Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition

Shahid Sadoughi University of Medical Sciences School of Public Health Nutrition Department

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2026; 11(2): 226-235 Website: jnfs.ssu.ac.ir

Fortification of Functional Yogurt with Dragon Fruit (Hylocereus polyrhizus) Pulp: A Nutritional Approach

Khondoker Moazzem Hossain; PhD[1], Narayon Chandro Sarker Nirob; MSc[*2], Anika Maharin Roza; MSc[2] & Nazmul Rahman Chowdhury; MSc[2]

[1] *Department of Genetic Engineering and Biotechnology, Daffodil International University, Daffodil Smart City (DSC), Birulia, Savar, Dhaka-1216, Bangladesh;* [2] *Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh.*

ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received: 22 May 2025
Revised: 12 Oct 2025
Accepted: 21 Nov 2025

****Corresponding author:***
nirobsarker488@gmail.com
Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh

Postal code: 9208
Tel: +880 1713638498

Keywords
Fortified yogurt; Dragon fruit pulp; Antioxidant properties; Antibacterial properties; Functional food.

ABSTRACT

Background: The incorporation of fruit pulp into yogurt has created a new dimension in the field of functional foods. Dragon fruit (*Hylocereus polyrhizus*) is a highly nutrient rich food which offers a range of health benefits, enriched in water-soluble fiber, vitamin C and antioxidants. The present study was aimed to evaluate antioxidant and antibacterial activities of both fresh and cold stored yogurts fortified with dragon fruit pulp. **Methods:** Laboratory made set yogurt was divided into four categories. The first one was used as control (T0) while the other three T1, T2 and T3 were fortified with 5, 7.5 and 10% dragon fruit pulp, respectively. Antioxidant activity was measured using DPPH method, pH was determined by a pH meter, syneresis was measured by centrifugation procedure, and antibacterial properties were assessed by disk diffusion method. **Results:** The experimental results showed that the pH of the yogurts decreased with the addition of dragon fruit pulp. Syneresis in yogurts increased with the addition of dragon fruit pulp. Treatments with 5%, 7.5%, and 10% dragon fruit pulp showed a significant increase in DPPH scavenging activity compared to the control group. Yogurt supplemented with 10% dragon fruit pulp demonstrated the most effective growth-inhibiting properties against the pathogens *A. hydrophila*, *Bacillus cereus*, *E. coli*, and *K. pneumoniae*. **Conclusion:** The results suggest that incorporating dragon fruit pulp to yogurt fortification improves its functional properties, especially its antibacterial and antioxidant activities, with the most significant benefits demonstrated at 10% fortification.

Introduction

One fermented beverage that utilizes lactic acid bacteria (LAB) in its production is yogurt. Yogurt is known for its tart flavor (Fitratullah *et al.*, 2019) and is considered a highly nutritious dairy product that promotes human health (Ahmad *et al.*, 2022), as it contains probiotics, which are live beneficial bacteria (Mohamed *et al.*, 2014). Probiotic bacteria reduce respiratory infections; prevent and treat urinary tract infections; treat food allergies, lactose intolerance, and peptic ulcers; reduce and treat kidney stones, and improve digestion. They also have antimicrobial, anti-carcinogenic, and immune-boosting qualities. These positive effects are accomplished by

This paper should be cited as: Moazzem Hossain K, Chandro Sarker Nirob N, Maharin Roza A, Rahman Chowdhury N. ***Fortification of Functional Yogurt with Dragon Fruit (Hylocereus polyrhizus) Pulp: A Nutritional Approach.*** *Journal of Nutrition and Food Security (JNFS)*, 2026; 11(2): 226-235.

