

Journal Pre-proofs

Full length article

Date juice addition to bio-yogurt: The effects on physicochemical and microbiological properties during storage, as well as blood parameters *in vivo*

Shayma Thyab Gddoa Al-Sahlany, Wafaa H. Khassaf, Alaa Kareem Niamah, Alaa Jabar Al-Manhel

PII: S1658-077X(22)00071-6
DOI: <https://doi.org/10.1016/j.jssas.2022.06.005>
Reference: JSSAS 525

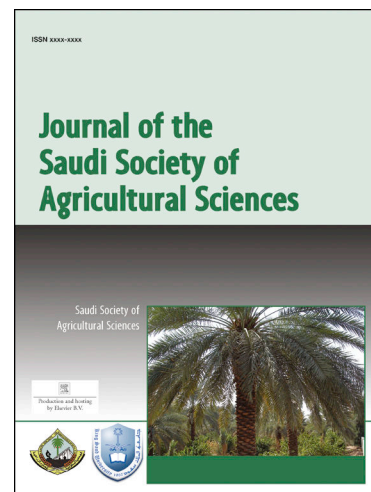
To appear in: *Journal of the Saudi Society of Agricultural Sciences*

Received Date: 3 April 2022
Revised Date: 8 June 2022
Accepted Date: 28 June 2022

Please cite this article as: Thyab Gddoa Al-Sahlany, S., Khassaf, W.H., Kareem Niamah, A., Jabar Al-Manhel, A., Date juice addition to bio-yogurt: The effects on physicochemical and microbiological properties during storage, as well as blood parameters *in vivo*, *Journal of the Saudi Society of Agricultural Sciences* (2022), doi: <https://doi.org/10.1016/j.jssas.2022.06.005>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2022 Published by Elsevier B.V. on behalf of King Saud University & Saudi Society of Agricultural Sciences.



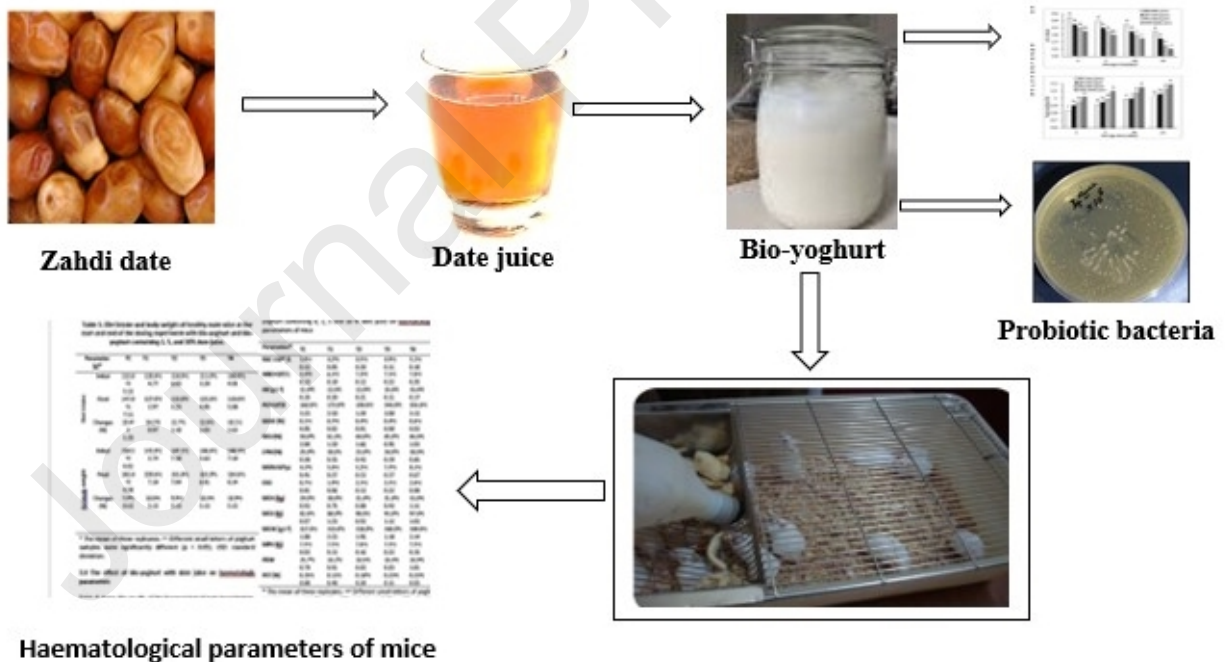
Date juice addition to bio-yogurt: The effects on physicochemical and microbiological properties during storage, as well as blood parameters *in vivo*

Shayma Thyab Gddoa Al-Sahlany¹, Wafaa H. Khassaf², Alaa Kareem Niamah^{1*} and Alaa Jabar Al-Manhel¹

¹Department of Food Science, College of Agriculture, University of Basrah, Iraq.

²Date Palm Research Centre, University of Basrah, Iraq.

*Corresponding author E-mail: alaa.niamah@uobasrah.edu.iq, Tel:009647709042069.



Abstract

This study aimed to investigate the impact of adding Zahdi date juice on the physicochemical characteristics and microbiological analysis of bio-yogurt with a probiotic starter (*St. thermophilus*, *Lb. acidophilus*, and *B. longum*) and haematological parameters in mice. The bio-yogurt was prepared by adding a 5% probiotic starter supplemented with date juice at 2, 5, and 10% (v/v). Hematological parameters: Mice (n = 30) were divided into five groups (Tc, T1, T2, T3, and T4) and treated with either a standard diet or a standard diet enriched with 3 ml/day of bio-yogurt containing 0, 2, 5, and 10% date juice for 30 days. The results showed that the addition of date juice increased the acidification rate, decreased pH values, and improved the syneresis of bio-yogurt. During the 21-day period, the viability of probiotic bacteria decreased, but a bio-yogurt sample containing 10% date juice had a higher number of probiotic bacteria than the other samples. The diet and body weight of the mice were affected by the daily administration of bio-yogurt or bio-yogurt with date juice. In addition, the groups treated with bio-yogurt, including date juice, typically had superior haematological parameters and better immunity levels than other groups ($P \leq 0.05$). Compared with the control group, mice administered bio-yogurt containing date juice showed reduced total cholesterol and triglyceride levels. In conclusion, the addition of date juice to the bio-yogurt improved the physicochemical properties and increased the viability of probiotic bacteria as well as improving the blood parameters of experimental animals.

Key words: Date juice, Bio-yogurt, Probiotic bacteria, blood parameters.

