

Effects of jackfruit puree supplementation on lactic acid bacteria (*Lactobacillus acidophilus* FTDC 1295) in terms of viability and chemical compositions of dadih

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Abstract

This research was conducted to evaluate the effects of supplementation of jackfruit puree on probiotic (*Lactobacillus acidophilus* FTDC 1295) in terms of cell count, viability and nutritional value of dadih. Four samples of dadih were prepared in this investigation; Control, Jackfruit dadih, Probiotic dadih and Jackfruit Probiotic dadih (Control, ConJD, ConPD and JPD respectively). Results revealed that dadih supplemented with jackfruit puree (JPD) directly improved the probiotic cell counts which are significantly higher than the dadih without jackfruit puree (ConPD). The high probiotic viability in dadih (ConPD 92%; JPD 96%) indicated that it can be an effective probiotic delivery vehicle. The chemical compositions (moisture, total solids, fat, protein, mineral, organic acid, and pH) showed variations in its pattern due to the differential in formulations and the incorporations of probiotic bacteria. In addition, the Total Phenolic Content and the antioxidant capacity were reported to be the highest in dadih supplemented with jackfruit puree and probiotic (JPD) as compared with other dadih samples. These are attributed by the presence of jackfruit puree and probiotic in the samples which effectively increased the total phenolic content which directly increase the antioxidant activity.

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Introduction

Dairy products as well as fermented dairies have become a major part of the people's diet throughout the world. Many of these dairy products have been enhanced with health beneficiary elements and designated as functional foods for the consumers. The inclusion of potential probiotic microorganisms in numerous food products have been one of the methods to value add and enhance the commercial value of a certain food product. In the dairy industry, a large variety of milk products and formulations have been developed in order to improve their role as probiotic carrier as well as delivery vehicle (Charalampopoulos *et al.*, 2003).

Probiotic bacteria, mainly Lactic Acid Bacteria (LAB) is used as food adjuncts in food to provide a wide variety of health benefits. Probiotics are live microbial food supplements which benefit consumer health by maintaining or improving their intestinal microbial balance (Sareela *et al.*, 2000). According to Sanders (1999), probiotics are microorganisms that have beneficial effects on the intestinal functions

and promote good health. Much scientific interests in the role of intestinal *Lactobacillus* in the prevention of human diseases has been derived from the observation of Metchnikoff in 1907, who has proven that longevity and good health of Bulgarian people resulted from high yoghurt consumption (Oliveira *et al.*, 2001). *Lactobacillus acidophilus* (LA) and *Lactobacillus rhamnosus* (LR) have been incorporated in the production of many fermented milk products with claimed to have probiotic values (Dave and Shah, 1998). The consumption of probiotics has been closely associated with their ability to aid lactose digestion (Oliviera *et al.*, 2001; Charalampopoulos *et al.*, 2002), prevent traveller's diarrhea (Guarino *et al.*, 1998), reduce the duration of rotavirus diarrhea (Klayraung *et al.*, 2008), exert antitumor activity (Kato *et al.*, 1994), enhance the activity of the immune system (Sareela *et al.*, 2002) and to aid in controlling serum cholesterol (Klayraung *et al.*, 2008). The consumption of products incorporated with probiotics have drastically increased in Europe, Asia Pacific and American countries and more than 90% of products containing *Lactobacillus acidophilus*

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or bifidobacteria are available worldwide. In order to achieve all the claimed health benefits, one of the most important requirements for manufacturing and marketing of any probiotic product is to maintain a high number of probiotic organisms which are ≥ 6 log CFU/g at the point of consumption as reported by several researchers (Dave and Shah, 1997; Oliviera *et al.*, 2001; Lucas *et al.*, 2004; Paseephol and Sherkat, 2009). Survival of the probiotic strains during gastric transit is also influenced by the physicochemical properties of the food used for probiotic delivery. Factors such as its buffering capacity and the pH of the food carrier plays significant roles, since the food formulations with pH ranging from 3.5 to 4.5 and high buffering capacity, such as yogurt, cheese, and skim milk, would increase the pH of the gastrointestinal tract and thus enhance the stability of probiotic strains (Charalampopoulos *et al.*, 2003). Prebiotics are nonviable food components that exert beneficial effects on the health of the host, associated with modulation of the intestinal flora (FAO/WHO, 2007). Prebiotics are usually carbohydrates that the host organisms or bodies are unable to digest and probiotics has the ability to digest it as well as use it for self-nourishment. Since the body cannot digest this particular food component, it travels down the body unaltered and only will be broken down by the probiotics when it reaches the colon where it resides. Most prebiotics are non-digestible oligosaccharides (short-chained sugar molecule) that help promote the growth and activity of probiotics. Products containing a combination of prebiotics and probiotics are known as synbiotics (Gibson and Roberfoid 1995).

