JMALS

Journal of Medical and Life Science
https://jmals.journals.ekb.eg/

SPBH

Therapeutic Effect of Functional Buttermilk Supplemented with Red Beetroot Juice and Microencapsulated Probiotics against High-Fructose Corn Syrup Disorders in Rats

[*1] Zeinab F. A. Mohamed & [2] Eman S. El-Ashaal

[1]Department of Dairy Microbiology, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt.

[*]**Corresponding Author:** zeinabfarouk55@gmail.com, zeinabfarouk55@arc.sci.eg, ORCID ID: https://orcid.org/0009-0003-9467-593X

[2]Nanotechnology and Advanced Material Central Lab, Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt

DOI:10.21608/jmals.2024.425311

ABSTRACT

Fructose, a widely used sweetener in food and pharmaceutical industries, is linked to alterations in gut microbiota, bacterial metabolic changes, and chronic diseases. Therefore, functional buttermilk (FB) supplemented with 5% Red Beetroot Juice (RBJ), microencapsulated *Lacticaseibacillus paracasei* subsp. *paracasei* B-4564 and/or *Bifidobacterium bifidum* B-41410 were developed as a dietary intervention against High-Fructose Corn Syrup (HFCS) disorders in rats. Results revealed that RBJ exhibited potent antioxidant activity, 69.11% and 78.91% according to DPPH and ABTS assays, respectively. Higher survival rate and excellent stability of microencapsulated *L. paracasei* B-4564 and *B. bifidum* B-41410, either individually or in combination form, were observed during the storage period. Counts of microencapsulated probiotics increased significantly, *L. paracasei* B-4564 counts reached 9.60 and 9.75 $\log_{10}$ CFU/ml in T1 (FBRP) and T3 (FBRM), whereas *B. bifidum* B-41410 reached 9.46 and 9.55 $\log_{10}$ CFU/ml in T2 (FBRB) and T3 (FBRM), respectively. Additionally, after 5 months of in vivo study, biochemical parameters, fecal microbial counts, and histopathological examination of liver & intestinal tissues were evaluated. FBRM showed the most improvements in metabolic markers, including glucose, IR, liver functions, and lipid profiles, compared to the other groups. Significant reduction of rats' fecal coliforms was found in contrast with the promotion of probiotic counts. Histopathological evaluation showed marked improvements to intestinal & liver tissues of rats receiving FBRM, followed by FBRP and FBRB, compared to the positive control. It could be recommended that daily administration of functional buttermilk supplemented with 5% RBJ and mixed microencapsulated *L. paracasei* B-4564 and *B. bifidum* B-41410 is an effective tool to overcome pathological features contributed to HFCS consumption.

Key words:

Functional buttermilk, Red Beetroot Juice, High Fructose Corn Syrup, Gastrointestinal microbiota, Microencapsulated probiotic.

Received: September 22, 2024. Accepted: November 18, 2024. Published: December 20, 2024

INTRODUCTION

In recent decades, High-Fructose corn syrup (HFCS) has become a dominant sweetener in most popular processed foods, baked goods, beverages, soft drinks, and sweets due to its advantages over other sweetener substitutes, including improved texture, taste, preparation methods, and cost-effectiveness (1). However, excessive fructose intake is linked to alterations in gut microbiota, which lead to dysbiosis "Imbalance of microbial species and reduction in originated bacterial diversity, bacterial metabolic changes, inflammation, metabolic dysregulation" and chronic conditions such as cardiovascular disease, cancer, reproductive system disorders, diabetes, obesity, kidney diseases, and inflammatory bowel disease (2).

Several studies examined fructose's pathophysiological impact on organs and gut microbiome proved that alternative sweetener consumption reduces probiotic bacteria counts, overgrows undesirable bacteria, and increases intestinal permeability causing bacterial translocation which has defined as "The passage of viable and non-viable bacteria and their metabolic products through the epithelial mucosa from the intestinal lumen to the mesenteric lymph nodes and eventually to other organs" (3).

On the other hand, fermented dairy products act as a suitable carrier of beneficial probiotic bacteria, including biologically active components that provide positive health benefits and /or reduce the risk of diseases in addition to their nutritive values, which are defined as "Functional Dairy Products" (4). Fermented Buttermilk is a valuable by-product with a unique composition obtained through butter manufacturing. It is a potential source of functional ingredients such as fractions of milk fat globule membrane, polar lipids, especially phospholipids, in addition to minerals, vitamins; that play an important role in many metabolic processes such as blood pressure reduction, amino and fatty acid synthesis, reducing total cholesterol and triglycerides (5).

Probiotics emerge as natural therapeutic agents that enhance gut microbial diversity, balance the gut microbiota, maintain epithelial cells, and increase levels of mucin production, resulting in improvement of the barrier functions (6). Additionally, it has positive physiological effects such as reduction of blood glucose, insulin resistance, total cholesterol, and triglycerides. Still, various factors can reduce their viability and stability, like unfavorable conditions of processing, storage, and the gastrointestinal tract (7). To overcome these issues, the Microencapsulation technique is employed to protect probiotic cells, which traps cells within a protective material to enhance probiotics' survivability in the gastrointestinal tract, improve their adhesion to mucosal surfaces, and colonize effectively to provide their intended health benefits (7).

Red Beetroot juice (RBJ) is a considerable source of several bioactive components such as nitrate, betalains, polyphenols, carotenoids, and vitamins (8). However, RBJ is used in the food industry on a limited scale as a natural pigment in ice cream, yogurts, and to enhance redness in tomato pastes, desserts, and jams. Recent research has found that RBJ has a biological activity against various diseases, such as cardiovascular disease, hypertension, diabetes, cancer, and hepatic steatosis (9). Also, RBJ improves oxygen kinetics in muscles for sports athletes (10). Interestingly, fermented RBJ has a broad spectrum of therapeutic properties, such as antimicrobial, antihypertensive, antioxidant, antianemic, immunomodulatory, hepatoprotective, anti-inflammatory, hypocholesterolemic, and anticancer, effectively than unfermented RBJ (11). Limited studies investigated the health effects of beetroot incorporated into dairy products. A previous study had illustrated the effective role of symbiotic camel milk containing beetroot extract to ameliorate the development of non-alcoholic fatty liver disease (12). Furthermore, stirred yoghurt fortified with beetroot powder and probiotics has a therapeutic role for type 2 diabetes (13). These plentiful health effects

of BRJ suggest its potential applications in the prevention and treatment of the negative health effects of HFCS consumption.

Therefore, introducing a functional buttermilk supplemented with probiotics and prebiotics (RBJ) could attract health-conscious consumers and expand its market presence. Additionally, to the best of our knowledge, the present study provides the first assessment of functional dairy product efficiency to ameliorate gastrointestinal disorders induced by HFCS.

Therefore, this study aimed to evaluate the potential therapeutic effect of functional buttermilk supplemented with red beetroot juice in addition to encapsulated *Bifidobacterium bifidium* B-41410 and/or *Lacticaseibacillus paracasei* B-4564 against physiological changes induced by high-fructose corn syrup in a rat model.

MATERIALS and METHODS

Raw Materials and Chemicals

Buttermilk was sourced from the Dairy Unit, Faculty of Agriculture, Cairo University (Giza, Egypt), while beetroot was procured from the local market (Giza, Egypt). Antioxidant reagents DPPH (1,1-diphenyl-2-picryl hydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) were obtained from Sigma-Aldrich (USA). Fructose was purchased from King M Company (Bader City, Egypt). Microbiological media (Biolife) were supplied by El-Badr Company (Egypt). Biochemical analysis kits were procured from Bio-Diagnostic and Research Reagents Company (Giza, Egypt). Additional chemicals, including sodium alginate, calcium chloride, lithium chloride, and L-cysteine HCl, were obtained from LOBA Chemie, Laboratory Reagents & Fine Chemicals, Pvt. Ltd. (Mumbai, India).

Starter Culture

Lacticaseibacillus paracasei subsp. *paracasei* B-4564 (*L. paracasei* B-4564) and *Bifidobacterium bifidum* B-41410 (*B. bifidum* B-41410) were obtained from the Northern Regional Research Laboratory (NRRL), Agriculture Research Service, National Center for Agriculture (Peoria, Illinois, USA). A commercial yogurt culture containing *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus (S. thermophilus)* were purchased from Chr. Hansen Laboratory (Copenhagen, Denmark).