Introduction

In recent years, functional foods have attracted great attention because of their advantages in preventing various health problems and enhancing certain physiochemical functions, in addition to their nutritional value. Food probiotics are a type of functional food product that has been widely recognised and accepted by consumers (Zommiti *et al.*, 2020). While probiotics have been defined

by the WHO and FAO in 2001 as useful living microorganisms (lactic acid bacteria are the most common probiotics) that provide several health functions to the host when consumed in sufficient amounts, such as balancing the intestinal microbiota (**Khaneghah *et al.*, 2020**), and most dairy products are currently used as carriers for probiotic bacteria (**Zókwicz *et al.*, 2020**), the consumption of some products is limited due to lactose intolerance and an increase in vegetarians. **Elsanhoty and Ramadan (2018)** investigated the development and metabolism of probiotic starter in β -glucan enriched yogurt and found that adding barley- β -glucan increased probiotic survivability and stability during cold storage. Therefore, the preparation of non-dairy based probiotic products or products fortified with other natural ingredients, including cereals, vegetables, and fruits, has been further developed (**Aspri *et al.*, 2020**).

The date palm (*Phoenix dactylifera* L.) is the oldest cultivated tree with a consumption history of more than 7,000 years, and it originates in Arabian countries. It belongs to the Palmae or Areaceae families (**El-Juhany, 2010**). Iraq is one of the major producers in the world (735.4 tonnes in 2020), which has 11 million trees, resulting in the overproduction of dates and other date-based products. The date fruit and date products (including date syrup and date paste) have a unique functionality with excellent nutritional value due to digestible sugars such as fructose (22.5–47.5%), glucose (42.3–51.8%), sucrose (3.2–7.4%), proteins (1.8–30.0%), dietary fibres (2.2%), lipids, pectin, vitamins, and mineral salts (**Mia *et al.*, 2020**). Therefore, date fruits are widely used to produce different products such as jelly, jam, candy, and chutney. Date syrup is a natural sweetener that contains fructose and glucose and is utilized in various food products and cultural media as a carbon source (**Khassaf *et al.*, 2019**). **Aydeniz-Güneşer (2022)** reported the average production of date fruit reached 8.53 million metric tonnes in 2018, and the use of waste date manufacturing residues into food products instead of discarding them is crucial in preventing environmental

problems. Furthermore, given the importance of dates and their products in our lives, the use of date syrup as an additive in some probiotic dairy products such as milk (Hariri *et al.*, 2020), yoghurt (Haneen, 2019), and cheese (El-Loly *et al.*, 2021) has increased in recent years (Abdeen, 2018).

On the other hand, despite the great importance of date products, there are few local investigations into the suitability of incorporating Zahdi type date juice into yogurt formulations as a prebiotic. Therefore, the current study aimed to evaluate the influence of added date juice at different concentrations on yoghurt as a prebiotic to enhance the bioavailability of lactic acid bacteria and improve haematological and immunological parameters *in vivo*.

2. Materials and methods

2.1. Materials

Dates (Zahdi) were obtained from a local market in Basrah, Iraq, and raw cow milk was obtained from the Agricultural Research Station, College of Agriculture, University of Basrah. All chemicals used in this study were of analytical grade and were obtained from OXOID, Merck, BDH (England), and Difco (USA).

2.2. Bacteria Strains

Streptococcus thermophilus (ST) was obtained from Sacco Company in Italy. *Bifidobacterium longum* (BB-536) and *Lactobacillus acidophilus* (La-5) were purchased from Chr. Hansen Company (Denmark). *Lactobacillus acidophilus* and *Bifidobacterium longum* cultures were activated in MRS broth (HiMedia, India) for 18–24 h at 37°C under anaerobic conditions, whereas *Streptococcus thermophilus* cultures were activated in M17 broth (HiMedia, India) for 24 h at 42°C under aerobic conditions.

2.3. Date juice preparation

Date juice was made from Zahdi dates. Date fruits were thoroughly cleaned to remove dust and other extraneous contaminants. Manual splitting was performed to separate the seeds from the fruit. To increase the amount of resources extracted, the mixture was heated at 85 °C for 3 hours. The extracted resources were separated by a centrifuge at 7000 RPM for 15 minutes and then filtered by passing filter paper through Whatman No. 1 (Whatman Limited Company, UK). Date juice extract is used as a prebiotic in yogurt production (**Al-Roomi and Al-Sahlany, 2022**). The extracted date juice was analyzed for total soluble solids (TSS) using a digital refractometer (Abbe 60 Refractometer, UK). Moisture, total sugar, reducing sugar, total protein, total fat, ash, the titratable acidity (as citric acid), pH, and ascorbic acid were determined by (**Nielsen , 2010**).

2.4. Bio-yogurt preparation

The bio-yogurt was prepared as follows: whole cow milk was divided into four groups in glass cups and mixed with 0, 2, 5, and 10% (v:v) date juice extract. The mixture was heated at 80 °C for 15 minutes (**Amerinasab et al., 2015**). After Subsequently, a cold incubation temperature (37°C) follows. The first group was inoculated with 5% (10^9 CFU/g) of probiotic starter (ST, La-5, and BB-535, 1:1:1) and served as a control yogurt. The second, third, and fourth groups were inoculated with 5% (10^9 CFU/g) of probiotic starter. The fermentation of bio-yogurt samples was stopped after 37°C incubation when they reached a pH of 4.6-4.8, and they were stored at 4°C for 21 days (**Niamah et al., 2016**). The bio-yogurt samples were examined every 1, 7, 14, and 21 days to perform physicochemical and microbiological tests.

Bio-yogurt analysis

pH and titratable acidity of bio-yogurt

The pH of the bio-yogurt sample was evaluated using a pH meter (SD300-Garmany), and the titratable acidity (TA) percentage was determined using 0.5 N NaOH with 0.5 ml phenolphthalein as an indicator, and is expressed as a percentage of lactic acid (Niamah, 2017).

Susceptibility to syneresis of bio-yogurt

Fifty grams of bio-yogurt was spread evenly on the Whatman No.1 filter paper in a funnel. The volume of bio-yogurt serum filtered (ml) was measured and expressed as the rate of syneresis after 6 h at 20°C (Al-Manhel & Niamah, 2017).

$$\text{Syneresis\%} = (\text{weight of whey} / \text{weight of yoghurt}) \times 100$$

Survival of starter bacteria

On days 1, 7, 14, and 21 of storage at 4°C, total lactic acid bacteria, La-5, BB-536, and ST in bio-yogurt samples were counted on MRS-CaCO₃ agar, MRS-sorbitol agar, MRS-NNLP agar, and M17-agar, respectively, and incubated at 37°C for 24-48 hours under anaerobic conditions. The number of colony-forming units (CFU) was calculated on plates containing 30-300 colonies. The number of lactic acid bacteria was calculated as log CFU/g of bio-yogurt (Niamah *et al.*, 2017).

