed the highest bacterial death. *Lactobacillus bulgaricus* LB-12 seems to be able to grow in presence of all the spices without showing a significant ($P < 0.05$) difference from control.

3.1.3. *Lactobacillus acidophilus* LAK

The growth of *Lactobacillus acidophilus* LAK as influenced by 1% of spice juice over 8 hours of incubation is shown in Figure 4. There was a significant effect for treatment, time and treatment * time (Table 9). All spices were significantly ($P < 0.05$) different at all incubation times when compared to control (Table 10).
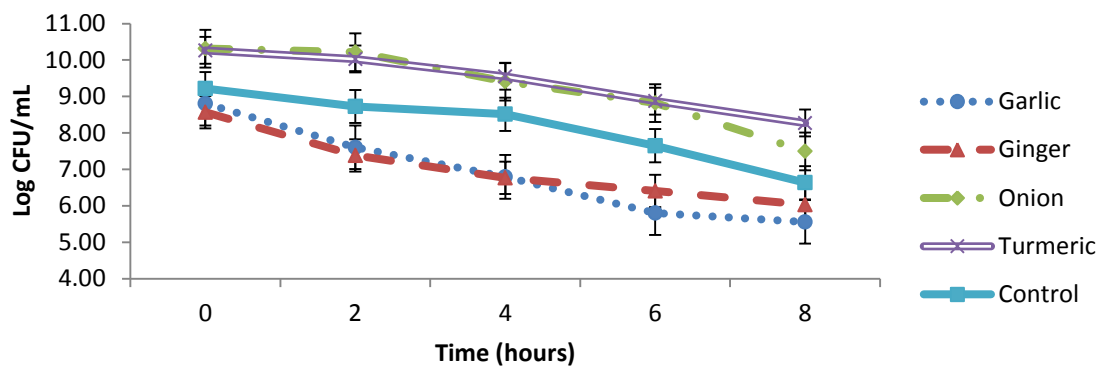
Figure 4. Growth of *Lactobacillus acidophilus* LAK as influenced by 1% of spice juice

Table 9. Probability > F (Pr > F) of fixed effects for the growth of *Lactobacillus acidophilus* LAK as influenced by 1% of spice juice

EFFECT	Pr > F
TREATMENT	<0.001
TIME	<0.001
TREATMENT * TIME	<0.001

Time = incubation period of 8 hours

Table 10. Least Square Means for growth of *Lactobacillus acidophilus* LAK as influenced by spice juice

Treatment	0 hrs	2 hrs	4 hrs	6 hrs	8 hrs
Control	9.21 [C]	8.72 [DE]	8.51 [EF]	7.64 [G]	6.63 [HI]
Garlic	8.80 [DE]	7.60 [G]	6.79 [H]	5.79 [JK]	5.55 [K]
Ginger	8.57 [DEF]	7.38 [G]	6.76 [H]	6.40 [I]	6.03 [J]
Onion	10.31 [A]	10.21 [A]	9.41 [BC]	8.82 [DE]	7.49 [G]
Turmeric	10.26 [A]	10.03 [A]	9.55 [B]	8.87 [D]	8.27 [F]

[ABC] LSMeans with different letter within the table are significantly different

Onion (10.31 Log CFU/mL) and turmeric (10.26 Log CFU/mL) had higher viable counts when compared to the other spices and the control at 0 hours of incubation and this trend can be observed throughout the 8 hours of incubation (Table 10 and Figure 4). After 2 hours of incubation, ginger (7.38 Log CFU/mL), garlic (7.60 Log CFU/mL) and control

(8.72 Log CFU/mL) decreased by 1 Log, while there were no significant changes in the viable counts of onion and turmeric (Table 10 and Figure 4). At 4 hours of incubation, all spices decreased their viable counts by 1 Log, while there was no significant difference with the viable counts of the control (8.51 Log CFU/mL) (Table 10 and Figure 4). At 6 hours of incubation, control and all spices, except ginger (6.40 Log CFU/mL) decreased their viable counts by 1 Log (Table 10 and Figure 4). At 8 hours of incubation, control (6.63 Log CFU/mL) and onion (7.49 Log CFU/mL) decreased their viable counts by 1 Log, while there was no significant difference with the viable counts of the rest of the spices (Table 10 and Figure 4).

Mean log reduction of the viable counts of *L. acidophilus* subjected to different spice juices were obtained by subtracting counts at 8 hours of incubation from 0 hours of incubation and are shown in Table 5.

Table 11. Mean Log reduction of the viable counts of *Lactobacillus acidophilus* LAK treated with 1% of spice juice

Treatment	Log CFU/mL
Control	2.58
Garlic	**3.25**
Ginger	2.54
Onion	2.82
Turmeric	**1.99**

In Table 11, a high number indicates high bacterial death and a lower number indicates low bacterial death. The log reduction of turmeric showed the lowest bacterial death when compared with the rest of the spices, while garlic showed the highest bacterial death. *Lactobacillus acidophilus* LAK seems to be able to grow better in presence of turmeric.

According to a study by Sutherland *et. al.* (2009), different spices had different effects on the growth of *Lactobacilli* bacteria. A contrasting example is turmeric: Sutherland *et. al.* (2009) reported that turmeric had a significant antibacterial effect against probiotic (*L. reuteri*, *L. rhamnosus* and *B. lactis*) and pathogenic bacteria (*E. coli O157:H7* and *E. coli LF 82*), in contrast with the results of this research. In addition, they reported that garlic enhanced the probiotic strains while inhibiting pathogenic strains. Ginger was reported to inhibit pathogenic strains while showing minimal probiotic activity (Sutherland *et. al.* 2009). A possible explanation for these results could be the form in which the spice was used (chemical extracts vs. pure juice). Furthermore, Mary Helen PA *et. al.* (2012) reported that oil, methanol, acetone and hexane extracts of turmeric had an inhibitory effect against *S. thermophilus*. According to Ibrahim and Bezkorovainy (1994): "most of the plant species naturally synthesize organic acids such as acetic, citric, malic, tartaric, benzoic and ascorbic, among others. Turmeric is known to produce tartaric acid. In addition, microorganisms including lactic acid bacteria and *Bifidobacteria* also produce acids as a result of fermentation. These organic acids inhibit the growth of both bacterial and fungal cells".

3.2. Acid Tolerance
3.2.1. *Streptococcus thermophilus* ST-M5

The acid tolerance of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice is shown in Figure 5. There was a significant ($P < 0.05$) effect for treatment, time and treatment * time (Table 12).
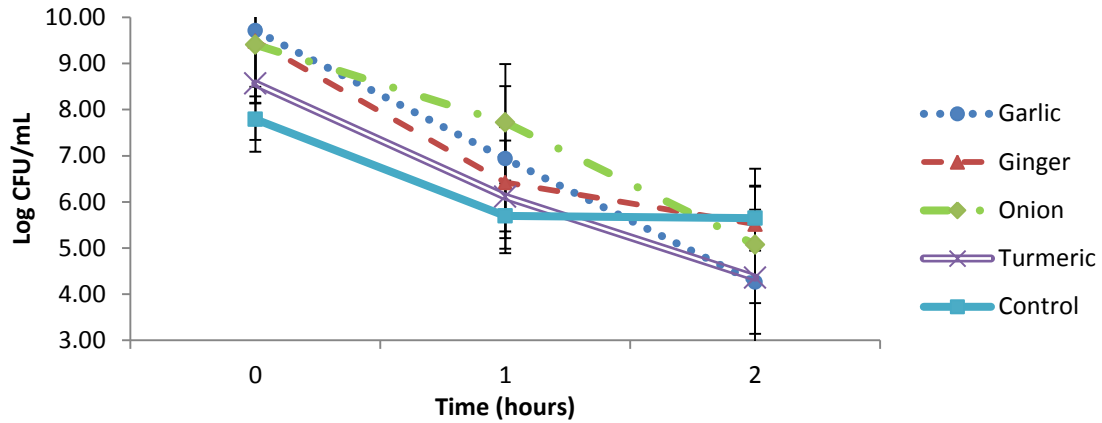
Figure 5. Acid tolerance of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice

Table 12. Probability > F (Pr > F) of fixed effects for the acid tolerance of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice

EFFECT	Pr > F
TREATMENT	<0.0001
TIME	<0.0001
TREATMENT * TIME	<0.0001

Time = incubation period of 2 hours

All spices showed a significant (P < 0.05) difference at 0 and 1 hours of incubation when compared to control (Table 13). Control (7.79 Log CFU/mL) and turmeric (8.56 Log CFU/mL), showed lower numbers in viable counts when compared to the rest of the spices at 0 hours of incubation (Table 13 and Figure 5).

Table 13. Least Square Means for acid tolerance of *Streptococcus thermophilus* ST-M5 as influenced by spice juice

Treatment	0 hrs	1 hrs	2 hrs
Control	7.79[C]	5.75[F]	5.64[F]
Garlic	9.71[A]	6.93[D]	4.25[H]
Ginger	9.48[A]	6.41[E]	5.51[F]
Onion	9.41[A]	7.72[C]	5.07[G]
Turmeric	8.56[B]	6.11[E]	4.35[H]

[ABC] LSMeans with different letter within the table are significantly different.

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At one hour of incubation, onion showed higher numbers in viable counts (7.72 Log CFU/mL) while control showed the lowest number in viable count (5.75 Log CFU/mL) (Table 13 Figure 5). All spices, except ginger showed a significant ($P < 0.05$) difference compared to control after 2 hours of incubation (Table 13). At 2 hours of incubation, control (5.64 Log CFU/mL), and ginger (5.51 Log CFU/mL) showed the highest numbers in viable counts, while there was slight, yet significantly lower counts for garlic (4.25 Log CFU/mL), onion (5.07 Log CFU/mL) and turmeric (4.35 Log CFU/mL) (Table 13 Figure 5).

Mean log reduction of the viable counts of *S. thermophilus* subjected to different spice juices were obtained by subtracting counts at 2 hours of incubation from 0 hours of incubation and are shown in Table 14.

Table 14. Mean Log reduction of the viable counts of *Streptococcus thermophilus* ST-M5 treated with 1% of spice juice in the presence of acidified broth

Treatment	Log CFU/mL
Control	**2.15**
Garlic	**5.46**
Ginger	3.97
Onion	4.33
Turmeric	4.21

In Table 14, a high number indicates high bacterial death and a lower number indicates low bacterial death. The log reduction of control showed the lowest bacterial death, while garlic showed the highest bacterial death when compared with the rest of the spices.

3.2.2. *Lactobacillus bulgaricus* LB-12

Lactobacillus bulgaricus LB-12 did not grow under the acidic conditions (pH 2.0) for the acid tolerance test, and no results were obtained.

3.2.3. *Lactobacillus acidophilus* LAK

Lactobacillus acidophilus LAK did not grow under the acidic conditions (pH 2.0) for the acid tolerance test, and no results were obtained.

An important characteristic of a probiotic is its survival at low pH (Brink *et. al.* 2006). A protective coating of exopolysaccharides (EPS) may allow the bacterium to better withstand stomach and bile salts (Roberts *et. al.* 1995). According to a study by Gulcin *et. al.* (2010), it was demonstrated that that EPS protected the bacteria in gastrointestinal conditions and they suggest that the bacterial EPS are thought to play a role in the protection of microbial cells against low pH, like that of the stomach. Mater *et. al.* (2005) reported that *S. thermophilus* by itself resisted a pH as low as 2 and 2.5 and maintained its viability. Furthermore, Lick *et. al.* (2001) found that *S. thermophilus* is able to survive gastrointestinal passage in vivo and detected viable counts in human duodenal samples after fresh yogurt ingestion.