[DOI: 10.18502/jnfs.v11i2.21432]

generating antimicrobial compounds, competing with pathogens for nutrition and adherence to the intestinal epithelium, increasing immunity, and halting the production of harmful bacterial toxins. Dairy products are important sources of isolating probiotic bacteria (Hosseiny and Nateghi, 2025). Additionally, numerous micronutrients, including calcium, zinc, potassium, magnesium, riboflavin, and vitamins, are present in yogurt. The amounts are greater than those of other dairy products (Tremblay and Panahi, 2017). Among fermented milk products, it is well-known and widely accepted for its nutritional and therapeutic benefits, including inhibiting the growth of pathogenic bacteria, treating intestinal disorders such as constipation, diarrhea, and dysentery, exhibiting anti-carcinogenic properties, and providing protection against osteoporosis and hypertension, as well as helping to lower blood cholesterol levels (Mckinley, 2005). The creation of novel functional and health-promoting foods has drawn a lot of interest, mostly because of the documented therapeutic effects of bioactive substances. A variety of natural ingredients are available for use in food formulations. For example, many biopolymers, probiotics, bioactive peptides, essential oils, and herbal extracts have demonstrated a number of functional properties. Functional foods with antimicrobial potential can reduce body infections other than their nutritional benefits (Jafarirad *et al.*, 2025). Naturally occurring compounds in food products have been demonstrated to exhibit potent antioxidant activity, making them potential candidates for use as functional foods or ingredients when only portions of the food are extracted and added to other food products (Paz *et al.*, 2015). Fruits are a natural source of phytochemicals, which have been used as natural antimicrobials and for human consumption (Suriyaprom *et al.*, 2022). Utilizing natural additives to develop new yogurt products that meet consumer demands for nutritional value, technological functionality, and 'clean label' standards—while also offering added benefits such as antioxidant potential, disease prevention, and the promotion of human health is currently a key focus in the yogurt industry (Gyebi *et al.*, 2021). Numerous studies have examined how the addition of vitamins such as C, B9, B12, A, and D (Keršienė *et al.*, 2020) and minerals such as chromium, iron, magnesium, manganese, molybdenum, selenium, and zinc affects the properties of yogurt (Achanta *et al.*, 2007). In addition to fortifying yogurt with vitamins and minerals, there is a growing trend of incorporating plant-based functional ingredients to enhance both its nutritional value and sensory qualities. Examples include the addition of 1–5% pomegranate juice powder (Swinburn *et al.*, 2019), 5-20% dried pomegranate seeds (Bchir *et al.*, 2020), 1-3% freeze-dried apple pomace powder (Wang *et al.*, 2020), 0-4% flaxseed (Mousavi *et al.*, 2019), 0-30% coconut-cake (Ndife *et al.*, 2014), 0.25-1% spirulina (Barkallah *et al.*, 2017), 1-5% aloevera gel (Azari-Anpar *et al.*, 2017), and small amounts of saffron or tea (e.g., 0.0125%) (Unal and Ozer, 2018). These additions aim to improve both the technological and health-promoting properties of yogurt. Recent studies also suggest that consuming yogurt in combination with fruits may offer synergistic health benefits, as this pairing provides probiotics, prebiotics, high-quality protein, essential fatty acids, and a wide range of vitamins and minerals (Fernandez and Marette, 2018). The unusual and visually striking dragon fruit (DF) is gaining popularity in both the agricultural industry and among consumers due to its vibrant color, unique shape, large size, and appealing flesh (Tarte *et al.*, 2023). The fruit is rich in vitamin C and lycopene, the latter of which has been associated with a reduced risk of high blood pressure, heart disease, and certain types of cancer (Foong *et al.*, 2012). The health advantages of dragon fruit are attributed to its abundance of bioactive phytochemicals, including flavonoids, phenols, anthocyanins, and betalains. The usefulness of dragon fruit as a functional food is further increased by these bioactive chemicals, which are well-known for their anti-inflammatory, anti-cancer, anti-microbial, and antioxidant qualities (Chen *et al.*, 2024). Furthermore, the seeds of dragon fruit are rich in stearic acid, oleic

[DOI: 10.18502/jnfs.v11i2.21432]

acid, myristic acid, palmitoleic acid, linolenic acid, linoleic acid, and palmitoleic acid (Rathi *et al.*, 2023). Extensive chemical research has also been conducted on betacyanin, the primary bioactive compound in red dragon fruit (Wijitra *et al.*, 2013). In addition to their health benefits, polyphenols possess notable antiviral and antibacterial properties. Based on this, researchers have shown interest in exploring the antibacterial effects of dragon fruit (*Hylocereus polyrhizus*) peel and pulp, particularly in inhibiting the growth of *Streptococcus mutans* (Rahayu *et al.*, 2019). This study aims to evaluate the antioxidant activity and antibacterial properties of yogurt fortified with the pulp of super red dragon fruit (*Hylocereus polyrhizus*).