Animal experiments and diets

For the *in vivo* study, 30 male BALB/c mice (10 weeks old, weighing 23-27 g) were purchased from the College of Veterinary Medicine, University of Basrah. Prior to use, all mice were checked for health and acclimated to the laboratory environment for 10 days. With a 12 h: 12 h light: dark photoperiod, the temperature was maintained at approximately 25±2°C and the relative humidity was approximately 50%. The five treatment groups (six mice each) were housed in five plastic cages with feeding and drinking sections. Each group was fed one diet during the study period of 30 days. The diets were as follows: treatment control (Tc) on a standard diet (containing 5% fat);

T1, T2, T3, and T4 on a standard diet plus 3 ml/day of bio-yoghurt containing 0, 2, 5, and 10% date juice. Diet intake was measured every two days, and the weight increase was tracked for six days (Evivie *et al.*, 2019). Changes in dietary intake or body weight were calculated using the following formula:

$$\text{Changes (\%)} = (A-B/B) \times 100$$

where A is initial dietary intake or body weight. B is final dietary intake or body weight.

Hematological parameters

After 30 days of feeding, blood samples were obtained from the retro-orbital plexus of all 12-hour fasted animals (mice) under anaesthesia with ketamine/xylazine (65:10 mg/kg IP). Heparinized tubes were used to collect blood samples and to estimate the red blood cell count (RBC), white blood cell count (WBC), haemoglobin (HB), platelet count test (PLT), basophils (BASO), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (ESO), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean platelet volume (MPV), platelet distribution width (PDW) and procalcitonin (PCT) using the haematological analyzer (CEE-DYN EM-2200, France).

Blood Serum Tests

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and total protein (TP) were measured using the Biolabo company kit, France.

Statistical analysis

Three replicates were used for the analysis. The results of this study were evaluated using one-way analysis of variance (ANOVA), and values were reported as mean standard deviation (\pm SD) using Gen Stat v6.0 software. P was set at $P < 0.05$.

Results and discussion

Date juice ingredients

Zahdi date juice has a total soluble solids content of 37.54%, a moisture content of 62.45%, a total sugar content of 32.15%, a reducing sugar content of 28.31%, a total protein content of 1.32%, a total fat content of 1.95%, an ash content of 2.12%, and ascorbic acid (15.00 mg/100 ml). The titratable acidity was 0.11% and the pH was 6.12 (**Table 1**). The date type and water-to-date ratio are clearly critical parameters in solid extraction for both technical and economic reasons. A high ratio will assist in rapid and thorough extraction. Fructose and glucose are the two main sugars present in date syrups. Compared to sucrose, these sugars offer more health benefits, are natural, and have a higher sweetness level. A previous study cooked date pulp for 20 minutes before combining it in a 1/3 ratio with heated water (80–85°C) to extract 96% of the sugar (**Ramadan, 1988**).

Bio-yoghurt analysis

pH and total acidity

Figure 1 shows the pH values and total acidity (%) of bio-yoghurt samples containing 0, 2, 5, and 10% date juice. Furthermore, the increase in total acidity or decrease in pH found in the fortified bio-yoghurt was higher than that found in the control sample. Increased date juice concentration resulted in a decrease in pH and an increase in total acidity throughout storage. Thus, the sugar in date juice (glucose and fructose) is a substrate for lactic acid bacteria. The acidification rate of bio-

yogurt prepared from cow's milk was accelerated. Furthermore, this indicates the activation of probiotic bacteria by adding date juice to yogurt samples during storage. These results are in agreement with those of **Al-Otaibi and El-Demerdash (2013)**. The acidity levels of fermented camel milk supplemented with 1, 2.5, and 5% date depis were higher than those of the control. Another study found that supplementing cow's milk to make bio-yogurt with 10 and 15% (w/w) rutub pieces of dates resulted in an increase in acidity and a decrease in pH (**Ismail, 2021**).

Syneresis of bio-yogurt

Syneresis is the determination of serum release from bio-yogurt gel when subjected to centrifugal force and is used to determine yogurt quality and the gel's water-holding capacity. **Table 2** shows the syneresis of bio-yogurts during storage. During the 21-day storage period, changes in the degree of syneresis were observed in all the samples. The addition of date juice, on the other hand, resulted in a decrease in syneresis. The lower syneresis rate of bio-yogurt containing date juice may be attributed to the increased water-holding capacity of date juices containing a high concentration of chemicals capable of binding with water, such as fiber. Increasing the concentration of date juice enhanced water-holding capacity and decreased syneresis.

During storage (1, 7, 14, and 21 days), the syneresis of bio-yogurt containing 10% date juice was 1.75, 1.66, 1.48, and 1.31, respectively, whereas that of the control sample (without date juice) was 3.11, 3.95, 4.53, and 4.29, respectively. Many studies have reported a lower rate of yogurt syneresis when plant juice is added (**Cakmakci et al., 2014; Dimitrellou et al., 2020; Zhao et al., 2021**). When 1-9 % of date liquid sugar was added, the rate of yoghurt syneresis increased, resulting in an increased water-holding capacity while decreasing whey syneresis values (**Amerinasab et al., 2015**).

Survival of starter bacteria

The survival of the bacterial starter in bio-yogurt samples during storage is shown in Figure 2. LAB, ST, La-5, and BB-536 counts of bio-yogurt without date juice (control sample) and other samples tended to decrease over the storage time, but when compared to other samples on days 1 and 7, the control sample was found to have similar numbers of bacterial starters. However, by the end of storage, bacterial starter counts in all bio-yogurts had reached different levels. The increase in probiotic bacteria counts may be attributed to the addition of date juice compared to the control sample. Date juice contains simple carbohydrates such as prebiotic compounds, which have been reported to stimulate the growth of probiotics (Al-Thubiani & Khan, 2017; Mirzaei *et al.*, 2020). The addition of 10% date juice to yogurt resulted in higher counts of *Lactobacillus acidophilus* (La-5) and *Bifidobacterium longum* (BB-536) compared with the control sample. After 21 days, the log. of probiotic bacteria (La-5 and BB-536) counts in bio-yogurt containing 10% date juice were 7.04 and 6.51 CFU/g, respectively, compared to 5.75 and 5.17 CFU/g in yoghurt with 0% date juice (control sample) (Figure 2 C and D). As a result, the highly acidic environment and inherent antibacterial activity of the accumulated organic acids influence the survival of probiotic bacteria. Lactobacilli can withstand and survive in food products with a pH of 3.7 to 4.3, better than bifidobacteria (Patel, 2017).

