Development of a New Milk Drink Containing Dates-Immobilized Probiotic Bacteria

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BY

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Dedicated to my beloved late Grandmother

Abstract

Date fruit has been a staple food in North Africa, the Middle East, and South Asian countries for thousands of years. Dates are rich in carbohydrates, mainly glucose and fructose, dietary fiber, vitamin B complex, minerals, carotenoids, phenolics, and antioxidants, but low in fats and proteins. Due to these essential functional compounds, dates provide a wide range of important nutrients and potential health benefits. In spite of attempts to develop functional foods containing dates and probiotics, this has not been done up until now. The aim of this study was to develop the best way to produce a novel probiotic date milk product and determine its microbiological and physicochemical properties. *Lactobacillus acidophilus* and mashed date fruits were encapsulated with calcium alginate using an extrusion technique. Two forms of dates were tested in the alginate beads: autoclaved and raw. In addition, the control beads, which were probiotic alginate beads without dates, were also examined to determine the effect of the dates on the product. The products were stored for 7 days at 4°C. The size of the beads was measured in millimeters and the morphology of the beads was measured using scanning electron microscopy. The texture properties of beads were examined using Texture Analyser (TA) apparatus. The survival of encapsulated *Lactobacillus acidophilus* was determined using a spread plate count on MRS agar. The color analysis of the milk and beads and the pH were also measured. The results showed that the microbial account and pH of the products were not affected by the date addition during a 7 days storage period at 4°C, compared to the control. *Lactobacillus acidophilus* was kept inside the beads without any release to the milk and the number of the bacterial cells remained in the range of the recommended dose of 10^6-10^7 CFU per g during the storage time. Moreover, there was no observation of pathogen growth caused by the Medjool dates in the product. Most of the beads were large in size (5-6 mm) with a teardrop shape, which affected the texture of the beads. The presence of the Medjool dates decreased the stability and the hardness of the beads. The colour of the autoclaved date beads was darker than raw date beads and the control beads, which was reflected in the colour of the milk in the same sample. The colour of autoclaved date beads was similar to the colour of the dates. From the results of this investigation, it is possible to develop date probiotic products by using the microencapsulation of probiotic bacteria with dates, and areas for future research are recommended.

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to be the best of my knowledge and belief, 'Development of a New Milk Drink Containing Dates-Immobilized Probiotic Bacteria' contains no material previously published or written by another person (any help that I have received in my research has been acknowledged), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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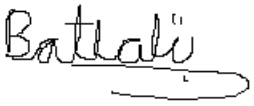
Signed: 

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Chapter. 1. Introduction

Dates constitute an important food consumed extensively in North Africa, the Middle East and South Asian countries. Dates are rich in nutrients including carbohydrates, salts and minerals, vitamins, fatty acids, dietary fiber, amino acids and proteins. Dates are processed into a variety of products including syrup, paste, dip, jam, honey and vinegar.

In the past two decades, there has been increasing interest in the role of probiotics in human health (Nagpal et al., 2012). Despite the fact that date palms have played an essential role in the lives of many people for more than 7,000 years, to the best of the author's knowledge, very little research has been conducted so far about using dates as a probiotic food. Aljasass et al. (2010) conducted research on producing date-flavoured probiotic yoghurt from both reconstituted whole milk powder and fresh cow's milk. It was noted that the viable number of Bifidobacteria, one of the most important probiotic bacteria, remained high to the end of the storage period in date syrup-flavoured probiotic yoghurt to a greater extent than needed for a beneficial effect. Similarly, Al-Otaibi and Saleh (2010) used some probiotic microorganisms (*Bifidobacterium lactis* Bb-12, *B. longum* Bb-46 and *Lactobacillus acidophilus* La-5) with dates to form a multi-functional product, improving the inherent nutrient value dates and the added functionality of probiotics.

Dates are considered a good carrier for transporting probiotic bacteria in the presence of nutrients and their micro-architecture. In recent years, there has been increasing interest in the effect of dates on the health of gut microbiota (Eid, N. et al., 2014; Eid, N. et al., 2015). These studies reported that date palm fruit intake may improve the health of the colon by enhancing beneficial gut flora growth and reducing colorectal cancer development. In addition, it has been suggested that date

fruits can be used as a good carbon source for the optimisation of probiotic *Lactobacillus casei* production (Shahravy, A, et. al., 2012).

Probiotic bacteria have to reach their site of action alive and in minimum numbers (10^6 CFU/g) to exert positive health effects. Immobilisation methods have been used to improve the survival of bacteria. Such methods have been developed for bacterial encapsulation for incorporation into products or in fermentation (Prakash, et al., 2016; De Prisco & Mauriello 2016). Calcium alginate has been widely used for immobilisation of lactic acid bacteria due to its non-toxic nature, ease of handling, and low cost (Ching, et al., 2015; Etchepare, et al., 2015).

The encapsulation of probiotic bacteria such as *Lactobacillus acidophilus* in sodium alginate gel has shown promise for ensuring the viability of these bacteria during storage and passing through the gastrointestinal tract (Etchepare, et al., 2015). The primary goal of probiotic microencapsulation research during the past years has been to improve probiotic survival during food processing, storage and consumption, as well as to mask the taste and aroma produced from metabolic compounds in fermented foods. Most of the functional foods available on the market are filled with free probiotic bacteria while a small portion consist of microencapsulated probiotics (De Prisco & Mauriello, 2016). More recent studies have confirmed the importance of expanding the types of foods containing probiotics so to reach to all consumers with their different acceptabilities and taste preferences (De Prisco & Mauriello, 2016; Gawkowski & Chikindas, 2012). Therefore, this study aimed to experimentally improve new functional foods combining dates and the probiotic bacteria *Lactobacillus acidophilus*. Dates are considered to be a remarkable fruit that provides the human body with all its daily nutrient needs (Assirey, E., 2015) and they have a very acceptable sweet taste. *Lactobacillus acidophilus* acts as a good enhancer of human health and assists recovery from gastrointestinal diseases (Nagpal el al., 2012). Dates provide an

ideal probiotic vehicle due to their nutritional composition. Moreover, the addition of probiotic dates to UHT milk enhanced the taste, color and appearance of the dates. Previous studies have argued that ultra-heat treatment (UHT) processing of raw milk develops a higher level of lactulose, which is a prebiotic that improves the growth of probiotic bacteria more than ordinary heat processing (Krasaekoopt et al., 2006). Moreover, dates enriched with potassium act well with calcium in milk to support bone growth and strength (Gad et al., 2010). Thus, the objective of this research was to develop the best way to produce a new probiotic date product.

Chapter. 2. Literature review

2.1 Date fruits as food

Dates, the fruit of the Date Palm *Phoenix dactylifera*, are one of the world's first cultivated fruit. Dates can provide many important nutrients and potential health benefits to the human. They are the essential food in North Africa and Middle East and have a significant role in the economy and social life of these regions. Furthermore, Date palms are honored and mentioned in the Holy Quran. So, people consume this fruit as a part of their religious beliefs and practices.

Date palms are cultivated in North Africa, the Middle East, parts of South and Central America, India, Pakistan and Southern Europe (Ashraf & Hamidi-Esfahani, 2011). In recent years, there has been an increasing interest in dates and the worldwide production of dates has risen from about 4.60 million tons in 1994 to 6.9 million tons in 2004 and it is expected that this will continue to increase. Saudi Arabia is one of the world's major date producers and exporters (Al Farsi and Lee, 2008).

The development of dates passes through four stages: Kimri, Khalaal, Rutab, and Tamer. At the Kimri stage there is a rise in weight, size and sugar content and the moisture content reaches up to 85%. The fruit starts changing to yellow or red depending on the variety. The sucrose starts to turn to glucose and fructose at the khalaal stage while the moisture content decreases. Tannins precipitate and lose their astringency during this stage. At the Rutab stage, the fruit starts ripening and turns brown accompanied by a reduction in weight with the moisture content decreasing to

35%. Finally, in the Tamer stage, the dates ripen completely on the palm or are sun dried. Thus, dates are different from other fruits in that they have three commercial maturation levels, the Khalaal, Rutab and Tamer stages (Ashraf & Hamidi-Esfahani, 2011). In addition, there are 2000 different date varieties depending on growth conditions (Al Farsi and Lee, 2008).

Process industries produce a variety of date products such as date syrup, date paste, date dip, date jam, date honey and date vinegar. Date paste is used in the preparation of different foods due to its mineral content; it contains potassium that is essential for human development and it is free of cholesterol and sodium. Date paste is widely used as a gelling and thickening agent in various types of processed food products like jellies, jams, yoghurts, soft cheeses and different confectionaries like cakes, cookies, muffins and bread. So, because it is rich in different nutrients, it has many uses from main dishes to beverages and desserts (Ashraf & Hamidi-Esfahani, 2011)

2.1.1 Nutritional and Chemical Composition

Dates are considered to be beneficial in human nutrition due to their rich content of significant nutrients which consist of carbohydrates, salts and minerals, vitamins, fatty acids, dietary fiber, amino acids and proteins. Table 1 shows that dates consist of sugar (63-66 g%), mainly glucose, fructose and sucrose and contain few fats and proteins (Al-Shahib and Marshall, 2002; Farsi & Lee, 2008). Therefore, it is a good source of energy owing to their high sugar content.

2.1.1.1 Sugars

Glucose and fructose are the major sugars in most varieties of date; they are present in equal amounts and are responsible for the sweetness of dates. Sucrose is found in smaller amounts. The sugar content of fresh dates is different from dried dates. The average content of glucose, fructose and sucrose in fresh dates is 38.3 g/100g, 25.2 g/100g, and 17.2 g/100g, respectively. In dried dates, the sugar content is higher and these differences depend on the cultivation area and moisture reduction at the maturation stage (Ashraf & Hamidi-Esfahani, 2011). Furthermore, when dates mature, the sugar content of fructose and glucose increases to the level of 38.47-40.04%. The fructose content increases by approximately three times, when the dates mature to the Tamer stage, while the sucrose content increases to reach its highest level (42.58%) at the Khalal stage (Al-Hooti et al., 1997). Recently, Ghfar, et. al. (2015) reported the determination of oligosaccharides in three date samples (Sefri, Mabroom, Ghassab). A number of studies have found that oligosaccharide can stimulate the growth of and/or activity of beneficial gut bacteria such as lactobacilli and bifidobacteria leading to positive effect on colonic health (Gibson, et al., 2010; Rastall, et al., 2005).

2.1.1.2 Minerals

Dates are the only fruit that provide a very good source of many minerals. A study by Hussein et al. (1976) found that the level of soil fertility and the amount of manure and chemical fertilizers applied to the palms affects the mineral composition of dates. The mineral content decreases when the dates mature to the Tamer stage but the change is small when compared with the changes in the sugar content. Al-Hooti et al (1997) studied the composition of five major date cultivars grown

in the UAE. In terms of mineral content, macro elements are found in much larger amounts in dates than microelements. These cultivars are high in potassium (K) (656-696 mg/100g) and low in sodium (Na) (1-2 mg/100g) (Table 1). In addition, Ragab et al (2001) noted that minerals, especially potassium, increase in dates during ripening. This sodium-potassium ratio makes the date a desirable fruit for people suffering from hypertension (Ashraf & Hamidi-Esfahani, 2011).

Dates were found to be a rich source of iron (Table 1). The iron content varies according to genetic differences between different dates. Furthermore, they are rich sources of selenium (0.24-0.40 mg/100g), copper (0.1–2.9 mg/100g) in the diet and the consumption of 100 g of dates supplies over 15% of the Recommended Dietary Allowance/ Adequate Intake (RDA/AI) of these minerals (USDA, 2014). However, selenium and copper are not present in Deglet Noor and Madjool dates. Manganese (43-54 mg/100g), phosphorus (62% mg/100g) and calcium (39-64 mg/100g) per 100 g of dates, supply over 7% of the daily RDA/AI (Table 1).

2.1.1.3 Vitamins

Dates are not a good source of vitamins but the small amounts of vitamins that are found in dates are essential for maintaining health. Moderate concentrations of vitamins B6, B9, B2, and B3 are found in 100 g of dates, which supply over 9% of the daily RDA/AI for adults while Vitamins B1, A and C are found in relatively low concentrations in dates, as 100 g of dates supply less than 7% of the daily RDA/AI. Dates contain mainly water soluble vitamins (B-complex and C). These are

not stored in the body and are eliminated in urine. However, fat-soluble vitamins (A, D, E and K) are absorbed in the blood stream to accomplish their functions. Vitamins C and B serve as coenzymes that promote the work of every cell in the body. They are very involved in carbohydrates, protein, fat metabolism and in the production of DNA in new cells. Vitamin C has an important role in fighting diseases by protecting cells from oxidative stress (Whitney and Rolfes, 2002).

2.1.1.4 Amino acids

Despite the small amounts of protein in dates, they contain essential amino acids that must be provided in the diet and that the body cannot make. The amino acid content varies significantly within the same stage of maturation in fresh and dried dates and it is increased in dried dates due to the water reduction (Ishurd et al., 2004). Furthermore, Al-Hooti et al (1997) reported that the crude protein content increases during the maturation process to reach its highest level at the Kimri stage (5.5-6.4 %) then decreases in the Tamer stage to 2.0-2.5 %. Table 1 shows that dates have significant amounts of protein, which is 1.81-2.45 g per 100 g. The amino acids found in dates include glutamic acid, lysine, aspartic acid, glycine, leucine and proline.

2.1.1.5 Dietary Fiber

In comparison with other fruits, dates are a very good source of dietary fiber, as 100 g of dates supply 32 % of the daily recommended intake of dietary fiber (Marlett et al., 2002). However, the dietary fiber content, which includes pectin, cellulose, hemicellulose, mucilages, gums, resistant

starch, and lignin, depends on the stage of maturity of the dates (Ashraf & Hamidi-Esfahani, 2011). The total dietary fiber decreases during the maturation process up to the Tamer stage where the fruit loses its texture and becomes soft.

A number of studies show that there are significant differences in the total fiber content of dates according to the method used in its determination. The Fibertec and Englyst methods identify resistant starch, whereas the Southgate method does not (Kirk and Sawyer, 1991). Lund et al. (1983) analyzed the total dietary fiber by the enzymatic method and found that of the total fiber content of 9.2%, 6.9% consisted of insoluble and 2.3 % of soluble fiber. However, Al-Shahib and Marshall (2002) reported that the total fiber content in some of the dates from Egyptian, Arabian, Irani and Iraqi cultivars, ranged from 8.1% to 12.7% and was determined by the Fibertec system.

In dates, the major proportion of dietary fiber is insoluble dietary fiber. This type of fiber plays a role in preventing serious diseases such as diverticular disease and bowel cancer (Marlett et al., 2002). The moisture reduction of dried dates causes the total fiber content in dried dates to decrease from 8.0 g/100 g to 7.5 g/100 where the enzymes gradually hydrolyse these substances to more soluble compounds during the ripening process (Al-Aswad, 1971). In addition, resistant starch that is found in fresh dates may provide an additional advantage in acting as a prebiotic, stimulating the growth of bifidobacteria in the gastrointestinal tract (Topping and Clifton, 2001).

Table 1. Nutritional value of Deglet Noor and Medjool dates (nutrient values and weights are for edible portion)

Nutrient	Unit	Value per 100 g	
		Deglet Noor.	Medjool
Proximates			
Water	g	20.53	21.32
Energy	kcal	282	277
Protein	g	2.45	1.81
Total lipid (fat)	g	0.39	0.15
Carbohydrate	g	75.03	74.97
Fiber, total dietary	g	8.0	6.7
Sugars, total	g	63.35	66.47
Minerals			
Calcium, Ca	mg	39	64
Iron, Fe	mg	1.02	0.90
Magnesium, Mg	mg	43	54
Phosphorus, P	mg	62	62
Potassium, K	mg	656	696
Sodium, Na	mg	2	1
Zinc, Zn	mg	0.29	0.44
Vitamins			
Vitamin C, ascorbic acid	mg	0.4	0.0
Thiamin	mg	0.052	0.050
Riboflavin	mg	0.066	0.060
Niacin	mg	1.274	1.610
Vitamin B 6	mg	0.165	0.249
Folate, DFE	µg	19	15
Vitamin A, RAE	µg	0	.7
Vitamin A, IU	IU	10	149
Vitamin E	mg	0.05	–
Vitamin K	µg	2.7	2.7

Source: US Department of Agriculture National Nutrient Database for Standard Reference, Basic Reports 9087 and 9421, accessed 18 Sept 2014

2.1.1.6 Carotenoids

Carotenoids are natural fat-soluble pigments that give a bright color to plants. They act as antioxidants to protect cells from the damaging effects of free radicals and they are an essential source of vitamin A (Di Mascio, et al., 1991). Boudries et al. (2007) indicated that dates consist of the carotenoids β-carotene, lutein and neoxanthin. In the three types of the Algerian fresh date, Tantebouchte, Deglet Noor and Hamraya, the β-carotene content is shown to be 3.3, 6.4 and 2.5 μg/100 g, whereas the lutein is reported to be 28, 156 and 33.6 μg/100 g respectively (Boudries et al., 2007).

The variation in carotenoid content is probably due to the differences in maturation, variety, analysis conditions and drying. They are found in different concentrations in the yellow and red colored dates. A significant decrease in the carotenoid levels shows through the transition from the Khalal to the Tamar stage during the ripening process (Boudries et al., 2007).

2.1.2 Dates application

2.1.2.1 Alternative fuel

The sugar extracts produced from surplus dates have been utilize for the production of alternative fuel. Zohri and Mostafa (2000) used date juice to produce ethanol by fermentation using *Saccharomyces cerevisiae* and *S. bayamus*. In the same way, Ghanim (2013) produced bioethanol from dates as a sugar yield substrate using hydrothermal extraction, fermentation and distillation. Recently, Chniti et al. (2014) reported that ethanol could be produced from the fermentation of date syrup by three yeasts, *Saccharomyces cerevisiae*, Zygosaccharomyces *rouxii* and *Candida pelliculosa*. It is evident from the findings that dates can be utilized as a good feedstock for some microorganisms to produce different alternative fuels.

2.1.2.2 Bacterial media

A number of studies have found that date syrup could be used as a source of carbon for the growth of several bacteria. Al-Bahry et al. (2013) showed that date syrup was used for biosurfactant production by *Bacillus subtilis* B20. Similarly, Omar et al. (2001) concluded that date syrup allowed optimum growth for *Bacillus megaterium* and they illustrated the essential effects of simple and complex nitrogen and carbon sources of date syrup on the growth of *Bacillus megaterium*. So far, however, there has been little discussion about examining the growth of other types of bacteria such as Bifidobacteria and Lactobacilli on the date syrup.

2.1.2.3 Yogurt manufacture

The date paste has been utilized as a value improver in the case of milk-based fermented products such as yogurt. In 2012, Trigueros et al.(2012) published a paper describing how they used the processing of two cultivars of dates that yield a high volume of blanching water to produce low fat yogurt, and the antioxidant activity of the date cultivars was determined. Considering the results, blanching water from dates, which is a good source of high sugars, organic acid and natural antioxidants has a promising future as a functional ingredient in food processing. Furthermore, Kale et al. (2011) argued that dates could be used as a novel ingredient in improving the characteristic quality of yoghurt.

2.1.2.4 Medicinal values of dates

Dates possess various health benefits and the medical activities of date fruit are dependant on its chemical composition. Many studies have shown that dates have many medicinal properties when they are consumed regularly. The antioxidant activity of dates has been stated by many researchers (Al-Turki et al., 2010; Amorós et al., 2009). In addition, components such as anthocyanins,

proanthocyanidins, *β*-carotene, selenium and phenolic acids present in dates have been found to have an antimutagenic effect (Ashraf & Hamidi-Esfahani, 2011). Furthermore, it has been shown that dates have gastro-protective, anti-inflammatory, hepato-protective, anticancer, nephroprotective and immunostimulant properties (De Lira Mota et al., 2009).

2.1.3 Microbial characterization

Although dates constitute an important food consumed extensively in North Africa, the Middle East and South Asian countries, few studies about microbial quality and composition are available. However, it was found that dates are mostly associated with a mixture of bacteria, yeasts and moulds. Microbial contaminants isolated from dates include lactic acid bacteria, yeasts, moulds and some potential pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Aspergillus flavus* and *A. parasiticus*. (Ashraf & Hamidi-Esfahani, 2011; Al Hazzani et al., 2014). Recently, Umar et al. (2014) detected a number of Salmonella-Shigella bacteria in date samples. However, there is no reliable evidence that these Salmonella-Shigella bacteria are naturally present in the dates and not as a result of contamination from the manufacturing process. In 2001, Ragab and others examined 40 date samples in Egypt and isolated *A. ochraceus, Penicillium chrysogenum, A. niger and A. flavus* with highest occurrence. Similarly, in 1999, in another study in Egypt, Abdel-Sater and Saber reported that *Aspergillus* was the most predominant genus isolated with a contamination rate of 100%, while *Penicillium* was a less predominant genus isolated with a contamination rate of only 30% of the date sample. Furthermore, Hasnaoui et al. (2010) found that *A. niger* was the most abundant species found in dates.

2.1.4 Dates as a Probiotic food

2.1.4.1 Probiotic bacteria

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). Recent research has reported the beneficial effects of probiotics on human health and the treatment of diseases (Daliri & Lee, 2015). Probiotics can be divided into three groups: lactic acid bacteria, non-lactic acid bacteria and yeasts (Table 2). Lactic acid bacteria are considered important probiotics known to provide beneficial health effects on the human gastro-intestinal (GI) tract (Burgain et al., 2011). In addition, Mortazavian et al. (2007) reported that lactic acid bacteria are most importantly used in the nutrition and food industries. Generally, *Lactobacillus* and *Bifidobacterium* are used widely as probiotics (Daliri & Lee, 2015), although, other genera such as *Escherichia, Streptococcus, Saccharomyces* and *Enterococcus* have also been known to be used as probiotics (Holzapfel et al., 2001).

Lactic acid bacteria are Gram positive, non-spore forming, rod shaped, anaerobic and strictly fermentative; lactic acid is the major end product of fermentation and these bacteria are acid tolerant. The bacteria usually grow in anaerobic conditions; however, they can also grow in aerobic environments. The most well-known probiotic lactic acid bacteria are *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus amylovorous, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus delbrueckii, Lactobacillus johnsonii, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus rhamnosus* and *Lactobacillus reuteri* (Anal & Singh, 2007). *Lactobacillus* play a vital role in treatment and prevention of infections caused by multi-resistant bacteria because they are part of the human microbial flora and have the ability to modulate the host immune system to protect the host against pathogens *via* competitive exclusion (Brachkova et al., 2010). *Bifidobacteria* are also rod shaped and Gram positive, but the most important feature

of these bacteria is strictly anaerobic growth. Moreover, these bacteria can grow at a pH range of 4.5-8.5. Acetic acid and lactic acid are their main end products from sugar fermentation. A few of the known *Bifidobacteria* that are used as probiotic bacteria are *Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve* and *Bifidobacterium lactis* (Holzapfel et al., 2001).

Probiotic bacteria should tolerate the manufacturing process without losing their viability or producing a negative impact on the sensory properties of food products. The stability and viability of probiotics, as well as a large number of probiotic cells (10^6-10^7 CFU/g product), are required during food processing and storage in order to exert their health effects on the host (Krasaekoopt et al., 2003). Although Sanders et al. (2007) argued that dead probiotic bacteria, endproducts of bacterial growth, or products derived from bacteria may also provide some health benefits, they cannot be considered as probiotics because they are not alive when consumed.

2.1.4.1.1 Health Benefits of Probiotics

In recent years, there has been an increasing amount of literature on the health effects of probiotic consumption. These studies provide strong evidence that probiotic consumption has beneficial effects on human health. (DiRienzo, 2014; Gilbert et al., 2013). Probiotics play essential therapeutic roles in human nutrition, However the main health benefits of probiotics include improving the immune system against gastrointestinal tract infections; prevention of upper intestinal diseases, diarrheal diseases, and hypercholesterolaemia; stabilisation of the gut mucosal barrier, and lactose metabolism (Daliri & Lee, 2015). For example, Salminen et al. (2005) found that probiotics can improve human health by modulation of the intestinal microbiota, i.e. several well-characterised strains of *Bifidobacteria* and *Lactobacilli* were used to decrease the risk of gastrointestinal (GI) infections or treat such infections. Parvez et al. (2006) also claimed that

probiotics can be considered as a food supplement that provides protection from the gastrointestinal (GI) infections and bowel syndromes by supplementing the healthy gut microflora.

Irritable bowel syndrome (IBS), one of the most prevalent gastrointestinal disorders, affects approximately 10% of the world population (Spiegel, 2009). As there is no effective therapy without side effects, probiotic bacteria may provide a valued alternative. In fact, a significant reduction in the symptoms of IBS has been detected after interventions with probiotics such as *Bifidobacteria infantis* 35624 or with probiotic combinations (O'Mahony et al., 2005). Another example of gastrointestinal discomfort— constipation—which is a common complaint among the adult population, was observed to be regulated by *Bifidobacterium animalis* DN-173 010 in probiotic fermented milk (Guyonnet et al., 2007). Furthermore, the treatment of colicky symptoms in newborn infants was achieved through the use of probiotic *Lactobacillus reuteri* ATCC 55730 (Indrio et al., 2008).