Materials and Methods

Preparation of dragon fruit pulp

Fresh dragon fruits were purchased from the local market in Khulna. The fruits were washed with clean water, then sliced using a knife. Any unwanted parts were manually removed. The chopped pieces of dragon fruit were then processed into a uniform pulp using an electric fruit processor. The resulting homogeneous pulp was then transferred into a sterile jar for future use.

Preparation of fruit-yogurt

The yogurt formulation procedure was employed according to Hamed (Hamed *et al.*, 2021) and Fitratullah (Fitratullah *et al.*, 2019). Cow's milk was poured into a clean beaker and heated to 85 °C for 15 minutes in a water bath. During the heating process, continuous stirring was performed to prevent the formation of cream. Afterward, the milk was rapidly cooled to 40 °C. The agar was added to distilled water (w/v) to a maximum concentration of 0.15%, and the mixture was then boiled. After boiling, the milk was inoculated with 1% starter culture of lactic acid bacteria. Following inoculation, dragon fruit was added along with agar, according to the respective treatment groups: T0=0% dragon fruit (w/v), T1=5% dragon fruit (w/v), T2=7.5% dragon fruit (w/v), and T3=10% dragon fruit (w/v). The inoculated milk was incubated at 30 °C for 12 to 15 hours. After incubation, the resulting yogurt was stored at 4 °C for 14 days.

Samples were collected at 1, 7, and 14 days following cold storage for all studies, which were performed in triplicate. All yogurt samples were analyzed periodically on the 1st, 7th, and 14th days after refrigeration, except for the antibacterial activity test, which was conducted using a water extract from fresh yogurt.

Yogurt water extract preparation

2.5 ml of distilled water was added to a 10 g yogurt sample, and the pH of the mixture was adjusted to 4.0 using 1M HCl. The yogurt was then centrifuged at 10,000 rpm for 20 minutes at 4 °C, following a 10-minute incubation at 45 °C. After collecting the supernatant, the pH was adjusted to 7.0 using NaOH. The neutralized supernatant was subsequently used for analysis after being recentrifuged at 10,000 rpm for 20 minutes at 4 °C (Zainoldin and Baba, 2009).

pH

The pH of the yogurt was measured by mixing 1 ml of yogurt with 3 ml of distilled water. pH measurements were taken using a pH meter (Hanna Instruments Model pH 211; Woonsocket, RI, USA) as described by Zainoldin and Baba (Zainoldin and Baba, 2009). The pH values were monitored over a 14-day storage period.

Syneresis measurement

Syneresis of the yogurt during storage at 4 °C was determined by centrifugation. A 20 g sample of yogurt was centrifuged for 15 minutes at 3500 rpm and 20 °C. After centrifugation, the clear supernatant was carefully collected and weighed (Aprodu *et al.*, 2012). The following formula was used to calculate syneresis:

$$\text{Syneresis} = \frac{\text{Weight of supernatant (g)}}{\text{Weight of yogurt sample (g)}} \times 100$$

Antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay

The antioxidant activity of yogurt water extract from both fresh and cold-stored yogurt samples was determined using the 1,1-diphenyl-2-

[DOI: 10.18502/jnfs.v11i2.21432]

picrylhydrazyl (DPPH) radical inhibition assay (Apostolidis *et al.*, 2007). For the assay, 250 µl of the homogenized yogurt water extract was mixed with 3 mL of a 60 µM DPPH solution in ethanol. Absorbance reduction at 517 nm was monitored until consistent measurements were obtained. The results were compared to the controls, which contained 250 µl of distilled water instead of the yogurt water extract.