Methods

Preparation of Red Beetroot Juice (RBJ)

Red Beetroot was washed, peeled, and cut into small pieces. One hundred grams of fresh beetroot was added to one liter of distilled water, then homogenized using a Philips blender (Model HR7627/00, U.K.) for 10 min/ 6000 rpm. The mixture was heated at 80ºC/10 min, kept for 24 h at 5 °C, and filtered using cheese clothes. The obtained crude extract was centrifuged at 6000 rpm for 30 min (Hermle Z206A, Germany) (14).

Antioxidant Activity of Red Beetroot Juice -DPPH Method

The free radical-scavenging activity of RBJ was assessed using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay (15). A 100 µL of RBJ was added to 4 mL of a DPPH solution prepared at a concentration of 5×10^6 in methanol. The mixture was thoroughly mixed and incubated at room temperature for 30 minutes in the dark. The absorbance of the sample was then measured at 517 nm.

$$\textbf{DPPH scavenging activity \%} = (A_0 - A_1 / A_0) \times 100$$

Where: A_0: Absorbance of the control reaction,

A_1: Absorbance in the presence of the tested RBJ.

- ABTS Method

For the ABTS method, the ABTS radical is prepared by mixing 7 mM ABTS with 2.45 mM ammonium persulfate and keeping it to react in the dark for 16 hours. Two hundred microliters of the resulting ABTS radical solution were dissolved in 10 mL of ethanol. To evaluate the antioxidant activity, a 20 µL sample of RBJ is mixed with 980 µL of this solution (16). Absorbance is measured after 6 minutes at 734 nm, and scavenging activity is calculated as:

$$\textbf{ABTS scavenging activity \%} = [(A_0 - A_1/A_0)] \times 100$$

Where: A_0: Absorbance of ABTS solution at time zero without the tested RBJ.

A_1: Absorbance in the presence of the tested RBJ after 6 min.

Determination of Betalain Compounds of Red Beetroot Juice

The contents of betacyanins and betaxanthins were determined spectrophotometrically at 538 nm and 480 nm, respectively, using a UV–Vis spectrometer, following modified methods from Stintzing *et al.*, (17). Betacyanin content (BC) was calculated using the equation:

$$\textbf{BC (mg/g)} = [(A \times DF \times MW \times 1000) / (\varepsilon \times L^* 1000)]$$

Where A = Absorption, DF = dilution factor, L = path length (1 cm) of the cuvette. Quantification of betacyanins: molecular weights (MW) equal 550 g/mol and molar extinction coefficients (ε) = 60,000 L/mol cm in H_2O.

The molecular weight (MW) for betacyanins is 550 g/mol, and the molar extinction coefficient (ϵ\epsilonϵ) is 60,000 L/mol. cm in water. For betaxanthins, the molecular weight is 308 g/mol, and the molar extinction coefficient is 48,000 L/mol. cm in water.

Microencapsulation of Probiotic Strains

B. bifidum B-41410 and *L. paracasei* B-4564 were enumerated on MRS broth medium and incubated at 45°C and 37°C for 24 h under anaerobic and aerobic conditions, respectively. For *B. bifidum* B-41410, MRS broth was supplemented with 0.5% L-cysteine HCl and 1% lithium chloride. This procedure was performed twice to prepare the cell suspension with the required cell count before microencapsulation (10^8-10^9). The obtained cell suspension was centrifuged separately at 4000 x g for 10 min at 4°C, then washed using sterile saline solution. The concentrated cells were mixed with 2% sodium alginate and extruded into 0.1 M calcium chloride to form beads. The formed alginate beads were rinsed with distilled water after 30 minutes, then stored in a sterile 0.1% peptone solution (18).

Evaluation of Microencapsulation Efficiency (ME%)

The following equation was used to calculate ME (19):

$$\textbf{ME\%} = (\text{Log}_{10}N/\text{Log}_{10}\,No) \times 100$$

Where: N: Number of the bacterial cells loaded in the alginate beads

No: Initial count of free bacterial cells used in microencapsulation

Viability of Microencapsulated Probiotic

The viability of *B. bifidum* B-41410 and *L. paracasei* B-4564 individually was assessed using the serial dilution method in 1% sodium citrate. The alginate beads were shaken for 20 minutes until completely dissolved, followed by regular 0.1% peptone water dilution. The resulting solution was plated on MRS agar and incubated at 37°C for 48 hours. The colonies were then counted and expressed as CFU/mL (20).

Production of Functional Buttermilk

The whole buttermilk was heated to 72°C for 15 seconds, cooled to 42°C, supplemented with 5% RBJ, and divided into four portions. Each portion was inoculated with 3% starter culture as follows:

The 1st portion (control): Inoculated with *L. bulgaricus* and *S. thermophilus* (YC), (FBR).

The 2nd portion (T1): Inoculated with YC + encapsulated *L. paracasei* B-4564 (1:1), (FBRP).
The 3rd portion (T2): Inoculated with YC + encapsulated *B. bifidum* B-41410 (1:1), (FBRB).
The 4th portion (T3): Inoculated with both encapsulated probiotic strains + YC (1:1:1), (FBRM).
The different inoculated buttermilk samples were placed in plastic cups and incubated at 40±2°C until the pH reached 4.6. The samples were then stored under refrigeration for 15 days to evaluate their microbiological, pH values, and organoleptic characteristics.

The pH Values of Functional Buttermilk

A digital pH meter (ADWA, AD11, Europe, Romania) was used to measure the pH of different functional buttermilk treatments at 1, 5, 10, and 15 days of storage.

Microbiological Analysis of Functional Buttermilk

Different functional buttermilk samples were microbiologically analyzed. Serial dilutions were prepared using 9 mL sterile NaCl (0.85%). Counts of *S. thermophilus* were enumerated aerobically using acidified M17 agar (adjusted to pH 6.8 with 1 M HCl), and the plates were incubated at 37ºC for 48 hours (21). *L. bulgaricus* was enumerated using acidified MRS agar (pH 5.4), with plates incubated at 37ºC for 48 hours (22). Counts of *B. bifidum* were enumerated under anaerobic conditions using MRS agar supplemented with 1% lithium chloride, 0.5% L-cysteine HCl, and a Gas Pak system, and plates were incubated at 37°C for 72 hours (23). *L. paracasei* counts were enumerated using MRS agar, with plates incubated at 37°C for 48 hours (22). Coliform bacteria were counted using Violet Red Bile agar, with plates incubated at 37ºC for 24 hours (24). Moulds and yeasts were counted using Malt Extract agar acidified to pH 3.5 with sterile lactic acid solution, and plates were incubated at 25ºC for 4-5 days (25). The results were recorded as the log number of colony-forming units per gram (log_{10} CFU/g).

Sensory Evaluation of Functional Buttermilk

A sensory evaluation of different treatments of functional buttermilk was conducted using a nine-point hedonic scorecard (26). The evaluation was performed by 12 personnel from the Animal Production Research Institute (Cairo, Egypt).

In vivo study

Biological experiment design

Thirty male Sprague-Dawley rats, each weighing approximately 250±5 grams, were housed under standard conditions in compliance with the Institutional Animal Care and Use Committee - Agricultural Research Center (ARC-IACUC). Over a five-month study, all rats were fed a normal diet with unrestricted access to food following AIN-93 (27). After a one-week acclimatization period, the rats were divided into six groups of five individuals each. The first group served as the normal control and had unrestricted access to water (Group 1) until the end of the experiment, while the other groups were provided with 30% high-fructose corn syrup (HFCS) (28). After three months, Group 2 was switched to water consumption, while groups 3 to 6 received daily oral administration of different treatments, functional buttermilk mentioned in the "Production of Functional Buttermilk" section as follows:

- Group 3: received FBR (FB Control)
- Group 4: received FBRP (T1)
- Group 5: received FBRB (T2)
- Group 6: received FBRM (T3)

After two months, the animals were sacrificed, and blood samples were taken from the retro-orbital venous plexus. These samples were centrifuged at 4000 rpm for 15 minutes (Hermle Z206A, Germany) to separate the serum for biochemical analysis. The rats' intestine and liver tissues were cleaned with saline, weighed, excised, and stored in 10% formalin for histopathological examination. Each animal's weight was recorded weekly and at the end of the experiment.