The influence of lactose hydrolysis and an increase in total acidity on the total LAB count was only observed within 14 days of storage. A value of 7.42 log CFU/g was observed in the control sample compared to 8.04, 8.52, and 8.87 log CFU/g found in bio-yogurt containing 2, 5, and 10% of date juice, respectively (Figure 2 A). According to Matijević *et al.* (2011), there is a link between lactose hydrolysis and the number of probiotic bacteria. These disparate results might indicate that the growth of bacterial cultures is dependent on the strain used and fermentation conditions (Niamah, 2019). When date juice is added to yoghurt, the quantity of probiotic bacteria

remains at a level beneficial to human health. WHO/FAO (2006) set the requirement that any food claiming to have a probiotic effect must contain at least 10^6 to 10^7 CFU/mL of live probiotic bacteria.

The effect of bio-yogurt on diet intake and animals weight

The average food intake rate was computed over a 48-hour period to determine whether there was any change in the amount of food consumed. When the data was analysed (**Table 3**), a substantial difference was observed in the mean value of food consumption between the control and experimental groups. Changes in diet intake (%) were 20.4, 14.1, 12.6, 12.6 and 10.1 for Tc, T1, T2, T3 and T4, respectively. Interestingly, compared with the control group, food intake tended to decrease as the ratio of date juice concentrations increased in bio-yogurt. At the start of the research, as compared to the control group, the bio-yogurt containing 10% date juice group exhibited lower food intake. While there was no significant difference in the initial body weight among all groups, after 30 days of bio-yoghurt dosing, the changes in weight gain were significantly higher in the T1, T2, T3, and T4 groups than in the control group (**Table 3**). Changes in weight (%) were 7.9, 10, 9.9, 10.4 and 10.9 for Tc, T1, T2, T3 and T4, respectively. Several studies have shown that long-term feeding of mice with normal diets supplemented with carbohydrates, such as prebiotic compounds, by 5-15% increases body weight (**Fabersani *et al.*, 2018; Sengupta *et al.*, 2019; El-Naggat *et al.*, 2020**). The bio-yogurt containing 10% date juice group gained the most weight, which might be related to the high sugar content and bacterial mass of the diet.

The effect of bio-yogurt with date juice on haematologic parameters

Table 4 shows the results of the haematological tests. Except for the MPV values, there was a significant difference in all test results between the control and treatment groups. Compared to the control sample and other experimental groups, the haematological parameter values significantly increased with an increase in date juice content in the bio-yogurt samples. In general, adding date juice to bio-yogurt treatments resulted in better results than the control sample and bio-yogurt without date juice sample (T1). In RBCs, for example, the TC, T1, T2, T3, and T5 groups gave readings (reported as $10^{12}/L$) of 3.9, 4.2, 4.5, 4.9, and 5.1, respectively, whereas the WBC values (reported as $10^9/L$) were 5.5, 6.3, 7.2, 7.3, and 7.8, respectively. The HB (g/L) value of the bio-yogurt containing a 10% date juice sample (T4) was 15, compared to 11 for the control sample (TC) and 12 for the bio-yogurt without a date juice sample (T1).

In general, foods containing probiotics increase immunoglobulin and other immune markers in the host (Evivie *et al.*, 2019). Improvements in the haematological criteria of experimental mice, as well as the standard of immunity, were also confirmed after feeding on food with probiotic bacteria (Niamah *et al.*, 2017). These recent *in vivo* discoveries, as well as the findings of this study, support the idea that beneficial bacterial strains with prebiotic compounds such as date juice might be included in food formulations to improve the health and well-being of consumers.

Because dates are high in carbohydrates, tannins, and minerals, and because they have antioxidant, antimutagenic, and free radical-scavenging properties (Kulkarni *et al.*, 2010), adding date juice to the yoghurt increased the nutritional value of the final product.

The effect of bio-yogurt with date juice on biochemical blood serum parameters

The consumption of bio-yogurt with 2, 5, and 10% of date juice (T2, T3, and T4) for 30 days reduced classical health parameters in mice, such as total cholesterol, triglycerides, and total

protein serum levels in comparison to the control and T1 groups, whereas the glucose level increased in T4 in comparison to the other groups (**Table 5**). After 30 days of dosing, the HDL value in the T3 and T4 groups increased to 55 (mg/dL) compared with the control sample (TC), which was 48 (mg/dL).

Increased cholesterol and triglyceride levels have been linked to the development of coronary heart disease (**Karamali *et al.*, 2016**). As a result, there is an increasing interest in probiotic foods that do not influence food intake while still improving lipid and glucose metabolism, as demonstrated in the current study using bio-yogurt with date juice. It is thought that if bio-yogurt consumption lowers these indicators in healthy animals, they may also be decreased when blood levels are raised (**Rerksuppaphol & Rerksuppaphol, 2015**). There has been no previous research on the impact of date juice yogurt on blood lipid profiles. In non-diabetic patients with mild to moderate hypertriglyceridemia, probiotic strains (*Lactilactobacillus curvatus* HY7601 and *Lactiplantibacillus plantarum* KY1032) resulted in an 18% decrease in blood triglyceride levels (**Ahn *et al.*, 2015**). Restoration of the gut barrier function by colonization has been linked to the improvement of glycemic and lipid indices by probiotic strains (**Wang *et al.*, 2020**).

Conclusion

Science and industry are responding to growing consumer knowledge about health and the role of food in promoting quality of life by continuously developing new functional foods. Dates and date juices are the major sources of monosaccharides, invert sugars, and fibre. The use of date juice in the manufacture of bio-yogurt improved the physicochemical characteristics and enhanced the survivability of probiotic bacteria, which promoted the growth of beneficial colon microbes.