There is significant evidence supporting the role of clinical applications of probiotics in the treatment and prevention of respiratory, gastrointestinal, and urinogenital tract diseases (Gardiner et al., 2002). Previous research has shown that blood serum cholesterol levels decreased significantly after consumption of yoghurt fermented with strains of *Lactobacillus* sp. (Mann & Spoerry, 1974). Further, Harrison et al. (1975) observed a significant reduction in levels of serum cholesterol due to the consumption of infant formula containing cells of *Lactobacillus acidophilus.* The ability of probiotics to enhance clinical outcomes via modulation of the immune response has been determined in subjects with acute and chronic diseases. For example, VSL#3 probiotic mixture was reported to reduce pouchitis relapse (Gionchetti et al., 2007) and to enhance clinical scores in ulcerative colitis patients through improvement of inflammation (Miele et al., 2009). The consumption of *Lactobacillus rhamnosus* LGG by infants through episodes of acute rotavirus

diarrhoea increased the specific anti-rotavirus antibodies and non-specific antibody-secreting cells in the circulation compared to the placebo group and decreased the duration of the diarrhoea (Majamaa et al., 1995). Moreover, beneficial immunomodulatory effects of probiotic bacteria have been shown for *Helicobacter pylori* associated gastritis (Pantoflickova et al., 2003). In addition, it has been reported that probiotics may reduce allergy symptom scores and developing allergies (Prescott & Bjorksten, 2007).

Lactic acid bacteria release various vitamins and enzymes into intestinal lumen. Probiotic bacteria and their secretions may provide synergistic effects to alleviate symptoms of intestinal digestion, and malabsorption. In addition, production of lactic acid by these bacteria lowers the pH of the intestinal tract and inhibits the invasion of pathogens such as strains of *E. coli* or *Salmonella* spp. (Mack et al., 1999). The enzymatic hydrolysis properties of probiotic bacteria might enhance the bioavailability of fat and protein and raise the production of short chain fatty acids (SCFA), free amino acids, propionic acid, butyric acid and lactic acid in the intestine. The absorption of SCFA may prevent pathological changes in the colonic mucosa (Leopold & Eileler, 2000) and contribute to the host's available energy pool. Moreover, SCFA help maintain an appropriate pH in the colonic lumen in which many bacterial enzymes are expressed (Rolfe, 2000).

However, the actions of probiotic bacteria are not limited to nutrient synthesis. During the preparation of probiotic food or in the digestive system, probiotic bacteria enhance the digestibility of some dietary nutrients. Several studies have shown evidence for the effect of lactic acid bacteria in relief of symptoms of lactose intolerance. These studies led to the addition of *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and other lactobacilli into fermented milk products to deliver enough bacterial lactase to the stomach and intestine to stop symptoms in lactase intolerant people (Rasic, 2003).

Probiotic effects depend on their ability of viable cells to survive and pass through the stomach, as well as their ability to compete with pathogens and prevent their adhesion to the intestinal wall (Tuomola et al., 1999). *Lactobacillus* and *Bifidobacterium* produce anti-microbial substances such as bacteriocin, hydrogen peroxide and diacetyl, which suppress the growth of various enteric and urinary pathogens (Hütt et al., 2006).

Table 2. Microorganisms considered as probiotics.

***Lactobacillus* species**	***Bifidobacterium* species**	**Other lactic acid bacteria**	**Non-lactic acid bacteria**
L. acidophilus	*B. adolescentis*	*Enterococcus faecalis*	*Bacillus cereus var. toyoi*
L. casei	*B. animalis*	*E. faecium*	*Escherichia coli strain nissle*
L. crispatus	*B. bifidum*	*Lactococcus lactis*	
L. gallinarum	*B. breve*	*Leuconostoc mesenteroides*	*Propionibacterium freudenreichii*
L. gasseri	*B. infantis*	*Pediococcus acidilactici*	*Saccharomyces cerevisiae*
L. johnsonii	*B. lactis*	*Sporolactobacillus inulinus*	*S. boulardii*
L. paracasei	*B. longum*	*Streptococcus thermophilus*	
L. plantarum			
L. reuteri			
L. rhamnosus			

Source: Holzapfel, W. H., Haberer, P., Geisen, R., Björkroth, J., & Schillinger, U. (2001).

Probiotic bacteria not only suppress the growth of pathogens in the human intestine, but also provide other beneficial effects to the host by balancing the intestinal microbiota (Kailasapathy & Chin, 2000).

Microbiota balance is determined as the oldest proposed probiotic health benefit. It is described as 'seeding' the intestinal tract with lactic acid bacteria that suppress harmful proteolytic bacteria

growth (Metchnikoff, 2004). A previous study found that it is possible to transiently modify the gut microbiota composition of healthy individuals with the assistance of *Bifidobacteria* and *Lactobacilli* species upon ingestion of probiotics (De Vrese et al., 2006). It is still difficult to link such changes with benefit of healthy populations, but it is well established that a microbial imbalance or dysbiosis is associated to conditions such as chronic inflammatory disorders (Manichanh et al., 2006), allergies (Penders et al., 2007), and obesity (Ley et al., 2006). According to Manichanh et al. (2006) the way in which microbiota dysbiosis influences health is seen in Crohn's disease (CD). A reduction in the biodiversity of intestinal bacteria, specifically within the phylum *Firmicutes*, has been detected in CD patients. In a different study, Sokol et al. (2008) investigated the effect of oral administration of *Faecalibacterium prausnitzii* and its culture supernatant in reducing the severity of trinitrobenzene sulfonic acid (TNBS)-produced colitis in mice by counterbalancing dysbiosis, which may be a promising strategy in the treatment of CD.

Certain members of *Lactobacillus* and *Bifidobacterium* reduce the level of carcinogenic enzymes produced by colonic microflora via normalisation of intestinal permeability, microflora balance, enhancement of the host's immune system, and production of antimutagenic organic acids (Kumar et al., 2010). Some probiotic health benefits that are variously claimed are summarised in Figure 1. The health benefits of probiotics are strain specific, therefore the health benefits are dependent on the genus and the species of probiotic bacteria. However, the exact mechanisms of probiotic actions are still not well understood (Mutukumira et al., 2015).

2.1.4.1.2 Probiotic food products

Probiotics are widely used in the production of fermented dairy products such as yoghurt, kefir and korut. however, evidence of their health benefits in humans and animals has resulted in the

development of other foods and pharmaceutical products containing them. Probiotic foods products are classified as functional foods and make up 60 to 70% of the functional food market (Tripathy & Giri, 2014). According to a recent worldwide industrial analysis, the probiotic market grew to over US $28 billion in 2015 and is expected to reach approximately US $34 billion by 2018 (Global Industrial Analysis, 2013). The dairy industry has started to introduce probiotics into its products such as fermented and non-fermented milk, yoghurt and cheese and are the most popular probiotic products (Sanchez, 2012). There are other types of probiotic foods such as chocolate-based products and fruit juices, but they represent a very small part of the market.

Nowadays, enhancing the functional product vehicles for probiotics could extend the types of probiotic food products, which would make probiotic foods available to groups of people that usually do not consume them because of their intolerance for milk or milk derivatives. Gawkowsky and Chikindas (2013) reported that non-dairy probiotic foods like fruit juices, soy-free and vegan-compliant foods are becoming important to consumers suffering from lactose intolerance, following specific diets, or avoiding high-cholesterol foods. Probiotic food products need to be safe and should contain the appropriate probiotic strains in adequate numbers when consumed. Accordingly, the probiotics selected must be sufficient for large-scale industrial production with the ability to survive and maintain their functionality throughout food processing operations and storage as dried or frozen cultures (Tripathi & Giri, 2014).

2.1.4.2 Application of dates in probiotic dairy products

Despite the fact that dates have played an essential role in the lives of many people for more than 7000 years, to the best of the author's knowledge, very little information has been found so far about using dates as probiotic foods. A recent study has reported the natural association of some

probiotic bacteria such as *B. subtilis*, *B. brevis*, and *B. megaterium* with dates **(**Al Hazzani et al., 2014). The genus *Bacillus* has been used as a probiotic to improve human health. (Cutting, 2011)

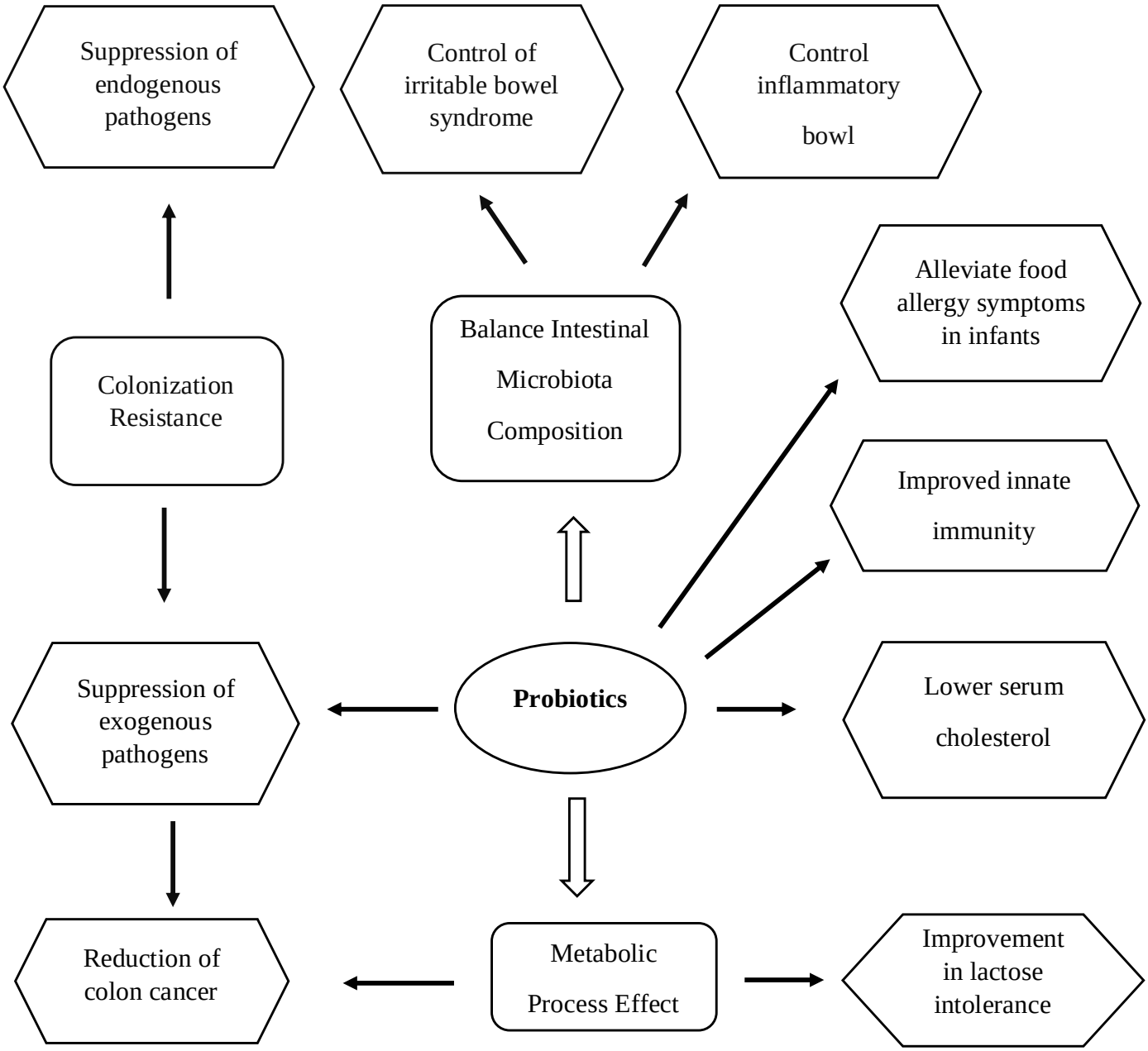

Figure 1. Probiotics consumption and health benefits
(Source: Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H. Y. (2006). Probiotics and their fermented food products are beneficial for health. Journal of Applied Microbiology, 100, 1171–1185.)

Aljasass et al. (2010) highlighted the need to use the date as a probiotic food by producing date-flavored probiotic stirred yoghurt from both reconstituted whole milk powder and fresh cow's milk. It was noted that the viable number of *Bifidobacteria* (as one of the important probiotic bacteria) increased to the end of the storage period in the date syrup-flavored probiotic yoghurt to a greater extent than the concentration of probiotics needed for a beneficial effect. In addition, the presence of date syrup in the probiotic yoghurt enhanced its survival in cool storage for up to 10 days and the count of *Bifidobacteria* was increased (Aljasass et al, 2010). These results may be explained on the basis that date syrup contains a lot of micronutrients, such as minerals and vitamins that might enhance the *Bifidobacteria* growth. Furthermore, the coliform bacteria, yeasts and moulds which are naturally present in the dates were not detected in any sample of date flavored probiotic stirred yogurt either during production or in refrigerated storage at 5 ± 1℃ for 10 days. This may be due to the effect of the probiotic bacteria protecting the products from spoilage by microorganisms by producing bacterial toxins against these pathogens. Researchers have argued that the addition of date syrup to the probiotic yoghurt improves the nutritional value of the yoghurt. This treatment resulted in an increase in total carbohydrates, total solids and total calorie content. On the other hand, this addition did not change the total protein, acidity and fat content or pH values (Aljasass et al, 2010). Al-Otaibi and Saleh (2010) used some probiotic microorganisms (*Bifidobacterium lactis* Bb-12, *B. longum* Bb-46 and *Lactobacillus acidophilus* La-5) with dates to form a multi-functional product, improving the inherent functionality of dates

and the added functionality of probiotics. The viable counts of all probiotic bacteria in the probiotic date products remained above the minimum number of probiotic organisms in food products, which is 10^6 cfu/g product (Al-Otaibi and Saleh, 2010). Therefore, all the organisms: *Lactobacillus acidophilus, Bifodobacterium lactis* and *B. longum,* were capable of surviving in dates

The addition of dates to probiotic fermented milk enhanced the taste, colour and appearance of probiotic date. There were critical differences in the odour of ordinary dates and probiotic dates. However, unlike other fruits, dates do not cause any off-flavors when mixed it with probiotic bacteria (Luckow et al. 2006).

The pH value of dates is pH 6.8 which is suitable for the activity and growth of most of probiotic bacteria. Probiotic dates have shown a slight decrease in pH during 12 weeks storage to pH 6.1 and this could be due to the activity of the probiotic bacteria in low acidity as a result of the low water content of probiotic dates and cold storage (Al-Otaibi and Saleh 2010). Moreover, it was observed that both *Lactobacillus acidophilus* or bifidobacteria have almost similar patterns of pH values in the probiotic dates over 12 weeks of storage. It was concluded that, *Bifidobacterium lactis, Lactobacillus acidophilus* and *B. longum* may be used as probiotic cultures to produce acceptable and healthy probiotic dates (Al-Otaibi and Saleh 2010)

Recently, Esteban et al. (2014) used diets enriched with probiotic bacteria and date extracts in different way. The antioxidant properties present in the probiotic dates and their effect on the gills

and skin of fish were examined. Probiotic dates could be considered as good natural antioxidants and may serve as a functional food ingredient for fish in fish farms.

2.1.5 Microencapsulation of probiotics

2.1.5.1 Definition and beneficial effects of microencapsulation

In recent decades, probiotics have been one of the major interesting research subjects due to significant evidence pointing to their beneficial health effects. However, these effects are strongly dependent on the type of probiotic strains and the ability of the bacteria to survive and multiply in the host. Accordingly, probiotic bacteria must be metabolically stable and active in food products, and survive through their movement in the stomach until reaching the intestine in large numbers (Sanz, 2007). Different factors including heat, the acidic pH of the stomach, bile salts, digestive enzymes, humidity and oxygen limit the viability and stability of probiotic bacteria during food processing and their passage through the gastrointestinal tract. Therefore, microencapsulation of probiotic bacteria has been required to protect them against adverse environmental factors (Rokka & Rantamäki, 2010; Sabikhi et al., 2010; Champagne et al., 2005).

Microencapsulation is a process in which liquid, solid or volatile (gaseous) materials are entrapped within a polymer matrix as small beads which then release controlled amounts of their contents under specific conditions (Champagne & Fustier, 2007). The encapsulated materials, referred to as the core materials, and which could be cells, enzymes, medicine, flavours or other active ingredients, are completely surrounded or dispersed in the matrix referred to as the coating or shell (Desai & Park 2005). There can be single or multiple coatings, which can be semi permeable, thin or strong membranes, while the core can be an emulsion, crystalline material, a suspension of microcapsules or a suspension of solids (Gharsallaoui et al., 2007). Morphologically,

microcapsules may be classified into three basic categories. The first one is monocored in which one core is coated by a matrix, polycored in which a few cores are inside the matrix, and matrix type in which entrapped material is dispersed within the matrix (Figure 2) (Zuidam & Shimoni, 2010). The coating material can be sugar, protein, polysaccharides or a combination of these. Alginate-based materials are largely used for microencapsulation of probiotic bacteria. Other polysaccharides such as maltodextrin, modified starch or prebiotics can be applied as a potential coating mixed with different proteins. Microencapsulation materials are used to immobilise or entrap probiotic bacteria inside the microcapsule as well as to protect the bacteria against the harsh external environment (Prakash et al., 2016).

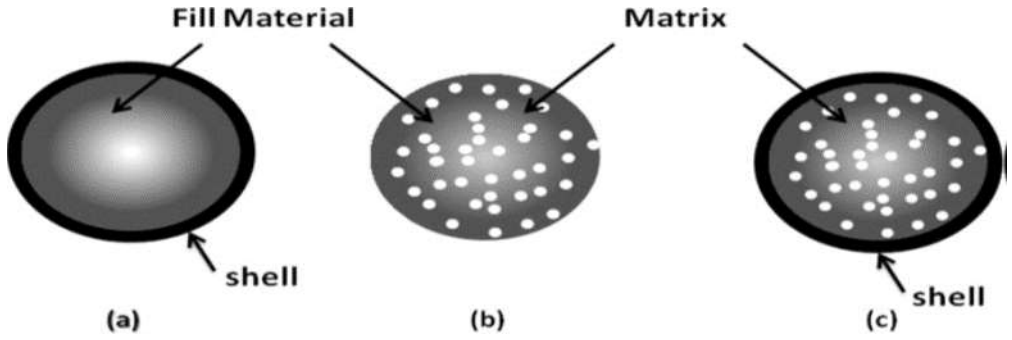

Figure 2.Schematic representation of microcapsule types: (a) monocored, (b) matrix, and (c) polycored. Source: Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. Journal of Food Engineering, 104, 467–483.

Microencapsulation has been applied in the food industry to mask flavours and odours, provide sustained and controlled release, control oxidative reactions, and protect sensitive ingredients (Champagne & Kailasapathy, 2008; Zuidam & Shimoni, 2010). The coating material should be food grade and able to protect the sensitive substance or living cells inside the capsule. Microencapsulation has provided different advantages to probiotics such as protection against bacteriophages and contamination. Steenson et al. (1987) reported that the microencapsulation of

probiotics into alginate beads prevented bacteriophage attacks due to the small pore size of the beads. In addition, probiotic microencapsulation has been found to preserve them from damaging environmental factors like high acidity, cold shocks caused by freeze drying and deep freezing, bile salts, oxygen, heat shocks induced by spray drying, and antimicrobial agents. Although other benefits such as enhancing sensory properties and probiotic homogeneous distribution all over the product can also be achieved (Yadav & Shukla, 2015). In fact, the main purposes of probiotic microencapsulation are the protection of the bacteria in the food process and from extreme environmental conditions, as well as consumption of lower amounts of encapsulated cells to gain the same probiotic free cell effects (Anal & Singh, 2007; Sohail et al., 2011).

2.1.5.1.1 Viability of Microencapsulated Probiotic Bacteria during Processing and Storage

Probiotic bacteria have been included in a range of foods, such as dairy products (e.g. cheese, yoghurt, ice cream, frozen dairy desserts) and non-dairy products (e.g. juices, chocolate, cereals) (Anal & Singh, 2007). Many factors could affect probiotic behaviour and vitality in the food environment, which can prevent their essential requirement to reach the intestines alive and in a metabolically active state. These factors include culture type, growth stage, osmotic or heat stress, food matrix composition, oxygen level, pH, food storage time, and manufacturing conditions (Soukoulis et al., 2014). De Vos et al. (2010) reported that certain factors negatively influence the viability of the probiotics in dairy products, namely the presence of hydrogen peroxide, the concentration of acetic acid and lactic acid, reduction in pH, and high oxygen content. The loss of probiotic viability during passage through the harsh gastrointestinal environment is an obstacle that must be overcome for probiotic bacteria to achieve their beneficial role. In this regard, the

main challenge of probiotic microencapsulation is to protect bacterial cells against hurdles that the bacteria may face.

The survival of probiotics in dairy products and the gastrointestinal tract can be improved by using microencapsulation (Krasaekoopt et al., 2003, Picot & Lacroix, 2004). A number of studies have found that the viability and survival of probiotic bacteria can be increased by microencapsulation; for example, Brinques and Ayub (2011) found that sodium alginate microcapsules and chitosan microcapsules enhance the survival of *Lactobacillus plantarum* in yoghurt during refrigerated storage. Divya and Nampoothiri (2015) reported that the survival of *Lactococcus lactis* when co-encapsulated in alginate and mannitol increased by 60% in stimulated gastrointestinal conditions in comparison to free cells. Similarly, it has been found that microencapsulation protected probiotic bacteria such as *L. casei, L. gasseri, B. bifidum, B. breve, B. longum, L. acidophilus, B. infantis* and *B. pseudolongum* against the harsh environment of simulated intestinal fluid when entrapped inside coating materials like alginates and chitosan compared to non-entrapped cells (Urbanska et al., 2007; Chávarri et al., 2010; Rao et al., 1989).

The above finding is consistent with the studies of other researchers. Krasaekoopt et al. (2006) discovered an increase in the number of *Bifidobacterium bifidum* ATCC 1994, *Lactobacillus casei*, and *Lactobacillus acidophilus* 547 by just about 1 log in yoghurt, after they encapsulated the bacteria within alginate beads coated with chitosan. Jiménez-Pranteda et al. (2009) and Ding and Shah (2007) carried out research on *L. rhamnosus* and *B. longum* in which they encapsulated the bacteria with a blend of gellan and xanthan gum. Recently, Dianawati et al. (2015) reviewed more specifically the survival of microencapsulated probiotic bacteria during food processing and storage.

2.1.5.2 Successful microencapsulation

Microencapsulation provides an appropriately small environment for probiotic bacteria to survive through food processing and storage until their release at a suitable location(s) in the gastrointestinal tract (Weinbreck et al., 2010). Besides the protection against the harsh environment, the aim of microencapsulation is to allow the release of probiotic cells, in the lower intestine, that are alive and metabolically active (Picot & Lacroix, 2004). However, successful probiotic microencapsulation relies upon various factors such the probiotic strain, its beneficial effects on the host, the bacterial number required to achieve viability, and the beneficial effects of probiotics cells on food processing, storage and sensory properties (Champagne & Fustier, 2007). In addition, the selection of coating materials and the methods adopted in probiotic microencapsulation play a vital role in the final functional and morphological properties of the capsules as well as in the release mechanism of the probiotic bacteria (Picot & Lacroix, 2004; Ding & Shah, 2007). Mostly, microcapsules release their contents due to enzymatic action, pH changes and chelating agents (Martín et al., 2015). The success of the protection and delivery of the probiotics to the right place(s) depends mainly on the morphology, size, texture and other related properties of the beads, of which the materials themselves determine these properties (De Prisco & Mauriello, 2016; Martín et al., 2015). In fact, the food industry looks for the balance between advantages and the cost of technologies because some of them may require expensive materials or devices (Martín et al., 2015). Therefore, different microencapsulation methods have been continuously developed to achieve successful probiotic bacteria entrapment.

2.1.5.3 Methods of microencapsulation

Microencapsulation techniques can be divided into the drying process and encapsulation process (Solanki et al., 2013). Probiotic bacteria can be immobilised by encapsulation processes, including extrusion, emulsion, spray drying, fluid bed coating and other methods. In the encapsulation process, bacterial cells are commonly entrapped in polymeric networks. Probiotic microencapsulation may offer many benefits in terms of metabolite production and biomass compared to non-capsulated cell systems, like high cell concentration, enhanced resistance to bacteriophage attack and contamination, reuse of biocatalysts, enhancement of plasmid stability, as well as chemical and physical protection of the bacteria (Champagne et al., 1994; Doleyres & Lacroix, 2005).

In general, encapsulation is carried out in three different stages. As found by Poncelet and Dreffier (2007), in the first stage, probiotic bacteria are incorporated in either a liquid or solid matrix. In the case of a liquid core, the incorporation consists of a dispersion in the matrix, while it is an adsorption or agglomeration in a solid core. The second stage of liquid matrix dispersion is achieved along with solution pulverisation on the solid matrix. The last step includes stabilisation using a process that could be chemical (polymerisation), physicochemical (solidification, evaporation or coalescence), or physicochemical (gelification). Probiotic microencapsulation can also be carried out using other processes like molecular inclusion coacervation, co-crystallisation or liposome. However, the cost of these processes is high and the large bacterial size limits their use (Champagne & Kailasapathy, 2008). This section discusses the preparation of probiotic microencapsulation by applying cell immobilisation techniques that are commonly used such as extrusion, emulsion, spray drying, fluid bed coating.