Biochemical analysis

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured to evaluate liver enzyme activity spectrophotometrically, along with lipid profiles, including total triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C), using the procedure outlined in Biodiagnostic kits. The following equations were used to calculate both very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C), according to Friedewald *et al.* (29):

VLDL (mg/dl) = TG / 5

LDL (mg/dl) = TC − (VLDL + HDL)

Microbiological Evaluation of Rats' Fecal

Fresh fecal samples were collected over 24 hours at the beginning of the biological experiment, after receiving HFCS, and at the end of the biological experiment. Serial decimal dilutions were prepared using liquid casein yeast medium, which consists of casein enzyme hydrolysate 2.0 g/L, yeast extract 2.0 g/L, NaCl 5.0 g/L, and KH_2PO_4 1.0 g/L, to evaluate microbial groups (30). *Lactobacilli* ssp. enumerated on MRS agar and incubated anaerobically at 37°C for 48 hours, while *Bifidobacterium* ssp were enumerated on MRS agar supplemented with 1% lithium chloride, 0.5% L-cysteine HCL, and incubated anaerobically at 37°C for 72 hours. Also, Coliforms were determined using Violet Red Bile agar, incubated at 37°C for 24 hours. Total bacterial counts were counted using Nutrient agar and incubated aerobically at 25°C for 48h (31).

Histopathology examination

The intestinal and liver tissues from each rat in all groups were embedded in a 10% formalin solution for 24 hours, followed by clearing in xylol. The specimens were then embedded in paraffin wax, cut into 4-micron-thick sections, collected on glass slides, and deparaffinized. The tissue sections were stained with hematoxylin and eosin for histopathological examination using an electric light microscope (X200) (32).

Ethical Approval

This protocol was approved by the Institutional Animal Care and Use Committee (ARC-IACUC) / Agricultural Research Center (Ethical Approval No ARC/AHRI/166/24).

Statistical Analysis

The statistical analysis was performed using one-way ANOVA with a significance threshold of 0.05 and conducted using CoStat (version 6.400) (33). The data were analyzed using a complete randomization method, and the least significant difference (LSD) test was used to evaluate the significance among the means of various samples.

RESULTS & DISCUSSION

Betalains Content and Antioxidant Activity of Red Beetroot Juice

Red beetroot, Betacyanin, and betaxanthin (vulgaxanthin I), have long been considered a unique source of these bioactive compounds. The content of betacyanins, betaxanthins, and the total betalains was determined spectrophotometrically (Table 1). The Red beetroot juice (RBJ) exposed a significant content of betalains (93.06 mg/g). Meanwhile, the betaxanthin content (70.45 mg vulgaxanthin I equivalents/g) was 3 times higher than betacyanin (23.25 mg betanin equivalents/g). A related study reported that total betalain, betaxanthin, and betacyanin content in red beetroot was 51.19, 30.86, and 20.19 mg/g, respectively (34). The higher betalain content found in the present study could be explained by differences in beetroot cultivar, soil quality, temperature, water availability, and ripening stage during harvest (35).

In terms of antioxidant activity, RBJ exhibited strong scavenging activity, with 69.11 ± 0.95% by the DPPH assay and 78.91 ± 0.30% by the ABTS assay, indicating potential capacity for neutralizing free radicals through both methods. The antioxidant activity of RBJ is significantly related to the content of the red pigments (betalains). A previous study mentioned significant antioxidant activity was 624.2 µmol Torolox/g extract by ABTS and 37.3 µg/mL

by DPPH (36). In agreement with a previous study that found total antioxidant activity of RBJ recorded 80.48% (37).

The antioxidant activity of RBJ is attributed to the phenolic and cyclic amine groups in betalains, which can donate electrons. This ability to donate electrons allows betalains, especially betacyanins like betanin, to quench free radicals and inhibit oxidative damage. Conjugated double bonds in betalamic acid help stabilize the molecule after donating electrons, thus increasing the antioxidant capacity of betalains (38). These findings agree with Diasari *et al.*, (39), who produced fermented soymilk with varying concentrations of RBJ and found that the 5% concentration exhibited the highest antioxidant activity among all treatments.

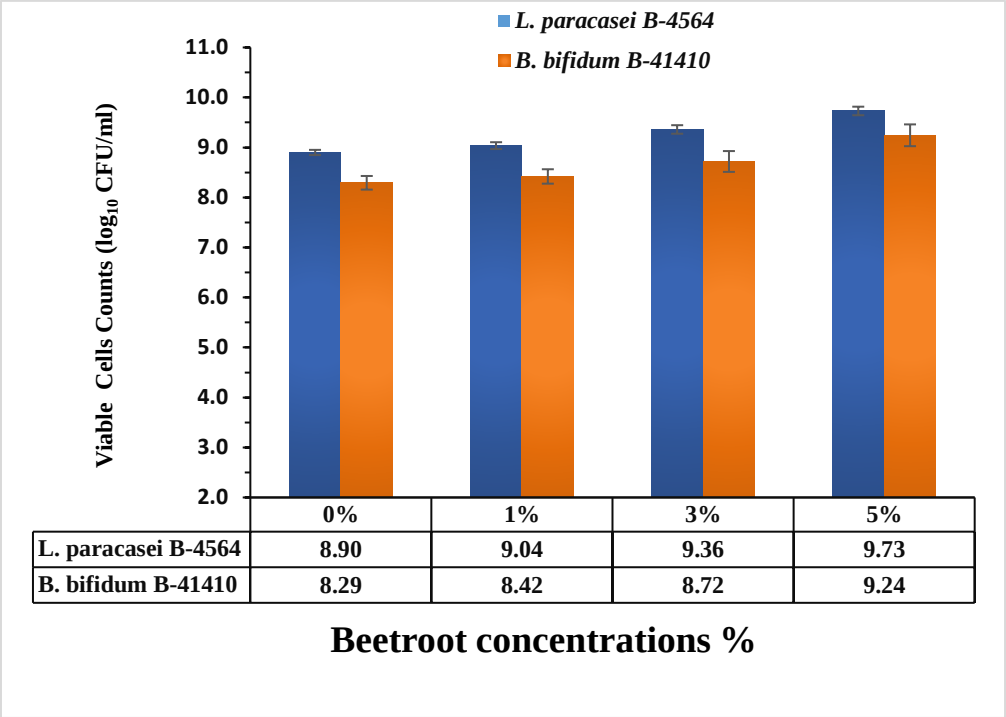
Effect of Red beetroot-juice concentrations on *B. bifidum* B-41410 and *L. paracasei* B-4564 growth (log10 CFU/ml)

The effect of different concentrations of RBJ on the proliferation of two probiotic strains, *B. bifidum* B-41410 and *L. paracasei* B-4564, was investigated. MRS medium was supplemented with 1%, 3%, and 5% RBJ, to enumerate the probiotic strains individually, then viable cells were counted (22, 23). As shown in Figure 1, counts of *L. paracasei* B-4564 and *B. bifidum* B-41410 significantly increased with increased concentrations of RBJ. However, no significant differences were observed at 1% RBJ compared to the control. At 5% RBJ, both *B. bifidum* B-41410 and *L. paracasei* B-4564 exhibited a significant increase, reaching 9.24 and 9.73 $\log_{10}$ CFU/mL, respectively. The increment rates also varied between the two strains. *L. paracasei* B-4564 showed a greater increase of 0.95 log cycles, compared to *B. bifidum*, which showed an increase of 0.83 log cycles at the same 5% RBJ concentration. In a related study, fermented soymilk was produced using 0, 5, 10, 15% RBJ and revealed that as RBJ concentration increased as pH decreased significantly, as an indirect indicator for stimulating bacterial growth (39). The efficiency of RBJ in enhancing probiotic growth can be attributed to its high content of carbohydrates, which is a suitable component for probiotic fermentation (40). Additionally, the nitrate content in RBJ could serve as a nitrogen source for both *B. bifidum* B-41410 and *L. paracasei* B-4564, aligning with Chen *et al.*, (41), who reported that optimized growth media with nitrogen sources significantly promoted *B. bifidum* growth.

Based on these results, 5% RBJ was selected for further use in functional buttermilk production with *B. bifidum* B-41410 and *L. paracasei* B-4564, either individually or in combination.