In general, diet intake, body weight, haematological, and serum biochemical parameters generally improved in animals fed bio-yogurt containing date juice-treated diets, and in some instances, the T4 group, which was fed bio-yogurt containing 10% date juice, was significantly superior to the other treatments. The results for bio-yogurt containing a date juice sample were better than those for the control samples or bio-yogurt without a date juice sample for all *in vitro* and *in vivo* parameters evaluated. We propose using date juice in bio-yogurt as a probiotic bacteria carrier meal and functional food.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Abdeen, E. S. M. M. (2018). Enhancement of Functional Properties of Dairy Products by Date Fruits. *Egypt. J. Food.*, 46:197- 206 .
2. Ahn, H. Y., Kim, M., Chae, J. S., Ahn, Y. T., Sim, J. H., Choi, I. D., ... & Lee, J. H. (2015). Supplementation with two probiotic strains, *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032, reduces fasting triglycerides and enhances apolipoprotein AV levels in non-diabetic subjects with hypertriglyceridemia. *Atherosclerosis*, 241(2), 649-656.
3. Al-Manhel, A. J., & Niamah, A. K. (2017). Mannan extract from *Saccharomyces cerevisiae* used as prebiotic in bio-yogurt production from buffalo milk. *International Food Research Journal*, 24(5), 2259-2264.
4. Al-Otaibi, M. M., & El-Demerdash, H. (2013). Nutritive value and characterization properties of fermented camel milk fortified with some date palm products chemical,

- bacteriological and sensory properties. *International Journal of Nutrition and Food Sciences*, 2(4), 174.
5. Al-Roomi, F. W., & Al-Sahlany, S. T. (2022). Identification and Characterization of Xanthan Gum Produced from Date Juice by a Local Isolate of Bacteria *Xanthomonas campestris*. *Basrah Journal of Agricultural Sciences*, 35(1), 35-49. <https://doi.org/10.37077/25200860.2022.35.1.03>
 6. Al-Thubiani, A. S., & Khan, M. S. A. (2017). The prebiotic properties of date palm (*Phoenix dactylifera* L.) seeds in stimulating probiotic lactobacillus. *Journal of Pure and Applied Microbiology*, 11(4), 1675-1686.
 7. Amerinasab, A., Labbafi, M., Mousavi, M., & Khodaiyan, F. (2015). Development of a novel yoghurt based on date liquid sugar: physicochemical and sensory characterization. *Journal of food science and technology*, 52(10), 6583-6590.
 8. Amerinasab, A., Labbafi, M., Mousavi, M., & Khodaiyan, F. (2015). Development of a novel yoghurt based on date liquid sugar: physicochemical and sensory characterization. *Journal of food science and technology*, 52(10), 6583-6590.
 9. Aspri, M., Papademas, P., & Tsaltas, D. (2020). Review on non-dairy probiotics and their use in non-dairy based products. *Fermentation*, 6(1), 30.
 10. Aydeniz-Güneşer, B. (2022). Valorization of date palm (*Phoenix dactylifera*) wastes and by-products. In *Mediterranean Fruits Bio-wastes* (pp. 391-402). Springer, Cham.
 11. Cakmakci, S., Tahmas-Kahyaoglu, D., Erkaya, T., Cebi, K., & Hayaloglu, A. A. (2014). β -Carotene contents and quality properties of set type yoghurt supplemented with carrot juice and sugar. *Journal of Food Processing and Preservation*, 38(3), 1155-1163.

12. Dimitrellou, D., Solomakou, N., Kokkinomagoulos, E., & Kandyliis, P. (2020). Yogurts supplemented with juices from grapes and berries. *Foods*, 9(9), 1158.
13. El-Juhany, L.I. (2010). Degradation of Date Palm Trees and Date Production in Arab Countries: Causes and Potential Rehabilitation. *Australian Journal of Basic and Applied Sciences*, 4(8): 3998-4010,
14. El-loly, M.M.; Farahat, E.S.A. and Mohamed, A.G. (2021). Novel Approach for Producing Processed Cheese Fortified with Date Syrup. *ACS Food Sci. Technol.* <https://doi.org/10.1021/acsfoodscitech.1c00012>.
15. El-Naggar, M. E., Hussein, J., El-sayed, S. M., Youssef, A. M., El Bana, M., Latif, Y. A., & Medhat, D. (2020). Protective effect of the functional yogurt based on Malva parviflora leaves extract nanoemulsion on acetic acid-induced ulcerative colitis in rats. *Journal of Materials Research and Technology*, 9(6), 14500-14508.
16. Elsanhoty, R. M., & Ramada, M. F. (2018). Changes in the physicochemical and microbiological properties of probiotic-fermented low-fat yoghurt enriched with barley β -glucan during cold storage. *Mljekarstvo/Dairy*, 68(4) 295-309.
17. Evivie, S. E., Abdelazez, A., Li, B., Bian, X., Li, W., Du, J., ... & Liu, F. (2019). In vitro organic acid production and in vivo food pathogen suppression by probiotic *S. thermophilus* and *L. bulgaricus*. *Frontiers in microbiology*, 10, 782.
18. Fabersani, E., Grande, M. V., Aráoz, M. V. C., Zannier, M. L., Sánchez, S. S., Grau, A., ... & Honoré, S. M. (2018). Metabolic effects of goat milk yogurt supplemented with yacon flour in rats on high-fat diet. *Journal of Functional Foods*, 49, 447-457.
19. Haneen, H. M. (2019). Physicochemical, microbiological and sensory evaluation of yogurt prepared with date paste. *Asian Journal of Applied science and Technology*, 3(1), 234Y48.

20. Hariri, A., Ouis, N., Ibri, K., Bouhadi, D., & Benatouche, Z. (2020). Technological characteristics of fermented milk product manufactured by milk-dates mixtures. *Acta Agriculturae Serbica*, 25(49), 27-35.
21. Ismail, M. M. (2021). Improvement of nutritional and healthy values of yoghurt by fortification with rutub date. *Journal of Microbiology, Biotechnology and Food Sciences*, 2021, 398-406.
22. Karamali, M. A. R. Y. A. M., Dadkhah, F., Sadrkhanlou, M., Jamilian, M., Ahmadi, S., Tajabadi-Ebrahimi, M., ... & Asemi, Z. (2016). Effects of probiotic supplementation on glycaemic control and lipid profiles in gestational diabetes: a randomized, double-blind, placebo-controlled trial. *Diabetes & metabolism*, 42(4), 234-241.
23. Khaneghah, A. M., Abhari, K., Eş, I., Soares, M. B., Oliveira, R. B., Hosseini, H., ... & Sant'Ana, A. S. (2020). Interactions between probiotics and pathogenic microorganisms in hosts and foods: A review. *Trends in Food Science & Technology*, 95, 205-218.
24. Khassaf, W. H. ., Niamah, A. K., & Al-Manhel, A. J. A. . (2019). Study of the optimal conditions of levan production from a local isolate of *Bacillus subtilis* subsp. *subtilis* w36. *Basrah Journal of Agricultural Sciences*, 32(2), 213–222. <https://doi.org/10.37077/25200860.2019.211>
25. Kulkarni, S. G., Vijayanand, P., & Shubha, L. (2010). Effect of processing of dates into date juice concentrate and appraisal of its quality characteristics. *Journal of food science and technology*, 47(2), 157-161.
26. Matijević, B., Lisak, K., Božanić, R., & Tratnik, L. (2011). Impact of enzymatic hydrolyzed lactose on fermentation and growth of probiotic bacteria in whey. *Mljekarstvo*, 61(2), 154.