The following formula was used to calculate the inhibition percentage:

$$\%Inhibition\ DPPH = \frac{\text{Absorbance of DPPH} - \text{Absorbance } of\ sample}{\text{Absorbance } of\ DPPH} \times 100$$

Antibacterial activity

Yogurt water extract was evaluated for its antibacterial activity against the pathogens *Aeromonas hydrophila*, *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The disc diffusion assay, as described by Correa (Corrêa *et al.*, 2011), was employed to assess whether extracts from fresh or cold-stored yogurt exhibited antibacterial properties. Nutrient broth was used to activate the indicator microorganisms, adjusted to a final concentration of 10^8 CFU/ml. After that, aliquots of 25 µl of the yogurt extract (at a concentration of 50 mg/ml) were applied to the prepared indicator strains. The plates containing the discs and bacterial cultures were then incubated at 37 °C for 24 hours.

The diameters of the growth inhibition zone (visible as clear areas) were measured and recorded in millimeters. Sterilized water was used as the negative control, while Erythromycin (500 mg) served as the positive control.

Ethical considerations

This study did not involve any human participants, animal subjects or personal data collection. All methods, including yogurt preparation, physicochemical analysis, antioxidant assay and antibacterial testing were carried out under laboratory conditions following the biosafety protocols and ethical standards.

Data analysis

The obtained data were statistically analyzed using two-way ANOVA in R software (version 4.3.3; R Core Team, 2024) within the RStudio environment (version 2023.12.1; RStudio, 2024) to identify significant differences between sample means, storage periods, and fresh yogurt in terms of antibacterial activity. All data are presented as mean ± standard deviation based on three replicates. A 95% confidence interval was applied, and mean comparisons were conducted using Tukey's test.

Results

pH

During the 14 days of cold storage, the pH of all yogurt samples gradually decreased, with the rate of reduction corresponding to the concentration of red dragon fruit pulp (*Hylocereus polyrhizus*) added (Error! Reference source not found.). The yogurt samples with 10%, 7.5%, and 5% red dragon fruit pulp showed statistically significant differences in pH values ($P<0.05$). Overall, the addition of red dragon fruit pulp resulted in pH values ranging from 4.48 to 4.00 over the 14-day storage.

Table 1. Changes in pH value of different treatments of yogurt during storage.

Group	Day 1	Day 7	Day 14
T0	4.48 ± 0.02^{a}	4.36 ± 0.03^{ab}	4.25 ± 0.04^{bc}
T1	4.41 ± 0.03^{a}	4.35 ± 0.03^{ab}	4.11 ± 0.03^{cde}
T2	4.25 ± 0.15^{bc}	4.18 ± 0.03^{cd}	4.06 ± 0.03^{de}
T3	4.09 ± 0.03^{de}	4.04 ± 0.02^{de}	4.00 ± 0.02^{e}

Data are presented a mean ± SD. Different letters indicate statistically significant differences (P<0.05)

Syneresis

The data presented in **Figure 1** indicate that whey separation in the tested samples significantly increased with longer cold storage time. All yogurt samples (T1, T2, and T3) exhibited higher syneresis percentages compared to plain yogurt. The syneresis values for the fresh samples were 30.48%, 34.26%, and 37.42% for yogurt containing 5%, 7.5%, and 10% (w/v) respectively, compared to the control yogurt, which had a syneresis value of 20.89%.

Antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay

[DOI: 10.18502/jnfs.v11i2.21432]

The incorporation of dragon fruit pulp increased the antioxidant activity, as shown in Error! Reference source not found.. On the first day of storage, the highest percentage of inhibition was observed in treatment T3 (32.60%), followed by T2 (27.35%) and T1 (24.44%), while the control yogurt (T0) exhibited the lowest value (15.07%). Although the percentage of inhibition decreased over the storage period, T3 consistently showed the highest values at each time point (23.96%, 21.08%, and 19.47%, respectively). Significant differences were observed among all treatments throughout the storage duration.