Table 1. Betalains Content and Antioxidant Activity of Red Beetroot Juice

The Betalain content (BC)	**Values**
Total betalains mg /g of RBJ	93.06±0.34
Total betaxanthin (mg vulgaxanthin I equiv. /g of RBJ)	70.45±0.11
Total betacyanin (mg betanin equiv. /g of RBJ)	23.25±0.79
Antioxidant activity %	
By DPPH	69.11±0.95
By ABTS	78.91±0.30



	0%	1%	3%	5%
L. paracasei B-4564	8.90	9.04	9.36	9.73
B. bifidum B-41410	8.29	8.42	8.72	9.24

Figure 1. Effect of RBJ concentrations on *B. bifidum* B-41410 and *L. paracasei* B-4564 growth (log_{10} CFU/ml)

Microencapsulation Efficiency of Microencapsulated Probiotic Strains

The microencapsulation efficiency (ME %) of *B. bifidum* B-41410 and *L. paracasei* B-4564, encapsulated in alginate beads, was evaluated to assess their viability and functionality. The microencapsulation efficiencies for both strains were high, with values of 92.14% for *B. bifidum* B-41410 and 95.30% for *L. paracasei* B-4564. These high efficiencies suggest that the encapsulation process effectively protects the probiotics, enhancing their stability and functionality. The high ME% can be attributed to the use of 2 % alginate, a linear heteropolysaccharide composed of D-mannuronic and L-guluronic acids, which provides effective protection against harsh conditions (42). In a similar study, the influence of different alginate concentrations (1%, 2%, and 3%) on the encapsulation efficiency of *L. plantarum* was investigated, with efficiencies of 81.10%, 82.58%, and 80.55%, respectively (19). Similar studies have reported high ME% for various lactic acid bacteria strains (43), including *L. plantarum*, *Pediococcus pentosaceus*, and *Pediococcus acidilactici*, with efficiencies ranging from 73.64% to 94.10%.

A higher ME % is critical for ensuring that sufficient numbers of viable probiotic bacteria reach the host colon, where they can exert their potential health benefits as well as increase their feasibility for use in food products and therapeutic applications (44).

The pH of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains

Figure 2 illustrates the impact of incorporating 5% RBJ and microencapsulated probiotics on the pH of functional buttermilk (FB) over the storage period. At the beginning of storage, significant differences were observed between the control and all

treatments, with pH values ranging from 4.40 to 4.68. However, no significant differences were observed among different functional buttermilk samples containing microencapsulated *B. bifidum* B-41410 and *L. paracasei* B-4564, whether used individually (T1, T2) or in combination (T3).

Throughout the storage period, all samples of different FB treatments and control showed a gradual decline in pH due to increased starter culture activity and the subsequent production of acidity. This trend aligns with the findings of similar work that observed similar pH changes in fermented beetroot beverages with 40% milk (8). Notably, there were no significant differences between T2 (FBRB) and T3 (FBRM) during the storage period, but significant differences were observed between the control and other treatments containing microencapsulated *B. bifidum* B-41410 and *L. paracasei* B-4564.

At the end of storage, the control sample exhibited the highest decline in pH (approximately 60%, with a final pH of 3.80 ± 0.17). In contrast, T3 (FBRM), which contained both encapsulated probiotics, retained the highest pH value (4.45 ± 0.15) with the lowest reduction in pH (23%) compared to the other treatments. This result suggests that the higher pH values observed in the treatments involving microencapsulated probiotics, either single or in combination, could be attributed to metabolic products such as peptides and amino acids, which help neutralize the acidity. This finding is consistent with Peteán *et al.*, (45), who reported that such metabolic products can mitigate acidity in fermented dairy products.

Additionally, the highest pH values observed in T1, T2, and T3 compared to the control during the storage period can be attributed to the microencapsulation process, which limits the diffusion of nutrients and metabolic products through the alginate beads, thus reducing the overall acidity. In contrast, the lowest pH in the control samples was due to the fermentation of lactose into lactic acid by *S. thermophilus* and *L. bulgaricus*, leading to increased acidity.

Similar observations were made by Diasari *et al.*, (39), who found that fermented soymilk with *S. thermophilus* and *L. bulgaricus* using red beetroot juice at different concentrations exhibited lower pH values compared to the control.

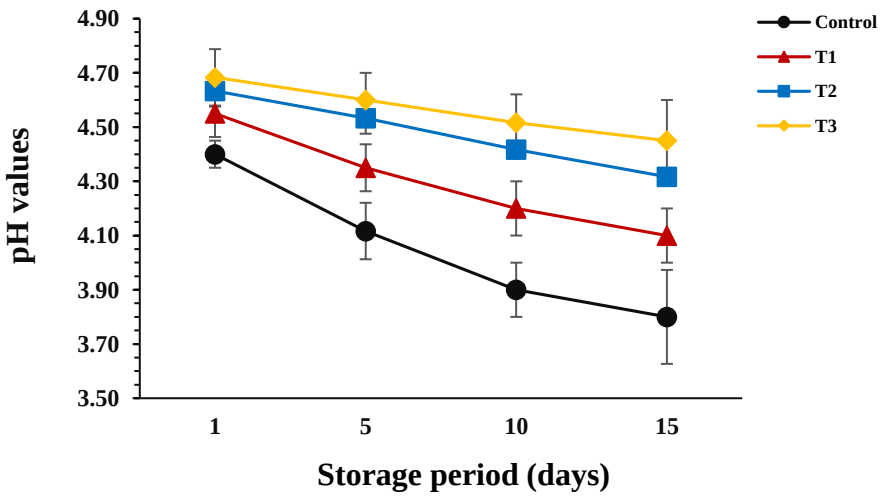


Figure 2. The pH of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains

Viability and Stability of Microencapsulated Probiotic Strains in Functional Buttermilk Supplemented with RBJ during Storage Period

As shown in Figure 3, the microencapsulated *B. bifidum* B-41410 population recorded 8.41 and 8.06 $\log_{10}$ CFU/ml in T2 (FBRB) and T3 (FBRM), respectively. Meanwhile, during the storage period, the counts of *B. bifidum* B-41410 increased significantly, while no statistically significant differences were observed between T2 (FBRB) and T3 (FBRM). On the other hand, the increment rate was observable in T3 with a 1.49 log cycle compared to T2 (1.05 log cycle). Similarly, microencapsulated *L. paracasei* B-4564 counts increased significantly, reaching 9.60 and 9.75 in T1 (FBRP) and T3 (FBRM), respectively. Furthermore, *L. paracasei* B-4564 counts increased by 1.06 and 1.38 log cycles in T1 and T3, respectively, at the end of storage.

A related study by El-Hameed *et al.*, (46), who produced a symbiotic buttermilk beverage using ABT-5 culture containing *S. thermophilus*, *L. acidophilus*, and *B. bifidum*, demonstrated that buttermilk is an appropriate medium for the viability enhancement of probiotic strains while developing a symbiotic beverage with desirable sensory properties. Abdo *et al.*, (8) produced fermented beetroot juice with 40% milk, found a significant increase in total probiotic counts, reaching 3.7×10^9 and 1.25×10^{10} for ABT-5 and LA-5, respectively. Also, a significant increase in *B. lactis* Bb12 and *L. acidophilus* CH-2 counts was found in stirred yoghurt fortified with 1% or 2% of beetroot powder (13). A higher survival rate of encapsulated *L. paracasei LBC-1e* was observed than free bacteria, with smaller log decreases in viability during storage in Mozzarella cheese (47).

The data further demonstrated a higher survival rate and stability of both *B. bifidum* B-41410 and *L. paracasei* B-4564, which remained within the recommended range of $\geq 10^7$ CFU/g, providing the desired health benefits to the host (48). The extrusion encapsulation procedure, which used 2% sodium alginate, significantly improved the viability and stability of *B. bifidum* B-41410 and *L. paracasei* B-4564 during the processing and storage of functional buttermilk. This aligns with Ayama *et al.*, (19), who observed similar results with *L. plantarum* and *B. bifidum* encapsulated in different concentrations of sodium alginate. Additionally, the improved growth and survival rates of the probiotics could be attributed to the beneficial ingredients in RBJ, which serve as a source of carbon and nitrogen, promoting the viability and stability of the encapsulated probiotics.