Zaika and Kissinger (1984) discuss that the extracts of some spices such as ginger and turmeric contain Manganese (Mn^{2+}) and Iron (Fe^{2+}), which increases the acid production of certain *Lactobacilli*. Gyawali and Ibrahim (2012) explain the role of Mn^{2+}: "Manganese competes against Iron (Fe^{2+}) for the binding site onto the surface of the cell membrane. Manganese stimulates the production of acid from *Lactobacilli*. Iron is known to be a growth-promoting factor for bacteria, while manganese has a strong inhibitory effect against iron which causes the bacteria to grow slower".

3.3. Bile Tolerance
3.3.1. *Streptococcus thermophilus* ST-M5

The bile tolerance of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice is shown in Figure 6. There was a significant effect for treatment, time, and treatment * time (Table 15).
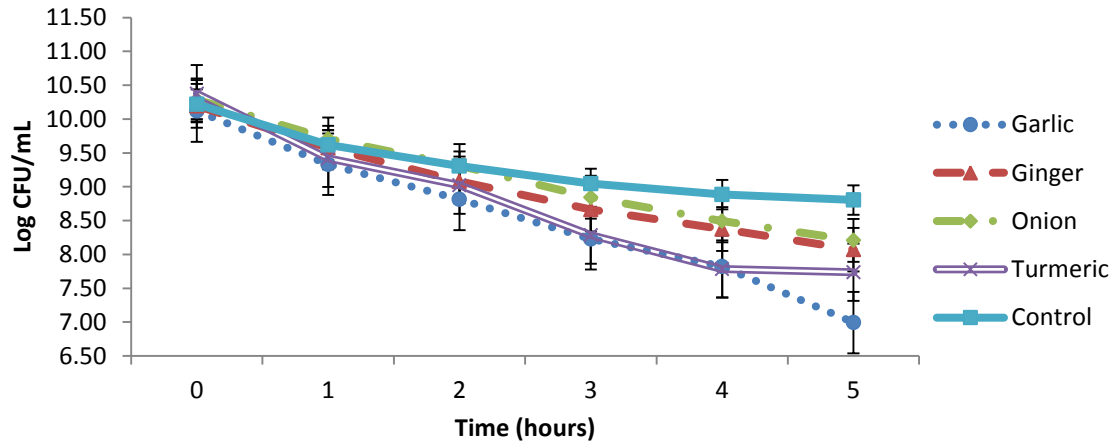
Figure 6. Bile tolerance of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice

Table 15. Probability > F (Pr > F) of fixed effects for the bile tolerance of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice

EFFECT	Pr > F
TREATMENT	<0.001
TIME	<0.001
TREATMENT * TIME	<0.001

Time = incubation period of 5 hours

None of the spices were significantly different at 0 and 1 hours of incubation when compared to control (Table 16). At 1 hour of incubation, there was a decrease of 1 Log in viable counts from all spices and control, with a range from 9.33 to 9.70 Log CFU/mL (Table 16 and Figure 6). At 2 hours of incubation, garlic was significantly (P < 0.05) different and showed a decrease of 1 Log (8.81 Log CFU/mL) in viable counts when compared to control while the other spices were not (Table 16 and Figure 6). All spices, except onion, showed a significant (P < 0.05) difference at 3 hours of incubation when

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compared to control (Table 16). In addition, ginger, onion and turmeric showed a decrease of 1 Log in viable counts, while control and garlic showed no significant difference (Table 16 and Figure 6). At 4 hours of incubation, all spices showed a significant (P < 0.05) difference when compared to control (Table 16). Also, control, garlic and turmeric showed a decrease of 1 Log (8.88 Log CFU/mL, 7.82 Log CFU/mL and 7.78 Log CFU/mL, respectively), while the rest of the treatments showed no significant difference (Table 16 and Figure 6). At 5 hours of incubation, all spices showed a significant (P < 0.05) difference when compared to control (Table 16) whereas garlic showed a decrease of 1 Log (6.99 Log CFU/mL), while the control and the rest of the treatments showed no significant difference (Table 16 and Figure 6).

Table 16. Least Square Means for bile tolerance of *Streptococcus thermophilus* ST-M5 as influenced by spice juice

Treatment	0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs
Control	10.21 [A]	9.61 [BC]	9.30 [CDE]	9.04 [DEF]	8.88 [FG]	8.80 [FGH]
Garlic	10.11 [A]	9.33 [BCDE]	**8.81** [FGH]	**8.22** [JK]	**7.82** [L]	**6.99** [M]
Ginger	10.19 [A]	9.57 [BC]	9.08 [DEF]	**8.66** [GHI]	**8.37** [IJK]	**8.07** [KL]
Onion	10.28 [A]	9.70 [B]	9.31 [CDE]	**8.84** [FGH]	**8.49** [HIJ]	**8.20** [JK]
Turmeric	10.37 [A]	9.41 [BCD]	9.02 [EFG]	**8.28** [IJK]	**7.78** [L]	**7.73** [L]

[ABC] LSMeans with different letter within the table are significantly different.

Mean log reduction of the viable counts of *S. thermophilus* subjected to different spice juices were obtained by subtracting counts at 5 hours of incubation from 0 hours of incubation and are shown in Table 17. In Table 17, a high number indicates high bacterial death and a lower number indicates low bacterial death. The log reduction of

control showed the lowest bacterial death when compared with the rest of the spices, while garlic showed the highest bacterial death.

Table 17. Mean Log reduction of the viable counts of *Streptococcus thermophilus* ST-M5 treated with 1% of spice juice in presence of bile (oxgall)

Treatment	Log CFU/mL
Control	**1.41**
Garlic	**3.12**
Ginger	2.12
Onion	2.08
Turmeric	2.64

3.3.2. *Lactobacillus bulgaricus* LB-12

The bile tolerance of *Lactobacillus bulgaricus* LB-12 as influenced by 1% of spice juice is shown in Figure 7.

Figure 7. Bile tolerance of *Lactobacillus bulgaricus* LB-12 as influenced by 1% of spice juice

There was a significant effect for treatment, time and treatment * time (Table 18). Ginger and turmeric showed a significant ($P < 0.05$) difference when compared to control at 0 hours of incubation while onion and garlic were not significantly different (Table 19). At 1 hour of incubation, ginger and onion showed a significant difference when

compared to control while turmeric and garlic did not (Table 19). In addition, control

and garlic showed a decrease in viable counts by 1 Log (7.77 Log CFU/mL and 7.98 Log

CFU/mL respectively) (Table 19 and Figure 7). All spices showed a significant (P <

0.05) difference when compared to control after 2 hours of incubation (Table 19) while

there was a decrease of 1 Log in viable counts from ginger, onion and turmeric (Table 19

and Figure 7). All spices showed a significant (P < 0.05) difference when compared to

control after 3 hours of incubation (Table 19), while there was no significant difference in

the viable counts from any of the spices or control (Table 19 and Figure 7). There was a

significant (P < 0.05) difference from all spices when compared to control at 4 hours of

incubation (Table 19), while control showed a decrease of 1 Log (6.68 Log CFU/mL) in

viable counts and the spices showed no significant difference in their viable counts

(Table 19 and Figure 7). At 5 hours of incubation, all spices were significantly (P < 0.05)

different when compared to control (Table 19). In addition, there was a decrease of 2

Logs in the viable counts of turmeric (5.42 Log CFU/mL) and a decrease of 1 Log in the

viable counts of onion (6.89 Log CFU/mL), while the rest of the treatments showed no

significant difference (Table 19 and Figure 7).

Table 18. Probability > F (Pr > F) of fixed effects for the bile tolerance of *Lactobacillus bulgaricus* LB-12 as influenced by 1% of spice juice

EFFECT	Pr > F
TREATMENT	<0.001
TIME	<0.001
TREATMENT * TIME	<0.001

Time = incubation period of 5 hours

Table 19. Least Square Means for bile tolerance of *Lactobacillus bulgaricus* LB-12 as influenced by spice juice

Treatment	0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs
Control	8.55[B]	7.77[EF]	7.20[IJKL]	7.14[JKLM]	6.68[NO]	6.60[O]
Garlic	8.63[B]	7.98[DE]	**7.70**[F]	**7.63**[FG]	**7.33**[HIJK]	**7.10**[KLM]
Ginger	**8.94**[A]	**8.05**[CD]	**7.52**[FGH]	**7.67**[F]	**7.24**[IJKL]	**7.31**[HIJKL]
Onion	8.70[AB]	**8.06**[CD]	**7.62**[FG]	**7.41**[GHI]	**7.07**[LM]	**6.89**[MN]
Turmeric	**8.30**[C]	8.02[DE]	**7.54**[FGH]	**7.41**[GHI]	**7.36**[HIJ]	**5.42**[P]

[ABC] LSMeans with different letter within the table are significantly different.

Mean log reduction of the viable counts of *L. bulgaricus* subjected to different spice juices were obtained by subtracting counts at 5 hours of incubation from 0 hours of incubation and are shown in Table 20.

Table 20. Mean Log reduction of the viable counts of *Lactobacillus bulgaricus* LB-12 treated with 1% of spice juice in presence of bile (oxgall)

Treatment	Log CFU/mL
Control	1.95
Garlic	**1.53**
Ginger	1.63
Onion	1.81
Turmeric	**2.88**

In Table 20, a high number indicates high bacterial death and a lower number indicates low bacterial death. The log reduction of garlic showed the lowest bacterial death when compared with the rest of the spices, while turmeric showed the highest bacterial death.

3.3.3. *Lactobacillus acidophilus* LAK

Lactobacillus acidophilus did not grow in presence of bile (0.3% w/v oxgall) for the bile tolerance test and no results were obtained.

It has been found that the bile that enters the duodenum decreases the survival rate of bacteria (Jin *et. al.* 1998). According to these authors, "this is probably due to the fact that all bacteria have cell membranes consisting of lipids and fatty acids which are very susceptible to destruction by bile salts". Therefore, the survival success of a probiotic strain depends on its bile resistance properties (Jin *et. al.* 1998). A protective coating of exopolysaccharides (EPS) may allow the bacterium to better withstand bile salts (Roberts *et. al.* 1995). According to Singh and others (2012), *L. bulgaricus* treated with "Indian spice blend" containing cumin, black pepper, ginger, long pepper, big cardamom, and clove, was not affected by the incubation in presence of 0.3% (wt/vol) bile salt. Additionally, Walker and Gilliland (1993) reported in their study that there was a considerable variation among different strains of *Lactobacillus acidophilus* and their ability to grow in presence of bile.