Dadih has been claimed to exist for decades and are believed to be a well known traditional food in the northern region of Peninsular Malaysia (Hamzah, 1993). Recently, there have been attempts by researchers and the local industry to modify and value-add dadih for commercial means. Dadih are now seen as a potential carrier and probiotic delivery vehicle. A recent approach in assisting the probiotic viability through storage is by incorporating prebiotic to support the growth and activity of probiotic (Shin *et al.*, 2000; Bruno *et al.*, 2002; Akalin *et al.*, 2004; Desai *et al.*, 2004).

Jackfruit (*Artocarpus heterophyllus*) is made of soft, easily digestible flesh (bulbs) with simple sugars like fructose and sucrose. It replenishes energy and revitalizes the body instantly. Jackfruit is rich in dietary fiber, which makes it a good bulk laxative. The fiber content helps to protect the colon mucous membrane by decreasing exposure time and binding to cancer causing chemicals in the colon. Fresh jackfruit fruit has small amounts of vitamin-A and

flavonoid pigments such as carotene- β , xanthin, lutein and cryproxanthin- β . Together, these compounds play vital roles in antioxidant and vision functions. Vitamin A is also required for maintaining integrity of mucus membranes and skin. Consumption of natural fruits rich in vitamin-A and carotenes has been found to protect from lung and oral cavity cancers. Jackfruit is also a good source of antioxidant vitamin-C where the consumption helps body to develop resistance against infectious agents and scavenge harmful free radicals. Jackfruit is also a good source of potassium, magnesium, manganese, and iron. Potassium is an important component of cell and body fluids that helps to control heart rate and blood pressure.

In this research, studies were conducted to determine the suitability of dadih as a delivery component for LAB. Further research was conducted to investigate the effects of jackfruit puree incorporation on the lactic acid bacteria (*Lactobacillus acidophilus* FTDC 1295) in terms of its total counts, viability, and on the chemical composition of jackfruit dadih.

Materials and Methods

Microbial cultures

Microbial culture of lactic acid bacteria strain *Lactobacillus acidophilus* FTDC 1295 (LA FTDC 1295) was obtained from the culture collection centre in Universiti Sains Malaysia. Methods for bacterial propagation and reactivation were adopted from Dave and Shah (1997). Pure strains were stored at -20°C suspended in concentrated form. The bacteria was thawed and reactivated for 72 hours at 37°C prior to incorporation inside the dadih samples. Initial counts of inoculum for the LA FTDC 1295 are 10.90 ± 6.29 log CFU ml $^{-1}$.

Dadih preparation and storage (standard dadih, jackfruit dadih, standard probiotic dadih and jackfruit probiotic dadih)

Sampling and jackfruit puree preparation

Jackfruit collections were obtained in local supermarkets in Bayan Baru, Pulau Pinang, Malaysia. Jackfruit puree was prepared before hand by blending 150 g of the fresh pulp with 20 ml of distilled water in a ratio (1:8) before it is incorporated into the dadih samples. The commercialized pasteurized cow's milk (Dutch Lady, Malaysia) were used in the making of all dadih samples. Commercialized gelatins were obtained from SIM Company Sdn Bhd in Georgetown, Pulau Pinang, Malaysia.

Standard dadih (control)

Gelatins (2 g) were incorporated to a volume

of 200 ml of pasteurized cow's milk in a sauce-pan at temperature 55°C and stirred continuously until dissolved. Sugar (18 g) and salt (0.8 g) were then added after the heat was turned off. The mixture was then stirred and dispensed into plastic cups (50ml/cup) which was immediately cooled and sealed, and kept at 4°C until further analysis

Standard probiotic dadih (ConPD)

The procedure was similar to the preparation of the standard dadih with the exception of the introductions of inoculum (LA FTDC 1295) in each cup at 40°C. Inoculated mix were sealed and stored at 4°C for further analysis.

Jackfruit dadih (ConJD)

Gelatins (2 g) and jackfruit puree (20 g) were incorporated to a volume of 200 ml of cow's milk at temperature 55°C and stirred continuously until dissolved. Sugar (18 g) and salt (0.8 g) were then added after the heat was turned off. The mixture was then stirred and dispensed into plastic cups (50ml/cup). The mixture was immediately cooled and sealed, and kept at 4°C for further analysis.

Jackfruit probiotic dadih (JPD)

The procedure was similar to the Jackfruit Dadih with the exception of the introductions of inoculum (LA FTDC 1295) in each cup at 40°C. Inoculated mix was sealed and stored at 4°C for further analysis.

Probiotic propagation/reactivation

Probiotic strain (LA FTDC 1295) was obtained from the culture collection centre of the Universiti Sains Malaysia. Method for bacterial propagation and reactivation were adopted from Dave and Shah (1997). The propagation and reactivation of strain were done by transferring 1 ml of the strains into 9 ml of deMann Rogosa Sharpe (MRS, Oxoid Ltd., Hampshire,UK) broth. The organisms were subcultures three times prior to use in sterile MRS broth using 10% inoculum and 72 hours incubation at 37°C.

