INTRODUCTION

In current years, local species of plant and fruit tree have been scientifically studied for their potential medicinal applications [1]. *Lansium domesticum* Corr is a fruit tree species derived from the family of Meliaceae. This popular tropical fruit found mainly in South-East Asia, especially in the Philippines, where it is known as *Lansium* and South Sumatra in Indonesia [2]. It also grows in Suriname, Puerto Rico and Australia [3]. Although many species have been ascribed to the genus *Lansium*, Correa. agg. [4] recognized only three species, *L. membranaceum* Kosterm, *L. domesticum* Corr. and *L. breviracemosum* Kosterm. In Peninsular Malaysia, the genus is represented by only one species, *L. domesticum* Corr. [4, 5]. There are numerous forms of the species, and these belong to four main types: Dokong, Duku, Langsat and Duku-langsat. There is a fairly clear distinction between the two major types of *L. domesticum*: Langsat and Duku. Langsat fruits are oblong, thin-skinned and possess latex, while Duku fruits are round, thick-skinned and latex-free. The Duku-langsat (or Duku Terengganu) is the intermediate type, generally regarded as superior to both Duku and Langsat [5]. Duku, Langsat and Duku-langsat are natives of Peninsular Malaysia while Dokong originates from southern Thailand, and has been cultivated in Peninsular Malaysia for more than 10 y. Langsat can be found throughout the peninsular, but predominates in the north, while *Lansium* can be found in Surinam, Puerto Rico and Australia [3]. Although many species have been ascribed to the genus *Lansium*, Mabberley [4] recognized only three species, *L. membranaceum* Kosterm, *L. domesticum* Corr. and *L. breviracemosum* Kosterm. In Peninsular Malaysia, the genus is represented by only one species, *L. domesticum* Corr. [4, 5]. There are numerous forms of the species, and these belong to four main types: Dokong, Duku, Langsat and Duku-langsat. There is a fairly clear distinction between the two major types of *L. domesticum*: Langsat and Duku. Langsat fruits are oblong, thin-skinned and possess latex, while Duku fruits are round, thick-skinned and latex-free. The **Duku-langsat** (or Duku Terengganu) is the intermediate type, generally regarded as superior to both Duku and Langsat [5]. Duku, Langsat and Duku-langsat are natives of Peninsular Malaysia while Dokong originates from southern Thailand, and has been cultivated in Peninsular Malaysia for more than 10 y. Langsat can be found throughout the peninsular, but predominates in the north, while Duku occurs in the southern region [6]. Duku-langsat is a popular fruit tree on the east coast and is mainly cultivated in the states of Terengganu and Kelantan.

Duku and langsat raw fruit are green in color, and the flavor is very sour gummy. As the fruit matures, the skin will turn into yellow and the flesh of the fruit will taste sweet. Most of the fruit is eaten fresh as Duku and Langsat fresh only. The nutritional composition of duku and langsat has reported that in every 100 grams of duku and langsat contained 70-74 calories, 1.0-1.5 g protein, 0.2-0.5 g fat, 13-15 g carbohydrates, 0.7-1.0 g minerals, 19-20 mg calcium, 9-11 g phosphorus and 0.9-1.5 mg of iron. For the caloric content, it shows that the level of mineral and iron found in duku and langsat are higher than imported fruits like apples and oranges [4]. Duku and langsat fruits are relatively high in dietary fiber, which gives great benefits for the digestive system in preventing cancer of the colon and act to cleanse the body from cancer-causing free radicals. In addition to healthy fresh fruit, the fruit peel and seeds are also useful for anti-diarrhea medicine raw materials as well as to reduce fever. Traditionally, the barks of duku and langsat tree are often used to treat venemous insect bites, dysentery medicine and eradicate cancer cells [7].