3.4. Protease Activity
3.4.1. *Streptococcus thermophilus* ST-M5

The protease activity of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice is shown in Figure 8. There was a significant effect for treatment, time and treatment * time (Table 21). Ginger and onion were significantly ($P < 0.05$) different when compared to control at 0 hours of incubation (Table 22). At 12 hours of incubation, ginger and turmeric showed a significant ($P < 0.05$) difference by showing an increase in their protease activity when compared to control while onion and garlic were not significantly different (Table 22 and Figure 8). At 24 hours of incubation, all four spices showed a significant ($P < 0.05$) difference when compared to control, with all of them showing an increase in their protease activity (Table 22 and Figure 8).
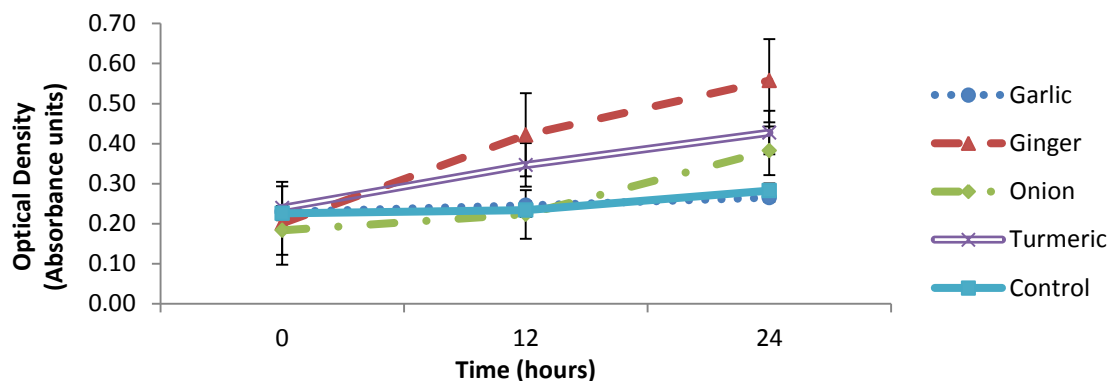
Figure 8. Protease activity of *Streptococcus thermophilus* ST-M5

Table 21. Probability > F (Pr > F) of fixed effects for the protease activity of *Streptococcus thermophilus* ST-M5 as influenced by 1% of spice juice

EFFECT	Pr > F
TREATMENT	<0.001
TIME	<0.001
TREATMENT * TIME	<0.001

Time = incubation period of 24 hours

Table 22. Least Square Means for protease activity of *Streptococcus thermophilus* ST-M5 as influenced by spice juice

Treatment	0 hrs	12 hrs	24 hrs
Control	0.22 [H]	0.23 [GH]	0.28 [E]
Garlic	0.23 [GH]	0.24 [G]	**0.26** [F]
Ginger	**0.20** [I]	**0.42** [B]	**0.55** [A]
Onion	**0.18** [I]	0.22 [H]	**0.38** [C]
Turmeric	0.23 [GH]	**0.34** [D]	**0.42** [B]

[ABC] LSMeans with different letter within the table are significantly different.

3.4.2. *Lactobacillus bulgaricus* LB-12

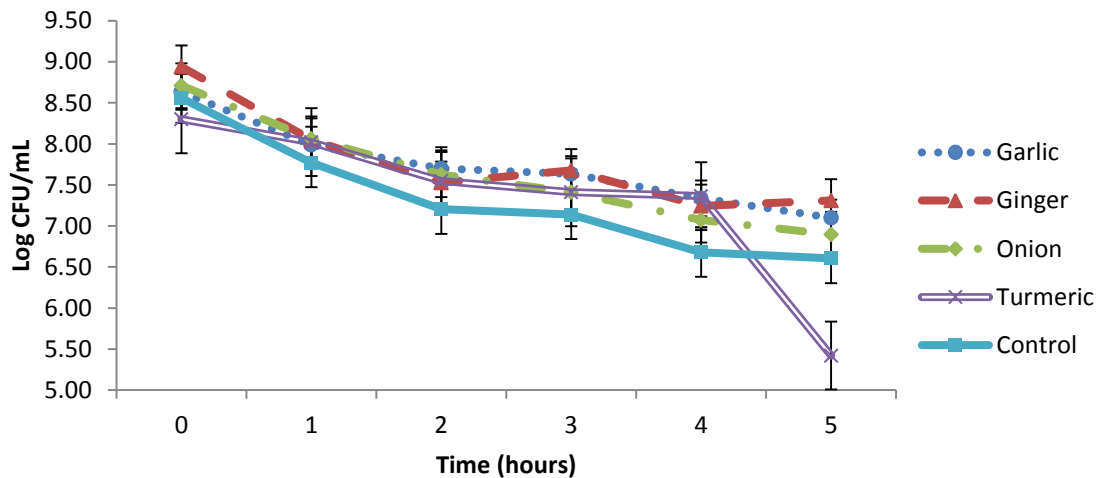The protease activity of *Lactobacillus bulgaricus* LB-12 as influenced by 1% of spice juice is shown in Figure 9. There was a significant effect for treatment, time and treatment * time (Table 23). Garlic and ginger were significantly (P < 0.05) different when compared to control at 0 hours of incubation, while onion and turmeric were not (Table 24). At 12 hours of incubation, all spices, except for garlic, were significantly (P

< 0.05) different from control (Table 24). In addition, all spices and control showed an

increase in their protease activity (Table 24 and Figure 9). At 24 hours of incubation, all

spices showed to be significantly (P < 0.05) different from control (Table 24) and also, all

spice and control showed an increase in their protease activity (Table 24 and Figure 9).
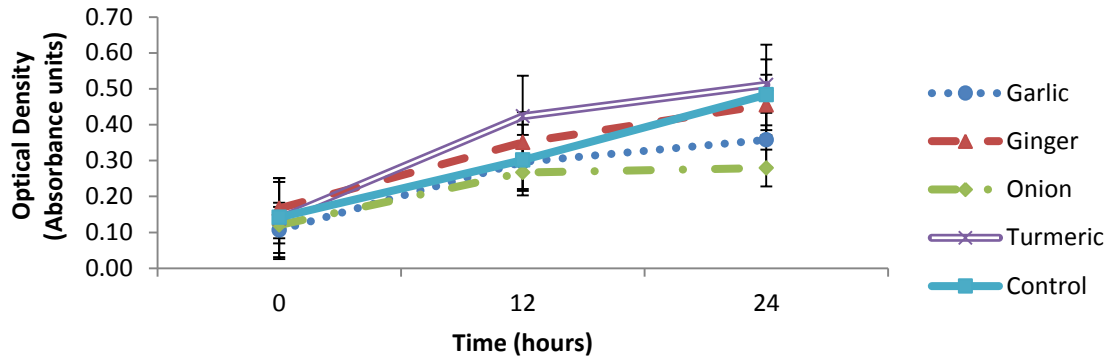
Figure 9. Protease activity of *Lactobacillus bulgaricus* LB-12

Table 23. Probability > F (Pr > F) of fixed effects for the protease activity of
Lactobacillus bulgaricus LB-12as influenced by 1% of spice juice

EFFECT	Pr > F
TREATMENT	<0.001
TIME	<0.001
TREATMENT * TIME	<0.001

Time = incubation period of 24 hours

Table 24. Least Square Means for protease activity of *Lactobacillus bulgaricus* LB-12 as
influenced by spice juice

Treatment	0 hrs	12 hrs	24 hrs
Control	0.14 [I]	0.30 [F]	0.48 [B]
Garlic	0.10 [J]	0.29 [F]	0.35 [E]
Ginger	0.16 [H]	0.35 [E]	0.45 [C]
Onion	0.12 [IJ]	0.26 [G]	0.27 [FG]
Turmeric	0.13 [I]	0.42 [D]	0.51 [A]

[ABC] LSMeans with different letter within the table are significantly different.

3.4.3. *Lactobacillus acidophilus* LAK

The protease activity of *Lactobacillus acidophilus* LAK as influenced by 1% of spice juice is shown in Figure 10. There was a significant effect for treatment, time and treatment * time (Table 25).
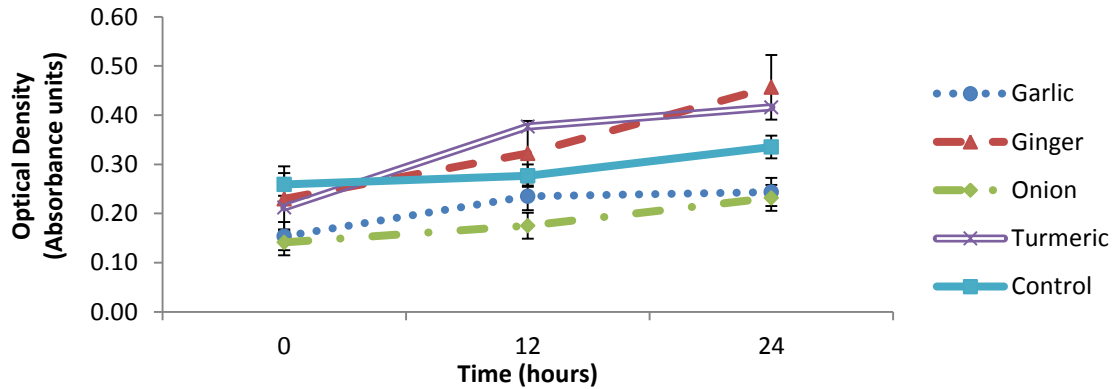
Figure 10. Protease activity of *Lactobacillus acidophilus* LAK

Table 25. Probability > F (Pr > F) of fixed effects for the protease activity of *Lactobacillus acidophilus* LAK as influenced by 1% of spice juice

EFFECT	Pr > F
TREATMENT	<0.001
TIME	<0.001
TREATMENT * TIME	<0.001

Time = incubation period of 24 hours

All spices were significantly (P < 0.05) different when compared with control at 0, 12 and 24 hours of incubation (Table 26), and all spices and control showed an increasing pattern in their protease activity at all incubation times (Table 26 and Figure 10). Protease activity is one of the features that have an impact on the taste, texture, and shelf life of cultured dairy products (Soda 1991). A crucial part of the proteolysis reaction in fermented products is the enzymes that are present in the intracellular components of the fermenting bacteria (Gatti *et. al.* 2004). The disruption of the bacterial cell wall is a crucial part of the protease enzyme release process (Wilkinson and Kilcawley 2005).

Table 26. Least Square Means for protease activity of *Lactobacillus acidophilus* LAK as influenced by spice juice

Treatment	L. acidophilus		
	0 hrs	12 hrs	24 hrs
Control	0.25 [EF]	0.27 [E]	0.33 [D]
Garlic	**0.15** [J]	**0.23** [G]	**0.24** [FG]
Ginger	**0.23** [GH]	**0.32** [D]	**0.45** [A]
Onion	**0.14** [J]	**0.17** [I]	**0.23** [GH]
Turmeric	**0.21** [H]	**0.37** [C]	**0.41** [B]

[ABC] LSMeans with different letter within the table are significantly different.

In a study by Thompson *et. al.* (1973), the proteolytic activity of ginger rhizome was studied with 3 % bovine serum albumin (BSA) as substrate. This substrate showed a relatively high proteolytic activity that occurred over a pH range of 4.5–6.0, with an optimum pH of 5.0, which for meat applications results in more tender meat.

Spices like turmeric are known to contain proteases and to have proteolytic activity (Nagarathnam *et. al.* 2010). In China, an oriental-style type of cheese, called "Jiangzhinai", is made with ginger proteases, and according to a study by Huang *et. al.* (2011), it is suggested that ginger has the potential to be a rennet substitute in the manufacture of cheeses.