The probiotic was enumerated on the MRS agar to determine the inocula initial counts. Prior to incorporating the strains into dadih samples, 1 ml from the stock solution that was reactivated was transferred to a Falcon tube and centrifuged for 15 minutes at 12,000 rpm. The pellet form was washed twice with phosphate buffer before incorporating it in 50 ml of dadih.

Enumerations of probiotic cell counts and viability

The method was adopted with slight modification from Paseephol and Sherkat (2009). For each run, the dadih samples were analysed at every 3 day interval for 21 days. The 0.15 % (w/v) of peptone solution were prepared by dissolving 0.75 g peptone (Oxoid Ltd., Hampshire,UK) in 500 ml of distilled water. MRS agar was prepared by dissolving 52.2 g of MRS in 500 ml of distilled water. All of the reagents and agar were autoclave at 121°C for 15 minutes to ensure sterilization and to avoid contamination. Dadih samples (1 ml each) were added to 9 ml of sterile peptone diluents (10 % w/v). Appropriate dilutions were made and subsequently pour-plated in duplicate into MRS media. Plates were incubated at 37°C for 48 hours.

Determination of organic acid (titrable acidity) and pH during storage

Titration acidity (TA) was determined using the method (number 16.023) of AOAC (1984) by titration with 0.01 N NaOH solutions with a few drops of phenolphthalein added to samples as an indicator. Results were expressed as percent lactic acid, while the pH of the samples was measured using a pH meter (Delta, Shanghai, China). Lactic acid percentage was calculated using the following formula:

$$\% \text{ Lactic acid} = \frac{\text{Volume of titration} \times 0.01 \times 90.08 \times 100}{10g}$$

Chemical analysis

Proximate

Moisture, ash, crude fat, crude fibre, crude protein and carbohydrate contents were determined according to the AACC method (2000). The moisture content of the samples was determined by oven-drying (method 44-15A), while ash was quantified by dry ashing (method 08-01). Crude fat was determined with the Soxhlet and Mojonnier method (method for dairy product), using petroleum ether extraction, followed by evaporation to constant weight (method 30-25). Crude fibre was determined according to the gravimetric method (method 32-10). Meanwhile, crude protein was analysed by the Kjeldahl method (method 46-13). Carbohydrate was determined as the remaining percent weight according to the formula: [100 – (moisture + ash + crude fat + crude protein)].

Determination of essential mineral content

Essential mineral contents [phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca)] in samples were determined by using atomic absorption

spectroscopy, AAS (Perkin Elmer 4100ZL, Massachusetts, USA). Absorbances were recorded and a standard curve was plotted. Results were expressed as mg/L sample. Phosphorus was quantified by colorimetry using spectrophotometer (PerkinElmer 550SE, Massachusetts, USA)

Antioxidant properties

Sample preparation

The pulps were cleaned and dried in oven at 70°C. The dried pulps were ground separately into fine powder using a dry grinder. For dadih samples, the samples were freeze dried to eliminate moisture and ground to fine powder texture.

Extraction

Extraction method described by Chun *et al.* (2005) was applied with slight modifications. A weight of 1 g of every sample (jackfruit or dadih) was added to 200 ml of distilled water and boiled until the water and samples were reduced to 100 ml. The solvents were stirred with magnetic stirrer for 24 hours. Solvents were centrifuged at 3500 x g for 30 minutes to obtain supernatant containing the water soluble extracts which was kept at 4°C for further analysis.

Total phenolic content

The total phenolic content was determined by using the Folin Ciocalteu's reagent following the colorimetric method adopted by Meot-Duros and Magné (2008) with slight modifications. In brief, 0.3 ml of extracts was introduced into the test tubes followed by 1.5 ml of the Folin Ciocalteu's reagent. The reagent was pre-diluted, 10 times with distilled water. After standing for 5 minutes at room temperature, 1.2 ml sodium carbonate (7.5% w/v) solution was added. The solutions were mixed and allowed to stand for 30 minutes at room temperature. The absorbance was measured at 765 nm, using a UV-visible spectrophotometer (Shimadzu UV-1601PC, Japan). A calibration curve was prepared, using a standard solution of gallic acid. Results were expressed as milligrams of gallic acid equivalents (mg GAE/g of dry weight).

Scavenging activity of DPPH radical

The scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by adopting the method described by Li *et al.* (2006) with slight modifications. Different dilutions of extracts (0.2 mg/ml - 1.0 mg/ml) amounting to 1.0 ml were added to 2.0 ml of DPPH solution. The samples solutions were mixed thoroughly and were left to stand in the dark for 30 minutes. The absorbance

was measured at 517 nm and against blank distilled water and DPPH solution with absence of samples was used as control. Synthetic antioxidants butylated hydroxyanisole (BHA) were used as positive control. Antiradical activity, as expressed by the inhibition percentage (IP %) of DPPH radical, following the equation :

$$\%IP = [(A \text{ control} - A \text{ sample})/A \text{ control}] \times 100$$

Statistical analysis

Data were analysed with SPSS version 17.0 (Illinois, U.S.A) using t-test and one-way analyses of variance (ANOVA). Significance was defined at $P < 0.05$ by using Duncan's Multiple Range test. At least three replications were made for chemical analysis and physical measurement.