*Bifidobacteria* is considered to be one of the most important genera of bacteria in terms of human health. They account nearly 85 to 99% of the intestinal flora in infants [7, 8]. All species derived from human are non-spore forming, non-motile, anaerobic Gram-positive bacteria. In a healthy adult, *Bifidobacteria* constitutes third to the fourth largest group of micro flora in the lower gastrointestinal tract, while *Clostridium* and *Lactobacillus* normally account for less than 15% of the intestinal flora [8]. Recently, there has been an increasing interest in the incorporation of the intestinal species *Lactobacillus acidophilus* and *Bifidobacterium* species into fermented milk products [9]. *Lactobacillus* colonizing in the human body such as oral cavity and gastrointestinal tract; in which fruits and fermented foods are the main sources of probiotics [10]. These species are frequently associated with health promoting effects in human and animal intestinal tract. These probiotic effects are generally related to healthy fresh fruit, the fruit peel and seeds are also useful for anti-diarrhea medicine raw materials as well as to reduce fever. Traditionally, the barks of duku and langsat tree are often used to treat venemous insect bites, dysentery medicine and eradicate cancer cells [7].

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to inhibition of pathogenic species, reducing the risk of colon cancer, increasing the immune response and decreasing the concentration of cholesterol in blood plasma [11]. Bifidobacteria is not true lactic acid bacteria in the sense of a Lactococcus or pediococcus [12]. Bifidobacteria produce both acetic and lactic acids as primary metabolites in the molar ratio of 2:3 [13]. Glucose is degraded characteristically by the fructose 6-phosphate shunt metabolic pathway [14].

Furthermore, milk is one of the best sources of nutrients for child growth. The previous study showed that Bifidobacterium spp. can be isolated mostly from the feces of infant milk feed the baby. These gut floras have to digest the milk-based food and compromise the major minerals and energy at the same time. Therefore, the objectives of the study were to screen the ability to add extracts of freeze-dried fruit extracts may enhance the growth of bifido culture in milk is not favorable to products since the flavor of bifido culture in milk is not favorable to test and provide consumers with certain nutrients especially minerals and energy at the same time. Therefore, the objectives of the study were to screen the ability to add extracts of freeze-dried duku and langsat fruits to stimulate the growth and stability of selected Bifidobacteria spp in skimmed milk.

MATERIALS AND METHODS

Fruits

Commercially matured Duku (Lansium Domesticum Corr) and Langsat (Lansium Domesticum Jack) fruits were obtained from a local plantation in Terengganu, Malaysia within a week of harvest. They were cleaned and peeled manually prior to extraction. Exposure to light was consciously avoided to reduce possible losses of nutrient.

Sample extraction procedure

The methods introduced by Xiaoli et al. [16] were used during the extraction procedure. The fruits were carefully washed under running tap water, dried with a soft cloth and the skin was carefully peeled. Lipid was removed from the sample (500 g) using petroleum ether (boiling range temperature of 37 ± 5 °C). The optimal conditions selected for the extraction of oligosaccharides in duku and langsat fruits were carried out as follows: Exactly 1.0 g of fruit sample was extracted 3 times with 10 ml/50% ethanol-water, at a ratio of 10:1 (solvent to fruit extracts) in a water bath at a temperature of 50 °C for 60 min. After each extraction, the samples were centrifuged at 2500 g for 20 min. Supernatants from the three cycles of extraction were combined and concentrated by using a rotary vacuum evaporator (Heidolph, Germany) then freeze-dried.

Bifidobacterial strain

Lyophilized B. longum (ATCC 15707) and B. breve (ATCC 15700) were purchased from American Type Culture Collection (Rockville, MD). Forty-eight hours prior to the start of each experiment, cultures were revived by a series of two inoculations into 10 ml of MRSL (MRS Broth added with 5% Lactose [12] and incubated at 37 °C for 30 min and cooled to 37 °C within 4 min. Each sample was divided into five portions and inoculated at a 5% level of B. longum and B. breve propagated in MRS medium with 5% lactose. Inoculated samples were incubated at 37 °C for 60 h. A total of 100 µl 15.8 M HNO₃ and 14.9 ml of 0.009 M H₂SO₄ were added to 1.5 ml of the sample and centrifuged at 4000 x g for 10 min using bench top centrifuge (Sigma-Aldrich, USA). The supernatant was filtered using 0.22 mm Millipore filters (Whatman, USA) and 2 ml aliquots were stored in HPLC vials at a temperature of 20 °C until analyzed.