3.5. Physico-chemical characteristics of yogurt
3.5.1. pH

The pH of the spicy blueberry yogurt as influenced by 0.05% of spice juice is shown in Figure 11. There was a significant effect for day, while the effects of treatment and treatment * day were not significant (Table 27).
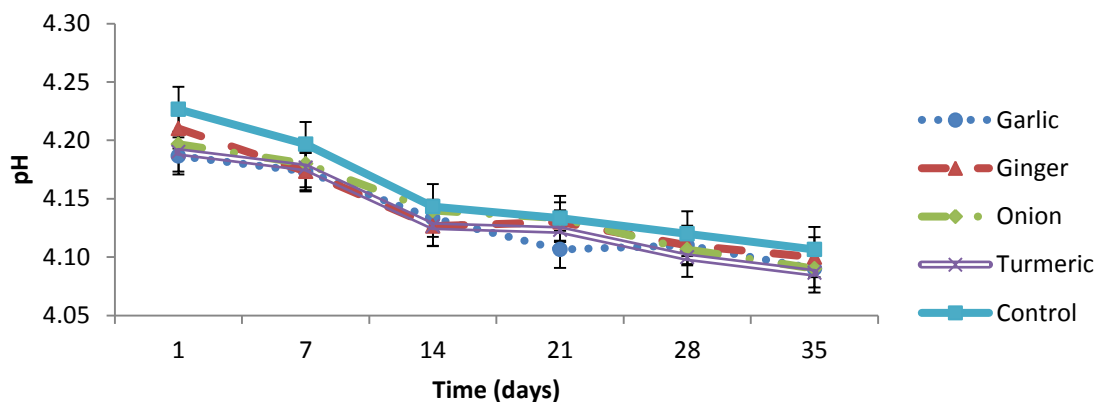
Figure 11. pH of spicy blueberry yogurt as influenced by 0.05% spice juice

None of the spices were significantly different when compared to control. The effect of the storage days shows that there was a significant difference among days 1 and 35, while there was no significant difference among the other days of storage (Table 28). There was a decrease in pH, with all four spices compared to control during the 35 days of storage (Table 28 and Figure 11).

Table 27. Probability > F (Pr > F) of fixed effects for the pH of the spicy blueberry yogurt as influenced by 0.05% of spice juice

EFFECT	Pr > F
TREATMENT	0.9681
DAY	**0.0277**
TREATMENT * DAY	1.0000

Day = once a week testing for 5 weeks (days 1, 7,14,21,28 & 35)

Table 28. Least Square Means for the pH of spicy blueberry yogurt as influenced by 35 days of storage

Days	pH
1	**4.20^{A}**
7	4.18^{AB}
14	4.13^{AB}
21	4.12^{AB}
28	4.10^{AB}
35	**4.09^{B}**

^{ABC} LSMeans with the same letter within the column are not significantly different

The changes in acidity and pH during storage time are due to the biochemical changes that take place in yogurt, where the lactic acid bacteria produce lactic acid from lactose (Simanca *et. al.* 2012). Amirdivani and Baba (2011), measured the pH on yogurts that were exposed to peppermint, dill and basil. According to their study, herbal-yogurts had faster rates of pH reduction than plain-yogurt. Behrad *et. al.* (2012) measured the pH on yogurts that were exposed to cinnamon and licorice and they reported on their study that there were no significant differences in pH between herbal-yogurts and plain-yogurt during fermentation and storage.

3.5.2. Titratable acidity

The titratable acidity of the spicy blueberry yogurt as influenced by 0.05% of spice juice is shown in Figure 12. There was a significant effect for day, while the effects of treatment and treatment * day were not significant (Table 29).
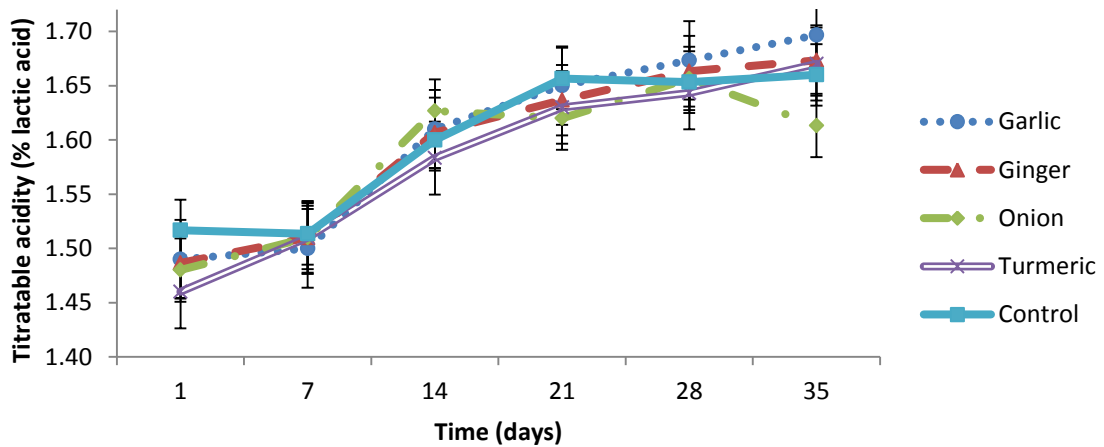
Figure 12. Titratable acidity of spicy blueberry yogurt as influenced by 0.05% spice juice

None of the spices were significantly different when compared to control. The effect of the storage days shows that there was a significant difference among days 1 and 14

(Table 30). There was an increase in titratable acidity, with all spices except onion above

the level of control (Table 30 and Figure 12).

Table 29. Probability > F (Pr > F) of fixed effects for the titratable acidity of spicy
blueberry yogurt as influenced by 0.05% of spice juice

EFFECT	Pr > F
TREATMENT	0.9721
DAY	**<0.0001**
TREATMENT * DAY	1.0000

Day = once a week testing for 5 weeks (days 1, 7,14,21,28 & 35)

Table 30. Least Square Means for titratable acidity (TA) of spicy blueberry yogurt as
influenced by 35 days of storage

Days	TA
1	**1.48^C**
7	1.50^{BC}
14	1.60^{AB}
21	**1.63^A**
28	**1.65^A**
35	**1.66^A**

[ABC] LSMeans with the same letter within the column are not significantly different

Gündoğdu *et. al.* (2009) measured the acidity of set and stirred yogurts that were exposed

to 1% and 0.05% of garlic. Their study showed that garlic addition had no effect on

acidity. Behrad *et. al.* (2012) measured the titratable acidity on yogurts that were

exposed to cinnamon and licorice and they reported on their study that there were no

significant differences in titratable acidity between herbal-yogurts and plain-yogurt

during fermentation and storage. According to Minto *et. al.* (2010), 1% (w/v) plant

extract (olive, garlic, onion and citrus with sodium acetate as a carrier) increased the

titratable acidity of yogurt when compared to the control.

3.5.3. Coliforms

The growth of coliform bacteria on the spicy blueberry yogurt as influenced by 0.05% of spice juice is shown in Figure 13. There was a significant effect for treatment, day and treatment * day (Table 31).
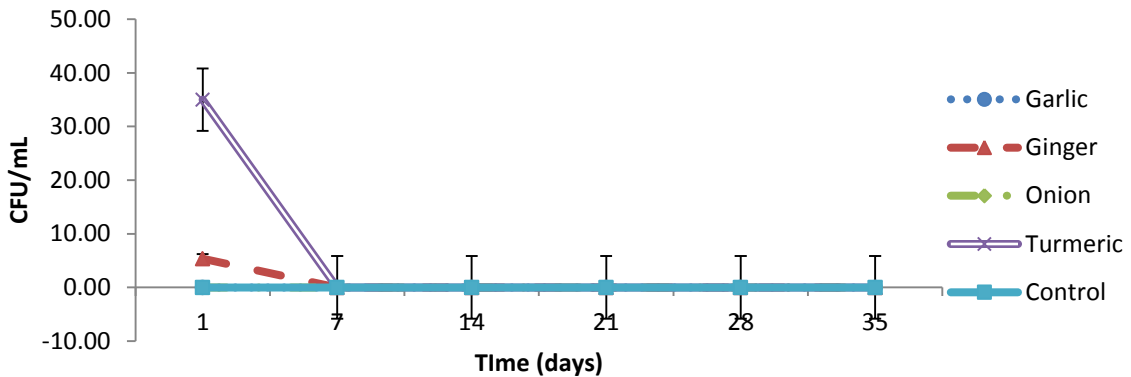

Figure 13. Coliform bacterial growth of spicy blueberry yogurt as influenced by 0.05% spice juice

Table 31. Probability > F (Pr > F) of fixed effects for the coliform bacterial growth of spicy blueberry yogurt as influenced by 0.05% of spice juice

EFFECT	Pr > F
TREATMENT	<0.0001
DAY	<0.0001
TREATMENT * DAY	<0.0001

Day = once a week testing for 5 weeks (days 1, 7,14,21,28 & 35)

Turmeric was significantly (P < 0.05) different when compared to control and the other spices at day 1 (Table 32 and Figure 13). None of the other spices were significantly different when compared to control during the other days of storage (Table 32). While turmeric and ginger showed coliform growth only at day 1 of storage, while there was no growth from the other spices and control, only turmeric was significant (Table 32 and Figure 13).

Table 32. Least Square Means for coliform bacterial growth of spicy blueberry yogurt as influenced by 0.05% of spice juice

Treatment (spice)	Coliforms					
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Control	0.00[B]	-0.00[B]	-0.00[B]	-0.00[B]	-0.00[B]	0.00[B]
Garlic	0.00[B]	-0.00[B]	-0.00[B]	-0.00[B]	-0.00[B]	0.00[B]
Ginger	5.33[B]	-0.00[B]	-0.00[B]	-0.00[B]	-0.00[B]	0.00[B]
Onion	-0.00[B]	-0.00[B]	-0.00[B]	-0.00[B]	-0.00[B]	0.00[B]
Turmeric	35.00[A]	0.00[B]	0.00[B]	0.00[B]	0.00[B]	-0.00[B]

[ABC] LSMeans with different letter within the table are significantly different.

Spices are grown and harvested in parts of the world where sanitation is poor, so they can harbor a wide variety of bacteria and fungi (Shelef 1983). Therefore, cleaning and sterilizing treatments are applied to species to avoid food poisoning (Fischettit 1980). According to a study by Gündoğdu *et. al.* (2009), coliforms did not grow in stirred and set yogurt exposed to garlic during the testing and storage period of 28 days.

3.5.4. Growth
3.5.4.1. *Streptococcus thermophilus* ST-M5

The growth of *Streptococcus thermophilus* ST-M5 in spicy blueberry yogurt as influenced by 0.05% of spice juice is shown in Figure 14. There was a significant effect for treatment, day and treatment * day (Table 33). At 1 day of storage, none of the spices were significantly ($P > 0.05$) different when compared to control (Table 34). At 7 days of storage, garlic, ginger and onion were significantly ($P < 0.05$) different when compared to control, while turmeric did not show any significant ($P > 0.05$) difference (Table 34). In addition, there was no significant difference in viable counts which ranged between 8.05 Log CFU/mL and 8.50 Log CFU/mL (Table 34 and Figure 14).