Results and Discussion

Chemical composition of jackfruit puree and dadih samples

Table 1 shows the chemical composition of jackfruit puree that was incorporated into the dadih samples. The inconsistency of chemical composition and mineral content in certain dairy products are influenced by a number of factors such as stage of lactation, nutritional status of the animal and environment, and genetic factors (Cashman, 2006). In addition, there were also studies conducted to observe the effect of fruit puree incorporation and the presence of lactic acid bacteria in commercialized fermented dairy products. Results from the studies showed that prior to the addition of fruit puree and lactic acid bacteria into dairy products leads to irregularity of the chemical composition such as fat, protein, carbohydrates, and minerals (Gambelli *et al.*, 1999).

As seen in Table 2, ConJD moisture content is significantly higher ($p < 0.05$) than control and this might be due to the addition of jackfruit puree. The moisture content of both ConPD and JPD were significantly ($p < 0.05$) higher than control. The total solid contents of the dadih samples ranged from 21.16-24.00%.

Crude fat content was significantly highest in ConJD compared to all the dadih samples. From Table 1, the trend showed that LAB containing samples had lower fat content. This might be due to the utilization of fat by the LAB. Previous research stated that the fatty acids were utilized by probiotics which enhanced their proliferation and assisted the probiotic's adhesion to mucosal surface once it enters the body system (Das, 2002).

Table 1. Chemical composition of jackfruit puree (JP)

Moisture (%)	88.20±0.58
Total solid (%)	11.80±0.58
Crude fat (%)	0.30±0.02
Crude protein (%)	0.72±0.26
Crude fiber (%)	1.34±0.03
Ash (%)	0.70±0.40
Sodium (mg/L)	ND
Potassium (mg/L)	2433.00±1.70
Phosphorous (mg/L)	75.60±1.71
Magnesium (mg/L)	79.51±0.57
Calcium (mg/L)	29.10±2.10

Means ± standard deviation (n=3)

JP = Jackfruit Puree

ND = Not Detected

Table 2. Chemical composition for dadih samples

	Control	ConJD	ConPD	JPD
Moisture (%)	76.00±0.50	77.13±0.41	78.53±0.46	78.84±0.36
Total solid (%)	24.00±0.50	23.13±0.11	21.46±0.46	21.16±0.30
Crude fat (%)	3.93±0.68	5.10±0.46	3.43±0.22	3.39±0.35
Crude protein (%)	3.45±0.09	3.83±0.13	3.17±0.22	3.10±0.11
Ash (%)	1.09±0.21	1.48±0.88	1.00±0.08	0.98±0.02
Sodium (mg/L)	2235.00±94.54	2291.00±14.00	1887.00±56.62	2236.00±57.71
Potassium (mg/L)	1141.00±52.32	1090.00±15.28	1003.00±6.11	1066.00±28.29
Phosphorous (mg/L)	965.00±12.10	888.00±3.06	902.00±44.66	866.00±5.77
Magnesium (mg/L)	106.00±7.55	106.67±8.50	95.00±4.04	103.66±6.51
Calcium (mg/L)	1123.00±22.74	1249.00±37.31	1109.00±36.86	1087.00±11.50

Means ± standard deviation (n=3)

Control=gelatin, ConJD= jackfruit puree+gelatin, ConPD=gelatin+LAB JPD=jackfruit puree+gelatin+LAB

Mean values in the same row with the same letter are not statistically significant (p>0.05)

Table 3. Mean values of the viable counts (log CFU/ml) of *Lactobacillus acidophilus* FTDC 1295 in dadih with and without jackfruit puree during storage at 4°C

Days	ConPD (log CFU/ml)	JPD (log CFU/ml)
0	8.88±5.12	9.02±5.21
3	8.07±4.65	8.75±5.06
6	8.52±4.92	9.43±5.45
9	8.65±5.00	9.45±5.46
12	8.63±4.98	8.98±5.18
15	8.47±4.89	8.90±5.14
18	8.53±4.92	8.88±5.12
21	8.24±4.76	8.88±5.12
Viability %	92.79±0.91	96.70±1.54

Data are means ± SD (n=2)

% viability = (CFU/ml after 3 weeks storage/initial CFU/ml) x 100

ConPD=gelatin+LAB, JPD= jackfruit puree+gelatin+LAB

Mean values in the same row with the same letter are not statistically significant (p>0.05)

Crude protein result showed significant difference between all four dadih samples. Crude protein content was significantly the highest in ConJD

(3.83%) and the pronounce increment on the protein content compared to control (3.45%) were attributed by the incorporation of jackfruit puree. The lower protein content observed in ConPD and JPD (3.17% and 3.10% respectively) were due to the probiotic presence in samples. Previous findings explained that the presence of milk nutrients and the incorporation of micronutrients to milk such as peptides and amino acids enhanced the proliferation of probiotic which used these ingredients for supplementation and growth (Dave and Shah, 1997; Oliveira *et al.*, 2001; Charalampopoulos *et al.*, 2003).