2.1.5.3.1 Extrusion method

The extrusion technique is the most common method due to its low cost, simplicity, and gentle formation conditions, which provide high cell viability but do not damage the probiotic cells (Krasaekoopt et al., 2003). Extrusion uses hydrocolloids such as alginate and carrageenan as encapsulating materials. In this method, the probiotic living cells are mixed with an alginate solution and are instantly incorporated in the form of alginate droplets in a hardening solution such as calcium chloride (Figure 3) (Burgain et al., 2011). The suspension of hydrocolloids and probiotic bacteria is projected *via* a syringe at high pressure for forming the microencapsules. When the probiotic-alginate solution flows out of the syringe nozzle opening, a droplet is formed at the tip of the needle. The droplet increases in size until the droplet falls towards the gelling bath. Spherical probiotic-alginate beads are formed during this time because of the surface tension of the liquid (Blandino et al., 1999; Chan et al., 2000; Chan et al., 2011 b). The interaction of ions like Ca^{2+} with the carboxyl groups of the alginate solution produces beads that are a few millimetres in diameter (Smrdel et al., 2008).

Sarao (2015) reported that this method does not include harmful solvents and can be carried out under both aerobic and anaerobic conditions. This advantage allows the use of anaerobic probiotics by placing extrusion equipment in a sterile chamber where oxygen is replaced by nitrogen (De Vos et al., 2010). The size of the beads or microencapsules is determined by the inner diameter of the nozzle used to drip the solution; the concentration and viscosity of the alginate solution; the exit flow rate of the alginate; and the distance between the syringe and calcium chloride solution (Burey et al., 2008). If the droplet formation is in a controlled manner, the approach is known as prilling. This is obtained by vibration of the nozzle or pulsation of the jet. Kailasapathy (2002) found another common technique for small droplet formation that uses an electrostatic field or coaxial

flow. In the application of an electrostatic field, the electrostatic forces disrupt the surface of the liquid at the needle tip, creating a charged stream of small droplets. It is easy to control the bead size by varying the applied potential, and it does not require organic solvents. Large-scale bead production can be achieved by using a multiple-nozzle system, jet-cutter techniques, or a spinning disc atomiser (De Vos et al., 2010; Kailasapathy, 2002).

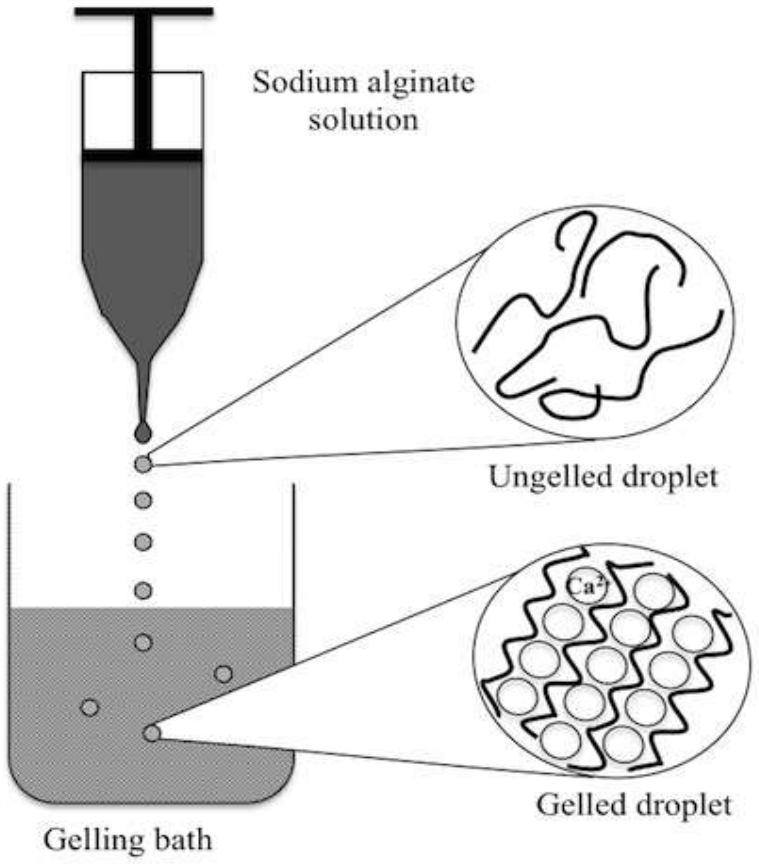

Figure 3. Alginate bead formation using the extrusion method.
Source: Ching, S. H., Bansal, N., & Bhandari, B. (2015). Alginate gel particles-a review of production techniques and physical properties. Critical reviews in food science and nutrition, (just-accepted), 00-00.

2.1.5.3.2 Emulsion method

Emulsion is a chemical method for microencapsulation of the living probiotic cells by using hydrocolloids such as alginate, carrageenan and pectin as encapsulating materials. This method is based on the addition of a solution of a cell polymer (such as alginate) into a vegetable oil (e.g. corn oil, soybean oil) to produce a water-in-oil emulsion (Burgain et al., 2011). The emulsion containing small droplets is achieved by agitating the mixture to form small gel particles inside the oil phase. Then, a cross-linking agent (e.g. calcium chloride) is added to form the solidified beads (Mutukumira, et al., 2015). A final step is needed for separating the beads from the oil phase. This usually involves centrifugation of the mixture and then removal of the excess oil by washing with solvents (Figure 4) (Sultana et al., 2000). The beads can be further added to a second polymer solution to form an encapsulating layer that gives more protection to the microencapsulated cells (Burgain et al., 2011).

In this method, the production of the beads of a desired size, ranging from 20-25 μm to 2 mm, is done by changing the water/oil ratio and agitation speed (Mortazavian et al., 2007). Chen and Chen (2007) argued that the emulsion method gives a high rate of bacterial survival and it is easy to scale up. However, the heat generation and high shear stress involved in the method may cause denaturation of biological cells and compounds (Zhao et al., 2007). Some emulsifiers such as Tween 80 may be added to make the emulsion technique more effective (Krasaekoopt et al., 2003). In most cases, hydrocolloids, such as alginate, gellan gum, κ-carrageenan and xanthan gum have been used as coating materials for microencapsulated probiotic bacteria.

Some probiotic cells may be very sensitive to shear pressure and force during homogenisation. Therefore, it is important to ensure that the selected method conditions are gentle and harmless to sensitive probiotic cells (Mutukumira et al., 2015). The disadvantages of this method are the large

size of the beads, which can influence the texture and mouthfeel, and the high cost due to the use of significant amounts of vegetable oil (Burgain et al., 2011; Mortazavian et al., 2007).

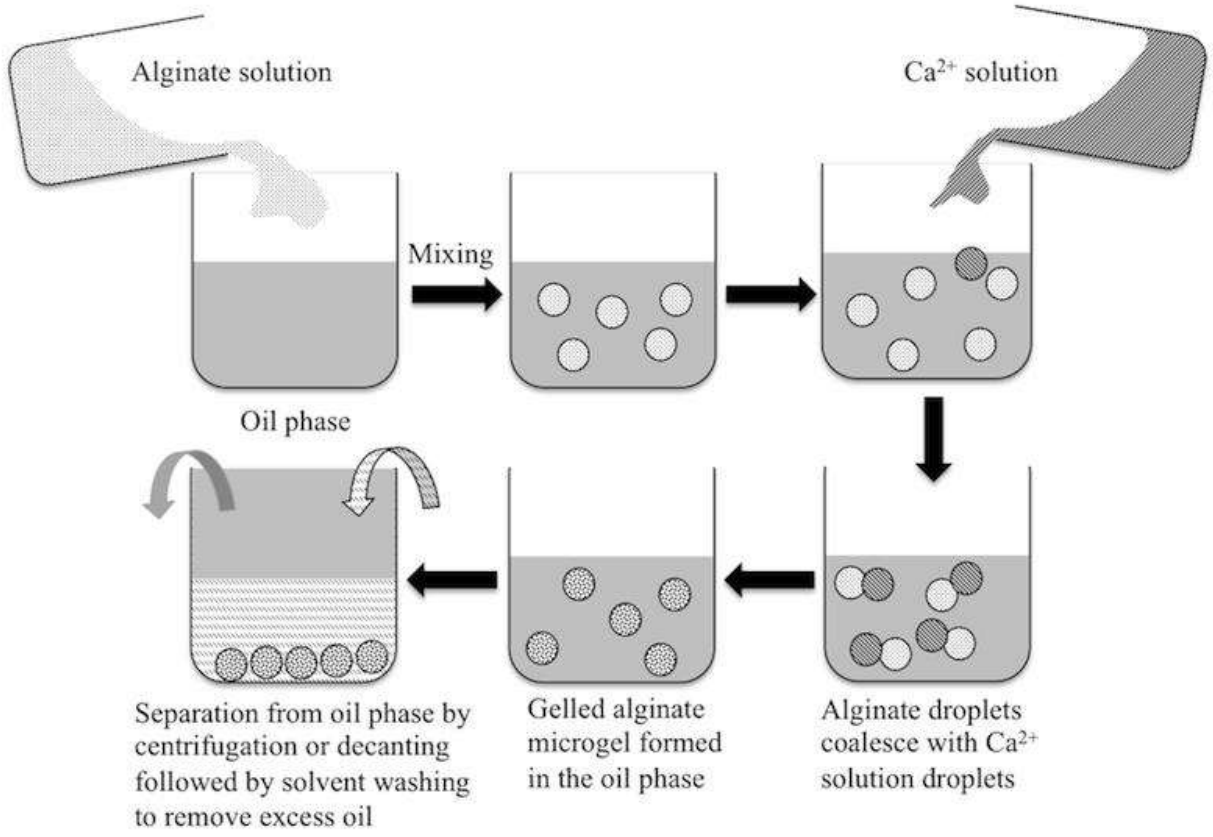

Figure 4. Alginate beads formation by emulsion method.
Source: Ching, S. H., Bansal, N., & Bhandari, B. (2015). Alginate gel particles-a review of production techniques and physical properties. Critical reviews in food science and nutrition, (just-accepted), 00-00.

2.1.5.3.3 Drying Method

2.1.5.3.3.1 Spray drying

Drying of bacterial cells in order to facilitate their transportation and storage, and without losing their viability has been investigated (Anal & Singh, 2007). Santivarangkna et al. (2007) defined spray drying as the removal of water from a solution of a non-volatile solid by vaporisation. Meng

et al. (2008) observed that large-scale production of bacterial cultures is an expensive technique with low yields. But, spray drying is considered a good alternative with a low cost that yields higher production rates.

Spray drying of probiotic cells accumulated in different protein solutions, with and without carbohydrates (oligosaccharides, maltodextrin, hydrocolloids), has been applied to the entrapment and drying of probiotic bacteria (Corcoran et al., 2004; Desmond et al., 2001). A matrix material is dissolved in a water-based continuous phase that surrounds the probiotic cells within the spray droplet. This solution is immediately vaporised in heated air and causes probiotic cells to shrink in an envelope (De Vos et al., 2010).

Microencapsulation can be carried out with biopolymers of different sources. However, low molecular weight carbohydrates, gelatin, soy or milk proteins, and hydrocolloids like gum arabic are typically used as wall material for microencapsulation by spray drying (Mutukumira et al., 2015). There are disadvantages to spray drying such as cellular membrane heat damage due to the high temperatures used (Anekella & Orsat, 2013). Moreover, it is more an immobilisation technique than an encapsulation technique because some of encapsulating core materials may be shown on the surface of spray-dried microcapsules, which can cause the leakage of bacterial cells into the product (Mutukumira et al., 2015). The viability and performance of probiotic bacteria through spray drying depends on several aspects, such as the type of probiotic bacteria strain, drying medium, and inlet and outlet temperatures (Mutukumira et al., 2015).

2.1.5.3.3.2 Spray Freeze Drying

This technique involves the spraying of a liquid solution into a freezing medium such as liquid nitrogen, and the resultant freezing of water within it (Figure 5). Then, the frozen material is

introduced to regular vacuum freeze-drying for water removal by sublimation (Kailasapathy, 2009; Semyonov et al., 2010; De Vos et al., 2010). Semyonov et al. (2010) reported that a shell coating can be added to provide more protection to the capsules against adverse environmental conditions. The coating materials can be milk powder, glucose, trehalose, whey protein or maltodextrin. Cryoprotectants may also be added to help in the probiotic cell adaptation to the environment (Basholli-Salihu et al., 2014; Capela et al., 2006). This technique has various advantages such as a larger specific surface area and controlled size as compared to spray-dried capsules, (Martín et al., 2015). In addition, Wang et al. (2004) found that the processing conditions of freeze drying are gentle in comparison with spray drying and also higher probiotic cell survival rates are obtained. However, Zuidam and Shimoni (2010) argued that the drawback of this method is the high energy input needed during the process.

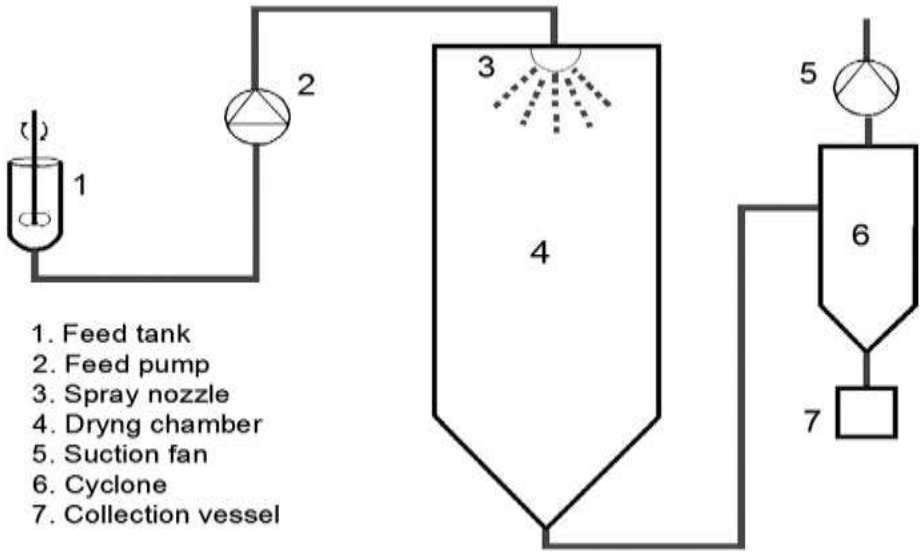

Figure 5. Spray drying technology.
Source: Martín, M. J., Lara-Villoslada, F., Ruiz, M. A., & Morales, M. E. (2015). Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. Innovative Food Science & Emerging Technologies, 27, 15-25.

2.1.5.3.4 Fluid bed coating

Fluid bed coating is a process in which core material (cell suspension) is sprayed and dried in a temperature and humidity-controlled chamber using a Wurster-based fluidised bed system. A liquid coating is sprayed via a nozzle covering the core material (Champagne & Fustier, 2007; De Vos et al., 2010). Before drying, the probiotic cell needs to be encapsulated in a supporting material such as calcium alginate, skimmed milk or fats. Shellac, a resin secreted by the insect *Kerria lacca* (*Coccoidea*), is also applied. The strain of the insect, refining methods and host trees are factors that determine the physicochemical properties of shellac (Buch et al., 2009). Shellac is considered an acceptable material for supplemental food products due to its natural origin. In addition, shellac has good resistance to intestinal fluid. However, shellac has low solubility when it used as an enteric coating for hydrophobic substances. Therefore, the use of shellac is limited as an enteric-coating polymer (Martín et al., 2015). Water-soluble polymers, such as sodium alginate, polyvinylpyrrolidone and hydroxypropyl methylcellulose, can be used to enhance the properties of shellac as an enteric-coating polymer, and glyceryl triacetate and glycerol as plasticisers (Stummer et al., 2010).

Optimal results of the encapsulated particles are achieved with sizes between 50 and 500 microns. The length of the time that the particles are in the chamber determines the amount of coating materials (Gibbs et al., 1999). Generally, 5-50% of coating is used, depending on the application of the encapsulate and the particle size of the core material (Zuidam & Nedovic, 2010). The advantages of this process are easy to scale up and total control on the temperature and lower cost. In addition, multiple layers may be added for density adjustments or controlled release, and enhance the viability of probiotics through processing shelf life and their passage in simulated gastric tract. Therefore, this method is one of the most common encapsulation technologies used

commercially for probiotics. The disadvantages are the difficult process and its need for a longer processing time (Martín et al., 2015; Dong et al., 2013). The characteristics, advantages and disadvantages of encapsulated technologies are shown in Table 3. The success of the coating process can be affected by the particle wettability of the coating liquid, the coating material stickiness, and the operating conditions (Dong et al., 2013). Burgain et al. (2011) reported that several companies have invented products using Duaolac® and Probiocap®.

Table 3. Characteristics, advantages and disadvantages of encapsulation methods

Methods	**Characteristics**	**Advantages**	**Disadvantages**
Spray drying	Cells encapsulated individually in the drying medium Medium cell load (10^{10}–10^{11} CFU g^{-1})	Many possibilities for coating materials Rapid cell release if ingredients dissolve rapidly	High temperatures in the process kill many strains
Emulsion	Homogenisation of aqueous and lipid phases	Easy to scale up, flexible adjustment of capsule size Lipid-based systems theoretically good for protection against acids and oxygen	Emulsifiers can be detrimental to viability High losses in liquid phase Liquid core may be unfavourable to long-term stability
Extrusion	Cells blended with various polymers and then extruded Low cell load (10^{9}–10^{10} CFU g^{-1})	Many possibilities for coating materials Particles can be air-dried Mild and simple preparation process	Difficult to be scaled up
Fluid bed coating	True coating: cells in core powder Coat generally lipid-based High cell load (> 10^{11} CFU g^{-1})	Easy to be scaled up Multiple layers can be added for controlled release or density adjustments	Phase separation in beverages if coating is lipid based Slow cell release at low temperatures

Source: Dong, Q. Y., Chen, M. Y., Xin, Y., Qin, X. Y., Cheng, Z., Shi, L. E., & Tang, Z. X. (2013). Alginate-based and protein-based materials for probiotics encapsulation: a review. International Journal of Food Science & Technology, 48(7), 1339-1351.

The materials used in the food industry for probiotic coating are mainly lipid based (fatty acid, waxes and specialty oil, etc.), carbohydrates or proteins. Champagne et al. (2010) applied fluid-bed coating technology for encapsulation probiotics by using a coating with two types of fats. In addition, Institut Rosell and Lal'food, a Canadian company, have created products containing probiotics using this method by applying fatty acids. Furthermore, A Danish-Korean company used a dual-coating method for probiotics made of soy peptides in the first layer and cellulose and gum in the second layer (Burgain et al., 2011).

2.1.5.4 Alginates as an encapsulating material

Alginate is a polysaccharide naturally derived from several brown seaweed species. The alginate polymer is composed of two monomeric units: β-(1 → 4)-linked D-mannuronic acid (M) residues and α-(1→4)-linked L-guluronic acid (G) residues (Figure 6). Different sequences and proportions of M and G residues determine the molecular weight and physical properties of the alginate and its derived structures. Furthermore, the composition of the polymer chain differs the sequential distribution and in the amount based on the source of the alginate. The functional properties of the alginate that are used as encapsulating material are influenced by these factors (Ching et al., 2015). Alginate has been used extensively as an encapsulating material as it is easily manipulated and innocuousness, as well as its ability to absorb water. It has other features such as being non-toxic; inexpensive and simple; biocompatible; gelling; stabilising; and thickening (Krasaekoopt et al., 2003). All these features have been of strong interest to the food industry, especially for probiotic cell microencapsulation. In 2011 Burgain et al. reported that alginate is the most widely used polysaccharide as an encapsulating material for probiotic bacteria due to its non-toxic nature, ease of handling and low cost, as well as the increased viability of bacterial cells when exposed to various conditions compared to non-encapsulated bacteria cells. Alginates are commercially

available in the form of sodium, potassium, or ammonium salts. In addition, it is available as a food additive, which is white or yellowish brown powder that is odourless and tasteless (Ching et al., 2015).

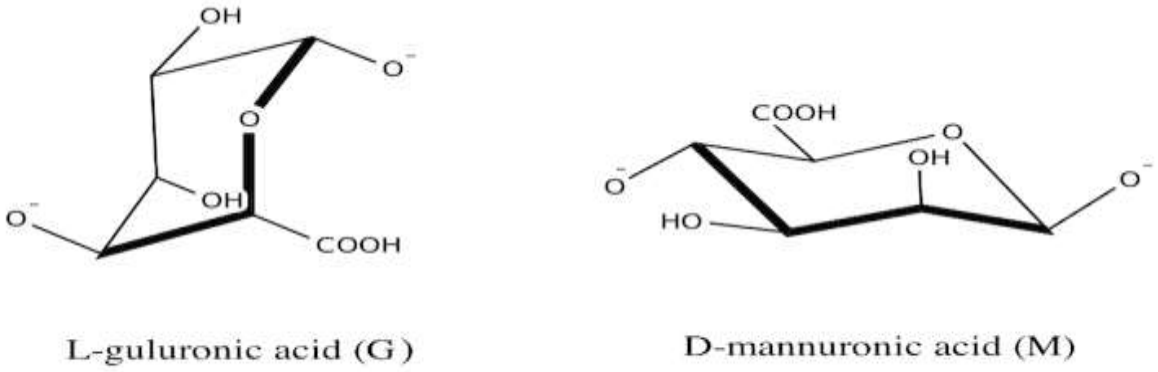

Figure 6 Chemical structure of alginate monomers: L-guluronic acid and D-mannuronic acid.
Source: Ching, S. H., Bansal, N., & Bhandari, B. (2015). Alginate gel particles-a review of production techniques and physical properties. Critical reviews in food science and nutrition, (just-accepted), 00-00.

Alginate is able to form a gel that is not affected by temperature compared to other polysaccharides like gelatin or agar. The formation of alginate gel can be done by two methods: acid precipitation (acidic gels) or ionic crosslinking with cations (ionic gels). Ionic gel is considered one of the most attractive properties of alginate for the food industry. The gel formed by the interaction of alginate with multivalent cations such as Ca^{+2} is widely used in the presence of bioactives in the food industry, for cell immobilisation in the biotechnology industry and in pharmaceutical drugs (Ching et al., 2015). Calcium is a non-toxic and widely used to form ionic alginate gels. The beads of calcium alginate are generally produced by two methods: extrusion by dripping a sodium alginate solution into a calcium salt solution, leading to the external ionic gelation; and the emulsification method leading to internal ionic gelation of alginate in a water/oil emulsion (Gombotz & Wee, 2012). The gelation happens when it is form a zone of union between blocks of acid α-L-guluronic

(G) of a molecule of alginate which it is physically connected to another block of acid α-L-guluronic (G) from another molecule of alginate by calcium ions. This structure is called the egg box model (Ching et al., 2015).

A number of studies have shown that a mixture of alginate and some other polymer compound could be used in the coating method of the microencapsulation process. The capsules or beads are coated by another compound or applying structural modification of the alginate by using different additives. For instance, mixing starch with alginate is widely used and it has been reported that this method enhanced the probiotic encapsulation effectiveness in protecting probiotic bacteria (Sultana et al., 2000; Truelstrup-Hansen et al., 2002; Krasaekoopt et al., 2003).

2.1.6 Conclusion

This literature review has shown that there is a lot of potential in developing a new functional food using a combination of dates and probiotics. Dates are considered to be a remarkable fruit that provide the human body with many of its daily needs of nutrients and they have a very acceptable sweet taste. Probiotic bacteria act as a good enhancer of human health and may aid recovery from disease. Dates provide a good vehicle to carry probiotics both because of the presence of nutrients and for their micro-architecture. These days, people want healthier products to ward off diabetes, heart disease, cancer, depression, obesity and other health conditions. So, more studies are required to know which is the best way to develop a probiotic date product.

Chapter. 3. Materials and Methods

This research was carried out in the food and microbiology laboratories in the WS Building at the Auckland University of Technology (AUT), Auckland.

3.1 Preparation of cultural media

Lactobacilli Difco MRS (de Man, Rogosa and Sharp) agar was prepared for *L. acidophilus* counts by adding 70 g of agar powder into 1 L of deionized water. The powder of the medium was completely dissolved by heating to boiling with agitation, then autoclaving at 121ºC for 15 min.

Lactobacilli Difco MRS (de Man, Rogosa and Sharp) broth was prepared for *L. acidophilus* enumeration with 55 g of the agar powder weighed and suspended in 1 L of deionized water. The medium was heated to boiling with frequent agitation to dissolve the powder before autoclaving at 121ºC for 15 min.

Mannitol Salt Agar (Oxoid) was prepared to detect staphylococci with 111 g of agar powder added to 1 L of deionized water. The medium was heated to dissolve the agar powder and boiled for 1 min. before autoclaving at 121ºC for 15 min.

Xylose-Lysine-odium Desoxycholate (XLD) agar (Oxoid CM469) was prepared for *Salmonella* detection, with 53 g of agar powder suspended in 1 L of deionized water. The medium was heated with frequent agitation to completely dissolve the powder. No autoclaving was needed.