Figure 14. Growth of *Streptococcus thermophilus* ST-M5 on spicy blueberry yogurt as influenced by 0.05% spice juice

Table 33. Probability > F (Pr > F) of fixed effects for the growth of *Streptococcus thermophilus* ST-M5 in spicy blueberry yogurt as influenced by 0.05% of spice juice

EFFECT	Pr > F
TREATMENT	<0.001
DAY	<0.001
TREATMENT * DAY	<0.001

Day = once a week testing for 5 weeks (days 1, 7,14,21,28 & 35)

Table 34. Least Square Means for the growth of *Streptococcus thermophilus* ST-M5 in spicy blueberry yogurt as influenced by 0.05% of spice juice

Treatment (spice)	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Control	8.73 [AB]	8.05 [DE]	7.68 [FGH]	6.66 [JK]	6.14 [L]	5.26 [N]
Garlic	8.95 [A]	**8.50** [BC]	7.65 [FGH]	**7.26** [I]	**6.63** [JK]	**6.50** [JKL]
Ginger	8.78 [AB]	**8.43** [BC]	7.95 [DEF]	**7.37** [HI]	**6.65** [JK]	**6.22** [L]
Onion	8.89 [A]	**8.45** [BC]	7.55 [GHI]	6.73 [J]	**5.65** [M]	5.26 [N]
Turmeric	8.76 [AB]	8.22 [CD]	7.79 [EFG]	**7.36** [HI]	6.30 [KL]	4.97 [N]

[ABC] LSMeans with different letter within the table are significantly different

At 14 days of storage, there was no significant (P > 0.05) difference among the spices when compared to control (Table 34) and there was a 1 Log decrease in viable counts from all spices and control with a range of 7.55 Log CFU/mL to 7.95 Log CFU/mL (Table 34 and Figure 14). At 21 days of storage, garlic, ginger and turmeric were

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significantly (P < 0.05) different when compared to control, while onion did not show any significant (P > 0.05) difference (Table 34). Also, there was a 1 Log decrease in viable counts from control (6.66 Log CFU/mL) and onion (6.73 Log CFU/mL) (Table 34 and Figure 14). All of the spices, except for turmeric, were significantly (P < 0.05) different when compared to control at 28 days of storage (Table 34), while there was a 1 Log decrease in viable counts from all spices which ranged from 6.65 Log CFU/mL to 5.65 Log CFU/mL) (Table 34 and Figure 14). At 35 days of storage, garlic and ginger were significantly (P < 0.05) different when compared to control, while onion and turmeric showed no significant (P > 0.05) difference (Table 34). In addition, the viable counts of control (5.26 Log CFU/mL) decreased by 1 Log, while the viable counts of turmeric (4.97 Log CFU/mL) decreased by 2 Logs (Table 34 and Figure 14). There was a decrease in viable counts, with garlic and ginger showing viable counts above control (Figure 14).

3.5.4.2. *Lactobacillus bulgaricus* LB-12

The growth of *Lactobacillus bulgaricus* LB-12 in spicy blueberry yogurt as influenced by 0.05% of spice juice is shown in Figure 15. There was a significant effect for treatment, day and treatment * day (Table 35). At 1 day of storage, garlic and ginger were significantly (P < 0.05) different when compared to control (Table 36), and also had lower viable counts with a difference of 1 Log (8.95 Log CFU/mL and 8.78 Log CFU/mL, respectively) (Table 36 and Figure 15). At 7 days of storage, garlic, ginger and onion were significantly (P < 0.05) different when compared to control, while turmeric was not (Table 36).
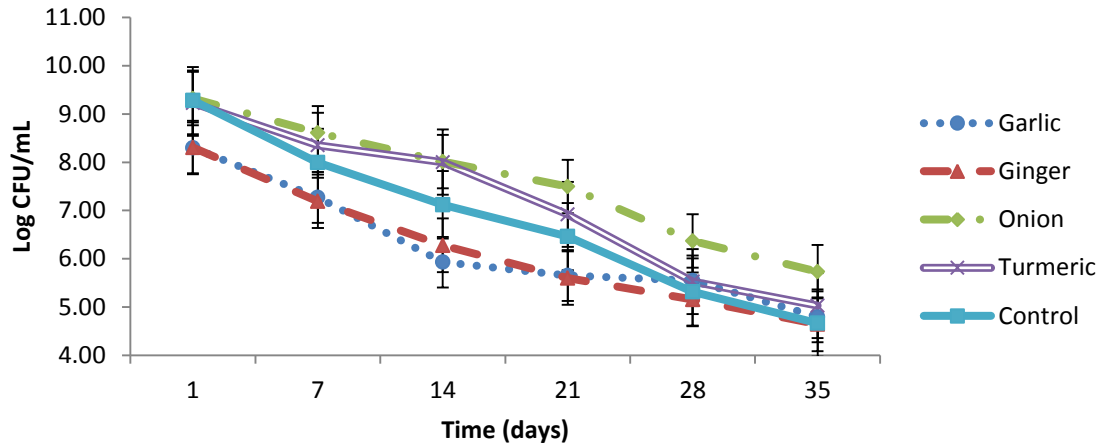
Figure 15. Growth of *Lactobacillus bulgaricus* LB-12 on spicy blueberry yogurt as influenced by 0.05% spice juice

Table 35. Probability > F (Pr > F) of fixed effects for the growth of *Lactobacillus bulgaricus* LB-12 in spicy blueberry yogurt as influenced by 0.05% of spice juice

EFFECT	Pr > F
TREATMENT	<0.001
DAY	<0.001
TREATMENT * DAY	<0.001

Day = once a week testing for 5 weeks (days 1, 7,14,21,28 & 35)

Table 36. Least Square Means for the growth of *Lactobacillus bulgaricus* LB-12 in spicy blueberry yogurt as influenced by 0.05% of spice juice

Treatment (spice)	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Control	9.28 [A]	7.99 [C]	7.12 [DE]	6.46 [F]	5.31 [JKL]	4.66 [NO]
Garlic	**8.29** [BC]	**7.27** [DE]	**5.92** [GH]	**5.65** [HIJ]	5.54 [HIJ]	4.79 [MNO]
Ginger	**8.30** [BC]	**7.19** [DE]	**6.27** [FG]	**5.60** [HIJ]	5.15 [KLM]	4.64 [O]
Onion	9.32 [A]	**8.61** [B]	**8.01** [C]	**7.49** [D]	**6.37** [F]	**5.73** [HI]
Turmeric	9.22 [A]	8.35 [BC]	**8.00** [C]	**6.92** [E]	5.53 [IJK]	5.03 [LMN]

[ABC] LSMeans with different letter within the table are significantly different

There was no significant difference in viable counts from any of the spices or control, which ranged in between 8.61 Log CFU/mL to 7.19 Log CFU/mL (Table 36 and Figure 15). All of the spices were significantly (P < 0.05) different at 14 days of storage when

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compared to control (Table 36) and there was a 1 Log decrease in viable counts from ginger (6.27 Log CFU/mL), while the viable counts of garlic (5.92 Log CFU/mL) decreased by 2 Logs (Table 36 and Figure 15). At 21 days of storage, all spices were significantly ($P < 0.05$) different when compared to control (Table 36) and the viable counts of control (6.46 Log CFU/mL), ginger (5.60 Log CFU/mL) and onion (7.49 Log CFU/mL) decreased by 1 Log, while the viable counts of turmeric (6.92 Log CFU/mL) decreased by 2 Logs (Table 36 and Figure 15). At 28 days of storage, onion was significantly ($P < 0.05$) different, while garlic, ginger and turmeric were not significantly ($P > 0.05$) different when compared to control (Table 36). In addition, there was a 1 Log reduction in viable counts from control (5.31 Log CFU/mL), onion (6.37 Log CFU/mL) and turmeric (5.53 Log CFU/mL) (Table 36 and Figure 15). At 35 days of storage, onion was significantly ($P < 0.05$) different, while the rest of the spices were not significantly different when compared to control (Table 36), while control (4.66 Log CFU/mL) and all spices except for turmeric (5.03 Log CFU/mL), decreased their viable counts by 1 Log (Table 36 and Figure 15). There was a decrease in viable counts, with onion and turmeric showing viable counts above control (Figure 15).

3.5.4.3. *Lactobacillus acidophilus* LAK

The growth of *Lactobacillus acidophilus* LAK in spicy blueberry yogurt as influenced by 0.05% of spice juice is shown in Figure 16. There was a significant effect for treatment, day and treatment * day (Table 37). At 1 day of storage, none of the spices were significantly ($P > 0.05$) different when compared to control (Table 38). At 7 days of storage, none of the spices were significantly ($P > 0.05$) different when compared to control (Table 38), while there was a 1 Log decrease in viable counts for all spices and

control which ranged from 6.77 Log CFU/mL to 6.66 Log CFU/mL) (Table 38 and Figure 16).
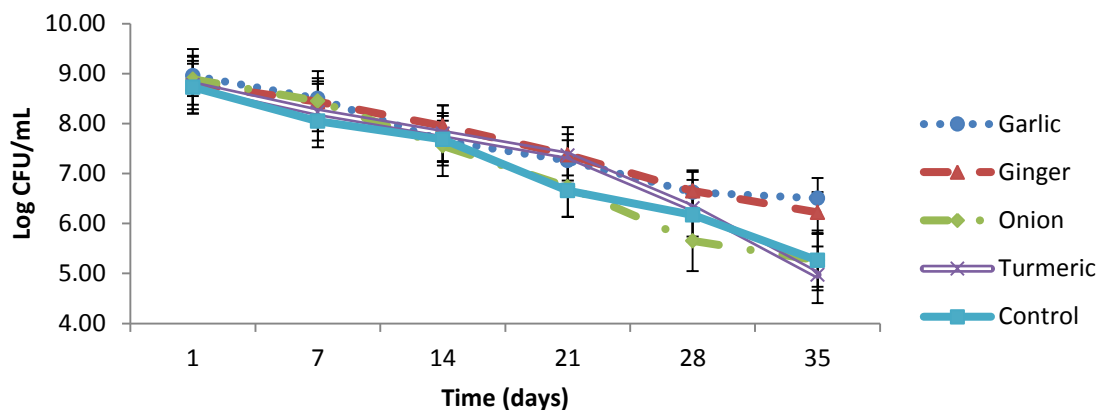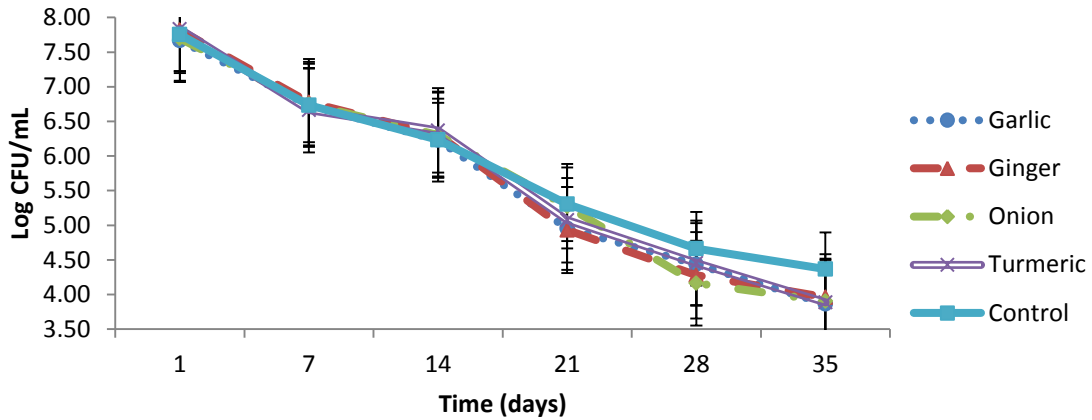

Figure 16. Growth of *Lactobacillus acidophilus* LAK on spicy blueberry yogurt as influenced by 0.05% spice juice

Table 37. Probability > F (Pr > F) of fixed effects for the growth of *Lactobacillus acidophilus* LAK in spicy blueberry yogurt as influenced by 0.05% of spice juice

EFFECT	*L. acidophilus*
	Pr > F
TREATMENT	<0.001
DAY	<0.001
TREATMENT * DAY	<0.001

Day = once a week testing for 5 weeks (days 1, 7,14,21,28 & 35)

Table 38. Least Square Means for the growth of *Lactobacillus acidophilus* LAK in spicy blueberry yogurt as influenced by 0.05% of spice juice

Treatment (spice)	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Control	7.75 [AB]	6.73 [C]	6.23 [D]	5.30 [E]	4.66 [G]	4.36 [HI]
Garlic	7.66 [B]	6.73 [C]	6.23 [D]	**4.95** [F]	**4.43** [HI]	**3.86** [K]
Ginger	7.82 [AB]	6.77 [C]	6.31 [D]	**4.93** [F]	**4.28** [IJ]	**3.96** [K]
Onion	7.70 [AB]	6.74 [C]	6.30 [D]	5.27 [E]	**4.16** [J]	**3.90** [K]
Turmeric	7.83 [A]	6.66 [C]	6.37 [D]	**5.07** [F]	**4.45** [H]	**3.89** [K]