The methods introduced by Ustunol and Gandi [17] were used for HPLC procedure with modification. The HPLC system consists of a pump and a 10A refractive index detector. A LiChrosart® 250-4 LiChrospher® NH₂ 5 µm column and a guard column with disposable cartridges (Merck, Germany) maintained at 65 °C for organic acids to be quantified. The standard solutions or organic acids (lactic and acetic acids; Sigma, St Louis, MO, USA) were prepared with water (HPLC grade) to establish election times and calibration curves. The retention time for lactic acid and acetic acid were 25.7 min and 16.2 min respectively. All experiments were replicated ten times.

Statistical analysis

Data were express as mean±standard deviation (SD) in 10 replicates. Statistical analysis was performed with a single factor and One-way ANOVA to identify the significant difference based on the effects of oligosaccharides from duku and langsat on the growth, viability, and activity of B. longum and B. breve.

RESULTS AND DISCUSSION

Clinical studies have associated other beneficial effects such as immune enhancement and anti-carcinogenicity with the presence of Bifidobacteria in the gastrointestinal tract. One approach for ensuring or increasing the presence of healthful colon bacteria is to utilize them as a prebiotic. A prebiotic is a live microbial food supplement, which beneficially affects the host organism by improving its intestinal microbial balance [14]. Another approach used for increasing the numbers of Bifidobacteria in the gastrointestinal tract is the incorporation of prebiotics in the diet. A prebiotic is a non-digestible dietary supplement that modifies the balance of the intestinal microflora stimulating the growth and activity of beneficial organisms and suppressing potentially deleterious bacteria [17]. However, not all dietary fibers are prebiotics, and certain criteria need to be recognized before sorting dietary carbohydrate as prebiotics [18]. Thus, it is important to select appropriate prebiotics to improve retention viability of Bifidobacteria in dairy food with an ultimate goal of delivering a large number of viable Bifidobacteria and stimulating Bifidobacteria growth in the colon.
**Bifidobacterial growth study evaluation**

In general, the mean doubling time was used to measure the efficacy of various carbon sources in modulating the growth rate of lactic acid bacteria known as probiotics. The growth of *B. longum* in skimmed milk was significantly stimulated by increasing the concentration of prebiotic extracts after being incubated anaerobically at the temperature of 37 °C for 72 h as shown in fig. 1. The growth promotion of *B. longum* by prebiotics of duku, langsat, inulin, GOS and FOS was obtained over the range either at 5% or 12% as evidenced by decreased mean doubling time with increased concentration of prebiotics indicating that *B. longum* strains grew faster in the presence of these carbohydrates compared to the controls.

![Fig. 1: Doubling time of *B. longum* in skimmed milk containing prebiotics](image)

Among the carbohydrate sources tested, a 12% supplementation of FOS, duku and GOS showed to be most effective in enhancing the growth rate of *B. longum* in skimmed milk. The mean doubling time of *B. longum* after 72 h of incubation was significantly reduced (p<0.05) compared to the controls. The addition of 12% FOS significantly decreased (p<0.05) the doubling time value as compared to the other samples. The supplementation of 12% GOS and duku also decreased the doubling time value after 72 h of fermentation. The supplementation of 5% and 12% oligosaccharides extracted from duku also helped in stimulating the growth of *B. longum* when added to skimmed milk. It showed a similar pattern of positive results as observed in the supplementation of 12% FOS. As compared with 12% supplementing of GOS, inulin and other carbohydrate sources tested, their mean doubling time was shorter than control except for the 12% supplementation of FOS.

![Fig. 2: Doubling time of *B. breve* in skim milk containing prebiotics](image)

In general, doubling time of *B. breve* grown with prebiotics significantly decreased (p<0.05) compared to the control as shown in fig. 2. From the results, the most effective supplements that enhanced the growth rate of *B. breve* in skim milk were 12% supplementation of FOS, duku and inulin. This study also found that the supplementation of inulin, GOS and FOS were equally enhancing the growth rate of *B. breve* in skim milk. The supplementation of 12% FOS provided the shortest mean doubling time, followed by 12% inulin, 12% GOS, 5% GOS, and 5% FOS. Both supplemented with 5% FOS and 5% GOS were found numerically higher, but not significant in stimulating the growth rate of *B. breve* in skim milk. For the fruit prebiotics tested, duku and langsat were significantly lower in enhancing the growth rate of *B. breve* in skim milk. The supplementation of 12% oligosaccharides from duku also helped stimulate the growth of *B. breve* when added to skim milk. It showed a similar pattern of positive results as with the supplementation of 12% FOS. As compared to supplementations of 12% GOS, 12% inulin and other carbohydrate sources tested, their mean doubling time was shorter than others except with 12% FOS. The concentration of more than 5% oligosaccharides from duku and langsat was needed to stimulate (p<0.05) the growth of *B. breve* strains.