Plate Count Agar (Difco) was prepared for the microbial content of food product with 23.5 g of agar powder weighed and suspended in 1 L of deionized water. The medium was heated to boiling with frequent agitation and boiled for 1 min to completely dissolve the powder then autoclaved at 121ºC for 15 min.

McConkey agar was prepared to detect *Escherichia coli* in the product with 50 g of the agar powder weighed and suspended in 1 L of deionized water. The medium was heated to boiling with frequent agitation and boiled for 1 min to completely dissolve the powder, then autoclaved at 121ºC for 15 min.

3.2 Preparation and enumeration of probiotic bacterium *L. acidophilus*

Pure probiotic culture of *L. acidophilus* (Dewinkel Co.NZ) was obtained from De Winkel Yoghurt (Fonterra Cooperative Group, New Zealand) and inoculated into MRS agar (de Man-Rogosa-Sharpe) and incubated anaerobically in GasPakTM EZ incubation chambers with anaerobe pouches at 37ºC for 72 h. Then, a loop full of the culture was inoculated into 100 ml MRS broth and incubated anaerobically in GasPakTM EZ incubation chambers with anaerobe pouches at 37ºC for 72 h to a final concentration of at least 106 CFU/ml. The bacteria were counted using a hemocytometer slide. Cultures were collected by centrifugation. The 100 ml MRS broth was divided into three (30 ml) centrifuge tubes and centrifuged at 4ºC (11180 g for 10 min - DuPont Sorvall Instruments RC5C), washed twice by centrifugation with 30 ml sterile deionized water, then rinsed with water. One ml of sterile deionized water was added to the tubes to suspend the pellet.

3.3 Preliminary immobilization experiment

The beads were prepared by the extrusion-dripping method (Smidsrød, & Skja, 1990; Puguan et al., 2014) and the prepared suspension contained *L. acidophilus* (10^6 CFU/ml), alginate and date fruit. Trials were performed to optimize the production of beads. Different concentrations of alginate and dates were examined to determine the appropriate concentrations for the mixture, which can be pumping easily through a needle without blockage. The concentrations of alginate

were 2%, 3%, 4% and 5% (w/v). The concentrations of date were 37%, 35% and 31%. Two forms of dates were tested in the alginate beads: autoclaved and raw. In addition, the control beads, which were probiotic alginate beads without dates, were also examined to determine the effect of the dates on the product.

3.4 Immobilisation using autoclaved dates

The dates were autoclaved to ensure that the colour of the date alginate beads was more similar to the colour of the dates. Three de-stoned mashed Medjool dates weighing 31 g were suspended in 100 ml of deionized water and autoclaved at 105ºC for 10 minutes. Sterile alginate powder (3 g) (Chemicals VWR BDH Prolabo) was added to the date palm suspension and dissolved. The culture sample was added to the date alginate mixture after cooling to room temperature. Ten ml of the alginate-date-bacteria mixture were withdrawn using a sterile syringe with a needle (1.20 × 38 mm) and dropped slowly into 20 ml of 10% calcium chloride dihydrate solution (Chemicals VWR BDH Prolabo) contained in a 250 ml Erlenmeyer flask. The calcium alginate was left to harden for 10 minutes. The excess calcium chloride was decanted off and discarded. The beads were washed twice with 30 ml of sterile deionized water. All experiments were done in triplicate. The weight of the beads ranged from 10-13 g in each Erlenmeyer flask.

3.5 Immobilisation using raw dates

Three grams of sterile alginate powder (Chemicals VWR BDH Prolabo) were dissolved in 100 ml of sterile deionized water. Three dry de-stoned mashed dates (31 g, Medjool) were mixed with the alginate solution, and the sample culture was added to this mixture. A full 10 ml syringe of the mixture was carefully dripped through (1.20 × 38 mm) needles into a 20 ml sterile 10% calcium

chloride dihydrate solution (Chemicals VWR BDH Prolabo) contained in a 250 ml Erlenmeyer flask. The calcium alginate was left to harden for 10 min. The excess calcium chloride was decanted off and discarded. The beads were washed with 30 ml of sterile deionized water, mixed and the water was discarded. The rinse in 30 ml of sterile distilled water was repeated. The beads were transfer into bottles. All experiments were performed in triplicate. The weight of 150 beads was approximately 10-13 g in each bottle (Figure 7).

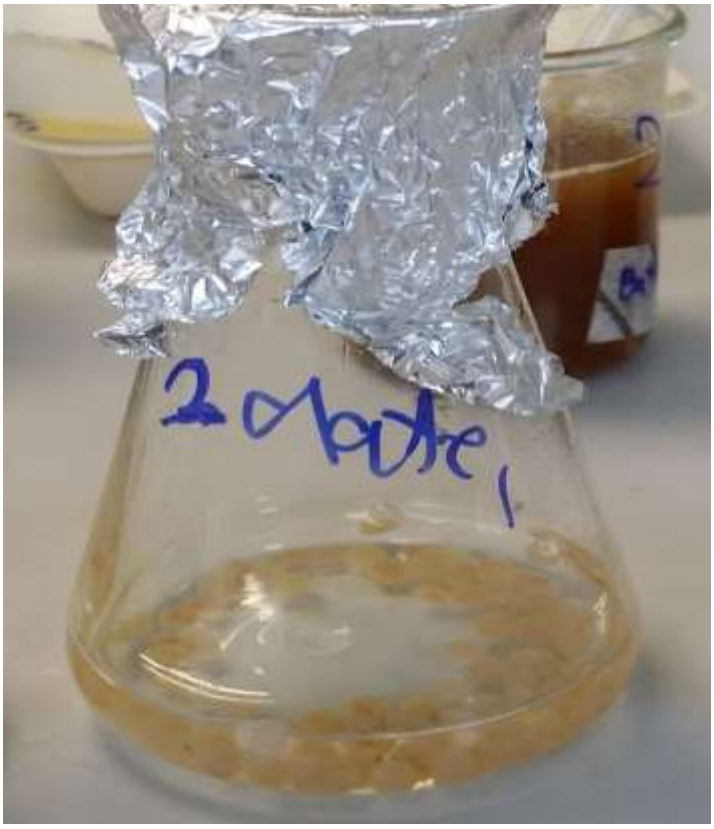

Figure 7 alginate beads of raw dates and *L. acidophilus*

3.6 UHT Milk with date-immobilised probiotic bacteria

Three bottles of 100 ml UHT trim milk (Anchor) were prepared and each bottle contained 10-13 g of the beads. The bottles were kept in the refrigerator at 4^{o}C for the physical, chemical and microbial analyses of the milk and the beads on days zero, two, four and seven.

3.7 Characterisation of beads

3.7.1 Bead size

The diameter of 10 beads, selected randomly, was measured in millimeters. The beads were dried on paper towels before measuring to remove excess solution.

3.7.2 Textural characterisation

The texture properties of beads were examined according to the method reported by Bhujbal et al. (2014) and Chan et al. (2011 a, b). A Texture Analyser (TA) apparatus (TA XT plus) equipped with a 5-kg load cell was used to determine the textural properties of the beads. Individual beads (n=10) were dried before the measurement. Individual beads were compressed in a vertical direction to 50% to determine the gel strength of the beads using the 25 mm diameter cylindrical aluminium probe at a speed of 2 mm/sec in a mode of compression, and the rupture distance was 3 mm. The peak force was measured in kilograms. Ten beads were randomly examined for each sample.

3.8 Viability of free and encapsulated *L. acidophilus* during storage

To determine viable counts of encapsulated cells, 1.4-1.6 g of beads were washed twice with sterile deionized water. The encapsulated cells were released by dissolving the beads in 10% of sodium citrate dihydrate solution (JT Baker Chemical Co., Phillipsburg, NJ) in a Stomacher-80 bag and they were homogenised using a Stomacher-80 laboratory blender (Seward Medical), at high speed

for 10 minutes at room temperature (Lee, & Heo, 2000). The number of encapsulated cells was detected by a spread plate count on MRS agar; serial dilutions of the released cells (10^{-4}-10^{-8}) were plated in triplicate and incubated for 72 h at 37ºC. The encapsulated cell number was counted at 0, 2, 4 and 7 days of storage. The results were reported as the colony-forming units (CFU/ g).

The number of free cells (released cells) in the milk was determined using a spread plate count on MRS agar. One ml of the milk sample was serially diluted (10^{-1}-10^{-6}) in 9 ml of sterile deionized water and spread onto pre-dried MRS agar. The number of colonies formed on the plates was counted after incubation for 72 hours at 37ºC. The cell number was counted at 0, 2, 4 and 7 days of storage. The results reported as the colony-forming units (CFU/ g).

3.9 pH of the milk

The pH values were determined using a pH meter (Meterlab@ Instrument) with a glass electrode standardised at room temperature over the range pH 4.0 to pH 7.0 (AOAC, 2000). The electrode was submerged into 10 ml of each milk sample and the pH reading was taken after stabilisation of the meter.

3.10 Colour analysis of the beads and the milk

Colour measurement was performed using a Lab Scan spectrophotometer (Hunter Lab, Colorflex). The spectrophotometer was calibrated with the standard white and black reference tiles that came with the instrument. The data collected include L* (lightness), a* (redness-greenness), and b* (yellowness-blueness). The beads were dried and placed on a small petri dish. Ten ml of the milk was poured into a small petri dish. Measurements were carried out in triplicate for each sample.

3.11 Microbial analysis of the dates

A 10 g sample of de-stoned (sterile knife and forceps) medjool dates was weighed in a sterile Stomacher-80 bag and 90 ml of sterile peptone water was added and homogenised (Stomacher-80 laboratory blender, Seward Medical) at high speed for 2 min. Then serial decimal dilution was prepared by transferring 1 ml of the stock solution into 9 ml of sterile deionized water. One ml from each dilution was pour plated onto sterile petri dishes.

The pour plate method was used for Total Viable Counts using Plate Count Agar at 37°C for 48 hours, for coliform bacteria (McConkey agar) at 37°C for 48 h, *Staphylococcus aureus* (Mannitol salt agar) at 37 °C for 48 h and lactic acid bacteria (MRS agar) for 30°C for 48 h. The results were reported as colony forming units (CFU per g). All experiments were done in triplicate.

3.12 Pathogen detection

Any contaminating pathogens in the product were detected by suspending 5 g of the product in 15 ml of 10% of sodium citrate dihydrate solution (JT Baker Chemical Co., Phillipsburg, NJ). The product was homogenised (Stomacher-80 laboratory blender, Seward Medical) at high speed for 10 min to dissolve the beads. One ml of the diluted (1:4) milk sample was transferred into 5 ml of sterile deionized water to dilute to 1:20.

Salmonella detection was performed by pouring 1 ml from each dilution onto Xylose-Lysine-sodium Desoxycholate (XLD) agar (Oxoid CM469) in triplicate. The pour plate method was used and plates were incubated aerobically at 37°C for 24 hours. The presence of *Salmonella* colonies is typically seen as yellow to red colonies with black centers (Ruby & Ingham, 2009).

Detection of *Staphylococcus aureus* was performed using Mannitol Salt Agar. One ml from each dilution was pour plated in triplicate. The plates were incubated aerobically at 37°C for 48 hours. *Staphylococcus aureus* colonies are typically seen as yellow colonies with yellow zones.

3.13 Scanning electron microscopy (SEM)

The microstructure of the beads was observed using a scanning electron microscope, SEM (Hitachi SU-70 Schottky field emission SEM, address of manufacturer). Random beads were air-dried for 48 hours on petri dishes at room temperature (Yeung et. al ,2016). Then, the beads were placed in a desiccator to remove all moisture for 24 hours. The beads were spattered with platinum in vacuum using a Hitachi E-1045 Ion Sputter Coater. The beads were loaded into the SEM and images of the morphological characteristics of the beads were recorded randomly.

3.14 Statistical analyses

Mean values of all collected data from the three groups of study (autoclaved dates, raw dates and control) were recorded with standard deviations (Mean ± standard deviation). The statistical significance of differences between the means of three groups was analysed using analysis of variance (ANOVA), followed by post hoc Turkey's test. P-values of < 0.05 defined statistical significance. In addition, the statistical significance of differences of each variable among the independent group during storage time was reported using analysis of variance (ANOVA), followed by post hoc Turkey's test. Statistical analyses were carried out using an SPSS statistics program (IBM SPSS version 23).

Chapter. 4. Results and discussion

4.1 Determination of gelling solution and bead preparation

The viscosity of 5% (w/v) alginate solution was high with all the concentrations (37% (w/v), 35% (w/v) and 31% (w/v) of date fruits. The mixture of 5% (w/v) alginate, *L. acidophilus* (10^6 CFU/ml) and the concentration of date fruits was difficult to pass and pump through the nozzle needle. Similarly, a 4% (w/v) alginate concentration had a high viscosity with bacterial culture and date fruits. The high viscosities of 4% (w/v) and 5% (w/v) were not suitable for encapsulation application due to difficulties in pumping and needle blockage in the bead forming process. Lee et al. (2013) gave a comprehensive review on the correlation between high concentrations of alginate solution (or viscosity) and the difficulty of the bead formation process, which is in line with the results of the present study. Consistent with findings by Seifert and Phillips (1997), it was found that a 2% (w/v) alginate solution would not form spherical-shaped beads due to its low viscosity. Thus, it was determined that 3% (w/v) alginate was the most appropriate concentration with 31% (w/v) date fruits and *L. acidophilus* (10^6 CFU/ml) to produce egg-shaped or drop-shaped beads.

4.2 Microbial Analysis, pH

4.2.1 Microbial analysis of the date fruits

It was found that there was no viable bacterial count in any sample of date fruit after autoclaving. Lactic acid bacteria were not detected in any sample. Neither coliform nor *Salmonella* was detected in the date fruits. The findings provided evidence that the autoclaved date fruits were sterile before being used in the encapsulation method.

4.2.2 Viability of *Lactobacillus acidophilus*

4.2.2.1 Viability of free *L. acidophilus* in the UHT milk

The viable bacterial count during the seven days of storage at 4°C is shown in Table 4. There was no increase in numbers of *L. acidophilus* in milk in any of the treatments (autoclaved date beads, raw date beads or control beads without dates) during the storage period. This finding indicates that microencapsulation in calcium alginate was able to maintain *L. acidophilus* inside the beads without release to the milk. Weinbreck et al. (2010) reported that microencapsulation maintains the bacteria in a microenvironment to survive during processing and storage until reaching a suitable location(s) in the gastrointestinal tract for release. Moreover, the release of probiotics in a food matrix can develop undesirable growth of probiotic bacteria, that may negatively influence the sensory properties and shelf life of food (Chen et al., 2015).

Table 4. Viable bacterial count of free *L. acidophilus* in milk CFU/ml during storage at 4°C

Samples	**Time (hours)**			
	0	24	96	168
Autoclaved dates	No Growth	No Growth	No Growth	No Growth
Raw dates	No Growth	No Growth	No Growth	No Growth
Control	No Growth	No Growth	No Growth	No Growth

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*. CFU/ml = colony-forming unit per milliliter

4.2.2.2 Viability of encapsulated *L. acidophilus*

The initial viable bacterial count in all three samples (autoclaved date, raw date and control) was in the range of the recommended dose of 10^6-10^7 CFU per g. The viable *L. acidophilus* count was measured at zero hours before being stored in a refrigerator for 168 h (7 days) at 4°C. Table 5 and

Figure 8 present the viable count of *L. acidophilus* during storage. There was no significant change in the viable count in all three samples throughout the 168 h of storage. The level of *L. acidophilus* count was maintained at more than 10^6 CFU per g after 168 h of storage.

The finding is consistent with findings of past studies by Ding & Shah (2009), which reported that alginate-encapsulated *L. acidophilus* survived during processing and storage. Furthermore, Ma et al. (2015) showed that microencapsulation can provide protection for *L. acidophilus*, which led to improved efficiency for its application. In addition, Krasaekoopt et al. (2006) reported on the survival and stability of encapsulated *L. acidophilus*, and that the bacterial count was kept above the recommended level (10^7 CFU/g) throughout storage. Research findings by Desai (2008) also point towards the important factor of survival and activity of probiotic bacteria until they reach their appropriate location in the lower small intestine. Several studies have shown that microencapsulation of *L. acidophilus* may increase the viability of probiotics throughout the storage period, confirming that encapsulated probiotics are more protected than nonencapsulated ones (Ann et al., 2007; Kim, 2008; Borges et al., 2012; Urbanska et al., 2007; Mandal, et al., 2006).

It is apparent from Table 5 and Figure 8 that there was a significant ($P < 0.05$) change in bacteria count between the three samples. The control sample had a lower *L. acidophilus* count than the autoclaved date sample and raw date sample. This could be explained on the basis that the dates could contain some micronutrients, such as minerals and vitamins, as well as their higher sugar content, which might stimulate the growth of *L. acidophilus* (Gad et al., 2010). In addition, Aljasass et al. (2010) reported increased growth of *L. acidophilus* in flavoured probiotic yogurt containing date syrup during 10 days of storage. Generally, the samples*time interaction was not significant.

The probiotic benefits are strongly dependent on their capability to survive and multiply in the intestine of the host. Therefore, probiotic bacteria should be active and metabolically stable in the product and survive in large number through their passage to the upper digestive tract (Gilliland, 1989). Generally, viability is necessary for the targeted probiotics to proliferate inside the human gut (Anal & Singh, 2007).

Table 5. Viable count of encapsulated *L. acidophilus* CFU/g during storage at 4°C

Samples	**Time (hours)**				**P values**		
	0	24	96	168	Samples	Time	Samples* Time
Autoclaved dates	6.0E6±4.3E6	6.1E7±1.1E8	2.0E7±1.9E7	4.1E7±5.9E7	0.017	0.404	0.183
Raw dates	2.3E7±2.9E7	1.3E7±1.5E7	1.5E7±2.1E7	2.1E7±4.2E7			
Control	2.6E6±2.9E6	5.2E6±1.4E6	4.1E6±3.5E6	4.6E6±4.5E6			

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*. CFU/g = colony-forming unit per gram

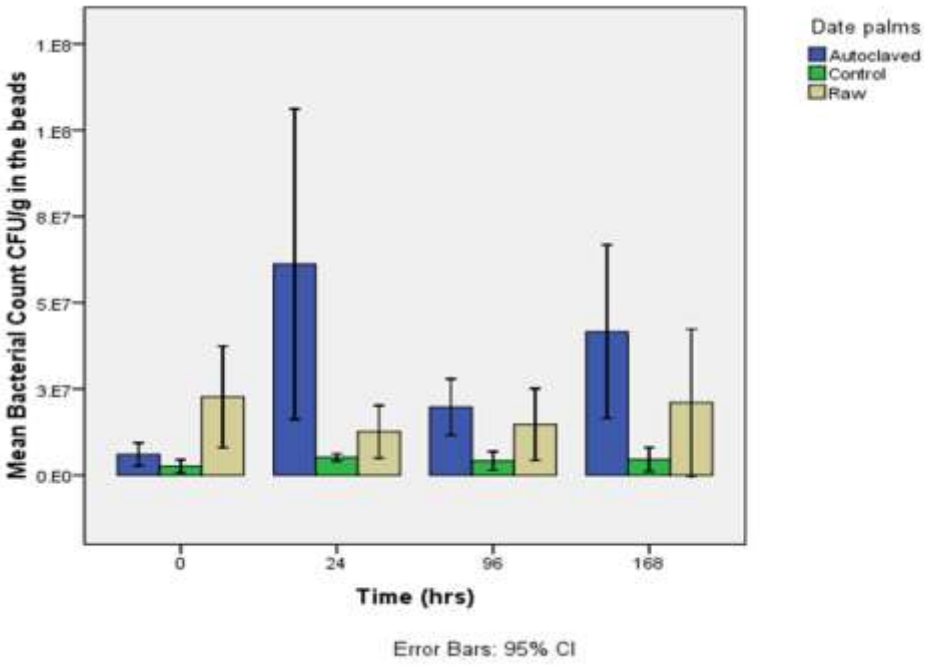

Figure 8 Viable count of encapsulated *L. acidophilus* during the 7 days (168 h) of storage at 4°C.
Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*. CFU/g = colony-forming unit per gram

4.3 Pathogen detection

Table 6 provides the viable pathogen counts after 0 and 168 h. Neither *Salmonella* nor *Staphylococcus aureus* was observed in any of the samples. The results indicate the safety of the product from any foodborne pathogens. Salmonella detection is important for assessing adherence to food safety standards and the consumer acceptability (Mrabet et al., 2008). *Staphylococcus aureus* and *Salmonella* are the most common pathogens in milk, causing foodborne disease (Oliver et al., 2005). A study by Ayachi et al. (2009) found that date fruit extracts showed an antibacterial effect against *Salmonella*. Furthermore, a number of studies have found that *L. acidophilus* has antagonistic activity against *Salmonella* (Hudault et al., 1997; Lin et al. 2007; Coconnier et al. 2000; Scapin et al., 2013; Sharma, 2014). It has been reported that probiotic cultures protect food products from spoiling (Anukam et al., 2006; Al-Otaibi, 2009). However, this may due to the strict hygienic precautions used during handling, production and storage of the product.

Recently, Chen et al. (2015) reviewed the safety requirements for developing encapsulated probiotics in food applications. Furthermore, Sarao (2015) reported the significance of focusing on the optimisation of the use of encapsulated probiotic cells with consideration of the safety of the product. An important issue emerging from the findings presented in Table 6 is that the production of this novel product is completely safe for consumers. The presence of dates and *L. acidophilus* in the product had no notable effect on the growth of potential pathogens.

Table 6. Viable count of pathogens after 0 and 168 h of storage at 4°C

	Salmonella		*Staphylococcus aureus*	
Samples	0h	168 h	0 h	168 h
Autoclaved date	No Growth	No Growth	No Growth	No Growth
Raw date	No Growth	No Growth	No Growth	No Growth
Control	No Growth	No Growth	No Growth	No Growth

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

4.4 pH

The pH values of the milk during the 168 h of storage are given in Table 7 and Figure 9. The initial mean pH value of the autoclaved date milk product and raw date milk product was 6.20 ± 0.17 and 6.14 ± 0.25 respectively. During the 168 h of storage, there were no significant differences in pH values ($P < 0.05$) for either the autoclaved date samples or the raw date samples. Similarly, the mean pH values of the control sample showed no significant changes during storage. The initial mean pH value of the control sample was 5.80 ± 0.26. This finding is consistent with the findings of an earlier study (Prakash et al., 2016), in which the encapsulation of probiotics had no effect on the pH of the orange juice during 35 days of storage at 4 °C. This result was expected and provides evidence that encapsulated *L. acidophilus* is stable inside calcium alginate beads (Krasaekoopt et al., 2006; Ma et al., 2015). The addition of date fruits had no effect on the activity of *L. acidophilus* inside the beads compared with the control sample.

However, this is contrary to a previous study conducted by Kailasapathy (2006). Kailasapathy (2006) observed the effect of free and encapsulated *L. acidophilus* on yoghurt pH over seven weeks of storage. The addition of *L. acidophilus* (free and encapsulated) decreased acid development in the yoghurt during the storage time. Interestingly, post-acidification in yoghurt with encapsulated

L. acidophilus was slower compared to yoghurt with free *L. acidophilus*. Furthermore, Rodrigues et al. (2012) found a decrease in pH in fruit juices during storage when using encapsulated *L. paracasei*.

There were significant differences ($P < 0.05$) in the pH values between the samples. The control sample had a lower pH value than the autoclaved date and raw date samples. The results obtained are compatible with the findings of the viability of encapsulated *L. acidophilus*. The number of encapsulated *L. acidophilus* in the autoclaved date and raw date samples was higher than in the control sample before and after storage.

Table 7. Changes in pH values in milk during storage at 4°C

Samples	**Time (hours)**				**P values**		
	0	24	96	168	Samples	Time	Samples*Time
Autoclaved date	6.20±0.17	6.26±0.25	6.22±0.19	6.23±0.18	0.001	0.968	0.994
Raw date	6.14±0.25	6.15±0.26	6.15±0.26	6.15±0.27			
Control	5.80±0.26	5.80±0.25	5.82±0.25	5.90±0.30			

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

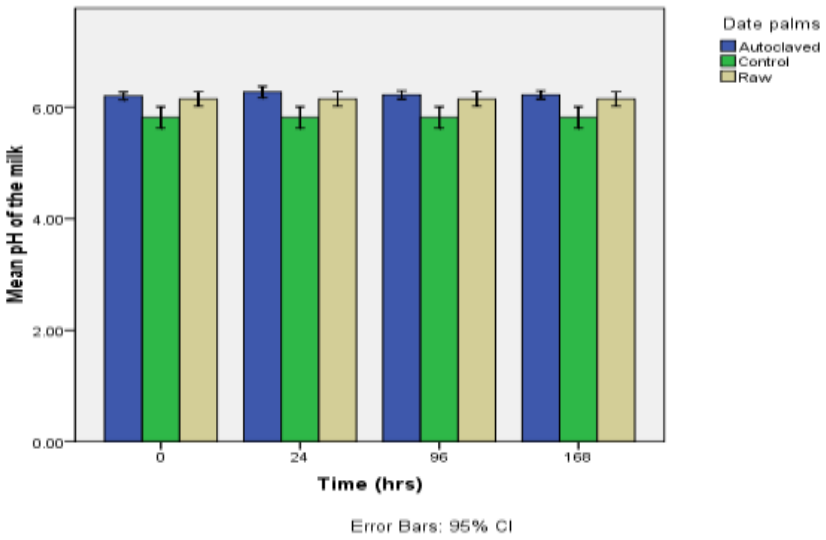

Figure 9. Changes in pH values in milk during storage at 4°C

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

4.5 Physical properties

4.5.1 Bead size and morphology

As illustrated in Table 8 and Figure 10, there were no significant changes ($P < 0.05$) in the size of the beads during storage time (samples*time) for any samples. The size of the beads remained the same after 168 h of storage. However, as shown in Table 8, there were significant differences ($P < 0.05$) in the size of the beads between the samples. The control sample had smaller bead sizes compared to the autoclaved date sample and raw date sample. The autoclaved date sample had the largest bead size and this could be explained by the lower viscosity of the alginate, autoclaved date and *L. acidophilus* solution. Lee et al. (2013) indicated that the size of the beads formed by the extrusion dripping method decreases when the viscosity of the alginate solution increases.