[ABC] LSMeans with different letter within the table are significantly different

At 14 days of storage, none of the spices were significantly (P > 0.05) different when compared to control (Table 38), while there was no significant difference in the viable counts from any of the spices or the control (Table 38 and Figure 16). At 21 days of storage, garlic, ginger and turmeric were significantly (P < 0.05) different when compared to control, while onion did not show any significant (P > 0.05) difference (Table 38). In addition, there was a 1 Log decrease in viable counts from control (5.30 Log CFU/mL), onion (5.27 Log CFU/mL) and turmeric (5.07 Log CFU/mL), while garlic (4.95 Log CFU/mL) and ginger (4.93 Log CFU/mL) decreased their viable counts by 2 Logs (Table 38 and Figure 16). At day 28 of storage, all spices were significantly (P < 0.05) different when compared to control (Table 38) and there was a 1 Log reduction in viable counts from control (4.66 Log CFU/mL), onion (4.16 Log CFU/mL) and turmeric (4.45 Log CFU/mL) (Table 38 and Figure 16). At 35 days of storage, all spices were significantly (P < 0.05) different when compared to control (Table 38) and there was a reduction of 1 Log in viable counts from garlic (3.86 Log CFU/mL), ginger (3.96 Log CFU/mL), onion (3.90 Log CFU/mL) and turmeric (3.89 Log CFU/mL) (Table 38 and Figure 16). There was a decrease in viable counts, with all spices showing viable counts below control (Figure 16).

A study by Behrad *et. al.* (2012), in which yogurt was exposed to some spices (cinnamon and licorice), reported a decrease in viable cell counts in the herbal-yogurts, but all yogurts contained acceptable level of probiotic bacteria by the end of the 28 days of refrigerated storage. Otaibi and El Demerdash (2008) reported that the viable counts of

Streptococcus thermophilus and *Lactobacillus bulgaricus* in labneh[10] treated with essential oils of thyme, marjoram and sage, increased and reached a maximum after 7 days of storage where they decreased until the end of the 21 days storage period.

3.5.5. Color
3.5.5.1.L*

The L*(lightness) of spicy blueberry yogurt as influenced by 0.05% of spice juice is shown in Figure 17. There was a significant (P < 0.05) effect for treatment and day (Table 39).
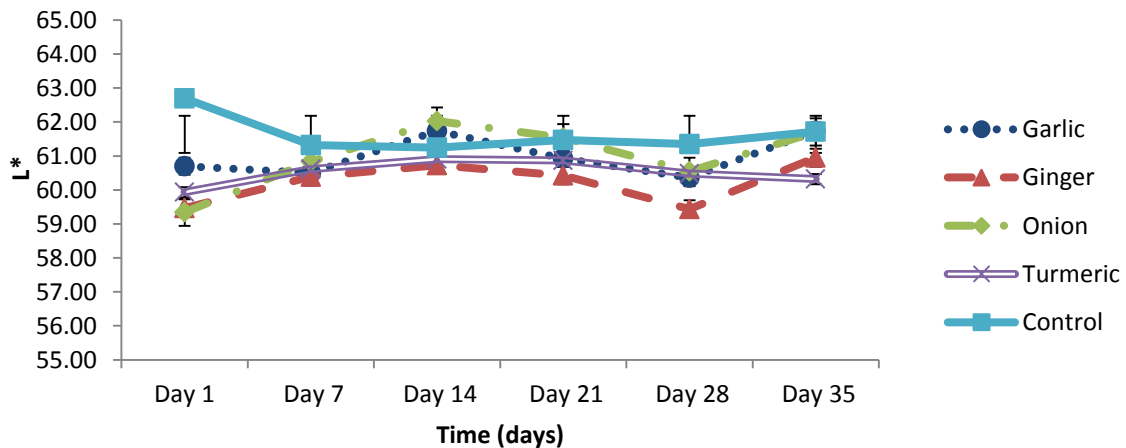
Figure 17. Measurement of L* of spicy blueberry yogurt as influenced by 0.05% spice juice

Table 39. Probability > F (Pr > F) of fixed effects for the color of spicy blueberry yogurt as influenced by 0.05% of spice juice

EFFECT	L*	a*	b*	C*	h*
	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
TREATMENT	0.0018	<0.0001	<0.0001	0.6555	<0.0001
DAY	0.0444	0.0016	<0.0001	0.0142	<0.0001
TREATMENT * DAY	0.4888	0.9103	0.2653	0.7753	0.6778

Day = once a week testing for 5 weeks (days 1, 7,14,21,28 & 35)

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[10] Labneh, a traditional fermented milk product that is consumed in Middle Eastern countries, is obtained from yoghurt after removal of part of its whey. In addition to having an acidic flavour and milky white colour, labneh is soft, smooth and spreadable with a consistency that resembles cultured cream (Otaibi and El Demerdash 2008).

Ginger and turmeric were significantly (P < 0.05) lower when compared with control (Table 40 and Figure 17). There were no significant differences throughout the 35 days of storage (Table 41). All spices showed values below control, indicating a decrease in lightness (Figure 17).

Table 40. Least Square Means for color of spicy blueberry yogurt as influenced by treatment

Treatment	L*	a*	b*	C*	h*
Control	61.63^A	9.40^A	-1.64^B	9.55^A	350.43^A
Garlic	61.03^{AB}	9.68^A	-1.36^B	9.70^A	351.75^A
Ginger	**60.23^B**	9.83^A	-1.69^B	10.00^A	350.66^A
Onion	61.00^{AB}	9.83^A	-1.90^B	10.02^A	349.37^A
Turmeric	**60.52^B**	**6.79^B**	**6.31^A**	9.67^A	**44.97^B**

^{ABC}LSMeans with the same letter within the column for a test are not significantly different

Table 41. Least Square Means for color of spicy blueberry yogurt as influenced by 35 days of storage

Days	L*	a*	b*	C*	h*
1	60.42^A	**10.05^A**	**-1.75^B**	**10.48^A**	**283.31^C**
7	60.73^A	9.06^B	-0.47^{AB}	9.65^{AB}	286.56^{BC}
14	61.33^A	8.86^B	-0.00^A	9.30^{AB}	288.86^{ABC}
21	61.04^A	8.74^B	0.56^A	9.78^{AB}	292.30^{AB}
28	60.43^A	9.18^{AB}	0.84^A	10.32^{AB}	**292.72^A**
35	61.34^A	8.74^B	0.46^A	**9.20^B**	292.30^{AB}

^{ABC}LSMeans with the same letter within the column for a test are not significantly different

3.5.5.2.a*

The a*(red- green axis) of spicy blueberry yogurt as influenced by 0.05% of spice juice is shown in Figure 18. There was a significant (P < 0.05) effect for treatment and day (Table 39). Turmeric was significantly lower from the other spices and control (Table 40 and Figure 18). As for the effect of day, day 1 showed to be significantly different from the rest of the storage days, showing the highest value (Table 41). Turmeric showed

values significantly lower than control, while all spices obtained positive numbers, which means they are in the red color space (Figure 18).
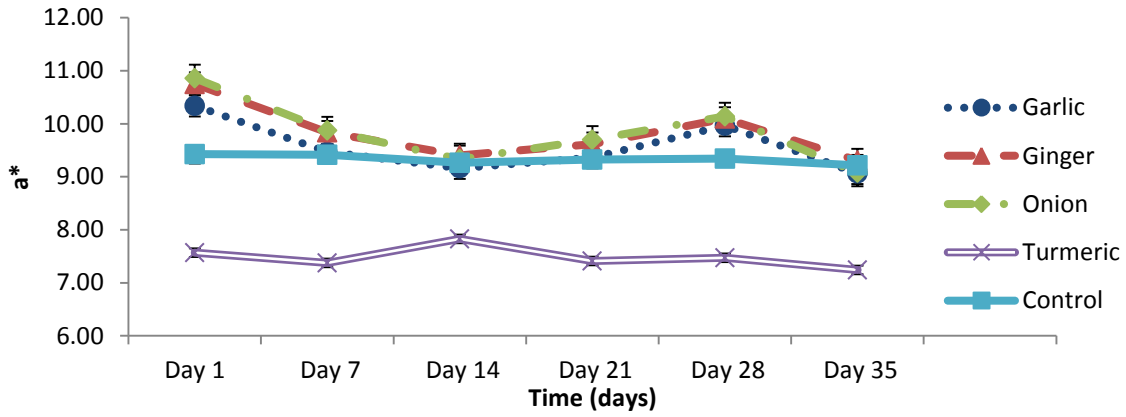
Figure 18. Measurement of a* of spicy blueberry yogurt as influenced by 0.05% spice juice

3.5.5.3.b*

The b*(blue- yellow axis) of spicy blueberry yogurt as influenced by 0.05% of spice juice is shown in Figure 19. There was a significant (P < 0.05) effect for treatment and day (Table 39). Day 1 showed a significant (P < 0.05) difference by obtaining the lowest value when compared to control and the other spices (Table 41). Turmeric showed values significantly higher than control (Table 40 and Figure 19). Garlic, ginger and onion obtained negative values, which indicate that they are in the blue color space, while turmeric obtained positive numbers, meaning it was in the yellow color space (Table 40 and Figure 19). The total color difference (ΔE) for garlic, ginger and onion compared to control can be seen in Table 42. The averages of the L*, a* and b* values of these three samples at days 1, 7, 14, 21, 28 and 35 were used to calculate the magnitude of the ΔE by means of the following equation (HunterLab 2001): $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$.
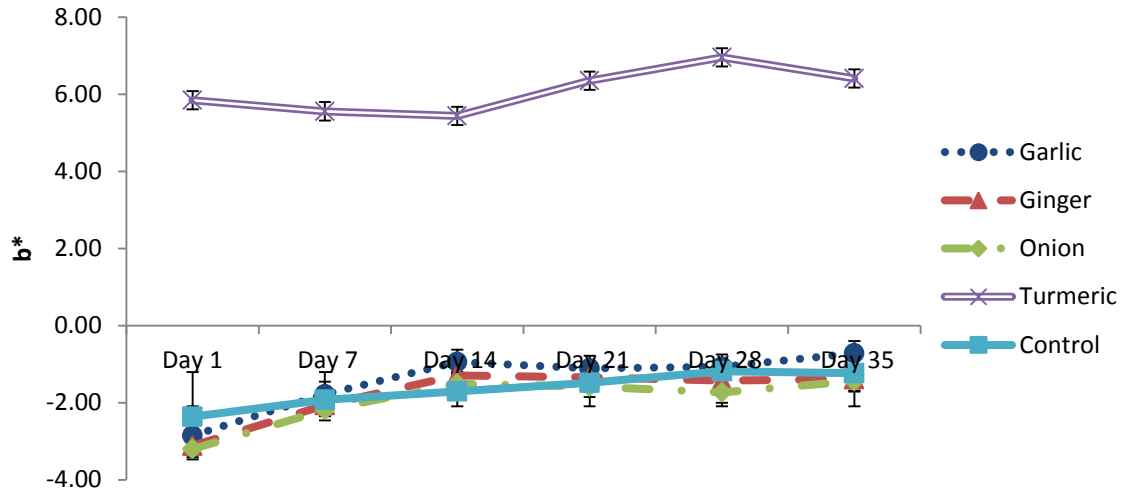
Figure 19. Measurement of b* of spicy blueberry yogurt as influenced by 0.05% spice juice

Garlic, ginger and onion yogurt looked similar in color when compared to control, whereas turmeric showed an obvious visual difference when compared to control. According to Caner and Cansiz (2008), ΔE values less than 3.0 cannot be easily detected by naked human eyes. According to table 42, there was no noticeable difference in the color of garlic yogurt when compared to control yogurt at any of the time points. There was a slight difference in the color of ginger yogurt when compared to control yogurt at day 1, but no noticeable color difference from days 7 to day 35. As for onion yogurt, there were slight color differences at days 1 and 28 when compared to control yogurt, but no discernible differences at the other time points.