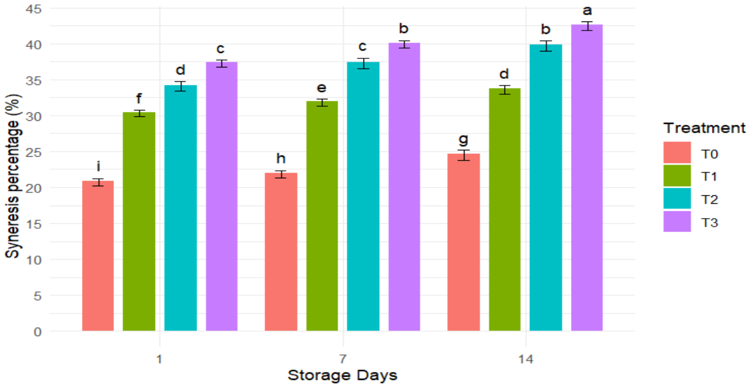

Figure 1. Syneresis measurement in yogurt. Data are presented as mean ± SD. Different letters indicate statistically significant differences (*P*<0.05).

Antibacterial activity

The antibacterial activities of yogurt water extracts from fresh yogurt samples were evaluated using the disk diffusion method, and the results are presented in **Figure 3** as the diameter of the inhibition zones (mm). Yogurt treatments enriched with dragon fruit pulp demonstrated higher antibacterial activity compared to the control yogurt. The concentration of dragon fruit pulp in the yogurt significantly (*P*<0.05) influenced the antibacterial effect. Furthermore, the highest zone of inhibition (24.08 mm) was observed with the control antibiotic erythromycin against *E. coli*. Among the yogurt treatments, T3 showed notably stronger antibacterial activity than the control treatment T1, with inhibition zones measuring 19.16 mm, 15.66 mm, 18.42 mm, and 11.76 mm against *A. hydrophila*, *Bacillus cereus*, *E. coli*, and *K. pneumoniae*, respectively. Treatment T3 and erythromycin exhibited similar antibacterial activity, with no significant differences in their effects on *A. hydrophila*, *Bacillus cereus*, *E. coli*, and *K. pneumoniae*.

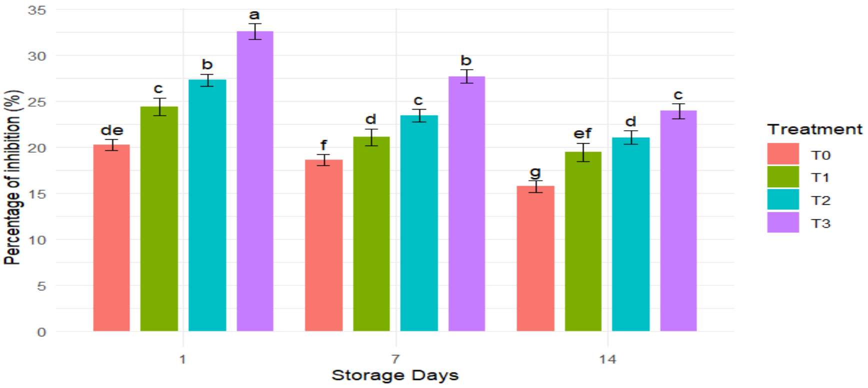

Figure 2. Percentage of antioxidant inhibition in yogurt. Data are presented as mean ± SD. Different letters indicate statistically significant differences (*P*<0.05).

[DOI: 10.18502/jnfs.v11i2.21432]