Overall the beads are considered large in size compared to other studies using the same extrusion dripping technique (Lee et al., 2013). Alginate beads produced by simple dripping are in the millimeter (1-2 mm) size range (Blandino et al., 1999; Chan et al., 2011 b). The present finding also supports earlier literature (Ching et al., 2015) that the extrusion method produced large gel particle size. In this study, the large bead shape was required to make the beads more similar in colour to the small pieces of dates. This view is supported by Champagne and Fustier (2007), who argued that the effect of the beads on sensory properties may become attractive if the consumer expects the existence of the particles. In food an average bead diameter of 30 μm is recommended to decrease the perception of graininess (Heidebach et al., 2012). Increased bead size is associated with grainy textures in food, which may be considered a limiting factor on sensory properties. However, decreasing the bead size is one of the biggest challenges in using encapsulation in food (De Prisco & Mauriello, 2016). In a different study, Lee and Heo (2000) argued that very large

beads may cause granularity of texture in food, but small beads may not provide enough protection for the probiotic bacteria. It has been reported that larger bead size (> 1 mm) provided more protection to probiotic bacteria (Sheu et al., 1993; Bhujbal et al., 2014). In addition, the viability of encapsulated bacteria in simulated gastric fluid increased with larger bead sizes (Anal & Singh, 2007; Truelstrup Hansen et al., 2002).

The bead shapes in the autoclaved date sample and raw date sample were uniform, but differed from the control bead shapes. The control sample had more spherical bead shapes than the other samples. Photographs in Figure 11 (a and b) show the bead shapes in both the autoclaved date sample and raw date sample. In general, the beads were teardrop shaped, as shown in Figure 11 (c). A similar bead shape as in the present study was observed by Krasaekoopt et al. (2004), Al-Hajry et al. (1999), Homayouni et al. (2007), Allan-Wojtas et al., (2007), Sultana et al. (2000), and Sheu & Marshall (1993).

It has also been shown that the shape and size of the beads are usually critically controlled. In the extrusion dripping method, the production of a spherical shape and desired size requires trial and error (Lee et al., 2013). Furthermore, the size and morphology of the alginate beads may vary depending on the diameter of the needle nozzle, alginate viscosity, alginate exit flow rate and the distance from alginate exit point to the gelling bath (Blandino et al., 1999; Chan et al., 2011 b). A number of studies have found that the low viscosity (or concentration) of alginate solution is less able to produce spherical-shaped beads (Lee & Heo, 2000; Lee et al., 2013). Moreover, Bhujbal et al. (2014) reported that the alginate viscosity determines the size and shape of beads.

Table 8. Size of the beads (mm) after 0 and 168 h of storage at 4°C

Samples	Time (hours)		P values		
	0	168	Samples	Time	Samples*Time
Autoclaved date	6.15±1.46	6.60±1.04	0.001	0.001	0.921
Raw date	5.36±0.64	5.85±0.659			
Control	4.54±0.64	4.90±0.61			

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

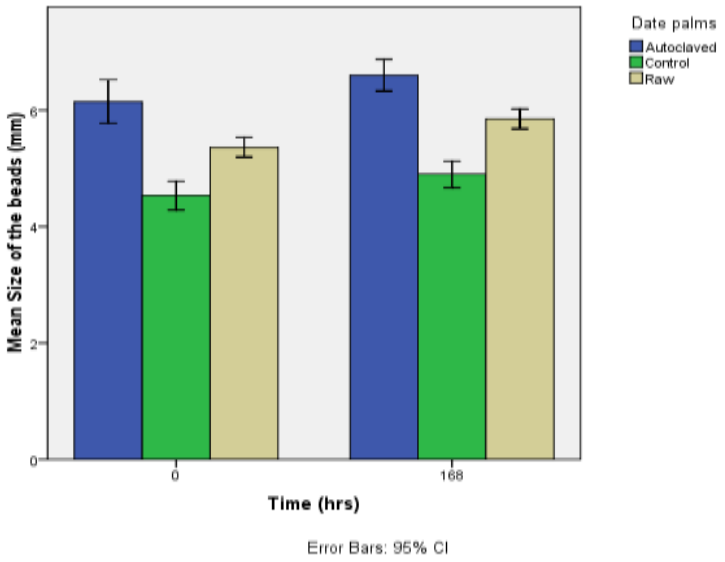

Figure 10. Changes in size of the beads (mm) after 0 and 168 h of storage at 4°C

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

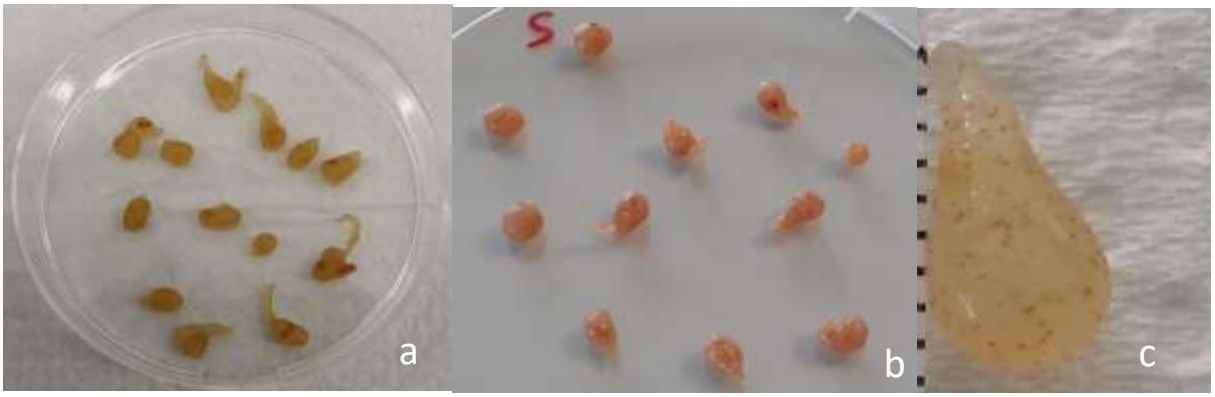

Figure 11. Shape of beads. (a) Raw date beads (b) Autoclaved date beads (c) Teardrop-shaped bead.

4.5.2 Textural analysis

The stability of the beads (texture) was measured as the force required to reach 60% compression. As the force increases stability increases. From the data in Table 9 and Figure 12, it is apparent that there was a significant change ($P < 0.05$) in the texture of the beads (force) during storage of all three samples. This finding indicates that the beads are not stable after 168 h of storage. There was a significant difference ($P < 0.05$) in the texture of the beads between the samples. The control sample had the highest F values, which indicates that it was most stable. The force required to compress the control sample was higher than other samples. The results revealed that the presence of dates decreased the stability of the beads.

The most likely explanation for this result is the relationship between shape and gel strength. Al-Hajry et al. (1999) discovered non-spherical-shaped beads decreased the gel bead strength compared to spherical beads. This finding is consistent with the present finding, which showed that the bead shape of the control sample was more spherical than the autoclaved date bead sample and raw date bead sample.

It has been demonstrated that the physical features of alginate hydrogels strongly depend on their content and the sequential structure of the polymer (Smidsrød, 1974; Martinsen et al., 1989; Smidsrød & Skja, 1990). In addition, Bhujbal et al. (2014) reported that there are several dominant factors that determine the final strength of the alginate-based beads. These factors include the size of the beads, the alginate viscosity, the alginate type, the cell load, the storage solution and the gelling time. The elasticity of the alginate-based beads is dependent on other factors such as the incubation time, the type of gelling ion and the presence of any additional materials. On the other hand, Simpson et al. (2004) demonstrated that the strength of the alginate gel network influences

the growth features of the encapsulated cell. Construction that is too rigid is not suitable for the proliferation and growth of cells.

Table 9. Texture of the beads (force Kg) after 0 and 168 h of storage at 4°C

Samples	Time (hours)		P values		
	0	168	Samples	Time	Samples*Time
Autoclaved date	0.13±0.05	0.22±0.17	0.001	0.256	0.001
Raw date	0.23±0.09	0.20±0.08			
Control	0.34±0.07	0.32±0.05			

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

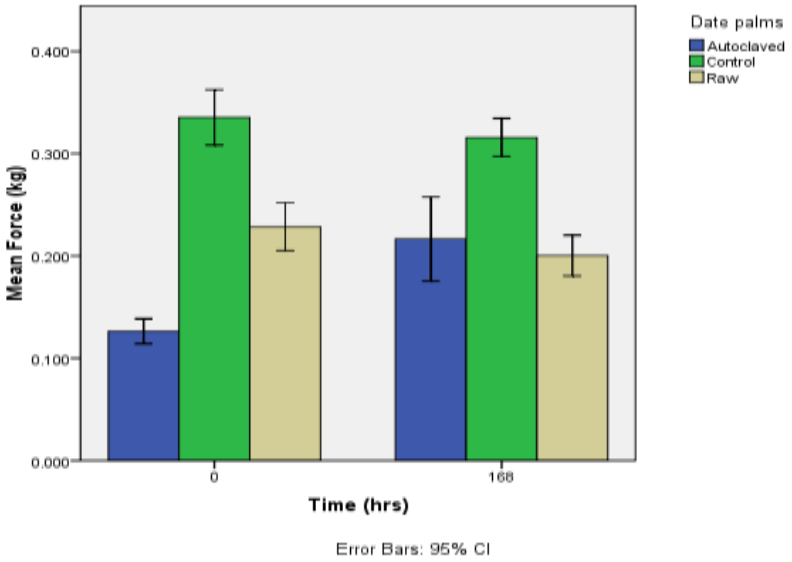

Figure 12. Changes in texture of the beads (force Kg) after 0 and 168 h of storage at 4°C
Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

4.5.3 Scanning Electron Microscope (SEM)

Morphological features of the microencapsulated *L. acidophilus* and the beads, after 0 and 168 h of storage at 4°C, were measured using Scanning Electron Microscopy (SEM). The results showed that dehydration of the samples led to approximately 50% shrinkage of the beads. The results presented here may be due to the high water content of the gel particles (Ching et al., 2015).

Similarly, Ouwerx et al. (1998) reported that the lyophilization and critical point drying of the beads caused shrinkage of the bead sizes. The shape of the beads changed after drying and had a rough surface (a spongy texture) (Figure 13), which was consistent with the observations of Stops et al. (2008) and Puguan et al. (2014). The beads were mostly teardrop in shape (Figure 13). There was a significant difference in the surface texture between the beads containing the dates and those without (i.e. the control sample). The surface of the beads containing dates was rough and wrinkled (Figure 14 a, b) whereas the beads without dates were smooth (Figure 14 c). However, there were no notable changes in the beads' shapes before and after the storage time (Figure 13). In addition, there were no significant difference between the beads of the autoclaved date sample and raw date sample.

SEM showed that *L. acidophilus* in all three samples (autoclaved date sample, raw date sample and control sample) was clearly observed under the surface of the alginate matrix of the beads and visible at high magnification (Figure 15, 16, 17). These findings revealed that *L. acidophilus* was completely entrapped and surrounded by the alginate matrix (Figure 15, 16, 17). It appears from Figure 14 b that the fibres of the date fruits are clearly on the surface of the bead of the autoclaved date sample.

When the bead of raw date sample was cut into cross section, *L. acidophilus* appeared inside the bead within the alginate matrix (Figure 18). This result is in agreement with the study performed by Sultana et al. (2000), which found *L. acidophilus* inside the hydrogel beads. However, a number of studies showed that encapsulated *L. acidophilus* was not clearly shown by SEM to be inside the alginate beads (Ma et al., 2015; Xing et al., 2014; Ma et al., 2014). It is confirmed according to these results that *L. acidophilus* was entrapped within the alginate-date fruit beads, and this type of microencapsulation is able to capture the probiotic bacteria during the 168 h of storage time.

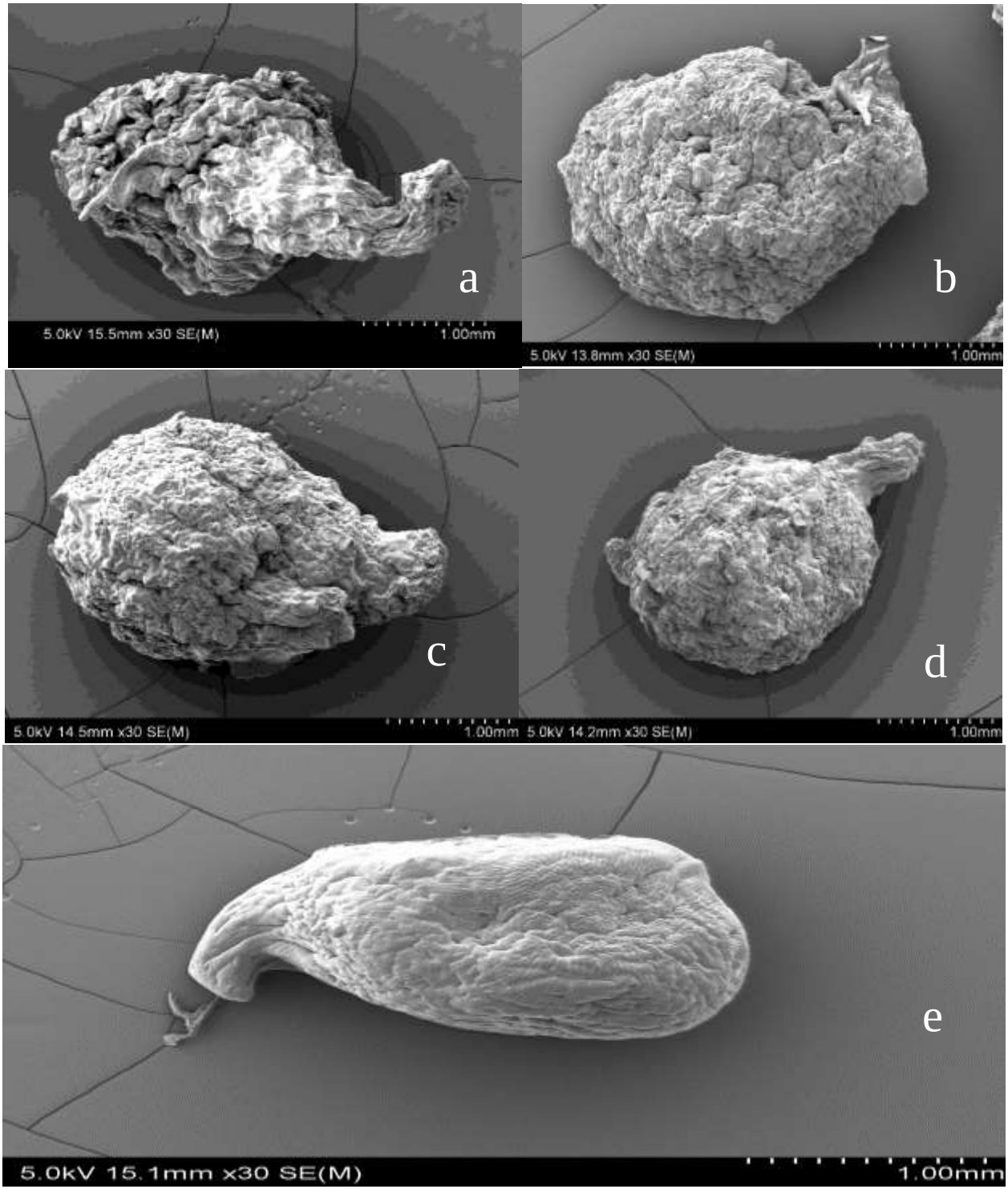

Figure 13.Scanning Electron Microscopy (SEM) photographs of alginate beads. (a) Whole alginate bead of raw date fruit sample (milk product containing alginate beads of raw dates and *L. acidophilus*) after 0 h of storage. (b) Whole alginate bead of raw date fruit sample (milk product containing alginate beads of raw dates and *L. acidophilus*) after 168 h of storage. (c) Whole alginate bead of autoclaved date fruit sample (milk product containing alginate beads of autoclaved dates and *L. acidophilus*) after 0 h of storage. (d) Whole alginate bead of autoclaved date fruit sample (milk product containing alginate beads of autoclaved dates and *L. acidophilus*) after 168 h of storage. (e) Whole alginate bead of control sample (milk product containing alginate beads of *L. acidophilus*)

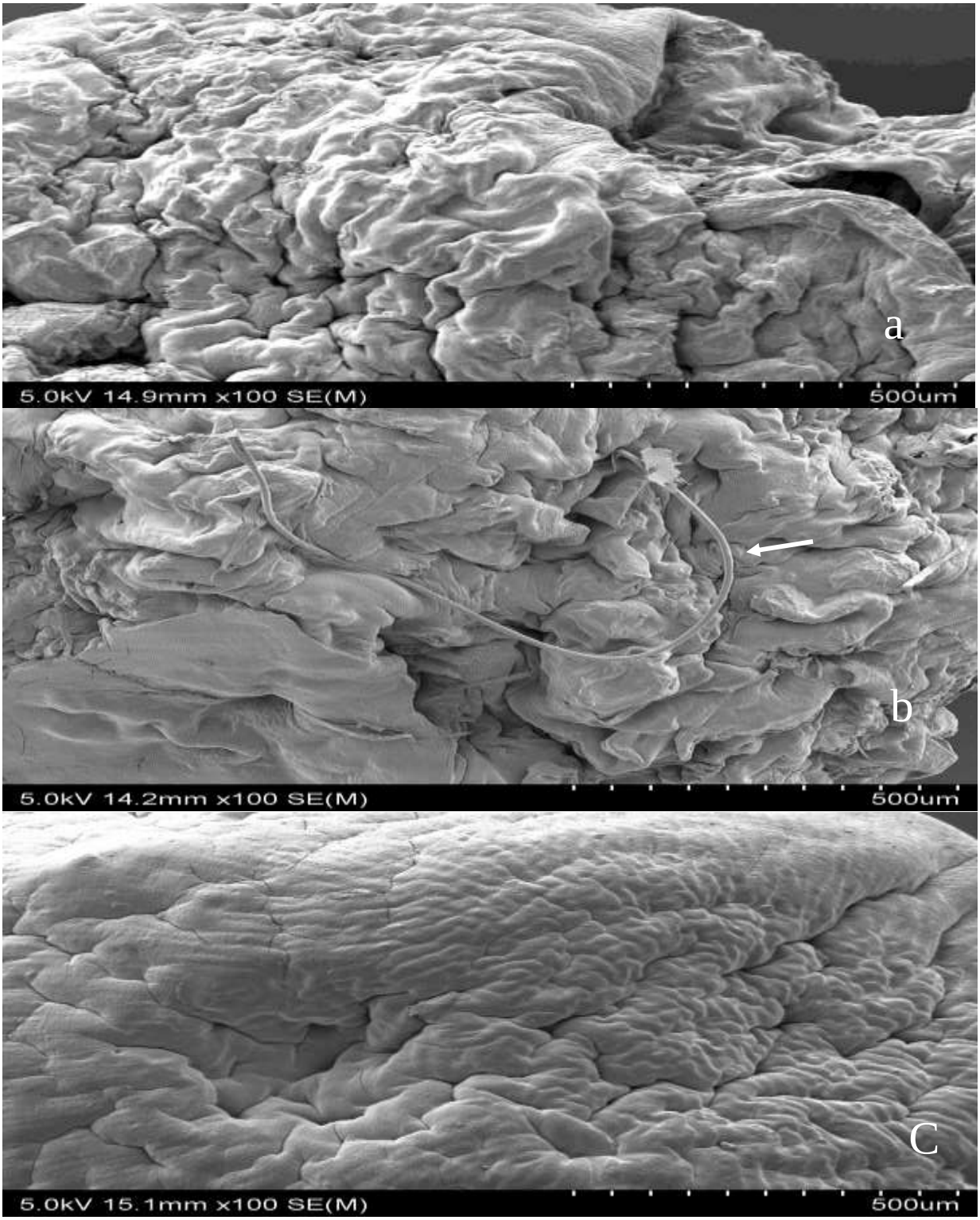

Figure 14. Scanning Electron Microscopy (SEM) photographs of alginate beads (a) Rough surface (a spongy texture) of the raw date sample bead (milk product containing alginate beads of raw dates and *L. acidophilus*). (b) Rough surface (a spongy texture) of the autoclaved date sample bead (milk product containing alginate beads of autoclaved dates and *L. acidophilus*) and shows fiber of the date fruit (arrow). (c) Less rough surface of the control sample bead (milk product containing alginate beads of *L. acidophilus*).

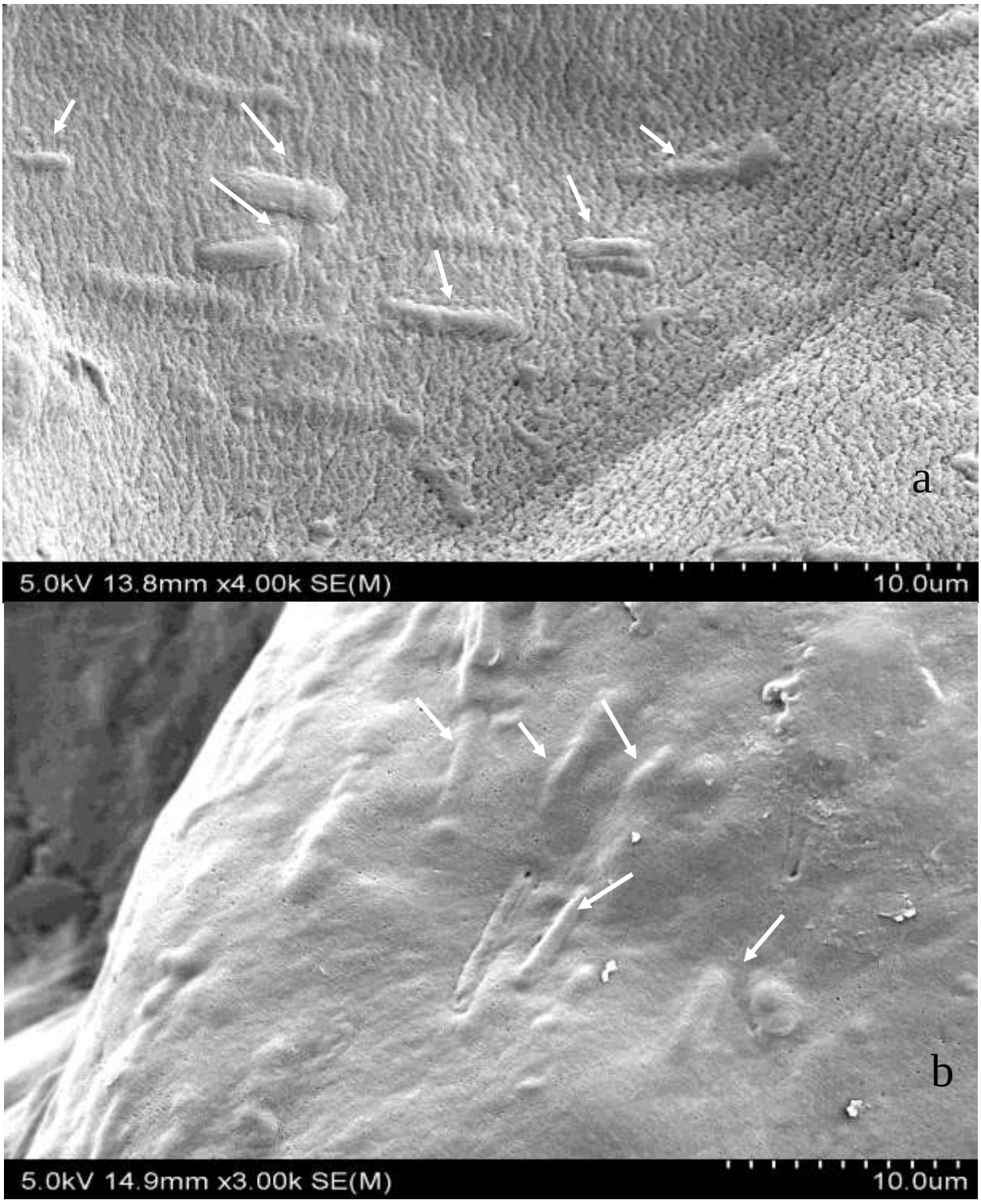

Figure 15. Scanning Electron Microscopy (SEM) photographs of alginate beads. (a) *L. acidophilus* (arrows) under the surface of the alginate matrix of the raw date sample beads (milk product containing alginate beads of raw dates and *L. acidophilus*) after 0 h of storage at 4°C. (b) *L. acidophilus* (arrows) under the surface of the alginate matrix of the raw date sample beads (milk product containing alginate beads of autoclaved dates and *L. acidophilus*) after 168 h of storage at 4°C.