Table 42. Total color difference (ΔE) of garlic, ginger and onion compared to control as influenced by 0.05% of spice juice and 35 days of storage

Days	Garlic	Ginger	Onion
1	2.25	**3.58**	**3.75**
7	0.83	1.03	0.73
14	0.92	0.68	0.81
21	0.67	1.09	0.40
28	1.18	2.07	**3.13**
35	0.53	0.79	0.25

3.5.5.4.C*

The C (chroma/saturation) of spicy blueberry yogurt as influenced by 0.05% of spice juice is showed in Figure 20. There was a significant (P < 0.05) effect for day (Table 39). None of the spices were significantly (P > 0.05) different when compared to control (Table 40). In addition, none of the spices were significantly different from each other or control (Table 40 and Figure 20). As for the effect of day, day 1and 35 showed a significant difference when compared to the other storage days (Table 41).
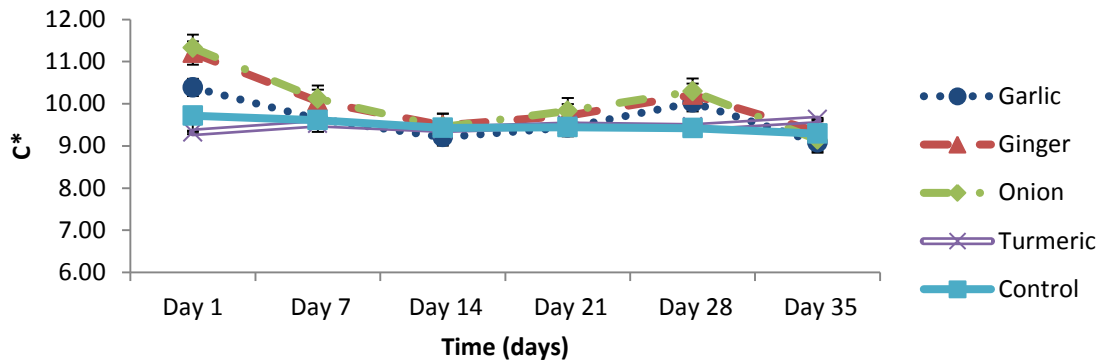
Figure 20. Measurement of C* of spicy blueberry yogurt as influenced by 0.05% spice juice

3.5.5.5.h*

The h (hue) of spicy blueberry yogurt as influenced by 0.05% of spice juice is showed in Figure 13B. There was a significant (P < 0.05) effect for treatment and day (Table 39). Turmeric was significantly (P < 0.05) lower when compared to control during all days of storage (Table 40 and Figure 21). As for the effect of day, days 1 and 28 were significantly (P < 0.05) different when compared with the other storage days (Table 41). Turmeric showed values significantly (P < 0.05) lower than control, meaning a shift in the overall true color (Figure 21).
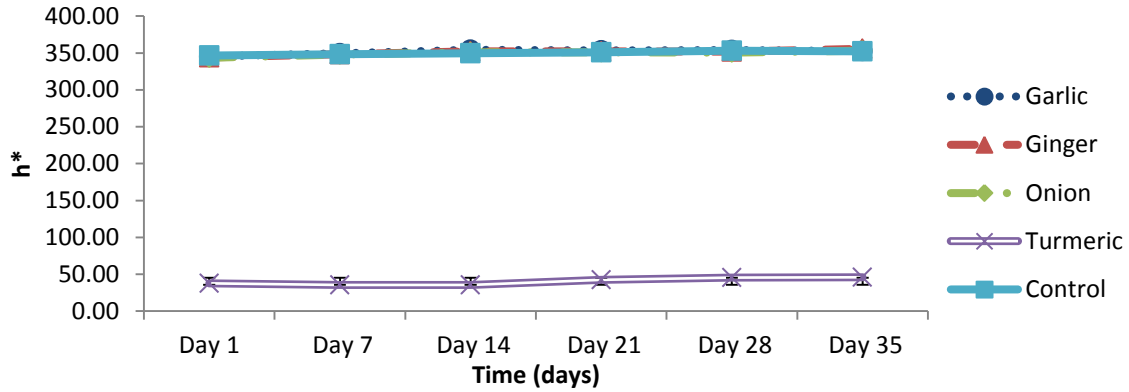
Figure 21. Measurement of h* of spicy blueberry yogurt as influenced by 0.05% spice juice

Spices can play an important role in the color of foods. Licón *et. al.* (2012) reported the effects that the addition of saffron spice had in hard cheese, where there was a significant change in color as the concentration of saffron spice increased, imparting a characteristic red color to the product. On the other hand, Tarakci *et. al.* (2011) reported that increasing the level of garlic in herbed pickled cheese decreased the L* (lightness) and the b* (blue-yellow axis), but the values of a* (red-green axis) increased. According to Chandan (2006) some of the natural colors that are preferred because of their heat stability during food processing include several spices, with turmeric among them.

3.5.6. Apparent viscosity

Apparent viscosity of spicy blueberry yogurt as influenced by 0.05% of spice juice is shown in Figure 22. There was a significant ($P < 0.05$) effect for day (Table 43). None of the spices were significantly ($P > 0.05$) different when compared to control. As for the effect of day, days 1and 35 were significantly different when compared to the rest of the storage days (Table 44). There was an increase in apparent viscosity, with all spices showing more apparent viscosity than control, especially onion and ginger (Figure 22).

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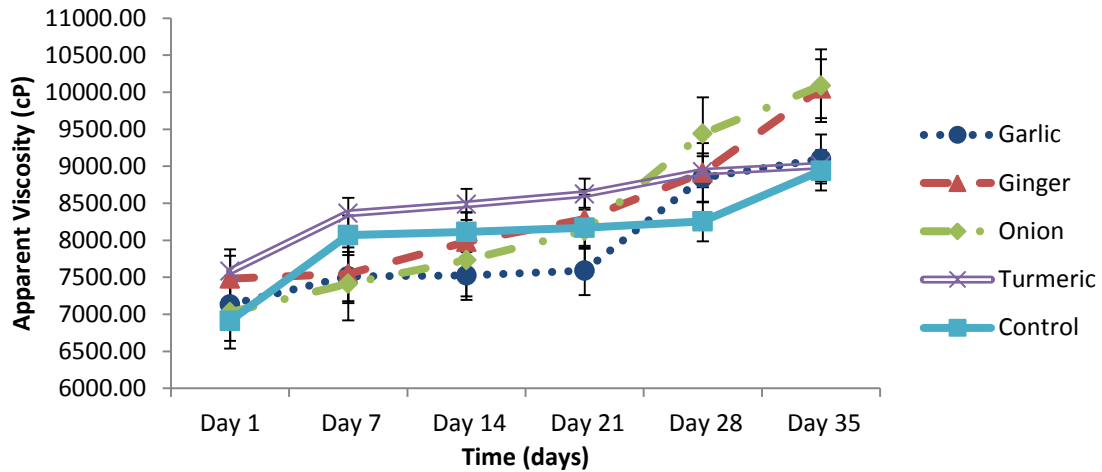
Figure 22. Measurement of apparent viscosity of spicy blueberry yogurt as influenced by 0.05% spice juice

Table 43. Probability > F (Pr > F) of fixed effects for the apparent viscosity of spicy blueberry yogurt as influenced by 0.05% of spice juice

EFFECT	Apparent Viscosity
	Pr > F
TREATMENT	0.5542
DAY	**<0.0001**
TREATMENT * DAY	0.9911

Day = once a week testing for 5 weeks (days 1, 7,14,21,28 & 35)

Table 44. Least Square Means for the apparent viscosity of spicy blueberry yogurt as influenced by 35 days of storage

Days	Apparent Viscosity
1	**7225.7^C**
7	7780.2^{BC}
14	7966.3^{BC}
21	8159.4^{BC}
28	8874.9^{AB}
35	**9436.2^A**

^{ABC}LSMeans with the same letter within the column are not significantly different

In a study by Yang *et. al.* (2012), the effects of the addition of ginger juice to milk was explored. It was found that there was a significant ($P < 0.05$) influence on the viscosity of yogurt exposed to concentrations of 2 to 10% of ginger juice. Hassan *et. al.* (2010) manufactured a yogurt using concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% of aqueous

garlic extract. According to their results, after 15 days of storage, the resultant yogurt in all different concentrations of garlic had higher values than control. Foda *et. al.* (2007) prepared a set yogurt using buffalo milk and supplemented with turmeric powder at concentrations of 0.1, 0.25, 0.50, 0.75 and 1%. Their study reported that the firmness and viscosity of the yogurt increased significantly as the concentration of turmeric increased.

3.5.7. Consumer testing/acceptance of spicy blueberry yogurt
3.5.7.1.Sensory test of spicy blueberry yogurt

A consumer acceptability of blueberry spicy yogurt as influenced by 0.05% of spice juice was done with 100 random people. The frequencies for the sensory and acceptability information are shown in Figures 23 and 24. There was a significant ($P < 0.05$) difference among treatment (Table 45). In terms of appearance and color, turmeric obtained significantly lowest scores when compared to the other spices and control (Table 46). In relation to aroma, garlic, onion and turmeric obtained significantly lower scores when compared to control, while ginger did not show a significant difference from control (Table 46). Regarding taste, garlic, onion and turmeric obtained significantly lower scores when compared to control, while ginger did not show a significant difference from control (Table 46). When evaluating thickness, it was noted that none of the spices showed a significant difference when compared to each other and to control (Table 46). When considering graininess, garlic and turmeric obtained significantly lower scores when compared to control, while ginger and onion showed no significant difference from control (Table 46). In overall like, ginger was not significantly different from control and both these treatments obtained the highest scores, while garlic, onion and turmeric obtained significantly lower scores from the rest of the spices (Table 46).