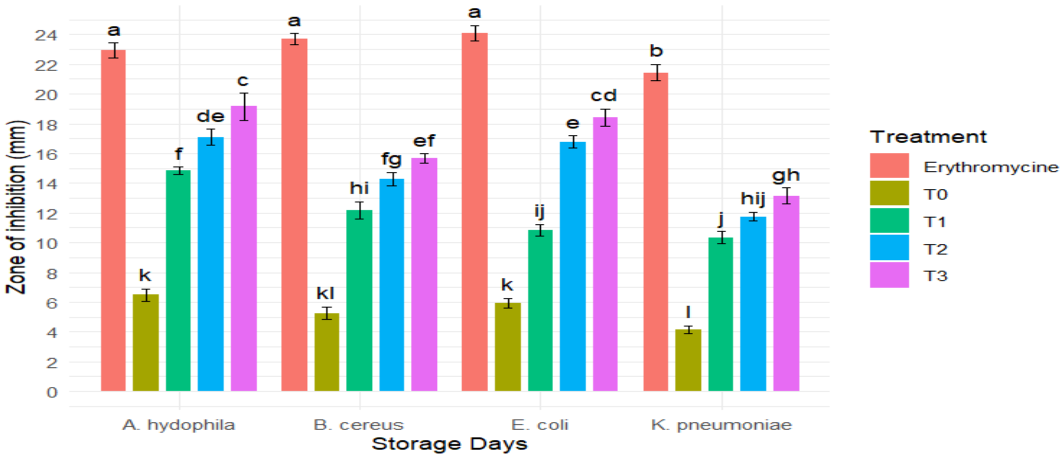

Figure 3. Comparison of antibacterial activity of yogurt. Data are presented as mean ± SD. Different letters indicate statistically significant differences (P<0.05).

Discussion

This study demonstrates that incorporating dragon fruit pulp into yogurt enhances its overall quality and functional properties. The addition of the pulp significantly altered key components of the yogurt, suggesting potential for the development of innovative dairy products. The pH of all yogurt samples tended to decrease progressively during cold storage, correlating with the concentration of incorporated dragon fruit pulp. This decline in pH is attributed to the growth of acid-forming bacteria over the storage period (Jayasinghe *et al.*, 2015). Lactic acid bacteria, which convert lactose in milk into lactic acid, are primarily responsible for this reduction in pH. The observed changes in pH in the present study are consistent with the findings of Zainoldin and Baba (Zainoldin and Baba, 2009). The findings of the pH changes in this investigation were comparable to (Jayasinghe *et al.*, 2015). Similarly, Joung (Joung *et al.*, 2016), reported a comparable trend in pH decline due to the incorporation of plant-based additives in yogurt formulations. Syneresis, also known as whey separation, is considered a textural defect in yogurt and serves as a key indicator of its quality during storage (Hamed *et al.*, 2021). The addition of dragon fruit pulp led to an increase in syneresis, which was proportional to the concentration of the pulp, showing significant differences between the treated and control yogurt samples. The addition of dragon fruit has been associated with an increase in yogurt whey separation, as reported by Zainoldin and Baba (Zainoldin and Baba, 2009). Across all treatments, a notable rise in syneresis was observed throughout the storage period. Yogurt containing 10% (w/v) red dragon fruit (T3) exhibited the highest syneresis, reaching 42.62% by the 14th day. A significant increase in syneresis was recorded not only in the control (T0) but also in all treated samples (T1, T2, and T3) during storage, which was consistent with the findings of Dhawi (Dhawi *et al.*, 2020). Compared to plain yogurt, one study mentions that a higher percentage of syneresis was observed in fruit yoghurt because of either a lower pH, which is indirectly associated to the contraction of coagulum; this reduces protein dehydration maybe because fruit has a higher water content (Tarte *et al.*, 2023). The addition of red and white dragon fruits did not raise the fiber content of the yogurt, which would have increased the syneresis by holding the water. This increase in syneresis is most likely the result of a decrease in water holding capacity, which increased whey discharges (Penna *et al.*, 2001). Additionally, Lucey (Lucey, 2002) demonstrated that low-solid yogurt tends to be more prone to syneresis than high-solid formulations. The addition of dragon fruit pulp to yogurt significantly increased its

[DOI: 10.18502/jnfs.v11i2.21432]