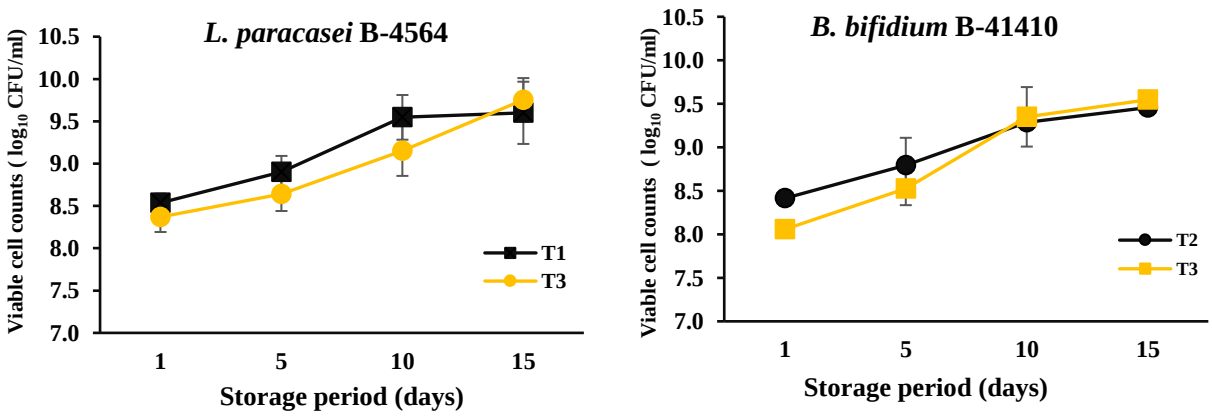


Figure 3. Viability and Stability of Microencapsulated Probiotic Strains in functional Buttermilk Supplemented with RBJ during the storage period

Microbiological Analysis of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains

Table 2 shows the impact of 5% RBJ and microencapsulated *B. bifidum* B-41410 and *L. paracasei* B-4564 on the microbiological properties of functional buttermilk over 15 days of storage. Significant differences were observed in the counts of *S. thermophilus* across all treatments during the storage period. It was observable that the counts of *S. thermophilus* increased significantly in T1, T2, T3, and control samples at the beginning of storage. However, throughout the storage period, *S. thermophilus* counts decreased non-significantly in T1, T2, and T3. While it decreased significantly in control samples due to the lower pH and increased acidity, which negatively affected bacterial growth. The increment rate values for *S. thermophilus* were 0.49, 0.54, and 0.39 log cycles for T1, T2, and T3, respectively, while the control showed a decline of -0.33 log cycle. These findings are consistent with Raghunath *et al.*, (49), who studied microbial properties of herbal yogurt and observed a significant reduction in *S. thermophilus* counts after the first week of storage.

Regarding *L. bulgaricus* counts, a significant increase was observed in the control and all treatments over the 15 days. The increment rates for *L. bulgaricus* were 0.76, 0.60, and 0.80 log cycles in T1, T2, and T3, respectively, compared to 0.41 log cycle in the control. This increase in *L. bulgaricus* counts could be attributed to the metabolic products of *S. thermophilus*, such as formic acid and CO_2, which promote the growth of *L. bulgaricus* (50). Notably, the counts of *S. thermophilus* and *L. bulgaricus* were slightly higher in the treatments containing microencapsulated *B. bifidum* B-41410 and *L. paracasei* B-4564 (T1, T2, and T3) compared to the control, suggesting a synergistic interaction that the metabolic products of the probiotics in these treatments stimulated the growth of the yogurt culture.

Interestingly, no coliforms, molds, or yeasts were detected in all different treatments of FB supplemented with RBJ and control during the 15-day storage period. This can be attributed to the high hygienic standards maintained during processing and storage. Additionally, the metabolic products of probiotics, such as lactic acid, acetic acid, and bacteriocins, as well as compete for available nutrients; inhibit the growth of undesirable microorganisms (51). This observation further supports the antimicrobial properties of probiotics in the functional buttermilk treatments. Also, it could be attributed to the antibacterial activity of RBJ in agreement with Ahmad *et al.*, (52), who examined the antibacterial properties of beetroot extracts in Rayeb milk; observed inhibition of *Pseudomonas aeruginosa* and *Escherichia coli* growth.

Sensory Evaluation of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains

Table 3 presents the sensory properties of control and various functional buttermilk treatments supplemented with RBJ. Functional buttermilk containing microencapsulated *B. bifidum* B-41410 and *L. paracasei* B-4564, either in individual forms (T1, T2) or as a combination form (T3), significantly improved the sensory attributes compared to the control sample. Notably, T3, which contained the mixed microencapsulated probiotic strains (FBRM), received the highest scores for flavor, color & appearance, body & texture, and overall acceptability.

The improved sensory properties of T3 (FBRM) could be attributed to the proteolytic and lipolytic actions of starter cultures during processing and storage, as well as the presence of RBJ, which enhanced the color & taste of the functional buttermilk. This finding aligns with Abbaszadeh *et al.*, (53), who reported that mild sourness was more favorable in fermented dairy products containing microencapsulated probiotics, which had lower acidity and higher pH than the control. Similar

studies have also shown that fermented beverages tend to have a better taste due to the lactic acid produced during fermentation, rather than acetic acid.

In terms of body & texture, significant differences were observed between control and functional microencapsulated probiotic buttermilk samples throughout the storage period. Among the samples, T3 (FBRM) achieved the highest score for body and texture, followed by T1 (FBRP), then T2 (FBRB), compared to the control. This texture improvement could be attributed to the ability of sodium alginate beads to absorb water at low storage temperatures, thereby increasing viscosity and enhancing the smooth, soft texture of the functional buttermilk. These results are consistent with the findings of Etchepare *et al.*, (42), who noted similar improvements in texture due to the water-retaining properties of alginate beads. All samples exhibited a favorable pink color due to the betalains, natural pigments in RBJ.

Table 2. Microbiological Analysis of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains

Treatments Days	Control	T1	T2	T3
		S. thermophilus		
1	$7.97^{Ba}\pm0.06$	$7.60^{Cb}\pm0.14$	$7.71^{Cab}\pm0.30$	$8.05^{Aa}\pm0.17$
5	$8.62^{Aa}\pm0.11$	$8.61^{Aa}\pm0.16$	$8.54^{ABa}\pm0.20$	$8.66^{Aa}\pm0.29$
10	$8.56^{Aa}\pm0.06$	$8.77^{Aa}\pm0.03$	$8.89^{Aa}\pm0.39$	$8.97^{Aa}\pm0.38$
15	$7.64^{Bb}\pm0.41$	$8.09^{Bab}\pm0.08$	$8.25^{Ba}\pm0.15$	$8.44^{Aa}\pm0.30$
		L. bulgaricus		
1	$8.29^{Aa}\pm0.34$	$8.13^{Cc}\pm0.20$	$8.18^{Cc}\pm0.27$	$7.99^{Bb}\pm0.22$
5	$8.39^{Aa}\pm0.02$	$8.35^{BCa}\pm0.14$	$8.29^{BCa}\pm0.09$	$8.33^{Ba}\pm0.16$
10	$8.54^{Aa}\pm0.21$	$8.60^{ABa}\pm0.13$	$8.57^{ABa}\pm0.15$	$8.74^{Aa}\pm0.20$
15	$8.70^{Aa}\pm0.16$	$8.89^{Aa}\pm0.18$	$8.78^{Aa}\pm0.17$	$8.79^{Aa}\pm0.13$

Different superscript capital letters within control and treatments (A, B……) are significantly different (P >0.05)
Different superscripts, small letters within different storage times (a, b……) are significantly different (P >0.05)

Table 3. Sensory Evaluation of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains

Treatments / Days	Control	T1	T2	T3
Flavor (9)				
1	$6.17^{Bc}\pm0.15$	$7.40^{Bb}\pm0.10$	$7.60^{Cb}\pm0.20$	$8.17^{Ca}\pm0.21$
5	$6.33^{ABc}\pm0.11$	$8.23^{Ab}\pm0.32$	$8.03^{BCb}\pm0.15$	$8.67^{Ba}\pm0.15$
10	$6.60^{Ab}\pm0.30$	$8.50^{Aa}\pm0.36$	$8.40^{ABa}\pm0.40$	$8.93^{Aa}\pm0.12$
15	$6.70^{Ab}\pm0.26$	$8.77^{Aa}\pm0.32$	$8.67^{Aa}\pm0.29$	$9.00^{Aa}\pm0.00$
Body & Texture (9)				
1	$5.40^{Cb}\pm0.36$	$8.00^{Ca}\pm0.20$	$7.93^{Ca}\pm0.12$	$8.20^{Ba}\pm0.26$
5	$5.67^{Cc}\pm0.06$	$8.47^{Bab}\pm0.21$	$8.40^{Bb}\pm0.17$	$8.67^{Ba}\pm0.060$
10	$6.43^{Bb}\pm0.40$	$8.77^{ABa}\pm0.25$	$8.73^{Aa}\pm0.21$	$8.83^{ABa}\pm0.21$
15	$7.10^{Ab}\pm0.26$	$9.00^{Aa}\pm0.00$	$9.00^{Aa}\pm0.00$	$9.00^{Aa}\pm0.00$
Color & appearance (9)				
1	$6.23^{Db}\pm0.21$	$6.43^{Dab}\pm0.15$	$6.40^{Dab}\pm0.17$	$6.60^{Ca}\pm0.10$
5	$6.77^{Cb}\pm0.12$	$7.00^{Cab}\pm0.10$	$7.03^{Cab}\pm0.12$	$7.20^{Ba}\pm0.26$
10	$7.30^{Bb}\pm0.26$	$7.50^{Bb}\pm0.10$	$7.47^{Bb}\pm0.06$	$8.17^{Aa}\pm0.31$
15	$7.67^{Ab}\pm0.15$	$7.80^{Ab}\pm0.17$	$7.77^{Ab}\pm0.15$	$8.60^{Aa}\pm0.36$
Overall acceptability (9)				
1	$5.80^{Cc}\pm0.26$	$8.17^{Bb}\pm0.5$	$7.50^{Ba}\pm0.28$	$8.33^{Ca}\pm0.28$
5	$6.07^{BCb}\pm0.21$	$8.83^{A}\pm0.5$	$8.50^{Aa}\pm0.29$	$8.83^{BCa}\pm0.00$
10	$6.27^{Bb}\pm0.25$	$9.00^{Aa}\pm0.3$	$8.83^{Aa}\pm0.00$	$9.00^{ABa}\pm0.29$
15	$6.90^{Ab}\pm0.10$	$9.00^{Aa}\pm0.0$	$9.00^{Aa}\pm0.00$	$9.00^{Aa}\pm0.00$

Different superscript capital letters within control and treatments (A, B……) are significantly different (P >0.05)

Different superscripts, small letters within different storage times (a, b……) are significantly different (P >0.05)

Biological Evaluation

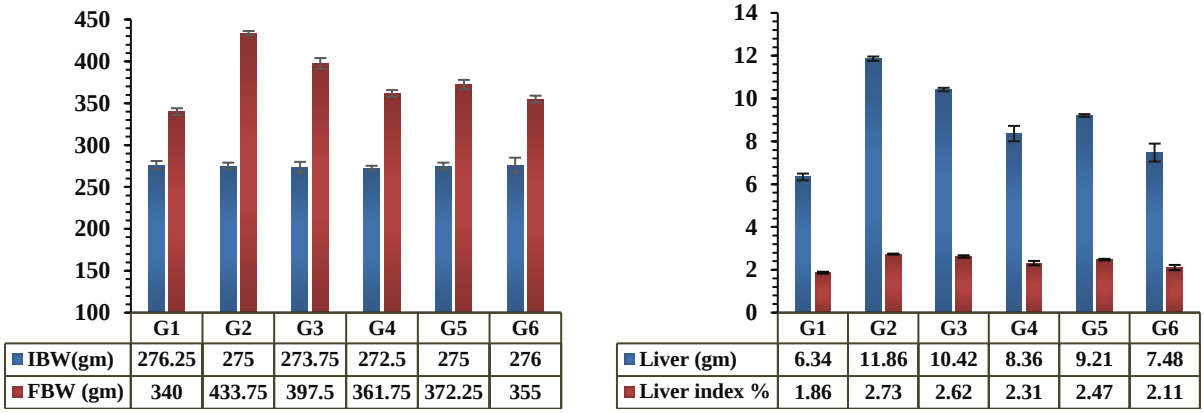
Effect of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains on liver and body Metrics

The impact of different treatments of FB supplemented with RBJ on various physiological parameters was investigated. Figure 4 shows that the initial body weights of all groups were similar, ranging from 272.5 to 276.0 g. On the contrary, the final body weights of all groups gradually increased. However, rats that received HFCS (G2) exhibited a marked increase in body weight, reaching a maximum weight of 433.75 g ± 9.53, representing a 57.73% increase from the baseline, which was significantly higher compared to the normal group (G1).

Interestingly, G6, which received FBRM (YC + encapsulated *L. paracasei* B-4564 + *B. bifidum* B-41410), showed the lowest weight gain percentage at 28.73%, with a final body weight of 355.0 g ± 4.08, which was closer to the weight gain percentage of the normal group (23.11%). Moreover, G4, which received FBRP (YC + encapsulated *L. paracasei* B-4564) and G5, which received FBRB (YC + encapsulated *B. bifidum* B-41410), display significant differences in their results compared to G2 (positive control, HFCS). While G3, which received FBR (YC + 5% RBJ), exhibited the lowest improvement.

In terms of liver weight and liver index percentage, similar trends were observed. G2 (positive control, HFCS) had the highest liver weight (11.86 g ± 0.59) and liver index (2.73% ± 0.16), significantly higher than G1 (normal group), which had the lowest values (6.34 g ± 0.30 and 1.86% ± 0.07, respectively). Conversely, G6 showed the most notable improvement in liver weight (7.48 g ± 0.42) and liver index (2.11% ± 0.12), closer to normal control values. Also, groups G4 and G5 showed significant differences in their liver weight and liver index% compared to G2, while G3 recorded the lowest improvements. These findings are in alignment with Sandeva *et al.*, (54), who concluded that HFCS increased body weight due to impairment of leptin secretion that regulates the appetite. Also, Wang *et al.*, (28) who observed higher liver and body weights of mice provided HFCS for 4 months compared to control. Moreover, most of the HFCS intake was metabolized in the small intestine and rapidly absorbed, causing hepatic lipid accumulation (3).

Previous studies have shown that RBJ significantly reduced body weight gain in obese, anemic rats due to its rich antioxidant content (55). Additionally, *L. paracasei* probiotic strains have been shown to reduce body fat and improve lipid metabolism in both animal models and humans (56). In conclusion, FB combined with RBJ and probiotics may offer an effective strategy for managing obesity-related complications, such as excessive weight gain and liver steatosis. The combined antioxidant effects of fermented RBJ, along with the bioactive compounds in FB and the gut-modulating properties of probiotics, provide holistic health benefits. Furthermore, probiotics have been shown to reduce hepatic steatosis and systemic inflammation (57).



	G1	G2	G3	G4	G5	G6
IBW(gm)	276.25	275	273.75	272.5	275	276
FBW (gm)	340	433.75	397.5	361.75	372.25	355

	G1	G2	G3	G4	G5	G6
Liver (gm)	6.34	11.86	10.42	8.36	9.21	7.48
Liver index %	1.86	2.73	2.62	2.31	2.47	2.11

Figure 4. Effect of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains on Liver and Body Metrics

Effect of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains on glucose, insulin, and insulin resistance levels

As presented in Figure 5 glucose, insulin, and IR levels were significantly higher in the HFCS rats (G2) (230.78 mg/dl, 8.19 µU/mL, and 78.33, respectively), as compared to normal rats (G1) which had a normal baseline of glucose, insulin, and IR levels at 103.11±3.36 mg/dL, 4.04±0.17 U/mL, and 18.49±0.64 respectively.

Interestingly, G3, which received FBR, showed slight improvements in these parameters, which recorded 190.22 ± 6.77 mg/dL for glucose, 6.39 ± 0.75 µU/mL for insulin, and 55.28 for IR, suggesting that the antioxidant and anti-inflammatory properties of RBJ may have contributed to these improvements. However, G6 (FBRM) demonstrated a more significant decrease in glucose (110.60 ± 4.68 mg/dL), insulin (4.10 ± 0.29 U/mL), and IR (22.79 ± 0.97), with results closest to the normal group (G1). G4 (FBRP) and G5 (FBRB) also demonstrated improvements; however, no significant differences were found between these groups compared to G6. These results align with (13), which observed a significant reduction of glucose along with enhanced insulin sensitivity in diabetic rats due to nourished on stirred yoghurt fortified with beetroot powder and *B. lactis* Bb12 + *L. acidophilus* CH-2. Also, Ban *et al.*, (58) found high counts of Lactobacillus ssp., and Bifidobacterium ssp. The consumption of synbiotic yogurt treatments led to an increase in SCFA levels, mainly propionic acid, butyric acid, and acetic acid, which alleviate diabetes and obesity.