There was no significant ash content between all dadih samples. The minerals selected for analysis were sodium, potassium, phosphorous, magnesium and calcium as they are the five major macrominerals that make up the milk and other dairy products composition (Cashman, 2002). As previously mentioned the variations found in the chemical compositions were affected by the addition of fruit puree and the presence of lactic acid bacteria in a certain product. One of the earliest studies conducted by MacLeod *et al.* (1947) were on essential minerals for lactic acid bacteria that listed K⁺, Na⁺, Mg²⁺, Mn²⁺, Fe²⁺, PO⁴⁻, SO⁴⁻, Cl⁻ and Ca ions, each of these elements had promoting effects on the growth lactic acid bacteria. Recent study done by Grayon *et al.* (2006) in wine production further supported that the specific metals and trace elements were very essential for lactic acid bacterial growth. These elements have been shown to be the key enzymes cofactors in many organism's and microorganism's metabolism.

Among all the minerals analysed, magnesium showed no significant difference between all the dadih samples. Sodium content in ConPD (1887.00 mgL⁻¹) was significantly lower than all the other 3 dadih samples. Comparing between ConPD and the control, observation on the reduction of sodium content might be ascribed to the presence of lactic acid bacteria, which utilized this element for nourishments.

Potassium content in the control was significantly the highest (1141.00 mgL⁻¹) as compared to other dadih samples. Slight reduction in the potassium content observed in ConPD and JPD (1003.00 mgL⁻¹ and 1066.00 mgL⁻¹) could have been attributed by the presence of bacteria in the sample that utilized potassium for nourishment.

Phosphorous content in the control was significantly higher as compared to ConJD (965.00 mgL⁻¹ and 888 mgL⁻¹ respectively). However, no significant difference observed between the control and ConPD. In addition, a lower phosphorous content shown by JPD (866.00 mgL⁻¹) than that of ConPD (902.00 mgL⁻¹) could have been ascribed to

higher probiotic counts in JPD than that of ConPD. Phosphorus is an important mineral as it is constituent of the nucleic acids, nucleotides, phospholipids and teichoic acids.

Calcium content in ConJD was significantly the highest as compared with the control, ConPD and JPD (1249.00 mgL⁻¹, 1123.00 mgL⁻¹, 1109.00 mgL⁻¹ and 1087.00 mgL⁻¹ respectively). The increment of calcium content in ConJD might be attributed by the presence of jackfruit puree where Table 1 shows the calcium content of the puree reached up to 29.10 mgL⁻¹. JPD calcium content (1087.00 mgL⁻¹) was significantly lower compared with ConJD. The reduction of calcium content could have been affected by the presence of probiotic in the samples. There was no significant difference between ConPD and JPD in term of the calcium content.

Enumeration of probiotic cell counts and viability

Lactic acid bacteria enumerations of LA FTDC 1295 were conducted on two samples that were inoculated with the bacteria that were ConPD and JPD. Table 3 shows the mean value of the probiotic counts throughout 21 days of storage. The preliminary studies conducted showed that the life span of dadih sample was only 21 days

The batch culture for LA FTDC 1295 that had been incorporated into the dadih samples with and without the supplementation of jackfruit puree had shown normal bacterial growth throughout 21 days of storage. Results indicated that the growth patterns followed the normal bacterial growth phases, which were lag, exponential, and stationary/death. After 21 days of storage the bacterial showed high percentage of viability which indicated that dadih condition was deemed suitable to act as the delivery vehicle for these probiotics.

The initial amount of LA FTDC 1295 inoculum that has been incorporated into dadih samples was 10.90 log CFU/g. It was observed there has been a slight decrease in the count after it has been incorporated into the ConPD and JPD which were 8.88 log CFU/g and 9.02 log CFU/g respectively. According to Klaver *et al.* (1993) probiotics grew slowly in milk due to their lack proteolytic activity and this might be a factor in the reduction of cell counts in the samples. According to Charalampopoulos *et al.* (2003), differences in the initial populations of the lactic acid incorporated and in samples could be ascribed to the difference in environment conditions during the preparation of inocula.