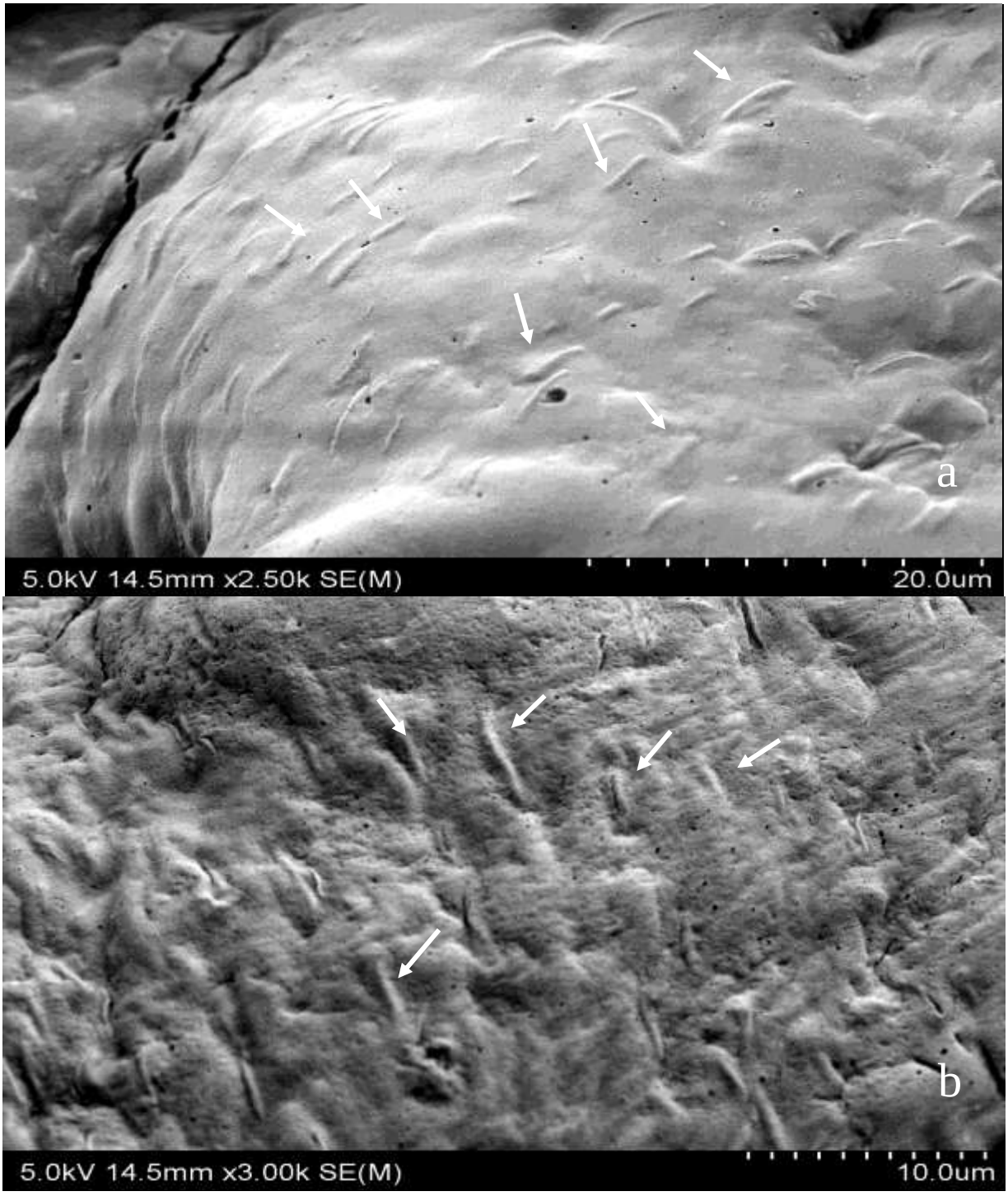

Figure 16. Scanning Electron Microscopy (SEM) photographs of alginate beads. (a) *L. acidophilus* (arrows) under the surface of the alginate matrix of the autoclaved date sample beads (milk product containing alginate beads of autoclaved dates and *L. acidophilus*) after 0 h of storage at 4°C. (b) *L. acidophilus* (arrows) under the surface of the alginate matrix of the autoclaved date sample beads (milk product containing alginate beads of autoclaved dates and *L. acidophilus*) after 168 h of storage at 4°C.

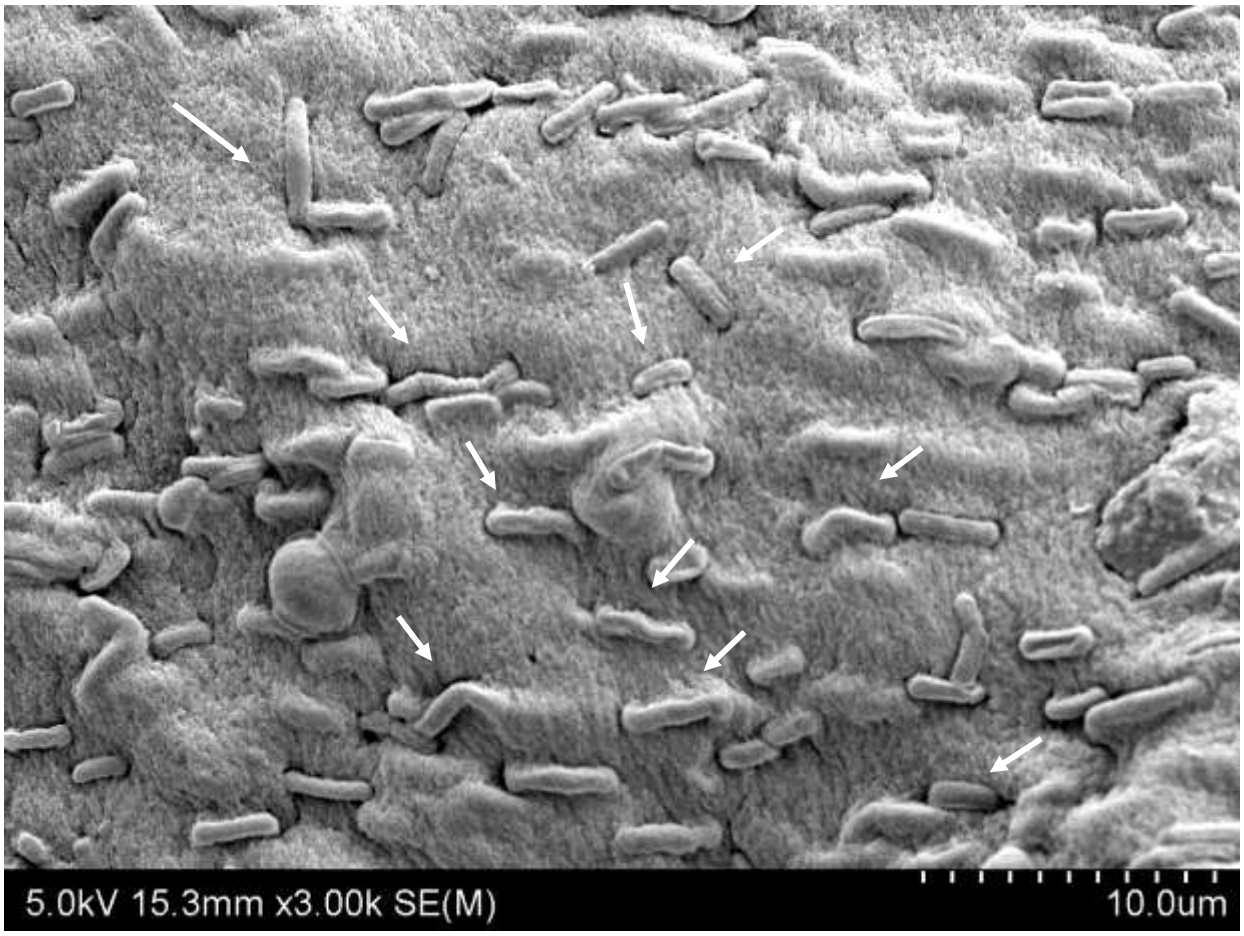

Figure 17. Scanning Electron Microscopy (SEM) photographs of *L. acidophilus*(arrows) under the surface of the alginate matrix of the control sample beads (milk product containing alginate beads of *L. acidophilus*) after 0 h of storage at 4°C.

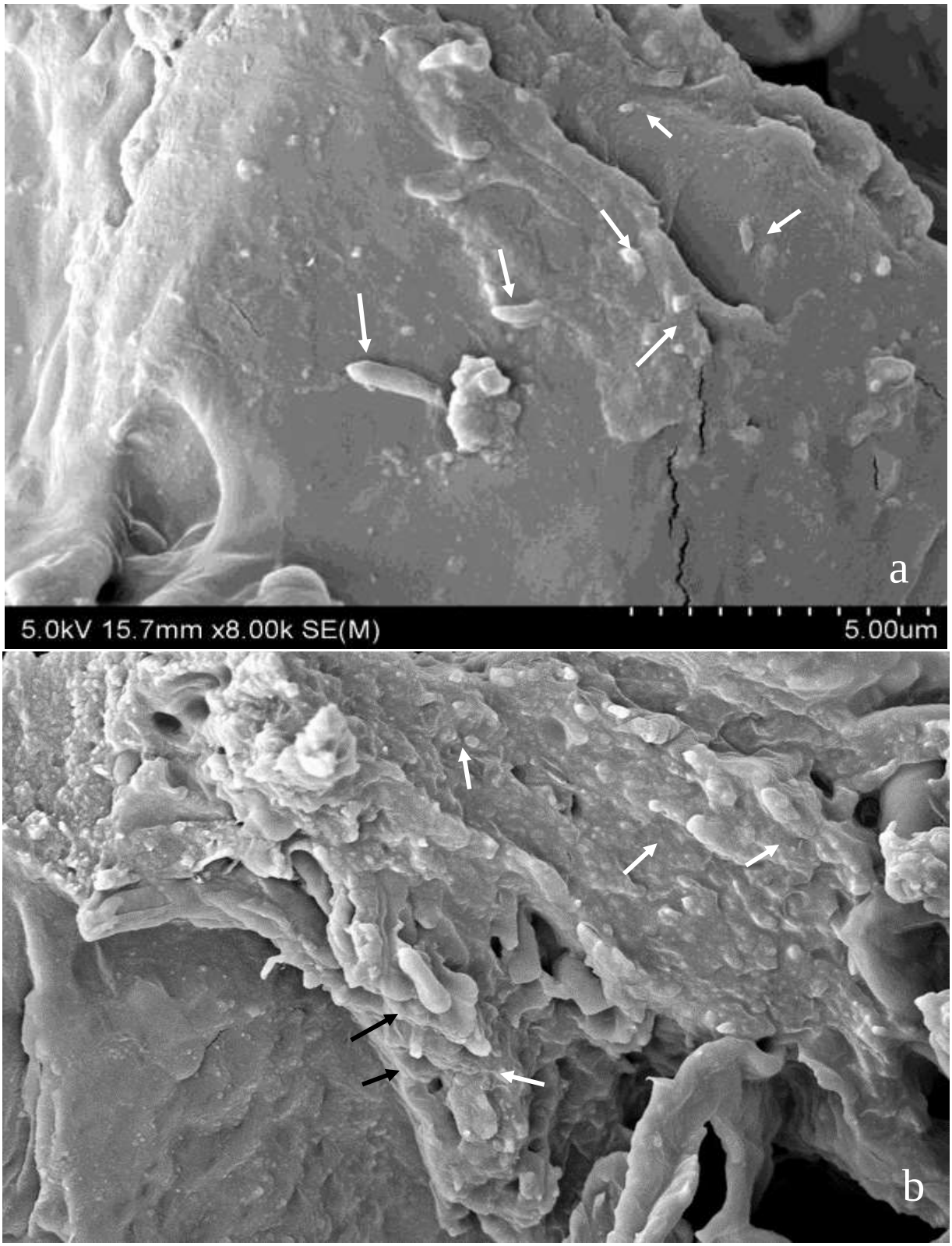

Figure 18. Scanning Electron Microscopy (SEM) photographs.(a) and (b) represent a cross-section of the raw date sample beads (milk product containing alginate beads of raw dates and *L. acidophilus*) showing entrapped *L. acidophilus* (arrows) surrounded by the alginate matrix and date fruits.

4.5.4 Colour analysis

Colour is considered a basic physical feature of food products and it corresponds to other chemical, physical and sensorial indicators of these products. Indeed, colour plays an essential role in the evaluation of food quality and it is normally the first feature the consumer observes (Saenz et al., 1993; Apruzzese et al., 2000). Colours are described using the CIELAB colour scale, where L* defines lightness, a* denotes the red/green value and b* the yellow/blue value. A more positive a* value indicates more redness, while a more negative value indicates a more greenish colour. A more positive b* value indicates more yellowish colour, while a more negative value indicates more blueness.

4.5.5 Colour of the milk

4.5.5.1 The L* (lightness) values

The L* value changed over the 168 h of storage as shown in Table 10 and Figure 19. There was a significant interaction ($P < 0.05$) between samples and storage time (Samples*Time). In addition, storage time alone and samples alone had significant effects on L* values. The raw date sample had significantly higher L* values, compared to the autoclaved date sample and control sample, which indicates a higher lightness value. In addition, the autoclaved date sample increased in L* value during the 168 h storage, while the control sample decreased in L* value by the end of storage.

Table 10. L* values in milk during storage at 4°C

Samples	Time (hours)				P values		
	0	24	96	168	Samples	Time	Samples*Time
Autoclaved date	2.96±1.80	2.42±0.74	2.81±1.3	4.20±2.3	0.001	0.023	0.001
Raw date	4.72±3.63	5.48±3.13	4.89±3.35	1.58±1.32			
Control	3.10±2.90	3.58±2.39	3.08±2.72	2.66±2.30			

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

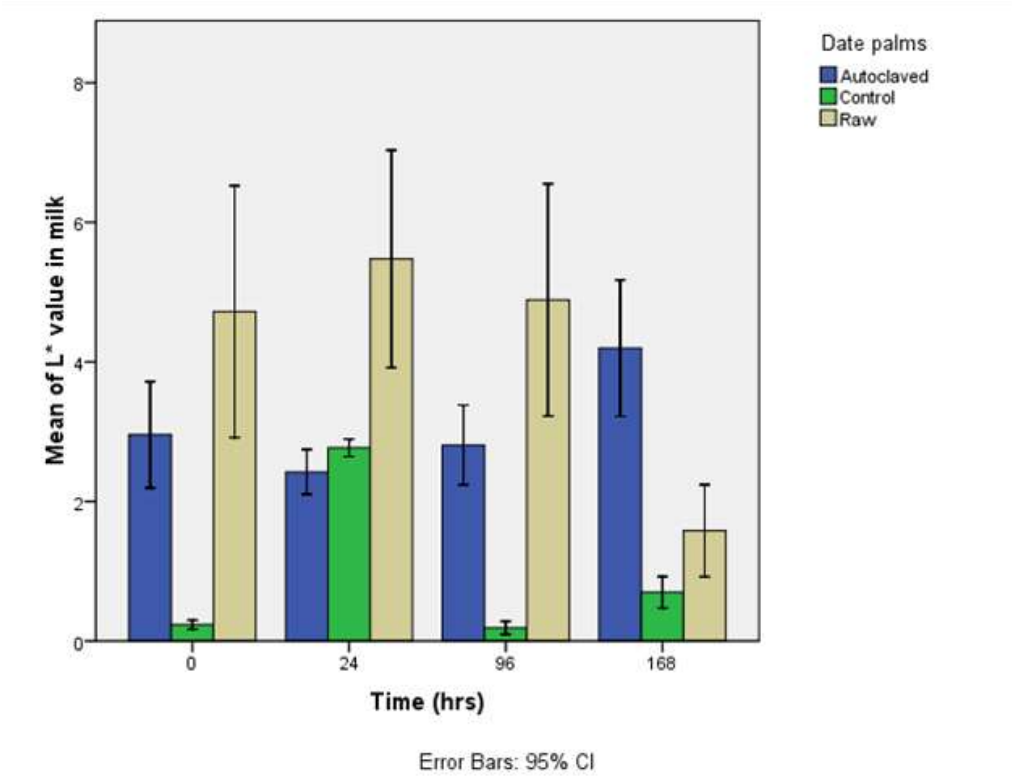

Figure 19. Changes in L* values in milk during storage time at 4°C
Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

4.5.5.2 The a* (red-green axis) values

The a* values of milk changed significantly ($P < 0.05$) over the 168 h of storage at 4°C for each sample (Samples*time). Furthermore, there was a significant difference ($P < 0.05$) in a* values

between samples. The autoclaved date sample had the highest a* values (red colour) compared to other samples, as shown in Table 11 and Figure 20. The control sample had the lowest a* values (green colour). The high redness value in the milk containing the autoclaved date sample was caused by the dark colour of the autoclaved date beads (Figure 24 b). The dates turned a dark brown colour during the autoclaving process. Ching et al. (2015) reported that the alginate gel is porous, allowing substrate release from the alginate beads by diffusion, and it is important for the gel immobilisation characteristics. In addition, Hariyadi (2011) found that the core materials are able to diffuse in or out of the alginate beads. Therefore, the redness was higher in the date fruit samples compared with the control sample. The finding provides evidence that the date fruits had a great effect on the milk colour. Generally, there were significant changes in a* values during storage at 4^{o}C.

Table 11. a* values in milk during storage at 4^{o}C

Samples	**Time (hours)**				**P values**		
	0	24	96	168	Samples	Time	Samples*Time
Autoclaved date	0.49±0.35	1.38±0.91	1.74±0.73	1.81±0.85	0.001	0.001	0.001
Raw date	0.55±1.05	0.53±0.81	0.80±0.56	0.45±0.21			
Control	0.03±0.05	-0.29±0.07	0.05±0.06	0.26±0.06			

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

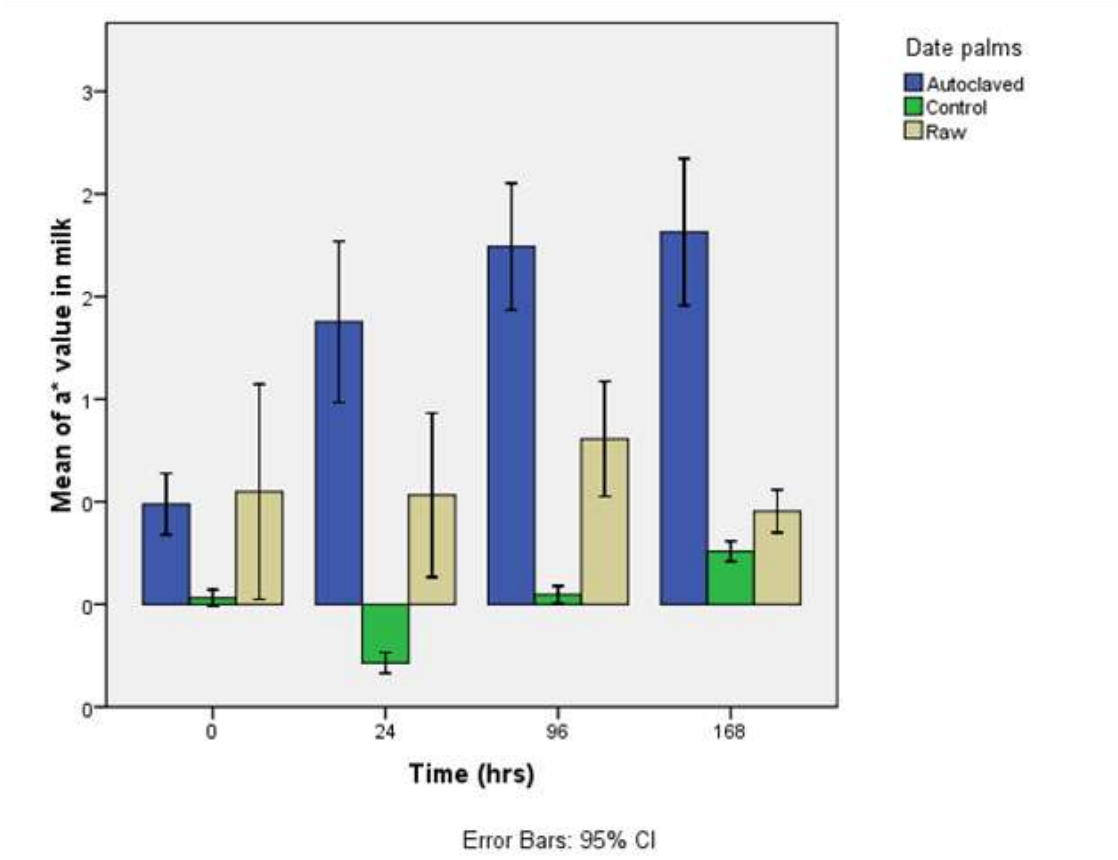

Figure 20. Changes in a* values in milk during storage at 4°C.
Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

4.5.5.3 The b* (yellow-blue axis) values

From the data in Table 12 and Figure 21, it is apparent that the b* values of milk changed over 168 h of storage at 4°C. The samples*time interaction was significant ($P < 0.05$), whereas there were no significant differences ($P > 0.05$) in the b* values between the samples. The addition of autoclaved dates or raw dates had no effect on the b* value of the milk. However, the b* values of all samples decreased significantly from 0 h to 168 h of storage. The colour of the milk in all samples became more blue by the end of storage.

Table 12. b* values in milk during storage at 4°C

Samples	Time (hours)				P values		
	0	24	96	168	Samples	Time	Samples *Time
Autoclaved date	-0.41±1.90	-1.97±1.85	-0.66±2.27	-0.78±1.75	0.146	0.001	0.001
Raw date	1.06±0.84	-0.10±0.65	-3.50±1.08	-2.71±0.70			
Control	-0.55±0.21	-0.09±0.24	-1.22±0.24	-1.35±0.26			

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

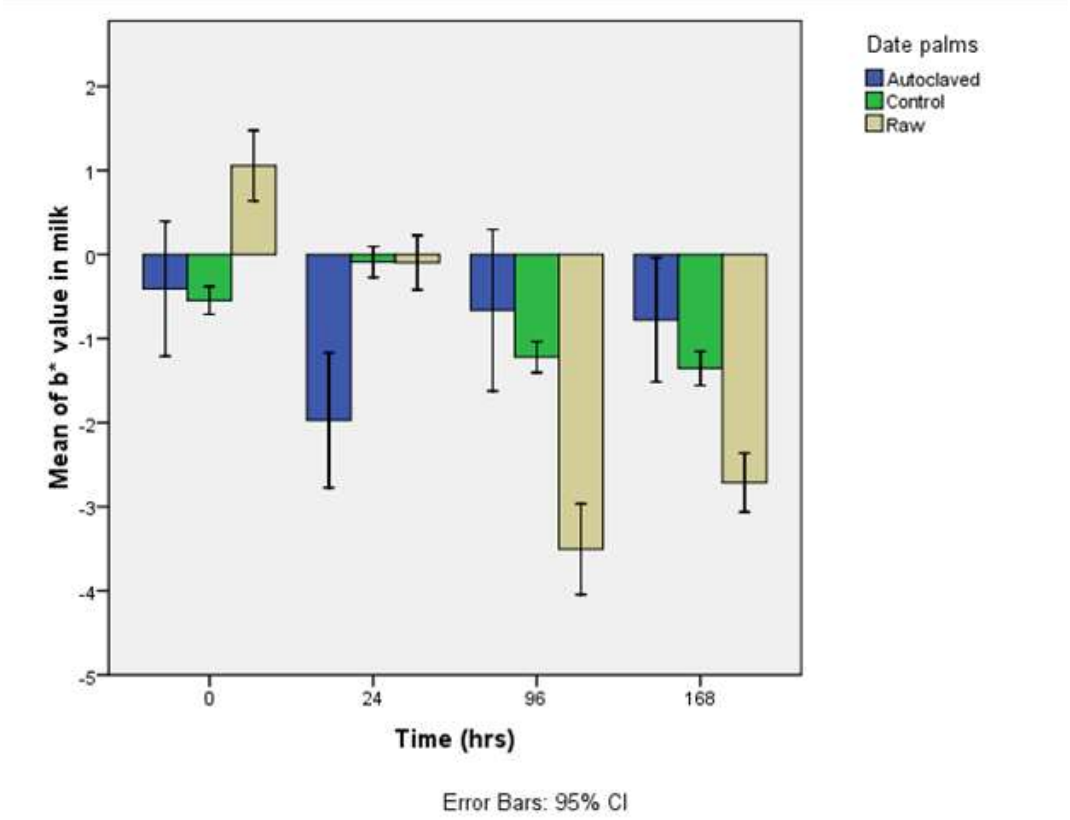

Figure 21. Changes in b* values in milk during storage at 4°C.