Fructose consumption has been shown to increase de novo lipogenesis (DNL), liver fat accumulation, insulin resistance, and fasting blood glucose levels. Conversely, the significant improvement observed in all FB treated groups can be attributed to the antidiabetic effect of RBJ (59). Furthermore, the antidiabetic effect of probiotics strains, which improve glucose metabolism, enhance insulin sensitivity, and modulate the gut microbiota activity (60).

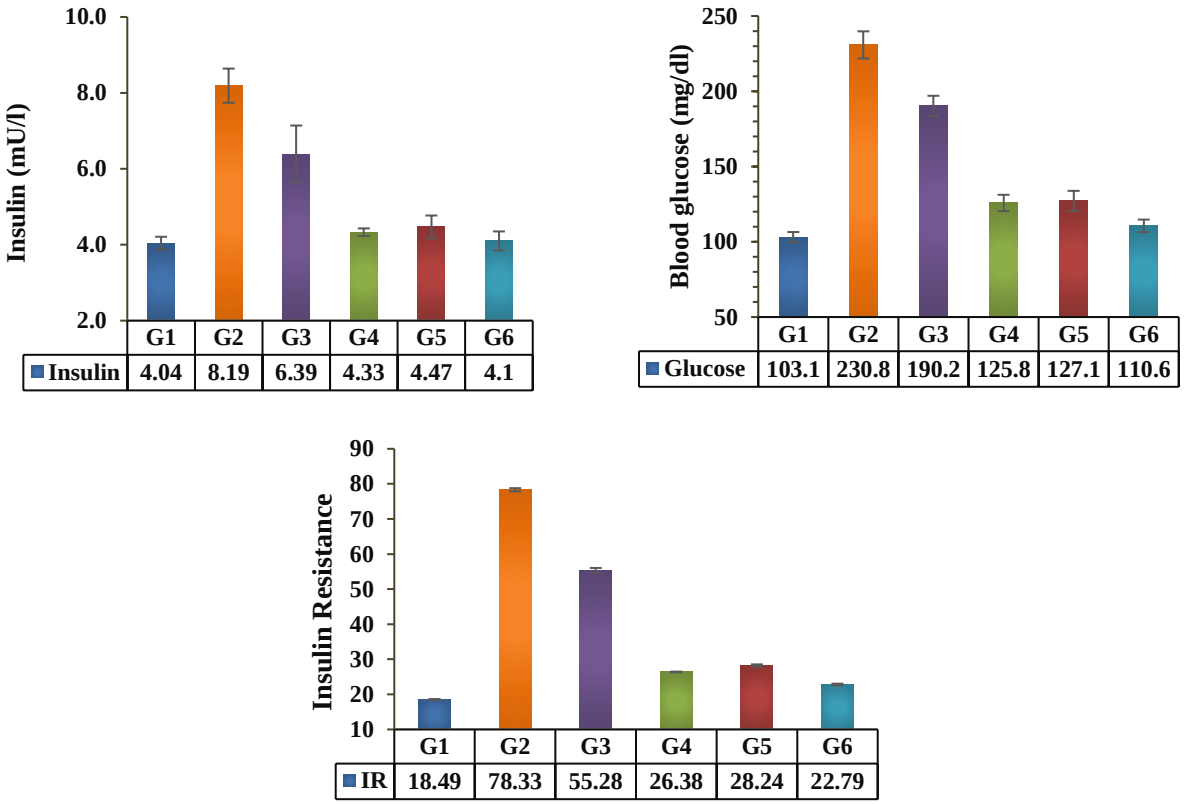


Figure 5. Effect of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains on glucose, insulin, and insulin resistance levels

Effect of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains on Liver Functions

The liver is a vital metabolic organ responsible for detoxification, sugar storage, and protein synthesis (61). The impact of FB incorporated with 5% RBJ and microencapsulated probiotic strains on liver functions after HFCS consumption was evaluated. The results, shown in Figure 6, indicate significant differences in ALT, AST, albumin, and total protein levels across all groups. G1 maintained normal levels for all the studied parameters, indicating healthy liver function. Meanwhile, G2 (HFCS) showed significantly elevated ALT (43.46 IU/L) and AST (67.2 IU/L) levels, along with reduced albumin (3.78 g/dL) and total protein (7.2 g/dL). Meanwhile, G6 (FBRM) exhibited the lowest ALT (28.28 IU/L) and AST (43.85 IU/L) levels and the highest total protein levels (9.72 g/dL), indicating the most therapeutic effect, closely approaching the values observed in the normal group. Interestingly, G4 (FBRP) and G5 (FBRB) showed improvements with non-significant differences compared to G6 (FBRM). These findings are consistent with a previous study (62), which demonstrated that green tea yogurt containing encapsulated *L. paracasei* reduced liver damage markers in the serum of rats fed on a high fructose diet.

The current results revealed that a combination of *L. paracasei* B-4564, *B. bifidum* B-41410, and RBJ into functional buttermilk offers significant liver protective effects, improves liver health,—and reduces inflammation caused by HFCS. These

combined effects contribute to lowering liver enzyme levels (ALT, AST), enhancing protein synthesis, and reducing liver damage; whereas microencapsulated probiotics aid in metabolizing bile acids and producing short-chain fatty acids that reduce liver fat (57). Encapsulated probiotics can also reduce liver inflammation by improving gut barrier function and reducing endotoxin leakage into the bloodstream (63). Furthermore, RBJ, rich in antioxidants, particularly betalains, improves liver functions, protects the liver from oxidative stress and inflammation. RBJ purifies the blood, regenerates the red blood cells, and provides oxygen to the body, being rich in iron content, as well as detoxifies the liver and prevents fat formation through betaine, as shown by Fateh *et al.*, (64).

Effect of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains on Lipid Profile

Lipid metabolism dysregulation, particularly due to excessive fructose intake, is a key factor in the development of cardiovascular diseases, hepatic steatosis, and metabolic syndrome. As shown in Figure 7, significant differences in blood lipid parameters were observed across the experimental groups. G1 displayed the lowest levels of total cholesterol (TC), triglycerides (TG), LDL, and VLDL (74.67, 87.45, 22.13, and 16.49 mg/dL, respectively), along with the highest HDL (40.05 mg/dL), indicating a healthy lipid profile. On the other hand, G2 (HFCS) exhibited a marked increase in TC (265.00mg/dL), TG (161.64 mg/dL), and LDL (185.45 mg/dL), while HDL levels decreased to 23.51 mg/dL, reflecting a disrupted lipid profile associated with metabolic disorders and the adverse effects of HFCS.

Whereas G3 to G6, which received FB with microencapsulated probiotic either single or mixed, showed improvements in lipid profiles, including reduced levels of TC, TG, LDL, and VLDL, along with increased HDL. Among these, G6 (FBRM) recorded the most significant improvements; thus, it recorded 85.36 mg/dL, 95.84 mg/dL, and 37.43 mg/dL for total cholesterol, triglycerides, and HDL levels, respectively; this indicates a restoration of healthier lipid levels compared to other groups. There were no significant differences between G4 (FBRP) and G5 (FBRB), while G3 (FBR) showed the least improvement among the treated groups.

High intake of HFCS increases total cholesterol and hepatic de novo lipogenesis (DNL), raising triglyceride levels. Additionally, HFCS consumption promotes the formation of small, dense LDL particles, which are more likely to contribute to arterial plaque formation. It also increases liver triglyceride synthesis and production of VLDL while lowering HDL levels, thereby elevating the risk of cardiovascular diseases (65). Briefly, HFCS consumption significantly impacts lipid metabolism, leading to dyslipidemia (66).

In contrast, different treatments of FB with microencapsulated probiotics have been shown to improve lipid profiles by modulating gut microbiota, enhancing bile acid metabolism, and modulating lipid metabolism. Furthermore, RBJ decreased LDL cholesterol, normalized blood pressure, and promoted cardiovascular health (13).