Growth of the probiotics LA FTDC 1295 supplemented with jackfruit puree has exhibited a significantly higher ($p < 0.05$) cell counts compared to

the probiotic cell counts without supplementation of jackfruit puree. The supplementation of jackfruit puree effectively increased the counts by approximately 0.14 log₁₀ cycles (N log₁₀ supplementation with jackfruit puree – N log₁₀ without supplementation of jackfruit puree) on 0 days and an average of approximately 0.7 log cycles for subsequent days. The addition of jackfruit puree might serve as a source of prebiotics to the cell counts which enhanced and maintained the viability of the probiotics during dadih storage time. There are many research approaches in supporting the growth of probiotics by supplementing with prebiotics substrates and the product containing the combinations of probiotics and prebiotics are known as synbiotics (Gibson and Roberfoed, 1995; Shin *et al.*, 2000; Bruno *et al.*, 2001; Akalin *et al.*, 2004; Desai *et al.*, 2004). This indicates that jackfruit puree might serve as a source of prebiotics that further supports probiotic growth and maintain probiotic viability. Prebiotics are non viable foods components that exert a benefit on the health of the host associated with modulation of the intestinal flora (FAO/WHO, 2007). According to Klaenhammer (2001) prebiotics are substances that stimulate the growth and activity of probiotics and the best known prebiotics are fructooligosaccharides extracted from food sources. Due to the significant difference on the bacterial growth in dadih supplemented with and without jackfruit puree containing carbohydrates and proteins (Table 1), it is believed that jackfruit puree provides prebiotics and micronutrients which would enhance and improve as well as to optimize the dadih condition to make it more suitable for the bacteria to live in. Moreover, the viability of both bacteria in both dadih samples showed high percentage of viability 92.79% for ConPD and 98.44% for JPD. Survival of probiotics strains during gastric transit is also influenced by the physicochemical properties of the food carrier used for delivery. The buffering capacity and the pH of the carrier medium are significant factors, since food formulations with pH ranging from 3.5 to 4.5 and high buffering capacity, such as yoghurt, cheese, skim milk and other dairy products, would increase the pH of the gastric tract and thus enhance the stability of the probiotic strains (Gardiner *et al.*, 1998 ; Kailasapathy and Chin, 2000 ; Zarate *et al.*, 2000). Moreover, dadih also exhibited good results in providing suitable condition for the bacterial to grow in as it met the minimum requirement on the total amount of bacteria presence in food products where the suggested minimum at consumption are 10⁵ to 10⁶ CFU/g (Dave and Shah, 1997 ; Kurmann and Rasic, 1991 and Robison, 1987). However, as time evolved the requirements has leveled up. In

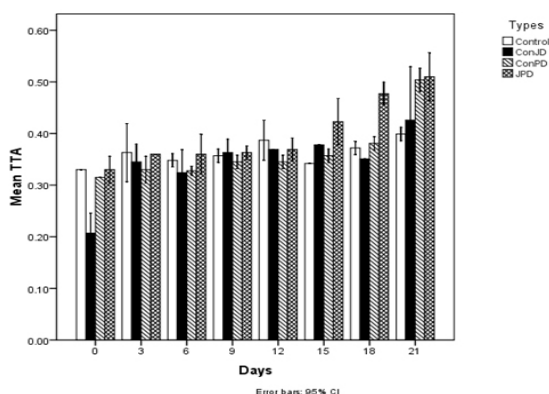


Figure 1. Mean values for titrable acidity (TTA) in dadih samples during storage at 4°C

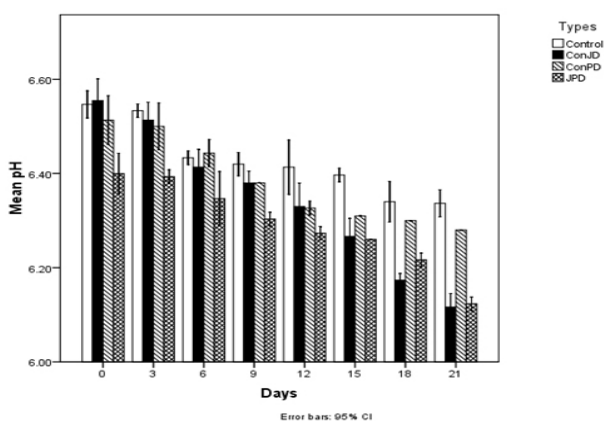


Figure 2. Mean pH values of dadih samples during storage at 4°C

achieving the claimed health benefits one of the most important requirement for the manufacturing and marketing probiotics products is to maintain a high number of probiotic organism $\geq 6 \log$ to $7 \log$ CFU/g at the point of consumption (Oliviera *et al.*, 2001 ; Lucas *et al.*, 2004 ; Paseephol and Sherkat, 2009).

Changes in pH and titrable acidity (TTA)

The bacteria traditionally used in yogurt and other dairy products are known as lactic acid bacteria because of their capacity to use lactose as their energy substrate and to produce lactic acid. The lactic acid bacteria are grouped as either homofermenters or heterofermenters based on the end products of their fermentation (Stiles and Holzappel, 1997). The homofermenters produce lactic acid as the major products of fermentation of glucose. The heterofermenters produce a number of products besides lactic acid, including carbon dioxide, acetic acid and ethanol from the fermentation of glucose.