84
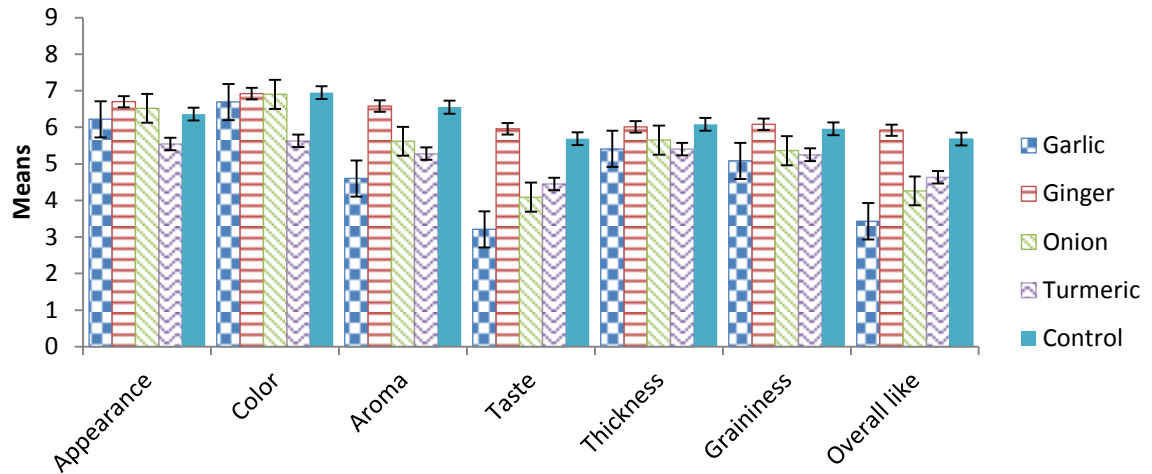
Figure 23. Spicy blueberry yogurt consumer test: means for sensory attributes
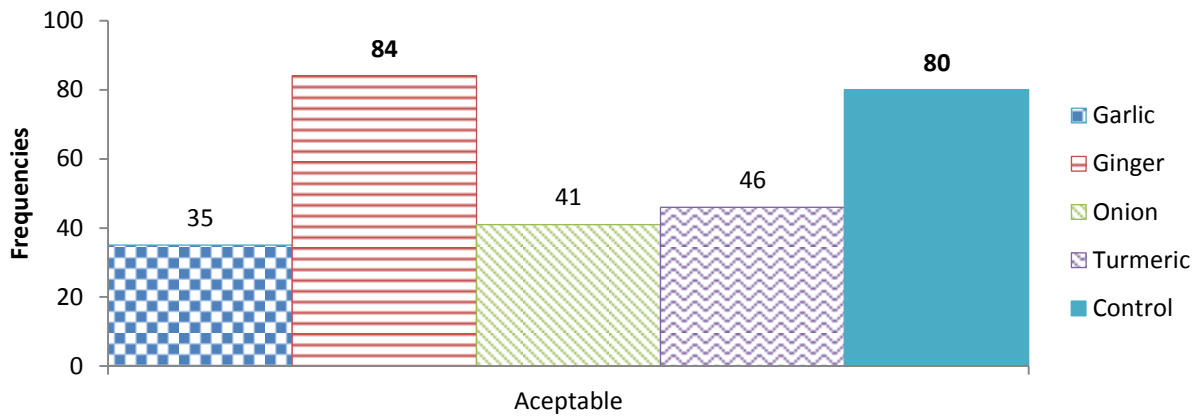
Figure 24. Spicy blueberry yogurt consumer test: frequency for acceptability of spicy blueberry yogurt

Table 45. Probability > F (Pr > F) of fixed effects for the sensory attributes of spicy blueberry yogurt as influenced by 0.05% of spice juice

EFFECT	Appearance	Color	Aroma	Taste	Thickness	Graininess	Overall like
	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
TREATMENT	<0.0001	<0.0001	<0.0001	<0.0001	0.0103	<0.0001	<0.0001

As for acceptability, control was accepted by 80% of the participants and ginger was accepted by 84% of the participants; as for garlic, onion and turmeric, they were rejected by 65%, 59% and 54% of the participants, respectively (Table 47 and Figure 24).

Table 46. Means and standard deviation for sensory attributes of spicy blueberry yogurt as influenced by 0.05% of spice juice

Treatment	Sensory attributes						
	Appearance	Color	Aroma	Taste	Thickness	Graininess	Overall like
Control	6.36 ± 1.64^A	6.95 ± 1.62^A	6.55 ± 1.83^A	5.69 ± 1.91^A	6.08 ± 1.75^A	$5.96 \pm 1.62^{A,B}$	5.68 ± 1.75^A
Garlic	6.22 ± 1.68^A	6.69 ± 1.48^A	$\mathbf{4.60 \pm 2.17^C}$	$\mathbf{3.21 \pm 2.13^C}$	5.41 ± 1.89^A	$\mathbf{5.08 \pm 1.84^C}$	$\mathbf{3.43 \pm 2.18^C}$
Ginger	6.70 ± 1.34^A	6.92 ± 1.35^A	6.58 ± 1.40^A	5.96 ± 1.72^A	6.01 ± 1.55^A	6.08 ± 1.52^A	5.92 ± 1.72^A
Onion	6.52 ± 1.34^A	6.90 ± 1.26^A	$\mathbf{5.62 \pm 1.82^B}$	$\mathbf{4.09 \pm 2.27^B}$	5.65 ± 1.76^A	$5.36 \pm 1.67^{B,C}$	$\mathbf{4.26 \pm 2.16^B}$
Turmeric	$\mathbf{5.54 \pm 1.89^B}$	$\mathbf{5.63 \pm 1.82^B}$	$\mathbf{5.28 \pm 2.06^{B,C}}$	$\mathbf{4.45 \pm 2.33^B}$	5.40 ± 1.83^A	$\mathbf{5.25 \pm 1.78^C}$	$\mathbf{4.63 \pm 2.23^B}$

[ABC] Means with the same letter within the row are not significantly different

Table 47. Frequency for acceptability of spicy blueberry yogurt

Treatment	Acceptability	
	Yes	No
Control	**80**	20
Garlic	35	65
Ginger	**84**	16
Onion	41	59
Turmeric	46	54

The intent of purchase for the spicy blueberry yogurt as influenced by 0.05% of spice juice was measured using a McNemar test (McNemar 1947), and it is shown in Table 48. When measuring the purchase intent of ginger, 46% of the participants indicated that they will purchase the sample before and after knowing about a health beneficial spice, and this percentage increased a 24% when participants changed their mind after they found out about the health beneficial spice for a total of 70% (Table 48). Garlic, onion and turmeric had low percentages of purchase intent, being them 33%, 27% and 34% respectively (Table 48). According to Gündoğdu and others (2009), the popularity and high consumption of yogurt is due to the nutritional value and the therapeutic effects of the starter culture bacteria during fermentation. Yang and others (2012), reported that the consumer results from external preference mapping indicated that yogurts made with the lower concentration of ginger juice ($\leq 4\%$) were preferred more than those made with the higher samples ($\geq 6\%$). Foda and others (2007) reported that yogurt made with 0.1% of

turmeric powder was better accepted than those made with higher concentrations (0.25% to 1% w/v).

Table 48. Frequency for intent of purchase of spicy blueberry yogurt

Treatment	Intent of purchase			
	Will purchase before knowing about spice and after knowing about spice	Will purchase before knowing about spice, but will not purchase after knowing about spice	Will not purchase before knowing about spice, but will purchase after knowing about spice	Will not purchase before knowing about spice and after knowing about spice
Garlic	5	0	28	67
Ginger	46	1	24	29
Onion	15	0	12	73
Turmeric	19	2	15	64

CHAPTER 4: CONCLUSIONS

The results from this study show that the juice in a concentration of 1% (v/v) of garlic, ginger, onion and turmeric did not have an inhibitory effect on the growth of *Streptococcus thermophilus* ST-M5, *Lactobacillus bulgaricus* LB-12 and *Lactobacillus acidophilus* LAK. Turmeric showed the lowest reduction in viable counts for *S. thermophilus* and *L. acidophilus*, while *L. bulgaricus* grew best in the presence of onion, when compared to control. All four spices decreased the acid and the bile tolerance of *S. thermophilus*, but still with viable counts. Garlic, ginger and onion improved the bile tolerance of *L. bulgaricus*, while turmeric decreased it when compared to control, but still with viable counts. Ginger, turmeric and onion had the best overall influence on the protease activity of *S. thermophilus*, when compared to control. Turmeric improved the protease activity of *L. bulgaricus* and alongside with ginger, it also improved the protease activity of *L. acidophilus*, when compared to control. The pH of the spicy blueberry yogurt was lower in the presence of all four spices when compared to control, while the titratable acidity was higher in the presence of garlic, ginger and turmeric, when compared to control. At day 1, coliform counts of turmeric were significantly higher compared to the rest of the spices and the control, while at all other storage time points there were no coliform counts. *Streptococcus thermophilus* obtained from the spicy blueberry yogurt grew better in presence of garlic and ginger when compared to control, while *L. bulgaricus* (from the spicy blueberry yogurt) grew better in the presence of onion and turmeric when compared to control. The counts of Lactobacillus acidophilus in the blueberry yogurt with all spices were about half a log lower when compared to control. The apparent viscosity of the spicy blueberry yogurt was particularly higher in

the presence of onion and ginger, when compared to control. The control yogurt obtained 80% acceptance and the ginger yogurt obtained 84% acceptance. Ginger yogurt obtained the highest intent of purchase. Ginger can be used in yogurts for direct consumption while all 4 spices can have potential for a new product line of yogurts for cooking, marinating and dips enabling potential for health benefits from some spices and some bacteria.

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APPENDIX A: RESEARCH CONSENT FORM

RESEARCH CONSENT FORM

I,_____, agree to participate in the research entitled "Sensory characteristics of mildly spicy blueberry yogurt" which is being conducted by the School of Animal Sciences at Louisiana State University, phone number (225)-578-4411.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty of loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. A total of 100 consumers will participate in this research. For this particular research, about a 10 minute participation will be required for each consumer.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigators any allergies I may have.
2. The reason for the research is to gather information on consumer attitude and their acceptance of yogurts with spice. The benefits that I may expect from it are a satisfaction that I have contributed to solution and evaluation of problems relating to such examinations.
3. The procedures are as follows: Coded samples of yogurts will be placed in front of me and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
4. Participation entails minimal risks: The only risk that can be envisioned is an allergic reaction to milk. However, because it is known to me beforehand what type of food to be tested, the situation can normally be avoided.
5. The results of this participation will be confidential and will not be released in any individual identifiable form without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the investigators. In addition, I understand that research at Louisiana State University, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. Philip Elzer, Assistant Vice Chancellor and Assistant Director of the LSU AgCenter 225 578 4182. I agree with the terms above and acknowledge I have been given a copy of the consent form.

_____
Signature of Investigator

Date:_____

_____
Signature of Participant

Witness:_____

APPENDIX B: QUESTIONNAIRE FOR SENSORY EVALUATION

Sample # _____   Date_____

PLEASE EVALUATE THE PRODUCT AND CHECK THE SPACE THAT BEST REFLECTS YOUR FEELING ABOUT THE   PRODUCT.

1. How would you rate the overall **APPEARANCE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

2. How would you rate the **COLOR** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

3. How would you rate the **AROMA** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

4. How would you rate the **TASTE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

5. How would you rate the **TEXTURE (THICKNESS)** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

6. How would you rate the **TEXTURE (GRAININESS)** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

7. OVERALL, how would you "**LIKE**" this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

8. Is this product **ACCEPTABLE**?   Yes   [ ]   No [ ]

9. Would you **BUY** this product if it were commercially available? Yes   [ ]   No [ ]

10. Would you **BUY** this product if you know it contained a spice with health benefits?   Yes   [ ]   No [ ]

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VITA

Margie Michelle Sánchez-Vega was born in Bayamón, Puerto Rico in February, 1981. In 1998 she graduated from Escuela Superior Católica de Bayamón in Bayamón, Puerto Rico. In May 2004 she received her Bachelor's Degree in Animal, Dairy and Poultry Science with a concentration in Science and Technology from Louisiana State University and Agricultural and Mechanical College. With the original intention of becoming a veterinarian, she explored that field by working as a veterinary technician in a few clinics in her natal Puerto Rico. She later decided to pursue another field of studies and in summer 2010 she received her Master's Degree in Public Health from University of Puerto Rico, Medical Sciences Campus in San Juan, Puerto Rico. In December 2013, she is set to obtain her degree of Doctor in Philosophy in Animal Sciences, with a concentration in dairy food sciences from Louisiana State University and Agricultural and Mechanical College.