27. Mia, A.; Mosaib, G.; Khalil, I.; Islam, A. and Gan, S.H. (2020). Potentials and Safety of Date Palm Fruit against Diabetes: A Critical Review. *Foods* 2020, 9, 1557; doi:10.3390/foods9111557.
28. Mirzaei, D., Nia, A. P., & Jalali, M. (2020). Effect of inulin and date syrup from Kaluteh variety on the qualitative and microbial properties of prebiotic ketchup. *Journal of Food Science and Technology*, 1-12.
29. Niamah, A. (2019). Ultrasound treatment (low frequency) effects on probiotic bacteria growth in fermented milk. *Future of Food: Journal on Food, Agriculture and Society*, 7(2), Nr-103.
30. Niamah, A. K. (2017). Physicochemical and microbial characteristics of yogurt with Added *Saccharomyces boulardii*. *Current Research in Nutrition and Food Science Journal*, 5(3), 300-307.
31. Niamah, A. K., Al-Sahlany, S. T. G., & Al-Manhel, A. J. (2016). Gum Arabic uses as prebiotic in yogurt production and study effects on physical, chemical properties and survivability of probiotic bacteria during cold storage. *World applied sciences journal*, 34(9), 1190-1196.
32. Niamah, A. K., Sahi, A. A., & Al-Sharifi, A. S. (2017). Effect of feeding soy milk fermented by probiotic bacteria on some blood criteria and weight of experimental animals. *Probiotics and antimicrobial proteins*, 9(3), 284-291.
33. Nielsen, S. S. (Ed.). (2010). *Food analysis laboratory manual* (p. 557). Springer Nature Switzerland.
34. Patel, A. R. (2017). Probiotic fruit and vegetable juices-recent advances and future perspective. *International Food Research Journal*, 24(5).

35. Peng, M., Tabashsum, Z., Anderson, M., Truong, A., Houser, A. K., Padilla, J., ... & Biswas, D. (2020). Effectiveness of probiotics, prebiotics, and prebiotic-like components in common functional foods. *Comprehensive Reviews in Food Science and Food Safety*, 19(4), 1908-1933..
36. Ramadan, B. R. (1998, March). Preparation and evaluation of Egyptian date syrup. In *First international conference on date palm. Al-Ain* (pp. 8-10).
37. Rerksuppaphol, S., & Rerksuppaphol, L. (2015). A randomized double-blind controlled trial of *Lactobacillus acidophilus* plus *Bifidobacterium bifidum* versus placebo in patients with hypercholesterolemia. *Journal of clinical and diagnostic research: JCDR*, 9(3), KC01.
38. Sengupta, S., Koley, H., Dutta, S., & Bhowal, J. (2019). Hepatoprotective effects of synbiotic soy yogurt on mice fed a high-cholesterol diet. *Nutrition*, 63, 36-44.
39. Wang, L., Shang, Q., Guo, W., Wu, X., Wu, L., Wu, L., & Chen, T. (2020). Evaluation of the hypoglycemic effect of probiotics via directly consuming glucose in intestines of STZ-induced diabetic mice and glucose water-induced diabetic mice. *Journal of Functional Foods*, 64, 103614.
40. WHO/FAO. 2006. "Probiotics In Food Health And Nutritional Properties And Guidelines For Evaluation. Rome: Food And Agriculture Organization Of The United Nations". Fao.Org. <http://www.fao.org/3/a-a0512e.pdf>
41. Zhao, J. L., Ma, C. M., Zhao, X. H., & Zhang, Q. (2021). Effects of yam (*Dioscorea opposita* Thunb.) juice on fermentation and textural attributes of set-style skimmed yoghurt. *Journal of Food Measurement and Characterization*, 15(3), 2220-2230.

42. Zólkiewicz, J.; Marzec, A.; Ruszczynski, M. and Feleszko, W. (2020). Postbiotics-A Step Beyond Pre- and Probiotics. *Nutrients* 2020, 12, 2189; doi:10.3390/nu12082189.

43. Zommiti, M.; Feuilleley, M.G.J. and Connil, N. (2020). Update of Probiotics in Human World: A Nonstop Source of Benefactions till the End of Time. *Microorganisms*. 8, 1907; doi:10.3390/microorganisms8121907.