This study also showed that a supplementation of oligosaccharides from duku and langsat could effectively stimulate the growth of *Bifidobacterium* species when added to skimmed milk and showed a similar pattern of results as supplementation of FOS and GOS. These data could have an important nutritional significance since they indicate that the fruit prebiotics, specifically red pitaya supplementation, showed potential in improving the bifidobacterial glucosidase activity during skimmed milk fermentation. The supplementation of FOS and GOS showed a sharp decrease in mean doubling time as compared to other prebiotic samples. These results were consistent with previous reports by Shin et al. [19] on the ability of FOS to stimulate *Bifidobacterium* spp (BF-1 and BF-6) growth in skimmed milk containing oligosaccharides and inulin. The results also indicated the ability of FOS to stimulate the proliferation
of *Bifidobacteria* relative to other intestinal microflora in *in vitro* culture models simulating the colon, as stated by Gibson and Wang [20]. The human colon was known to have over 400 distinct species of bacteria as resident flora (a population of up to 10^{10} bacteria per gram of colonic contents) [21]. The ingestion of GOS was also demonstrated to promote the increase number of fecal *Bifidobacteria* in human feeding trials [22, 23].

A previous study was done by Shin et al. [19] showed that inulin was found to be less effective in stimulating the growth of *Bifidobacterium* spp. However, in this study, inulin was shown to be effective in stimulating the growth of *Bifidobacteria*. The results of this study were similar and consistent with previous reports by Bruno et al. [22] on the ability of prebiotics from *H. maizae*, lactulose, raffinose and inulin in stimulating growth, viability, and activity of *Bifidobacterium* spp in skimmed milk. Gibson and Warg [20] reported on the ability of inulin to stimulate the proliferation of *bifidobacteria* relative to other intestinal microflora. Roberfroid [23] also reported positive outcomes in *in vitro* fermentation of inulin by human fecal bacteria when molecules had DP>10. Overall, the results showed that there was a significant difference between the effect of oligosaccharides from duku and langsat on the growth activities of *B. longum* and *B. breve* in skimmed milk.

**Determination of Bifidobacterial activity**

According to Shin et al. [17], the *Bifidobacteria* fermentation pathway resulted in 5 moles of acetic acid and 2 moles of lactic acid per 2 moles of glucose in an ideal synthetic medium. The yields of the theoretical molar ratio (acetic:lactic) should be 1.5 as proposed by Scardovi and Trovatelli [24]. Although lactic acid production is desired in fermented dairy foods, a high concentration of acetic acid can result in distinct vinegar flavor in products, thus a high acetic to lactic acid ratio is typically undesirable in fermented dairy foods. In accordance with growth stimulation, both acetic and lactic acid production by *B. longum* and *B. breve* were enhanced by the presence of both fruit prebiotics and commercially available probiotics (table 1) in skimmed milk as compared to the controls. For the production of acetic acid by *B. longum*, the highest production level of acetic acid was produced with a supplementation of 12% oligosaccharides from duku followed by 12% oligosaccharides from langsat, 12% FOS, 12% GOS, 12% inulin, 5% oligosaccharides from duku, 5% oligosaccharides from langsat, 5% inulin, 5% FOS, while the lowest acetic acid level was produced by 5% GOS.