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

4.5.6 Colour of the beads

4.5.6.1 The L* (lightness) values

L* values of the bead samples over 168 h of storage at 4°C are presented in Table 13 and Figure 22. There was a significant interaction ($P < 0.05$) between the bead samples and storage time (Samples*Time). Furthermore, storage time alone had an effect on L* values. Table 13 illustrates the significant changes in the L* values between the beads of the samples. The beads of the autoclaved date sample had significantly higher L* values, which indicated a higher lightness compared to other samples. In addition, the lowest L* value was the control sample that contained alginate and *L. acidophilus*. To the best of knowledge, there have been no previous studies in which the colour of alginate beads has been sufficiently documented and measured.

Table 13. L* values of bead samples during storage at 4°C.

Samples	Time (hours)				P values		
	0	24	96	168	Samples	Time	Samples* Time
Autoclaved date	8.32±0.65	10.98±1.42	11.35±0.43	11.00±1.14	0.001	0.001	0.001
Raw date	3.23±7.40	10.57±0.71	10.62±1.75	8.50±0.22			
Control	4.43±0.53	1.19±0.19	1.29±0.67	3.54±1.32			

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

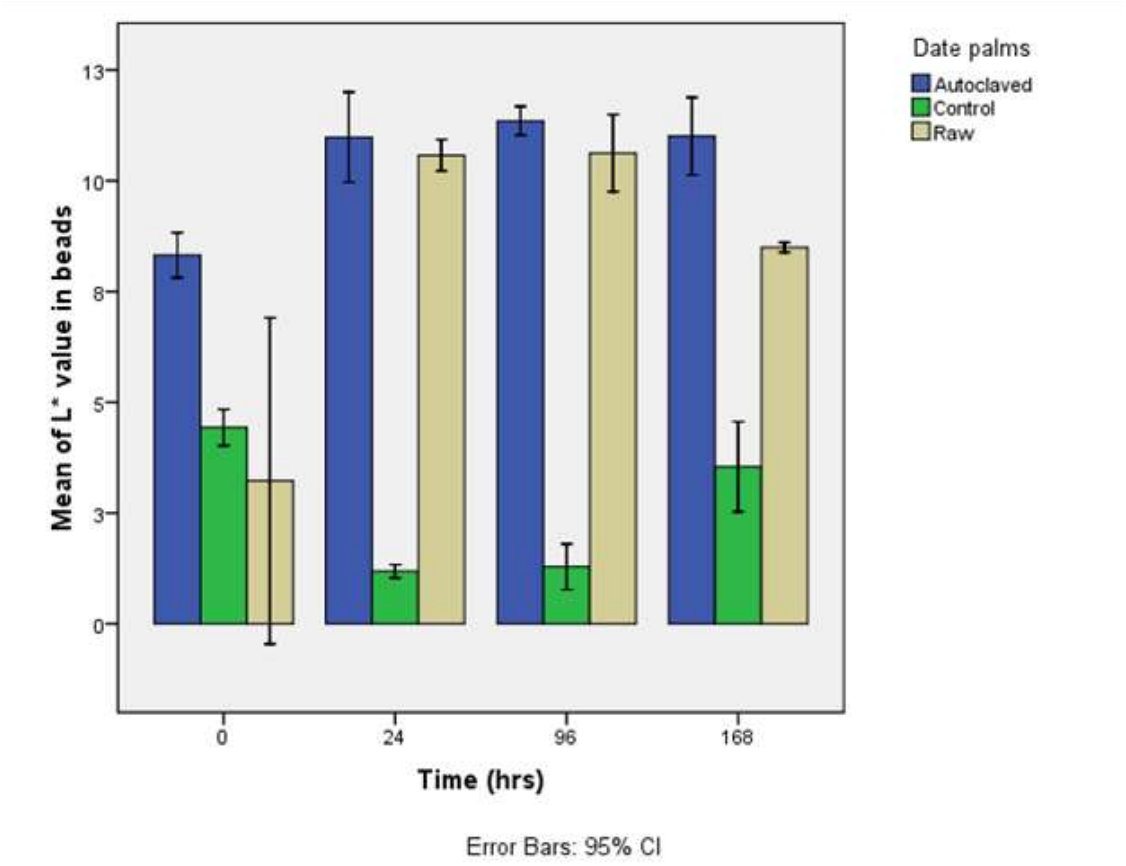

Figure 22. Changes in L* values of bead samples during storage at 4°C.
Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

4.5.6.2 The a* (red-green axis) values

The a* values of the bead samples changed over the 168 h of storage as shown in Table 14 and Figure 23. Samples*Time interaction, storage time effect and samples were all significant ($P < 0.05$). The beads of the autoclaved date sample had significantly higher a* values (red colour) compared to other samples. From Figure 24 the red colour of the autoclaved date beads can be seen compared to the raw date beads. This finding is consistent with the finding of a* values in the milk of the autoclaved date sample (Table 11). Therefore, the autoclaved date beads were more similar to the colour of the date fruits than were the raw date beads. On the other hand, the control

sample had significantly lower a* values, whereby the sample turned a green colour by the end of storage. Interestingly, the a* values increased gradually in the raw date beads during storage.

Table 14. a* values of the sample beads during storage at 4ºC.

Samples	Time (hours)				P values		
	0	24	96	168	Samples	Time	Samples* Time
Autoclaved date	10.30±0.80	9.64±1.04	9.48±1.05	10.25±1.27	0.001	0.013	0.001
Raw date	4.97±1.62	6.37±0.20	6.54±0.19	7.44±0.49			
Control	0.33±0.69	-0.36±0.09	-0.14±0.14	-0.31±0.15			

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

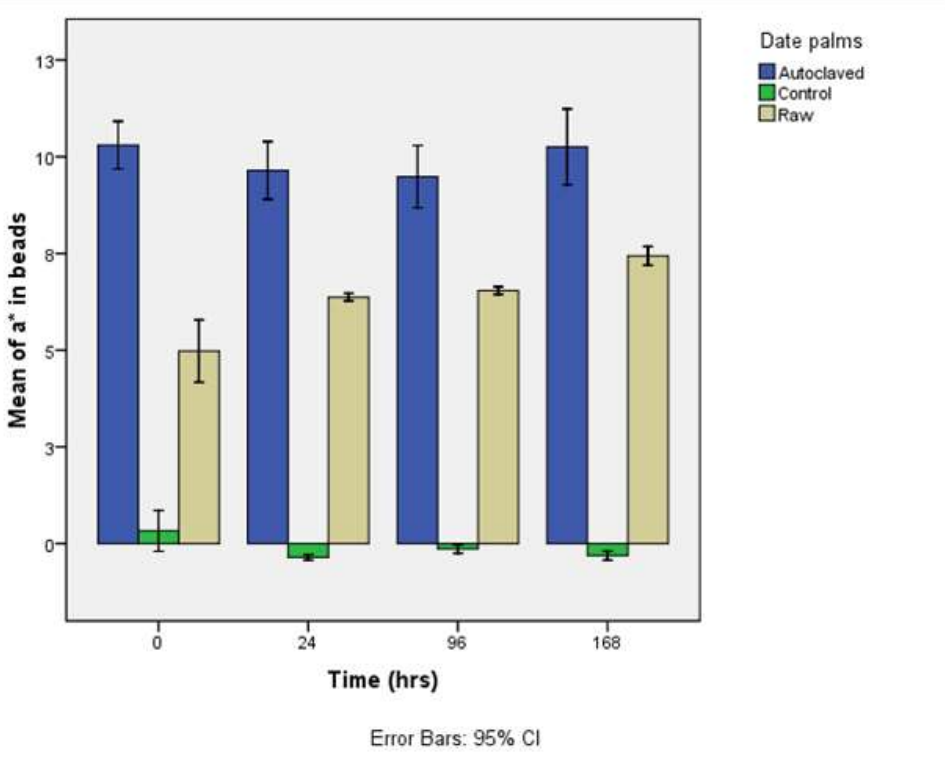

Figure 23. Changes in a* values of sample beads during storage at 4ºC.
Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

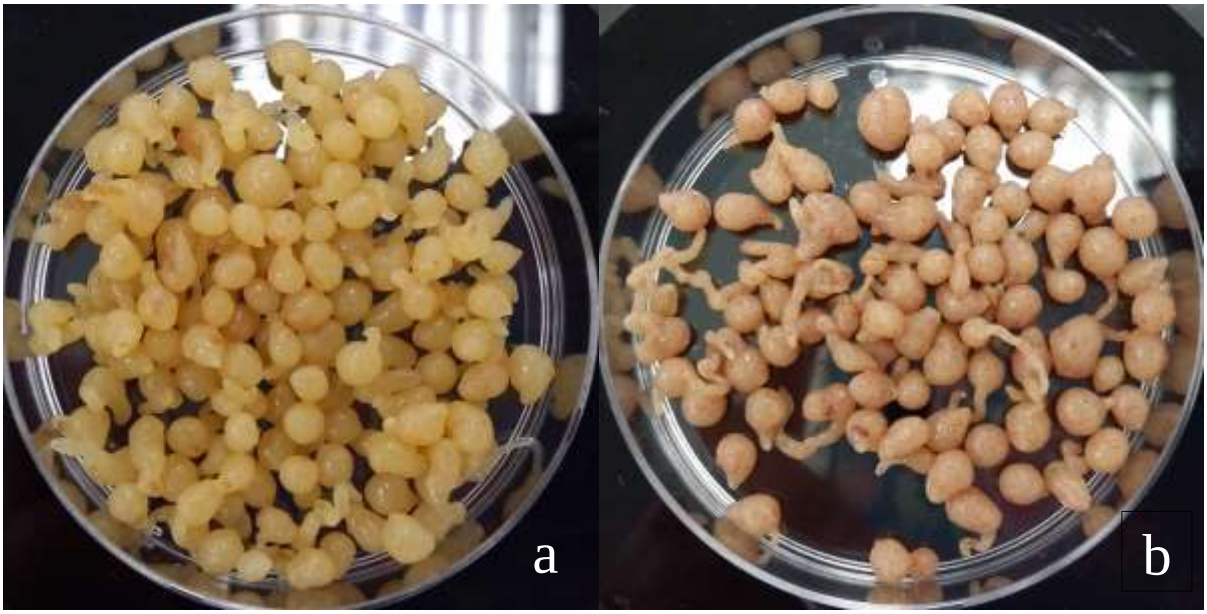

Figure 24. The morphology and the colour of the beads. (a) Beads of the raw date sample (milk product containing alginate beads of raw dates and *L. acidophilus*). (b) Beads of the autoclaved date sample (milk product containing alginate beads of autoclaved dates and *L. acidophilus*).

4.5.6.3 The b* (yellow-blue axis) values

The b* values of the bead samples changed over the 168 h of storage as illustrated in Table 15 and Figure 25. There were significant changes in the Samples*Time interaction ($P < 0.05$) and samples. The storage time did not significantly ($P < 0.05$) affect the b* values. The beads of the raw date sample had the highest b* values, whereby the beads were yellow in colour (Figure 24 a). However, the beads of the control sample had significantly lower b* values (blue colour) compared to other samples. This finding highlights the effect of the dates on the b* values of the beads.

Table 15. b* values of bead samples during storage at 4°C.

Samples	Time (hours)				P values		
	0	24	96	168	Samples	Time	Samples*Time
Autoclaved date	10.38±0.50	7.60±0.58	8.50±0.29	10.05±0.32	0.001	0.185	0.002
Raw date	13.72±4.81	15.06±0.33	14.08±0.77	14.48±0.82			
Control	1.64±1.07	3.09±0.17	3.16±0.13	3.64±0.47			

Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

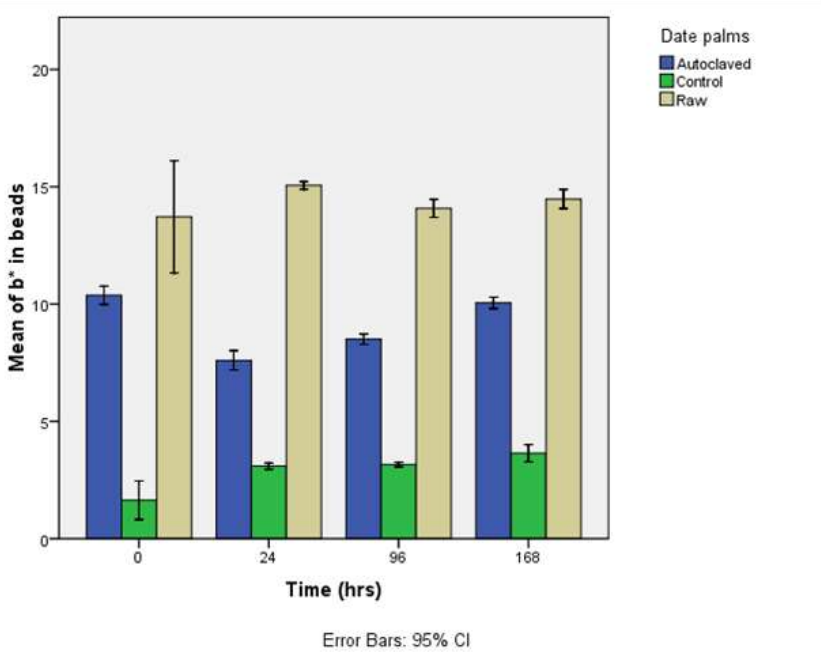

Figure 25. b* values of bead samples during storage at 4ºC.
Autoclaved dates = milk product containing alginate beads of autoclaved dates and *L. acidophilus*; Raw dates = milk product containing alginate beads of raw dates and *L. acidophilus*; Control = milk product containing alginate beads of *L. acidophilus*.

Chapter. 5. Conclusion

Dates are one of the richest foods, providing a large range of essential nutrients such as carbohydrates, minerals and salts, fatty acids, vitamins, dietary fiber, proteins and amino acids. In addition, the most effective antioxidants in this fruit appear to be phenols and flavonoids. Based on the above properties of dates, it is one of the most appropriate substances for the production of probiotic functional foods using the microencapsulation technique. As benefits provided by *L. acidophilus* are now well documented, it is considered one of the most effective probiotic bacteria and is commonly used in food products (Kechagia et al., 2013; Nagpal, et al., 2012). However, most probiotic bacteria are incorporated into an unsuitable food matrix such as one with low pH or competing microbes. The sweetness of dates could attract consumers to probiotic date milk products, including children who show no desire toward milk consumption or individuals who do not have a preference for probiotic milk products. The purpose of the current study was to investigate the development of a novel way to produce a new probiotic date product.

This study indicated that the production of a probiotic date product using the microencapsulation technique is possible and can improve the viability of *L. acidophilus* in the product. Using 3% alginate and 31% Medjool date beads (autoclaved or raw), viable *L. acidophilus* survived during storage and met the basic criteria for probiotic products. The results of this study show that the probiotic bacteria were kept inside the beads without releasing to the milk. Also the pH of the milk remained stable during storage at 4^{o}C, which indicates that the milk was not affected by microbial activity. Furthermore, there was no observation of pathogen growth caused by the Medjool dates in the product. Therefore, the production of this novel probiotic product is completely safe for consumers. The bead shape and size of the autoclaved date sample and raw date samples were critically controlled. Most of the beads were large in size (5-6 mm) with a teardrop shape, which

may affect the texture of the product from a consumer's perspective and make it more attractive if the consumers expect the existence of the beads. The finding revealed that the presence of the Medjool dates decreased the stability or the hardness of the beads. The colour of the autoclaved date beads was similar to the colour of the dates, which was reflected in the colour of the milk in the same sample. From the outcome of the investigation it is possible to conclude that the production of date probiotic products can be achieved by using the microencapsulation of probiotic bacteria with dates. Therefore, this study contributes to food science and its finding can be applied by the dairy industry to develop new functional foods.

More research is needed to evaluate the viability of other probiotic strains by applying different microencapsulation techniques and other biopolymers into the coating materials. In addition, using other types of dates is also needed to better understand to the effects of the date fruits on the product. The spherical shapes of the beads may be produced by using other coating materials. Further investigations are required on sensorial factors that could influence consumers' attitudes toward probiotic date products by using product-oriented tests. The acceptance of the probiotic product by the consumer is one of the essential criteria to determine the success and the efficacy of the product. Hansen et al. (2002) reported that the existence of microencapsulated *Bifidobacteria lactis* and *Bifidobacteria longum* in milk resulted in unacceptable bitter flavours not found in samples with free cells. Therefore, further study of the flavour issue using microencapsulated *L. acidophilus* would be of interest.

Chapter. 6. References

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Chapter. 7. Appendices

Appendix A. Statistical analysis of variance of encapsulated *L. acidophilus* CFU/g during storage at 4°C

Descriptive Statistics

Dependent Variable: Bacterial Count CFU/g in the beads

Time (hrs)	Samples	Mean	Std. Deviation	N
0	Autoclaved dates	6044444.44	4313383.565	9
	Control	2588888.89	2526580.913	9
	Raw dates	22700000.00	2.963E7	18
	Total	13508333.33	2.281E7	36
24	Autoclaved dates	61178750.00	1.064E8	24
	Control	5233333.33	1387443.693	9
	Raw dates	12612222.22	1.527E7	18
	Total	34164901.96	7.719E7	51
96	Autoclaved dates	19761666.67	1.931E7	24
	Control	4144444.44	3531328.047	9
	Raw dates	14719444.44	2.092E7	18
	Total	15226078.43	1.883E7	51
168	Autoclaved dates	41615000.00	5.954E7	24
	Control	4555555.56	4490019.797	9
	Raw dates	21048333.33	4.281E7	18
	Total	27816274.51	4.962E7	51
Total	Autoclaved dates	36984320.99	6.902E7	81
	Control	4130555.56	3213704.533	36
	Raw dates	17770000.00	2.877E7	72
	Total	23406719.58	5.004E7	189

Tests of Between-Subjects Effects

Dependent Variable: Bacterial Count CFU/g in the beads

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.221E16	11	5.655E15	2.450	.007
Intercept	5.178E16	1	5.178E16	22.428	.000
Timehrs	6.785E15	3	2.262E15	.980	.404
Samples	1.938E16	2	9.690E15	4.197	.017
Timehrs * Samples	2.066E16	6	3.444E15	1.492	.183
Error	4.086E17	177	2.309E15		
Total	5.744E17	189			
Corrected Total	4.708E17	188			

a. R Squared = .132 (Adjusted R Squared = .078)

Between-Subjects Factors

		N
Time (hrs)	0	36
	24	51
	96	51
	168	51
Samples	Autoclaved dates	81
	Control	36
	Raw dates	72

Time (hrs) and Samples Interaction

Dependent Variable: Bacterial Count CFU/g in the beads

Time (hrs)	Samples	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	Autoclaved dates	6044444.444	1.602E7	-2.556E7	3.765E7
	Control	2588888.889	1.602E7	-2.902E7	3.420E7
	Raw dates	2.270E7	1.133E7	350370.321	4.505E7
24	Autoclaved dates	6.118E7	9807837.257	4.182E7	8.053E7
	Control	5233333.333	1.602E7	-2.637E7	3.684E7
	Raw dates	1.261E7	1.133E7	-9737407.456	3.496E7
96	Autoclaved dates	1.976E7	9807837.257	406319.600	3.912E7
	Control	4144444.444	1.602E7	-2.746E7	3.575E7
	Raw dates	1.472E7	1.133E7	-7630185.234	3.707E7
168	Autoclaved dates	4.162E7	9807837.257	2.226E7	6.097E7
	Control	4555555.556	1.602E7	-2.705E7	3.616E7
	Raw dates	2.105E7	1.133E7	-1301296.345	4.340E7

Time (hrs)

Multiple Comparisons

Bacterial Count CFU/g in the beads

Tukey HSD

(I) Time (hrs)	(J) Time (hrs)	Mean Difference (I-J)	Std. Error	Sig.
0	24	-20656568.63	1.046E7	.201
	96	-1717745.10	1.046E7	.998
	168	-14307941.18	1.046E7	.521
24	0	20656568.63	1.046E7	.201
	96	18938823.53	9514999.757	.195
	168	6348627.45	9514999.757	.909
96	0	1717745.10	1.046E7	.998
	24	-18938823.53	9514999.757	.195
	168	-12590196.08	9514999.757	.549
168	0	14307941.18	1.046E7	.521
	24	-6348627.45	9514999.757	.909
	96	12590196.08	9514999.757	.549

Based on observed means.

The error term is Mean Square(Error) = 2308648119813244.000.

Multiple Comparisons

Bacterial Count CFU/g in the beads

Tukey HSD

(I) Time (hrs)	(J) Time (hrs)	95% Confidence Interval	
		Lower Bound	Upper Bound
0	24	-47783828.49	6470691.24
	96	-28845004.96	25409514.76
	168	-41435201.04	12819318.69
24	0	-6470691.24	47783828.49
	96	-5739323.63	43616970.69
	168	-18329519.70	31026774.61
96	0	-25409514.76	28845004.96
	24	-43616970.69	5739323.63
	168	-37268343.23	12087951.08
168	0	-12819318.69	41435201.04
	24	-31026774.61	18329519.70
	96	-12087951.08	37268343.23

Based on observed means.

The error term is Mean Square(Error) = 2308648119813244.000.

Bacterial Count CFU/g in the beads

Tukey HSD[a,,b,,c]

Time (hrs)	N	Subset 1
0	36	13508333.33
96	51	15226078.43
168	51	27816274.51
24	51	34164901.96
Sig.		.168

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2308648119813244.000.

a. Uses Harmonic Mean Sample Size = 46.189.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Samples

Multiple Comparisons

Bacterial Count CFU/g in the beads

Tukey HSD

(I) Samples	(J) Samples	Mean Difference (I-J)	Std. Error	Sig.
Autoclave	Control	32853765.43*	9624497.030	.002
	Raw	19214320.99*	7782440.868	.038
Control	Autoclaved	-3.29E7	9624497.030	.002
	Raw	-13639444.44	9807837.257	.348
Raw	Autoclaved	-1.92E7	7782440.868	.038
	Control	13639444.44	9807837.257	.348

Based on observed means.

The error term is Mean Square(Error) = 2308648119813244.000.

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Bacterial Count CFU/g in the beads

Tukey HSD

(I) Samples	(J) Samples	95% Confidence Interval	
		Lower Bound	Upper Bound
Autoclaved	Control	10105323.40	55602207.47
	Raw	819759.23	37608882.74
Control	Autoclaved	-55602207.47	-10105323.40
	Raw	-36821229.08	9542340.19
Raw	Autoclaved	-37608882.74	-819759.23
	Control	-9542340.19	36821229.08

Based on observed means.

The error term is Mean Square(Error) = 2308648119813244.000.

Bacterial Count CFU/g in the beads

Tukey HSD[a,,b,,c]

Samples	N	Subset 1	Subset 2
Control	36	4130555.56	
Raw dates	72	17770000.00	17770000.00
Autoclaved dates	81		36984320.99
Sig.		.295	.091

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2308648119813244.000.

a. Uses Harmonic Mean Sample Size = 55.543.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

b. Alpha = .05.

Appendix B. Statistical analysis of variance of pH

Between-Subjects Factors

		N
Time (hrs)	0	54
	24	54
	96	54
	168	54
Samples	Autoclaved dates	108
	Control	36
	Raw dates	72

Descriptive Statistics

Dependent Variable: pH of the milk

Time (hrs)	Samples	Mean	Std. Deviation	N
0	Autoclaved dates	6.2030	.17448	27
	Control	5.8178	.24692	9
	Raw dates	6.1478	.26347	18
	Total	6.1204	.25629	54
24	Autoclaved dates	6.2741	.25028	27
	Control	5.8178	.24692	9
	Raw dates	6.1478	.26347	18
	Total	6.1559	.29791	54
96	Autoclaved dates	6.2222	.18820	27
	Control	5.8178	.24692	9
	Raw dates	6.1478	.26347	18
	Total	6.1300	.26427	54
168	Autoclaved dates	6.2222	.18820	27
	Control	5.8178	.24692	9
	Raw dates	6.1478	.26347	18
	Total	6.1300	.26427	54
Total	Autoclaved dates	6.2304	.20134	108
	Control	5.8178	.23610	36
	Raw dates	6.1478	.25785	72
	Total	6.1341	.26959	216

Levene's Test of Equality of Error Variances[a]

Dependent Variable: pH of the milk

F	df1	df2	Sig.
.791	11	204	.649

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Timehrs + Samples + Timehrs * Samples

Tests of Between-Subjects Effects

Dependent Variable: pH of the milk

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.692[a]	11	.427	7.959	.000
Intercept	6501.437	1	6501.437	121306.404	.000
Timehrs	.014	3	.005	.085	.968
Samples	4.617	2	2.308	43.069	.000
Timehrs * Samples	.038	6	.006	.117	.994
Error	10.933	204	.054		
Total	8143.028	216			
Corrected Total	15.625	215			

a. R Squared = .300 (Adjusted R Squared = .263)

The time (hrs) and samples interaation

Dependent Variable: pH of the milk

Time (hrs)	Samples	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	Autoclaved dates	6.203	.045	6.115	6.291
	Control	5.818	.077	5.666	5.970
	Raw dates	6.148	.055	6.040	6.255
24	Autoclaved dates	6.274	.045	6.186	6.362
	Control	5.818	.077	5.666	5.970
	Raw dates	6.148	.055	6.040	6.255
96	Autoclaved dates	6.222	.045	6.134	6.310
	Control	5.818	.077	5.666	5.970
	Raw dates	6.148	.055	6.040	6.255
168	Autoclaved dates	6.222	.045	6.134	6.310
	Control	5.818	.077	5.666	5.970
	Raw dates	6.148	.055	6.040	6.255

Time (hrs)

Multiple Comparisons

pH of the milk
Tukey HSD

(I) Time (hrs)	(J) Time (hrs)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	24	-.0356	.04455	.855	-.1510	.0799
	96	-.0096	.04455	.996	-.1250	.1058
	168	-.0096	.04455	.996	-.1250	.1058
24	0	.0356	.04455	.855	-.0799	.1510
	96	.0259	.04455	.937	-.0895	.1413
	168	.0259	.04455	.937	-.0895	.1413
96	0	.0096	.04455	.996	-.1058	.1250
	24	-.0259	.04455	.937	-.1413	.0895
	168	.0000	.04455	1.000	-.1154	.1154
168	0	.0096	.04455	.996	-.1058	.1250
	24	-.0259	.04455	.937	-.1413	.0895
	96	.0000	.04455	1.000	-.1154	.1154

Based on observed means.
The error term is Mean Square(Error) = .054.

pH of the milk

Tukey HSD[a,,b]

Time (hrs)	N	Subset 1
0	54	6.1204
96	54	6.1300
168	54	6.1300
24	54	6.1559
Sig.		.855

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .054.

a. Uses Harmonic Mean Sample Size = 54.000.

b. Alpha = .05.