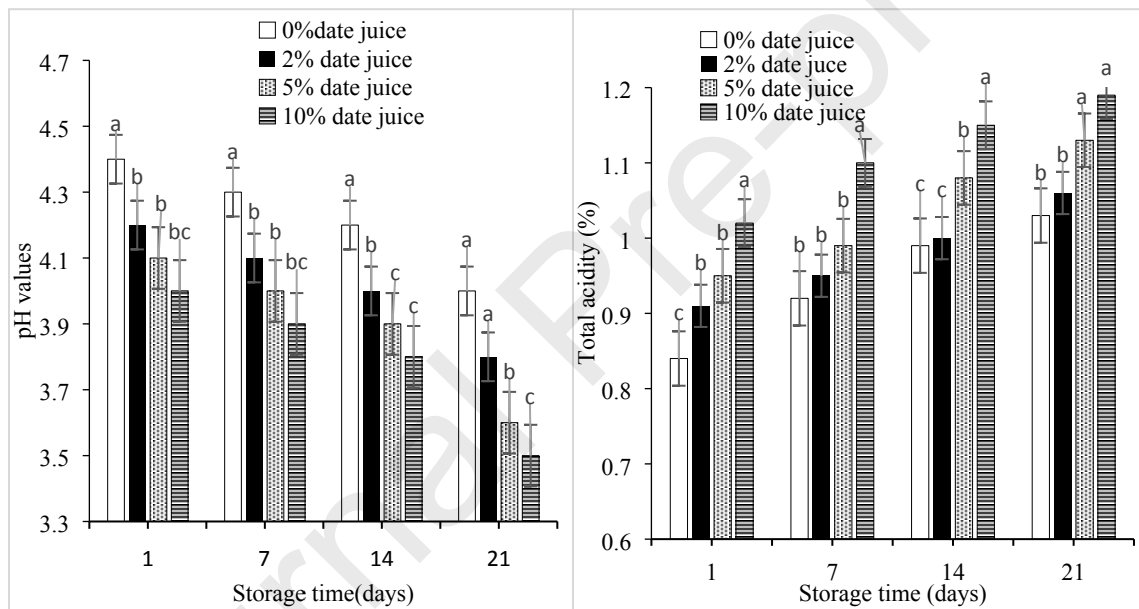
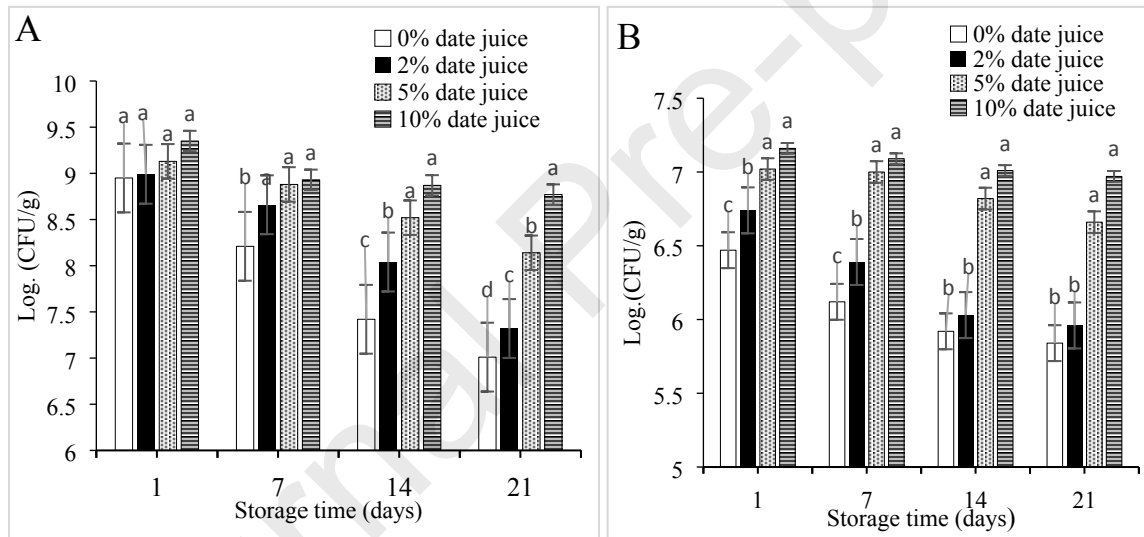


Figure 1. pH values and total acidity (TA) percentage of bio-yogurt containing 0, 2, 5 and 10 % of date juice. ^{a-c} Different small letters of bio-yogurt samples were significantly different ($p < 0.05$).



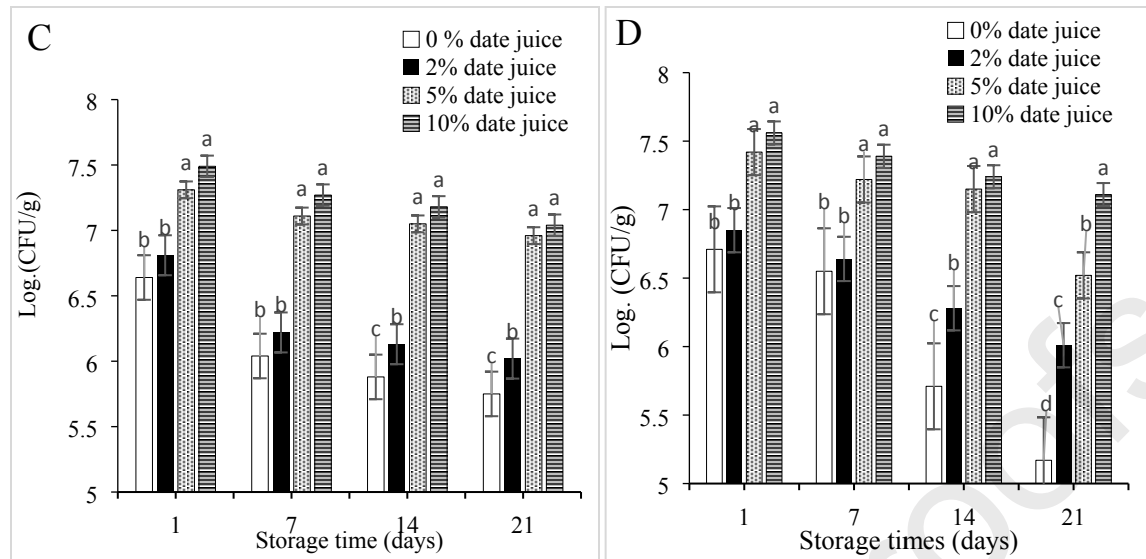


Figure 2. Lactic acid bacteria count of bio-yogurt sample during storage at 4°C, (A) total Lactic acid bacteria, (B) *Streptococcus thermophilus* (ST), (C) *Lactobacillus acidophilus* (La-5), (D) *Bifidobacterium longum* (BB-536). ^{a-d} Different small letters of bio-yoghurt samples were significantly different (p < 0.05).

Table 1. Chemical composition* of Zahdi date syrup

Chemical elements	value
Total soluble solid (%)	37.54±1.21
Moisture (%)	62.45±2.15
Total sugar (%)	32.15±1.18
Reducing sugar (%)	28.31±1.52
Total protein (%)	1.32±0.06
Total fat (%)	1.95±0.01
Ash (%)	2.12±0.05

Total acidity (%)	0.11±0.03
pH	6.23±0.12
Ascorbic acid (mg / 100g)	15.00 ±0.51

* The mean of three replicates. ±SD: standard deviation.

Table 2. Syneresis (%) of bio-yogurts during storage times at 4°C

Date juice concentrates (%)	Syneresis (%) [*]			
	1 day	7 day	14 day	21 day
0	3.11 ^a ±0.23	3.95 ^a ±0.41	4.53 ^a ±0.56	4.29 ^a ±0.72
2	3.00 ^a ±0.51	3.46 ^b ±0.11	3.30 ^b ±0.61	3.21 ^b ±0.33
5	1.96 ^b ±0.18	1.86 ^c ±0.25	1.50 ^c ±0.37	1.43 ^c ±0.31
10	1.75 ^c ±0.28	1.66 ^d ±0.19	1.48 ^c ±0.22	1.22 ^d ±0.10

* The mean of three replicates. ^{a-d} Different small letters of yoghurt samples were significantly different ($p < 0.05$).
±SD: standard deviation.