For lactic acid production by *B. longum*, the highest production level of lactic acid was produced with a supplementation of 12% oligosaccharides from langsat followed by 5% oligosaccharides from duku, 12% GOS, 5% oligosaccharides from langsat, 5% inulin, 5% FOS, 5% GOS, 12% FOS, 12% inulin and the lowest lactic acid production level was produced by supplementation of 12% oligosaccharides from duku. Acetic acid concentration produced by *B. longum* when combined with prebiotics of different concentrations were only significant (p<0.05) with 12% concentrations of supplementation of duku, langsat, inulin, GOS and FOS, compared to the controls. Lactic acid concentration produced by *B. longum* was significantly lower (p<0.05) compared to the controls when 12% oligosaccharides from duku, 12% inulin and 12% FOS were used. This study also observed a higher average molar ratio of acetic to lactic acid production by *B. longum* when 12% oligosaccharides from duku was added, followed by 12% FOS, 12% inulin, 12% GOS, 12% oligosaccharides from duku, 5% FOS, 12% oligosaccharides from langsat, 5% oligosaccharides from langsat, 5% inulin and 5% GOS. The control samples containing *Bifidobacteria* with no added prebiotics produced an average ratio of 1.27:1.

**Table 1: Acetic and Lactic Acid Production by five strains of *Bifidobacterium* spp. in skimmed milk containing 5% and 12% fruit and commercial prebiotics**

| Prebiotics/Species | *B. longum* (ATCC15707) | *B. breve* (ATCC15700) |
|--------------------|--------------------------|------------------------|
| **Control**        |                          |                        |
| Acetic Acid (mM)   | 54.90±1.50\(^a\)         | 39.90±1.20\(^a\)       |
| Lactic Acid (mM)   | 43.20±1.08\(^b\)         | 30.40±1.07\(^b\)       |
| Ratio\(^c\)        | 1.27                     | 1.31                   |
| **5% Duku**        |                          |                        |
| Acetic Acid (mM)   | 59.40±0.70\(^a\)         | *91.90±0.60\(^b\)      |
| Lactic Acid (mM)   | 32.30±0.90\(^c\)         | 45.60±0.20\(^c\)       |
| Ratio\(^d\)        | 1.84                     | 2.01                   |
| **12% Duku**       |                          |                        |
| Acetic Acid (mM)   | *99.40±1.70\(^d\)        | *91.90±1.15\(^d\)      |
| Lactic Acid (mM)   | *22.03±1.35\(^d\)        | *22.98±1.32\(^d\)      |
| Ratio\(^e\)        | 4.51                     | 3.99                   |
| **5% Langsat**     |                          |                        |
| Acetic Acid (mM)   | 63.21±1.10\(^d\)         | *62.98±1.50\(^e\)      |
| Lactic Acid (mM)   | 32.90±1.00\(^d\)         | *21.90±1.04\(^d\)      |
| Ratio\(^f\)        | 1.73                     | 1.72                   |
| **12% Langsat**    |                          |                        |
| Acetic Acid (mM)   | *96.78±1.01\(^d\)        | *97.56±1.50\(^d\)      |
| Lactic Acid (mM)   | 38.45±0.80\(^d\)         | 43.90±1.09\(^d\)       |
| Ratio\(^g\)        | 2.22                     | 2.22                   |
| **5% Inulin**      |                          |                        |
| Acetic Acid (mM)   | 59.21±0.90\(^d\)         | 54.2±0.30\(^d\)        |
| Lactic Acid (mM)   | 32.78±0.20\(^d\)         | 33.20±0.78\(^d\)       |
| Ratio\(^h\)        | 1.81                     | 1.63                   |
| **12% Inulin**     |                          |                        |
| Acetic Acid (mM)   | *89.56±0.45\(^d\)        | *84.67±0.80\(^d\)      |
| Lactic Acid (mM)   | *22.90±0.20\(^d\)        | *23.55±0.35\(^d\)      |
| Ratio\(^i\)        | 3.91                     | 3.64                   |
| **5% GOS**         |                          |                        |
| Acetic Acid (mM)   | 40.98±0.50\(^d\)         | 42.9±0.90\(^e\)        |
| Lactic Acid (mM)   | *23.09±1.10\(^d\)        | *28.67±1.04\(^d\)      |
| Ratio\(^j\)        | 1.77                     | 1.50                   |
| **12% GOS**        |                          |                        |
| Acetic Acid (mM)   | *90.21±0.10\(^d\)        | *92.67±0.35\(^d\)      |
| Lactic Acid (mM)   | 33.89±0.30\(^d\)         | *32.56±0.50\(^d\)      |
| Ratio\(^k\)        | 2.66                     | 2.85                   |
| **5% FOS**         |                          |                        |
| Acetic Acid (mM)   | 58.97±0.50\(^d\)         | 54.5±1.02\(^b\)        |
| Lactic Acid (mM)   | *23.20±0.70\(^d\)        | *23.67±0.10\(^d\)      |
| Ratio\(^l\)        | 2.54                     | 2.31                   |
| **12% FOS**        |                          |                        |
| Acetic Acid (mM)   | *95.23±0.10\(^d\)        | *94.9±0.50\(^d\)       |
| Lactic Acid (mM)   | *21.98±0.50\(^d\)        | *25.24±0.90\(^d\)      |
| Ratio\(^m\)        | 4.33                     | 3.76                   |
| **Average**        |                          |                        |
| Acetic Acid (mM)   | *7.13±0.20\(^d\)         | *71.57±1.07\(^d\)      |
| Lactic Acid (mM)   | *30.61±0.78\(^d\)        | *29.74±1.82\(^d\)      |
| Ratio\(^n\)        | 2.22                     | 2.41                   |