Figure 1 shows the mean value for titrable acidity (lactic acid) in control, ConJD, ConPD and JPD during 21 days of storage. On day 0, the addition of jackfruit puree in ConJD resulted in slightly lower TTA percentage compared with the control (0.33% and 0.19% respectively). The TTA percentages of ConPD and JPD were slightly lower compared

with the control. Throughout storage duration, the percentage of TTA slightly increased in all dadih samples. However, a drastic TTA percentage change in ConPD and JPD observed after 21 days of storage were due to the presence of probiotic (LA FTDC 1295). Kendlar (1983) had classified *lactobacillus* as homofermenters and homofermenters produce lactic acid as the major products of fermentation of glucose. The relationship of lactic acid production is directly proportional to the days of storage which further explained that the presence of probiotic in the samples (i.e. ConPD and JPD) utilized the sugar and carbohydrate storage provided by their carrier. Both ConPD and JPD showed different amounts in lactic acid percentage (TTA) increment from day 0 to day 21 (0.12% and 0.17% respectively). The higher increment of the TTA percentage in JPD compared to ConPD might be ascribed to the fact that the cell counts in JPD was higher than that of ConPD, which leads to higher lactic acid production in JPD. Previous studies showed that the lactic acid production were effected by the number of probiotic present in the sample (Dave and Shah, 1998; Oliveira *et al.*, 2001; Paseephol and Sherkat, 2009).

The TA percentage is closely linked to the pH condition of a sample. Samples with high TA showed low pH values due to the presences of LAB which produces lactic acid resulting in an acidic condition in a sample or food products (Dave and Shah, 1997; Paseephol and Sherkat, 2009). Figure 2 shows the decrease in pH throughout the storage and this was due to the lactic acid production. Control and ConJD on day 1 showed highest pH value of 6.54 followed by ConPD and JPD (6.49 and 6.39 respectively). However during storage, drastic change in pH means were observed in all dadih samples, control, ConJD, ConPD and JPD (drop points 0.21, 0.43, 0.21 and 0.27 respectively). The drop points seen in ConPD and JPD were closely linked to high percentage of lactic acid production by the LAB present in the sample. However, the lactic acid production and pH condition of the dadih samples were shown to have no effects on the viability of probiotics inside the dadih samples (ConPD and JPD) during the refrigerated storage and this was further supported by previous research findings (Dave and Shah 1998; Oliveira *et al.*, 2001; Paseephol and Sherkat 2009).

Total phenolic content and antioxidant activity

Phenolic compound as products of secondary metabolism in plants are good sources of natural antioxidants in human diets (Chun *et al.*, 2004). Phenolic compound have the ability to bind to proteins *in vitro* forming soluble and insoluble complexes

Table 4. Total phenolic content in samples

	Total Phenolic content (mg GAE /g extract)
Control	1.07±0.08 ^a
ConJD	1.47±0.04 ^b
ConPD	1.44±0.09 ^c
JPD	1.71±0.01 ^d
Jackfruit Puree	0.92 ± 0.003

Expressed on dry basis, and means ± standard deviation (n=3)

Control=Control, ConJD=control+jackfruit puree+gelatin, ConPD=control+gelatin+LAB, JPD=control+jackfruit puree+gelatin+LAB

Mean values in the same row with the same letter are not statistically significant (p>0.05)

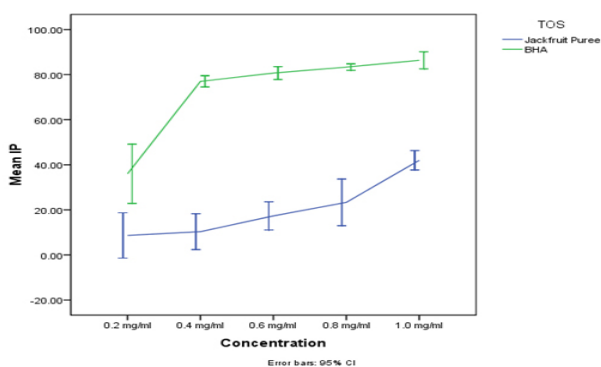


Figure 3. Inhibition Percentage (IP) mean values for jackfruit puree using BHA (synthetic antioxidant) as the positive control

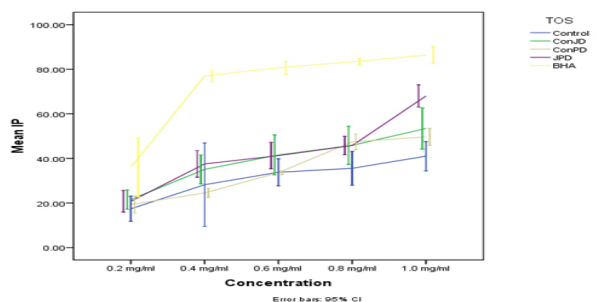


Figure 4. Inhibition Percentage mean values in different types of dadih (Control, ConJD, ConPD and JPD) and BHA as the positive control

and these phenolics through protein interactions are believed to be responsible for the putative function of phenolics as plant defense compound (Torti *et al.*, 1994). Hypothetically, phenolic compounds are believed to possess strong antioxidant capacity *in vitro* and some of them have been demonstrated to be significantly bioavailable *in vivo* (Li *et al.*, 2006). This further leads to a theory that these phenolic compounds and antioxidant activities are directly proportional to one another. Antioxidant can be defined as compounds that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation of propagation of an oxidizing chain (Lim *et al.*, 2007). Dietary phenolic compound has shown to play an important role in delaying development of chronic disease such as cardiovascular disease (CVD) cancer, inflammatory bowel syndrome and Alzheimer disease (Ajila *et al.*, 2007; Chun *et al.*, 2005; Li *et al.*,

2006). In plants the antioxidant activity in phenolic compounds is due to their redox properties and chemical structure, which can play an important role in neutralizing free radicals, chelating transitional metal and quenching singlet and triplet oxygen by delocalizing or decomposing peroxides (Haliwell, 1995).