Table 3. Diet intake and body weight of healthy male mice at the start and end of the dosing experiment with bio-yogurt and bio-yogurt containing 2, 5, and 10% date juice.

Parameter (g)*		TC	T1	T2	T3	T4
Diet intake	Initial	122.0 ^a ±5.13	120.0 ^a ±4.77	118.0 ^a ±6.03	111.0 ^b ±3.29	108.0 ^b ±4.95
	Final	147.0 ^a ±7.11	137.0 ^b ±3.97	133.0 ^b ±5.23	125.0 ^c ±4.85	119.0 ^c ±5.08
	Changes (%)	20.4 ^a ±1.15	14.1 ^b ±0.97	12.7 ^c ±1.10	12.6 ^c ±1.03	10.1 ^c ±1.63
Body weight	Initial	150.5 ^a ±6.81	145.0 ^a ±5.74	147.1 ^a ±7.10	146.6 ^a ±5.63	148.3 ^a ±7.19
	Final	162.4 ^a ±6.58	159.6 ^a ±7.19	161.8 ^a ±7.04	161.9 ^a ±6.91	164.6 ^a ±8.14

Changes (%)	7.9 ^b ±0.13	10.0 ^a ±5.13	9.9 ^a ±5.13	10.4 ^a ±5.13	10.9 ^a ±5.13
--------------------	------------------------	-------------------------	------------------------	-------------------------	-------------------------

* The mean of three replicates. ^{a-c} Different small letters of yoghurt samples were significantly different ($p < 0.05$).

±SD: standard deviation.

Table 4. The effects of 30-days feeding of bio-yogurt and bio-yogurt containing 0, 2, 5 and 10 % date juice on haematological parameters of mice

Parameters*	TC	T1	T2	T3	T4
RBC ×10¹² /L	3.9 ^c ±0.12	4.2 ^b ±0.05	4.5 ^a ±0.20	4.9 ^a ±0.11	5.1 ^a ±0.18
WBC×10⁹/ L	5.5 ^d ±0.13	6.3 ^c ±0.10	7.2 ^b ±0.12	7.3 ^a ±0.22	7.8 ^a ±0.25
HB(g/L)	11.0 ^d ±0.15	12.0 ^c ±0.20	13.0 ^b ±0.21	15.0 ^a ±0.11	15.0 ^a ±0.17
PLT×10⁹/L	160.0 ^e ±3.23	173.0 ^d ±2.50	199.0 ^c ±1.29	243.0 ^b ±2.00	255.0 ^a ±3.13
BASO (%)	0.1 ^c ±0.05	0.3 ^b ±0.02	0.4 ^b ±0.01	0.4 ^b ±0.00	0.6 ^a ±0.03
NEU (%)	50.0 ^d ±2.00	61.0 ^c ±1.50	64.0 ^b ±1.66	65.0 ^b ±0.95	66.0 ^a ±1.03
LYM (%)	25.0 ^d ±0.28	30.0 ^c ±0.55	33.0 ^b ±0.43	34.0 ^b ±0.39	38.0 ^a ±0.65
MON×10³μL	4.1 ^d ±0.41	5.8 ^c ±0.27	5.2 ^c ±0.53	7.5 ^b ±0.37	8.1 ^a ±0.67
ESO	0.7 ^c ±0.01	1.9 ^b ±0.06	2.3 ^a ±0.13	2.5 ^a ±0.22	2.6 ^a ±0.08
MCH (Pg)	29.0 ^b ±0.93	30.0 ^b ±0.75	31.0 ^b ±0.88	31.0 ^b ±0.43	33.0 ^a ±1.11
MCV (fL)	82.0 ^c ±0.97	88.0 ^d ±1.23	90.0 ^c ±0.93	93.0 ^b ±1.12	97.0 ^a ±1.03
MCHC (g/L)	327.0 ^c ±1.88	333.0 ^b ±2.23	338.0 ^b ±1.95	340.0 ^b ±1.18	349.0 ^a ±3.14

MPV (fL)	7.5 ^a ±0.03	7.5 ^a ±0.13	7.6 ^a ±0.16	7.5 ^a ±0.22	7.5 ^a ±0.15
PDW	15.7 ^b ±0.79	16.2 ^b ±0.91	16.5 ^a ±0.83	16.4 ^a ±0.83	16.9 ^a ±1.01
PCT (%)	0.15 ^c ±0.08	0.13 ^c ±0.40	0.18 ^b ±0.20	0.23 ^a ±0.11	0.23 ^a ±0.03

* The mean of three replicates. ^{a-d} Different small letters of yoghurt samples were significantly different ($p < 0.05$).

±SD: standard deviation.

Table 5. The effects of 30-days feeding of bio-yogurt and bio-yogurt containing 0, 2, 5 and 10 % date juice on biochemical blood serum parameters of mice

Parameters*	TC	T1	T2	T3	T4
TC (mg/dL)	145.0 ^a ±3.62	141.0 ^a ±4.45	137.0 ^a ±4.66	136.0 ^a ±5.94	131.0 ^b ±3.88
TG (mg/dL)	108.0 ^a ±2.24	100.0 ^a ±3.16	93.0 ^b ±2.55	90.0 ^b ±2.96	85.0 ^b ±2.11
HDL (mg/dL)	48.0 ^b ±1.44	50.0 ^a ±1.53	52.0 ^a ±2.15	55.0 ^a ±2.03	55.0 ^a ±1.28
LDL (mg/dL)	75.4 ^a ±2.97	72.0 ^a ±2.83	66.4 ^b ±3.24	63.0 ^b ±2.98	59.0 ^c ±3.04
VLDL (mg/dL)	21.6 ^a ±1.22	20.0 ^a ±1.10	18.6 ^b ±2.00	18.0 ^b ±2.15	17.0 ^b ±1.86
TP (mg/dL)	5.7 ^a ±0.11	5.6 ^a ±0.10	5.5 ^a ±0.11	5.5 ^a ±0.09	5.5 ^a ±0.13
Glucose (mg/dL)	135.0 ^a ±3.29	128.0 ^{ab} ±4.11	130.0 ^b ±3.90	132.0 ^a ±3.67	136.0 ^a ±4.71

* The mean of three replicates. ^{a-c} Different small letters of yoghurt samples were significantly different ($p < 0.05$).

±SD: standard deviation.