\(^{a}\)Significantly different (p<0.05) from the control, \(^{b}\)Variation in the following letters between acetic acid indicates significant of difference by Duncan’s test at 5% level (p<0.05%), \(^{c}\)Variation in the following numbers between lactic acid indicates significant of difference by Duncan’s test at 5% level (p<0.05%).
The supplementation of fruits or commercially available prebiotics were influenced by the production of acetic and lactic acids. It was similar in all prebiotics supplementation (p<0.05) and the results showed it did not depend on prebiotic concentration. In the case of B. longum, acetic and lactic acids were significantly enhanced (p<0.05) when 12% oligosaccharides from duku and langsat was added to skimmed milk. The results from this study showed a certain stimulatory effect upon Bifidobacterium spp. in skimmed milk and it was more effective exhibiting similar result patterns as supplementations of FOS, GOS and inulin. The concentration of more than 5% oligosaccharides from duku and langsat was needed to stimulate (p<0.05) the viability of B. bifidum in skimmed milk. Overall, the result showed there is a significant difference between the effect of oligosaccharides from duku and langsat on the growth activities of B. longum and B. breve in skimmed milk. The addition of 12% oligosaccharides from duku supplementation had greatly influenced the viability of B. longum as compared to other fruit prebiotic samples. The authors also showed that a supplementation of oligosaccharides from duku significantly stimulated the growth of B. longum and B. breve when added to skimmed milk and it was more effective exhibiting similar result patterns as supplementations of FOS, GOS and inulin. The concentration of more than 5% oligosaccharides from duku and langsat was needed to stimulate (p<0.05) the viability of B. bifidum in skimmed milk. Other than that, results of lactic acid showed that the production had significantly enhanced (p<0.05) when B. longum, B. breve, B. infantis, B. bifidum and B. adolescentis were grown in the presence of 12% oligosaccharides from red pitaya with the product ion of lactic acid ranging from 3.29–4.51 and nearly 2.5 times greater as compared to inulin, GOS and FOS. Thus, this study reveals that milk supplementation with oligosaccharides from duku and langsat had significantly improved the survival and viability of Bifidobacterium spp. cells and it showed potential in improving the bifido-bacterial glucosidase activity during skimmed milk fermentation.

ACKNOWLEDGEMENT

The authors would like to thank Universiti Sultan Zainal Abidin (UniSZA) for the financial aid (RAGS) and the Faculty of Medicine and Health Sciences for providing the facilities. The authors also would like to acknowledge all staffs from Teaching Laboratory 1, Faculty of Bioresources and Food Industry, UniSZA.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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How to cite this article
Norhayati AH, Mohd Adzim Khalili R, Zetty Hulwany MZ, Intan Suhana Munira MA, Atif Amin B, Muralidhara DV, Ahmad Zubaidi L. Potential effects of duku (Lansium domesticum corr) and langsat (Lansium domesticum jack) extracts on the growth of Bifidobacteria spp. Int J Pharm Pharm Sci 2016;8(11):69-74.