antioxidant activity compared to the control group (*P*<0.05). This enhanced antioxidant property may improve the therapeutic benefits of the yogurt. The increase is likely due to the presence of bioactive compounds in dragon fruit, such as vitamins, Phyto albumins, and lycopene, which are known for their antioxidant effects (Wu *et al.*, 2006). Moreover, higher concentrations of dragon fruit pulp resulted in greater inhibition values, indicating a dose-dependent enhancement in antioxidant activity. It was demonstrated that all fruit-enriched yogurts showed an increase in the percentage of inhibition compared to plain yogurt, as measured by the DPPH radical scavenging method, which is consistent with the findings reported by Zainoldin and Baba (Zainoldin and Baba, 2009). However, this increase in inhibition decreased over the course of cold storage, a trend also noted by Dhawi (Dhawi *et al.*, 2020), who reported a reduction in antioxidant activity during storage. The breakdown of phenolic molecules due to changes in microbial activity during storage may contribute to the reduction in antioxidant activity over time (Yildiz *et al.*, 2011). Additionally, an increase in milk protein–polyphenol interactions may also play a role (Yuksel *et al.*, 2010). The results also indicated that the inhibition levels in all fruit-enriched yogurts were significantly different from those in plain yogurt. It is hypothesized that the addition of dragon fruit to yogurt may influence or enhance this inhibitory effect. Moreover, dragon fruit contains a variety of antimicrobial substances that could potentially exert antibacterial properties in yogurt. One of the key compounds responsible for these antibacterial effects is betalains, specifically betacyanin and betanin, which are known for wide range of therapeutic properties (Chen *et al.*, 2024). The antibacterial activity of dragon fruit-enriched yogurt increased with the fortified concentrations. This is supported by the findings of Fitratullah (Fitratullah *et al.*, 2019) which show an increased zone of inhibition against *E. coli*, and these results are consistent with those reported by (Dhawi *et al.*, 2020). According to Said et al.(Said *et al.*, 2019) and Davis and Stout (Davis and Stout, 1971), inhibition zones are categorized as follows: a zone less than 5 mm is considered a low inhibitory value, between 5 and 10 mm is medium inhibitory, between 10 and 19 mm is high inhibitory, and beyond 20 mm is very high inhibitory. Therefore, the results can be classified as showing moderate to high inhibitory activity. These outcomes point to clear strengths of the study. In addition to improving antioxidant and antibacterial properties, using super red dragon fruit pulp as a fortifier offered natural color and possible nutritional appeal without the need for synthetic additions. The method is applicable to the development of functional foods since it is straightforward, reasonably priced, and compatible with current yogurt production practices. These qualities suggest potential for developing innovative dairy products with added health benefits. However, certain limitations should be acknowledged. The analysis was confined to a single fruit variety and pulp portion, and storage stability was only observed for 14 days. Furthermore, the precise bioactive substances responsible for the antimicrobial activities were not found. Future studies that focus on these areas may offer a more thorough comprehension and useful applications of yogurt fortified with dragon fruit pulp as a functional food.

Conclusion

Yogurt without fortification and three fortified yogurt variants (T2, T2 and T3) containing 5%, 7.5%, and 10% red dragon fruit pulp, respectively, have been successfully formulated. Lactic acid bacteria are able to utilize the carbohydrates in red dragon fruit (H. polyrhizus) as an energy source to produce lactic acid. The enhanced antioxidant and antibacterial properties of the fortified yogurt can be attributed to the addition of dragon fruit pulp. The yogurt sample supplemented with 10% dragon fruit pulp (T3) demonstrated the highest antioxidant activity compared to the control yogurt. However, the antioxidant activity in the fortified yogurt decreased during cold storage. In all yogurt samples, the pH value decreased, and syneresis increased as the storage duration lengthened. Additionally, the yogurt enriched with

[DOI: 10.18502/jnfs.v11i2.21432]

10% dragon fruit pulp (T3) showed significantly higher antibacterial activity against the pathogenic microorganisms tested, compared to the control yogurt. Yogurt with dragon fruit can be stored in the refrigerator at 4 °C for up to 15 days without compromising its quality. By incorporating dragon fruit pulp, it is possible to create novel fermented milk products with enhanced nutritional and functional benefits. In conclusion, it is recommended to develop unique functional yogurt with improved properties by using dragon fruit pulp, a cost-effective and natural source of antioxidants.

Authors' contributions

Chandro Sarker Nirob N and Moazzem Hossain designed the research. Chandro Sarker Nirob N, Maharin Roza A, and Rahman Chowdhury N Chowdhury conducted the research under the supervision of Moazzem Hossain, who also provided essential research inputs and facilities. Chandro Sarker Nirob N, Maharin Roza A, and Rahman Chowdhury N Chowdhury analyzed the data. Chandro Sarker Nirob N and Rahman Chowdhury N Chowdhury drafted the manuscript, while Moazzem Hossain and Chandro Sarker Nirob N edited the final version. All authors reviewed and approved the final manuscript.

Conflict of interests

The authors declared no conflict of interest.

Funding

This research received no external funding.

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