Based on the results obtained in Table 4, the highest phenolic content was shown in JPD as compared with the control, ConPD, and ConJD. The control showed the lowest phenolic content among all samples. Comparison between control and ConJD, showed that ConJD exhibited higher phenolic content as this was due to the incorporation of jackfruit puree. ConPD possessed higher phenolic content than the control due to the presence of LAB. Further comparisons between ConJD and JPD showed that, JPD also indicated higher phenolic content due to the presence of LAB. Higher phenolic content in JPD than that of ConPD were ascribed to the incorporation of jackfruit puree and higher number of LAB counts in the samples. According to Wang *et al.*, (2006), the liberation of aglycone genistein and diadzein through catalytic action of β -glucosidase during fermentation by the LAB may affect the phenolic content and antioxidative activity of a certain sample.

Figure 3 shows the mean for the inhibition percentage of jackfruit puree. Results indicated that there were traces of antioxidant activity in jackfruit puree due to the presence of phenolic compound that had been previously discussed. The figure further explained that the higher phenolic content the higher the level of antioxidant activity. The highest antioxidant activity could be observed in a jackfruit puree at the concentration of 1 mg/ml that reached up to 40%. The antioxidant activity performances in jackfruit puree were efficient as it reached almost half of the performance of butylated hydroxyanisole (BHA-synthetic antioxidant) at 1mg/g which is 86%. BHA was used as positive control to act as a benchmark of the antioxidant activity performance as this synthetic antioxidant gives out excellent antioxidant activity and performed well even at a low concentration.

Previous studies mentioned the direct relationship between phenolic content and antioxidant activity which were directly proportional to one another (Li *et al.*, 2006; Budrat and Shotipruk, 2008; Papoulias *et al.*, 2009; Ahmed and Beigh, 2009; Isabelle *et al.*, 2010). As reported earlier, JPD showed the highest phenolic content which also expressed the highest antioxidant activity that reached up to 66%, followed by ConJD, ConPD and control. The results of this research further supported the previous findings, which mentioned

the direct relationship between phenolic content and antioxidant activities. The antioxidant performance in JPD exceeded more than half of the positive control where the percentage of inhibition reached up to 86%. All samples showed the highest antioxidant activity at a concentration of 1 mg/ml. ConJD expressed higher antioxidant activity compared to the control due to the addition of jackfruit puree. Further comparisons made between the control and ConPD showed that ConPD had higher antioxidant activity which resulted from the addition of LAB. JPD expressed the highest antioxidant activity which could be attributed to the synergistic effect of jackfruit puree and the presence of LAB. Referring to the previous result, it indicated that the cell counts of JPD exhibited higher counts compared with ConPD causing an effect in the increment of phenolic content of the dadih samples hence resulting in higher antioxidant activity in JPD. Similar finding was shared in a research done by Wang *et al.* (2006) that involved the incorporation of lactic acid bacteria in soymilk so as to observe the antioxidative properties. The production of the enzyme superoxide dismutase during LAB fermentation was believed to be responsible for the autoxidative properties. Furthermore, super oxide dismutase enzyme production along with fermentation time increased the antioxidant activity alongside with fermentation time. Among the various samples of fermented soymilk tested, soymilk fermented with *Lactobacillus acidophilus* and *Bifidobacterium longum* simultaneously exhibited the highest inhibition rate. Lin and Yen (1999) indicated that cells of lactic acid bacteria exhibited antioxidative activity thus further explaining that the ascorbate autoxidation inhibition and super oxide anion scavenging will increase alongside fermentation time.

Conclusions

Present result showed that supplementation of jackfruit puree affects the *Lactobacillus acidophilus* FTDC 1295 in terms of its viability and chemical composition of dadih samples. The addition of jackfruit puree further enhanced and optimized the dadih condition which resulted in the effectiveness of increasing the LAB (LA FTDC 1295) cell counts. The incorporation of jackfruit puree assisted in probiotic proliferation and improved the probiotic viability by maintaining a high number of LAB organism $\geq 8 \log$ CFU/g throughout storage. The supplementation of jackfruit puree and the inclusion of LAB (LA FTD 1295) also affected the chemical compositions of the dadih. The supplementation of jackfruit puree and inclusion of LAB had significantly increased the

overall phenolic content which directly affected the overall antioxidant activity performance.

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