Samples

Multiple Comparisons

pH of the milk

Tukey HSD

(I) Samples	(J) Samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Autoclaved	Control	.4126*	.04455	.000	.3074	.5178
	Raw	.0826	.03522	.052	-.0006	.1658
Control	Autoclaved	-.4126*	.04455	.000	-.5178	-.3074
	Raw	-.3300*	.04726	.000	-.4416	-.2184
Raw	Autoclaved	-.0826	.03522	.052	-.1658	.0006
	Control	.3300*	.04726	.000	.2184	.4416

Based on observed means.

The error term is Mean Square(Error) = .054.

*. The mean difference is significant at the .05 level.

pH of the milk

Tukey HSD[a,,b,,c]

Samples	N	Subset	
		1	2
Control	36	5.8178	
Raw dates	72		6.1478
Autoclaved dates	108		6.2304
Sig.		1.000	.131

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .054.

a. Uses Harmonic Mean Sample Size = 58.909.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Appendix C. Statistical analysis of variance of size of the beads

Between-Subjects Factors

		N
Samples	Autoclaved dates	121
	Control	58
	Raw dates	118
Time (hrs)	0	147
	168	150

Descriptive Statistics

Dependent Variable: Size of the beads (mm)

Samples	Time (hrs)	Mean	Std. Deviation	N
Autoclaved dates	0	6.15	1.459	61
	168	6.60	1.045	60
	Total	6.37	1.285	121
Control dates	0	4.54	.637	28
	168	4.90	.607	30
	Total	4.72	.643	58
Raw dates	0	5.36	.641	58
	168	5.85	.659	60
	Total	5.61	.692	118
Total dates	0	5.53	1.212	147
	168	5.96	1.035	150
	Total	5.75	1.145	297

Levene's Test of Equality of Error Variances[a]

Dependent Variable: Size of the beads (mm)

F	df1	df2	Sig.
10.002	5	291	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Samples + Timehrs + Samples * Timehrs

Tests of Between-Subjects Effects

Dependent Variable: Size of the beads (mm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	125.278[a]	5	25.056	27.746	.000
Intercept	8199.400	1	8199.400	9079.832	.000
Samples	111.231	2	55.615	61.587	.000
Timehrs	12.515	1	12.515	13.858	.000
Samples* Timehrs	.149	2	.074	.082	.921
Error	262.783	291	.903		
Total	10199.000	297			
Corrected Total	388.061	296			

a. R Squared = .323 (Adjusted R Squared = .311)

Appendix D. Statistical analysis of variance of beads texture

Between-Subjects Factors

		N
Samples	Autoclaved dates	128
	Control	56
	Raw dates	116
Time (hrs)	0	144
	168	156

Descriptive Statistics

Dependent Variable: Force (kg)

Samples	Time (hrs)	Mean	Std. Deviation	N
Autoclaved dates	0	.12631	.047415	61
	168	.21664	.167977	67
	Total	.17359	.133331	128
Control	0	.33538	.067289	26
	168	.31567	.049840	30
	Total	.32482	.058876	56
Raw dates	0	.22846	.088805	57
	168	.20025	.076285	59
	Total	.21411	.083521	116
Total	0	.20449	.103800	144
	168	.22949	.128417	156
	Total	.21749	.117721	300

Levene's Test of Equality of Error Variances[a]

Dependent Variable: Force (kg)

F	df1	df2	Sig.
17.421	5	294	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Samples+ Timehrs + Samples * Timehrs

Tests of Between-Subjects Effects

Dependent Variable: Force (kg)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.182[a]	5	.236	23.470	.000
Intercept	14.709	1	14.709	1460.238	.000
Samples	.922	2	.461	45.748	.000
Timehrs	.013	1	.013	1.298	.256
Samples * Timehrs	.246	2	.123	12.216	.000
Error	2.962	294	.010		
Total	18.334	300			
Corrected Total	4.144	299			

a. R Squared = .285 (Adjusted R Squared = .273)

Appendix E. Statistical analysis of variance of colour (L* values) of milk

Between-Subjects Factors

		N
Samples	Autoclaved dates	95
	Control	36
	Raw dates	72
Time (hrs)	0	51
	24	50
	96	51
	168	51

Descriptive Statistics

Dependent Variable: L* values of milk

Samples	Time (hrs)	Mean	Std. Deviation	N
Autoclaved dates	0	2.96	1.803	24
	24	2.42	.744	23
	96	2.81	1.354	24
	168	4.20	2.312	24
	Total	3.10	1.768	95
Control	0	.23	.082	9
	24	2.77	.163	9
	96	.19	.121	9
	168	.70	.292	9
	Total	.97	1.085	36
Raw dates	0	4.72	3.629	18
	24	5.48	3.134	18
	96	4.89	3.349	18
	168	1.58	1.327	18
	Total	4.17	3.309	72
Total	0	3.10	2.900	51
	24	3.58	2.394	50
	96	3.08	2.716	51
	168	2.66	2.306	51
	Total	3.10	2.592	203

Levene's Test of Equality of Error Variances[a]

Dependent Variable: Colour analysis of milk

F	df1	df2	Sig.
37.656	11	191	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Samples + Timehrs + Samples * Timehrs

Tests of Between-Subjects Effects

Dependent Variable: L* values of milk

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	493.022[a]	11	44.820	9.902	.000
Intercept	1299.105	1	1299.105	287.003	.000
Samples	244.828	2	122.414	27.044	.000
Timehrs	44.129	3	14.710	3.250	.023
Samples * Timehrs	226.389	6	37.732	8.336	.000
Error	864.552	191	4.526		
Total	3311.280	203			
Corrected Total	1357.574	202			

a. R Squared = .363 (Adjusted R Squared = .326)

Time (hrs) and samples interaction

Dependent Variable: L* values of milk

Samples	Time (hrs)	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Autoclaved dates	0	2.956	.434	2.100	3.813
	24	2.423	.444	1.548	3.298
	96	2.808	.434	1.951	3.665
	168	4.197	.434	3.340	5.053
Control	0	.233	.709	-1.166	1.632
	24	2.769	.709	1.370	4.168
	96	.190	.709	-1.209	1.588
	168	.698	.709	-.701	2.097
Raw dates	0	4.721	.501	3.731	5.710
	24	5.477	.501	4.488	6.466
	96	4.887	.501	3.898	5.876
	168	1.581	.501	.592	2.570

Post Hoc Tests
Time (hrs)

Multiple Comparisons

L* values of milk

Tukey HSD

(I) Time (hrs)	(J) Time (hrs)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	24	-.49	.423	.660	-1.58	.61
	96	.02	.421	1.000	-1.07	1.11
	168	.44	.421	.720	-.65	1.53
24	0	.49	.423	.660	-.61	1.58
	96	.50	.423	.633	-.59	1.60
	168	.93	.423	.129	-.17	2.03
96	0	-.02	.421	1.000	-1.11	1.07
	24	-.50	.423	.633	-1.60	.59
	168	.42	.421	.746	-.67	1.52
168	0	-.44	.421	.720	-1.53	.65
	24	-.93	.423	.129	-2.03	.17
	96	-.42	.421	.746	-1.52	.67

Based on observed means.

The error term is Mean Square(Error) = 4.526.

L* values of milk

Tukey HSD[a,,b,,c]

Time (hrs)	N	Subset 1
168	51	2.66
96	51	3.08
0	51	3.10
24	50	3.58
Sig.		.127

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 4.526.

a. Uses Harmonic Mean Sample Size = 50.746.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Appendix F. Statistical analysis of variance of colour (a* values) of milk

Between-Subjects Factors

		N
Time (hrs)	0	51
	24	50
	96	51
	168	51
Samples	Autoclaved dates	95
	Control	36
	Raw dates	72

Descriptive Statistics

Dependent Variable: a* values of milk

Time (hrs)	Samples	Mean	Std. Deviation	N
0	Autoclaved dates	.4883	.35367	24
	Control	.0311	.05159	9
	Raw dates	.5483	1.05310	18
	Total	.4288	.68582	51
24	Autoclaved dates	1.3768	.90780	23
	Control	-.2856	.06598	9
	Raw dates	.5322	.80474	18
	Total	.7735	.99680	50
96	Autoclaved dates	1.7438	.73142	24
	Control	.0467	.05568	9
	Raw dates	.8061	.56358	18
	Total	1.1133	.88550	51
168	Autoclaved dates	1.8146	.84656	24
	Control	.2578	.06431	9
	Raw dates	.4539	.21049	18
	Total	1.0596	.93102	51
Total	Autoclaved dates	1.3556	.90229	95
	Control	.0125	.20484	36
	Raw dates	.5851	.72461	72
	Total	.8442	.91700	203

Levene's Test of Equality of Error Variances[a]

Dependent Variable: a* values of milk

F	df1	df2	Sig.
13.113	11	191	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Timehrs + Samples + Timehrs * Samples

Tests of Between-Subjects Effects

Dependent Variable: a* values of milk

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	83.936[a]	11	7.631	16.962	.000
Intercept	73.111	1	73.111	162.518	.000
Timehrs	7.843	3	2.614	5.812	.001
Samples	54.597	2	27.298	60.681	.000
Timehrs * Samples	14.310	6	2.385	5.302	.000
Error	85.924	191	.450		
Total	314.522	203			
Corrected Total	169.860	202			

a. R Squared = .494 (Adjusted R Squared = .465)

Post Hoc Tests

Time (hrs)

Multiple Comparisons

a* values of milk

Tukey HSD

(I) Time (hrs)	(J) Time (hrs)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	24	-.3447	.13348	.051	-.6907	.0013
	96	-.6845*	.13282	.000	-1.0288	-.3403
	168	-.6308*	.13282	.000	-.9750	-.2865
24	0	.3447	.13348	.051	-.0013	.6907
	96	-.3398	.13348	.056	-.6858	.0062
	168	-.2861	.13348	.143	-.6321	.0599
96	0	.6845*	.13282	.000	.3403	1.0288
	24	.3398	.13348	.056	-.0062	.6858
	168	.0537	.13282	.978	-.2905	.3980
168	0	.6308*	.13282	.000	.2865	.9750
	24	.2861	.13348	.143	-.0599	.6321
	96	-.0537	.13282	.978	-.3980	.2905

Based on observed means.

The error term is Mean Square(Error) = .450.

*. The mean difference is significant at the .05 level.

a* values of milk

Tukey HSD[a,,b,,c]

Time (hrs)	N	Subset	
		1	2
0	51	.4288	
24	50	.7735	.7735
168	51		1.0596
96	51		1.1133
Sig.		.050	.055

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .450.

a. Uses Harmonic Mean Sample Size = 50.746.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Appendix G. Statistical analysis of variance of colour (b* values) of milk

Between-Subjects Factors

		N
Time (hrs)	0	51
	24	50
	96	51
	168	51
Samples	Autoclaved dates	95
	Control	36
	Raw dates	72

Descriptive Statistics

Dependent Variable: b* values of milk

Time (hrs)	Samples	Mean	Std. Deviation	N
0	Autoclaved dates	-.4069	1.90381	24
	Control	-.5456	.21402	9
	Raw dates	1.0567	.84370	18
	Total	.0852	1.56341	51
24	Autoclaved dates	-1.9708	1.85221	23
	Control	-.0878	.23790	9
	Raw dates	-.0961	.65242	18
	Total	-.9570	1.60955	50
96	Autoclaved dates	-.6625	2.27229	24
	Control	-1.2200	.23948	9
	Raw dates	-3.5039	1.08444	18
	Total	-1.7637	2.12358	51
168	Autoclaved dates	-.7775	1.74693	24
	Control	-1.3544	.26316	9
	Raw dates	-2.7117	.70550	18
	Total	-1.5620	1.53724	51
Total	Autoclaved dates	-.9437	2.01464	95
	Control	-.8019	.56906	36
	Raw dates	-1.3137	2.04572	72
	Total	-1.0498	1.85927	203

Levene's Test of Equality of Error Variances[a]

Dependent Variable: b* values of milk

F	df1	df2	Sig.
12.415	11	191	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Timehrs + samples + Timehrs * Samples

Tests of Between-Subjects Effects

Dependent Variable: b* values of milk

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	300.880[a]	11	27.353	13.146	.000
Intercept	180.580	1	180.580	86.789	.000
Timehrs	93.203	3	31.068	14.932	.000
Ssmples	8.086	2	4.043	1.943	.146
Timehrs * Samples	187.065	6	31.178	14.984	.000
Error	397.408	191	2.081		
Total	922.021	203			
Corrected Total	698.288	202			

a. R Squared = .431 (Adjusted R Squared = .398)

Post Hoc Tests
Time (hrs)

Multiple Comparisons

b* values of milk

Tukey HSD

(I) Time (hrs)	(J) Time (hrs)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	24	1.0421*	.28707	.002	.2981	1.7862
	96	1.8489*	.28565	.000	1.1085	2.5892
	168	1.6471*	.28565	.000	.9068	2.3875
24	0	-1.0421*	.28707	.002	-1.7862	-.2981
	96	.8068*	.28707	.028	.0627	1.5508
	168	.6050	.28707	.154	-.1390	1.3490
96	0	-1.8489*	.28565	.000	-2.5892	-1.1085
	24	-.8068*	.28707	.028	-1.5508	-.0627
	168	-.2018	.28565	.894	-.9421	.5386
168	0	-1.6471*	.28565	.000	-2.3875	-.9068
	24	-.6050	.28707	.154	-1.3490	.1390
	96	.2018	.28565	.894	-.5386	.9421

Based on observed means.

The error term is Mean Square(Error) = 2.081.

*. The mean difference is significant at the .05 level.

b* values of milk

Tukey HSD[a,b,c]

Time (hrs)	N	Subset 1	Subset 2	Subset 3
96	51	-1.7637		
168	51	-1.5620	-1.5620	
24	50		-.9570	
0	51			.0852
Sig.		.895	.153	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2.081.

a. Uses Harmonic Mean Sample Size = 50.746.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Appendix H. Statistical analysis of variance of colour (L* values) of the beads

Between-Subjects Factors

		N
Time (hrs)	0	36
	24	37
	96	36
	168	36
Samples	Autoclaved dates	37
	Control	36
	Raw dates	72

Descriptive Statistics

Dependent Variable: L* values of the beads

Time (hrs)	Samples	Mean	Std. Deviation	N
0	Autoclaved dates	8.3222	.65953	9
	Control	4.4328	.53207	9
	Raw dates	3.2261	7.40383	18
	Total	4.8018	5.59358	36
24	Autoclaved dates	10.9830	1.42014	10
	Control	1.1883	.19378	9
	Raw dates	10.5744	.71145	18
	Total	8.4018	4.23922	37
96	Autoclaved dates	11.3511	.42576	9
	Control	1.2872	.67070	9
	Raw dates	10.6244	1.75048	18
	Total	8.4718	4.40690	36
168	Autoclaved dates	11.0044	1.13770	9
	Control	3.5450	1.32085	9
	Raw dates	8.4956	.22153	18
	Total	7.8851	2.87330	36
Total	Autoclaved dates	10.4305	1.55532	37
	Control	2.6133	1.61978	36
	Raw dates	8.2301	4.81711	72
	Total	7.3971	4.59521	145

Levene's Test of Equality of Error Variances[a]

Dependent Variable: L* values of the beads

F	df1	df2	Sig.
312.532	11	133	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Timehrs + Samples + Timehrs * Samples

Tests of Between-Subjects Effects

Dependent Variable: L* values of the beads

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1993.732[a]	11	181.248	23.025	.000
Intercept	6573.540	1	6573.540	835.057	.000
Timehrs	134.506	3	44.835	5.696	.001
Samples	1210.128	2	605.064	76.863	.000
Timehrs * Samples	455.414	6	75.902	9.642	.000
Error	1046.972	133	7.872		
Total	10974.688	145			
Corrected Total	3040.703	144			

a. R Squared = .656 (Adjusted R Squared = .627)

Post Hoc Tests
Time (hrs)

Multiple Comparisons

L* values of the beads

Tukey HSD

(I) Time (hrs)	(J) Time (hrs)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	24	-3.6000*	.65683	.000	-5.3089	-1.8910
	96	-3.6700*	.66131	.000	-5.3906	-1.9494
	168	-3.0833*	.66131	.000	-4.8039	-1.3627
24	0	3.6000*	.65683	.000	1.8910	5.3089
	96	-.0700	.65683	1.000	-1.7790	1.6389
	168	.5166	.65683	.860	-1.1923	2.2256
96	0	3.6700*	.66131	.000	1.9494	5.3906
	24	.0700	.65683	1.000	-1.6389	1.7790
	168	.5867	.66131	.812	-1.1339	2.3073
168	0	3.0833*	.66131	.000	1.3627	4.8039
	24	-.5166	.65683	.860	-2.2256	1.1923
	96	-.5867	.66131	.812	-2.3073	1.1339

Based on observed means.

The error term is Mean Square(Error) = 7.872.

*. The mean difference is significant at the .05 level.

L* values of the beads

Tukey HSD[a,b,c]

Time (hrs)	N	Subset 1	Subset 2
0	36	4.8018	
168	36		7.8851
24	37		8.4018
96	36		8.4718
Sig.		1.000	.810

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 7.872.

a. Uses Harmonic Mean Sample Size = 36.245.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Appendix I. Statistical analysis of variance of colour (a* values) of the beads

Between-Subjects Factors

		N
Time (hrs)	0	36
	24	37
	96	36
	168	36
Samples	Autoclaved dates	37
	Control	36
	Raw dates	72

Descriptive Statistics

Dependent Variable: a* values of the beads

Time (hrs)	Samples	Mean	Std. Deviation	N
0	Autoclaved dates	10.3000	.80196	9
	Control	.3289	.68638	9
	Raw dates	4.9733	1.62290	18
	Total	5.1439	3.78772	36
24	Autoclaved dates	9.6437	1.04169	10
	Control	-.3567	.09474	9
	Raw dates	6.3689	.19510	18
	Total	5.6180	3.74152	37
96	Autoclaved dates	9.4833	1.05118	9
	Control	-.1367	.14335	9
	Raw dates	6.5400	.19626	18
	Total	5.6067	3.61532	36
168	Autoclaved dates	10.2556	1.27320	9
	Control	-.3111	.15415	9
	Raw dates	7.4378	.48604	18
	Total	6.2050	4.05086	36
Total	Autoclaved dates	9.9132	1.07381	37
	Control	-.1189	.44220	36
	Raw dates	6.3300	1.22263	72
	Total	5.6432	3.78072	145

Tests of Between-Subjects Effects

Dependent Variable: a* values of the beads

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1967.304[a]	11	178.846	261.371	.000
Intercept	3785.213	1	3785.213	5531.825	.000
Timehrs	7.704	3	2.568	3.753	.013
Samples	1904.785	2	952.392	1391.855	.000
Timehrs * Samples	42.482	6	7.080	10.347	.000
Error	91.007	133	.684		
Total	6675.969	145			
Corrected Total	2058.311	144			

a. R Squared = .956 (Adjusted R Squared = .952)

Levene's Test of Equality of Error Variances[a]

Dependent Variable: a* values of the beads

F	df1	df2	Sig.
45.190	11	133	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Timehrs + Samples + Timehrs * Samples

Post Hoc Tests
Time (hrs)

Multiple Comparisons

a* values of the beads

Tukey HSD

(I) Time (hrs)	(J) Time (hrs)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	24	-.4741	.19365	.073	-.9780	.0297
	96	-.4628	.19497	.087	-.9701	.0445
	168	-1.0611*	.19497	.000	-1.5684	-.5538
24	0	.4741	.19365	.073	-.0297	.9780
	96	.0114	.19365	1.000	-.4925	.5152
	168	-.5870*	.19365	.015	-1.0908	-.0831
96	0	.4628	.19497	.087	-.0445	.9701
	24	-.0114	.19365	1.000	-.5152	.4925
	168	-.5983*	.19497	.014	-1.1056	-.0911
168	0	1.0611*	.19497	.000	.5538	1.5684
	24	.5870*	.19365	.015	.0831	1.0908
	96	.5983*	.19497	.014	.0911	1.1056

Based on observed means.

The error term is Mean Square(Error) = .684.

*. The mean difference is significant at the .05 level.

a* values of the beads

Tukey HSD[a,,b,,c]

Time (hrs)	N	Subset 1	Subset 2
0	36	5.1439	
96	36	5.6067	
24	37	5.6180	
168	36		6.2050
Sig.		.075	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .684.

a. Uses Harmonic Mean Sample Size = 36.245.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Appendix J. Statistical analysis of variance of colour (b* values) of the beads

Between-Subjects Factors

		N
Time (hrs)	0	36
	24	37
	96	36
	168	36
Samples	Autoclaved dates	37
	Control	36
	Raw dates	72

Descriptive Statistics

Dependent Variable: b* values of the beads

Time (hrs)	Samples	Mean	Std. Deviation	N
0	Autoclaved dates	10.3767	.50301	9
	Control	1.6356	1.06719	9
	Raw dates	13.7200	4.80890	18
	Total	9.8631	6.05602	36
24	Autoclaved dates	7.5969	.58013	10
	Control	3.0911	.17489	9
	Raw dates	15.0556	.33010	18
	Total	10.1294	5.14216	37
96	Autoclaved dates	8.5022	.29359	9
	Control	3.1556	.13192	9
	Raw dates	14.0778	.77335	18
	Total	9.9533	4.63537	36
168	Autoclaved dates	10.0500	.32125	9
	Control	3.6400	.47379	9
	Raw dates	14.4778	.82299	18
	Total	10.6614	4.54616	36
Total	Autoclaved dates	9.0900	1.23789	37
	Control	2.8806	.94886	36
	Raw dates	14.3328	2.47346	72
	Total	10.1516	5.08588	145

Tests of Between-Subjects Effects

Dependent Variable: b* values of the beads

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3290.203[a]	11	299.109	91.551	.000
Intercept	10095.233	1	10095.233	3089.946	.000
Timehrs	16.006	3	5.335	1.633	.185
Samples	3200.381	2	1600.191	489.786	.000
Timehrs * Samples	72.767	6	12.128	3.712	.002
Error	434.527	133	3.267		
Total	18667.842	145			
Corrected Total	3724.730	144			

a. R Squared = .883 (Adjusted R Squared = .874)

Levene's Test of Equality of Error Variances[a]

Dependent Variable: b* values of the beads

F	df1	df2	Sig.
169.564	11	133	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Timehrs + Ssmples + Timehrs * Samples

Post Hoc Tests
Time (hrs)

Multiple Comparisons

b* values of the beads

Tukey HSD

(I) Time (hrs)	(J) Time (hrs)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	24	-.2664	.42315	.922	-1.3673	.8346
	96	-.0903	.42604	.997	-1.1987	1.0182
	168	-.7983	.42604	.244	-1.9068	.3101
24	0	.2664	.42315	.922	-.8346	1.3673
	96	.1761	.42315	.976	-.9249	1.2770
	168	-.5320	.42315	.592	-1.6329	.5690
96	0	.0903	.42604	.997	-1.0182	1.1987
	24	-.1761	.42315	.976	-1.2770	.9249
	168	-.7081	.42604	.348	-1.8165	.4004
168	0	.7983	.42604	.244	-.3101	1.9068
	24	.5320	.42315	.592	-.5690	1.6329
	96	.7081	.42604	.348	-.4004	1.8165

Based on observed means.

The error term is Mean Square(Error) = 3.267.

b* values of the beads

Tukey HSD[a,,b,,c]

Time (hrs)	N	Subset 1
0	36	9.8631
96	36	9.9533
24	37	10.1294
168	36	10.6614
Sig.		.241

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 3.267.

a. Uses Harmonic Mean Sample Size = 36.245.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.