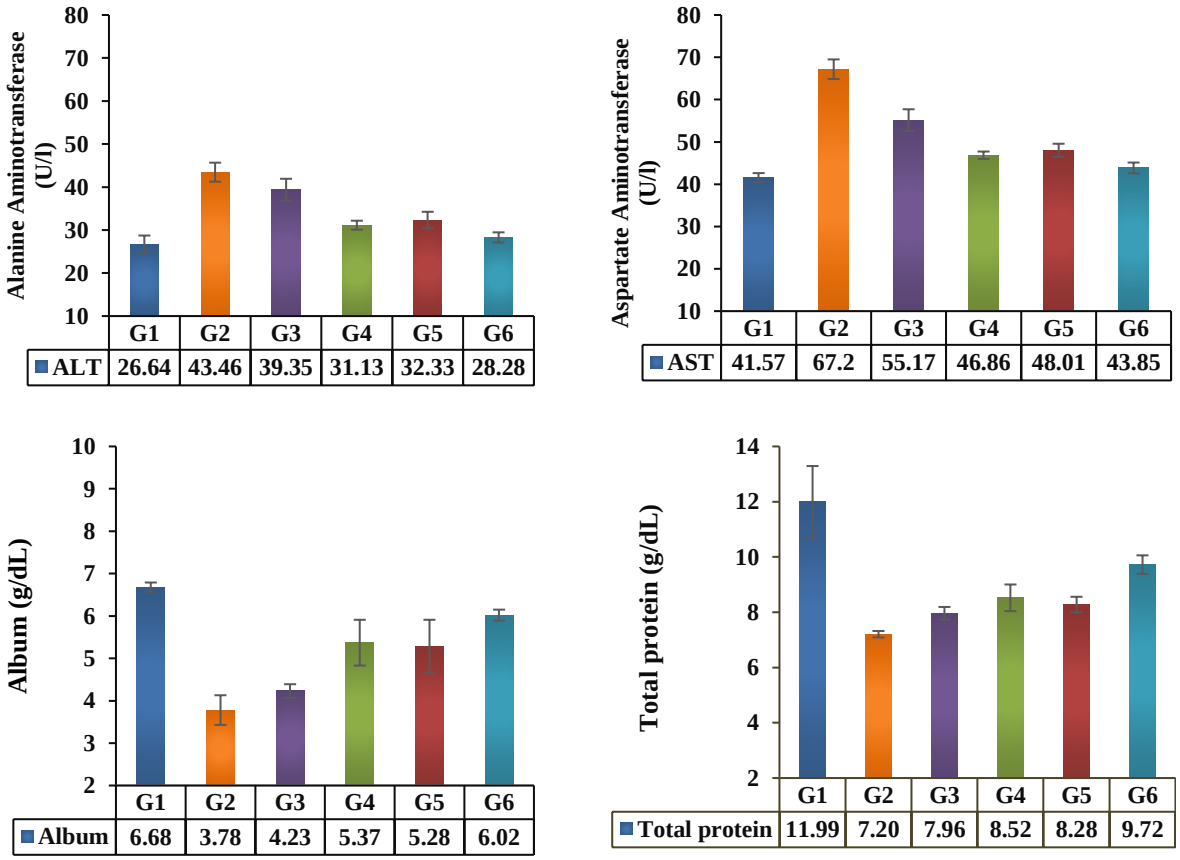


Figure 6. Effect of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains on Liver Functions

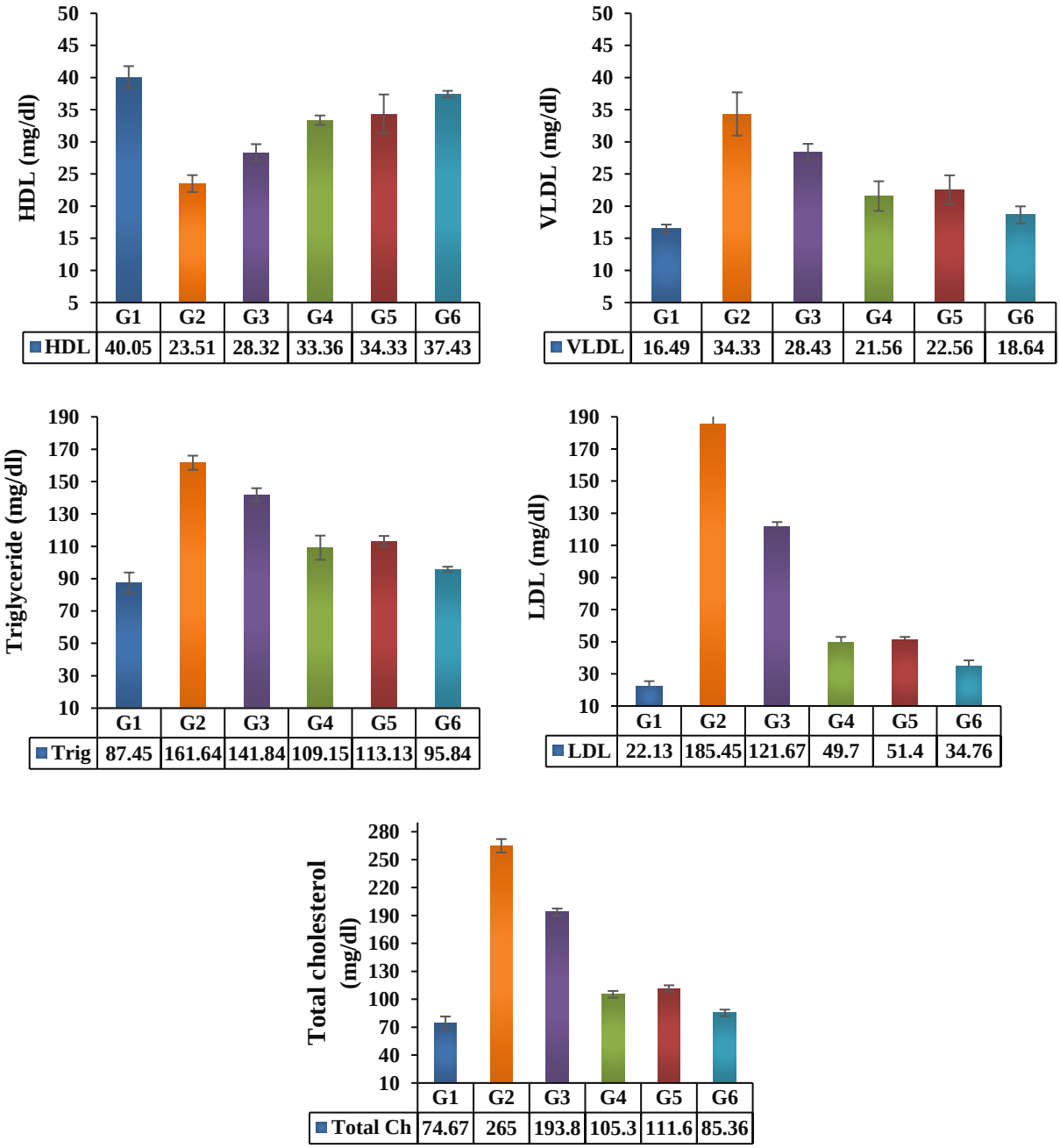


Figure 7. Effect of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains on Lipid Profile

Effect of Functional Buttermilk Supplemented with RBJ and Microencapsulated Probiotic Strains on Rats' Fecal Microbiology:

As shown in Figure 8, significant differences in various microbial genera were observed across the experimental groups, reflecting the impact of HFCS consumption and treatment interventions on the gut microbial community. Different gut microbiota in the fecal samples were determined at the beginning of the biological experiment, after consumption of HFCS (3 months), and at the end of the biological experiment (5 months).

At the beginning of the experiment, non-significant differences in microbial counts were observed among all the different rat groups. However, after three months, a significant reduction of probiotics (Bifidobacterium ssp. and lactobacilli ssp.) occurred in all rats (G2 to G6) that consumed 30% HFCS compared with the normal group (G1), which still at the same $\log_{10}$ CFU/ml. While total bacterial counts and coliform group exhibited the highest counts in G2 to G6 compared to their counts at the beginning of the experiment.

Previous studies have shown an observable reduction of butyrate-producing bacteria counts, meanwhile increase gram gram-negative bacteria, such coliform group, occurred due to HFCS, which causes the microbial imbalance (2). Additionally, Sánchez-Terrón *et al.*, (3) observed a reduction in the expression of probiotic bacteria in contrast with promoting the expression of sulfate-reducing bacteria and their harmful metabolites in rats provided HFCS for 4 months. Following Wang *et al.*, (28) who reported that most of HFCS intake was metabolized in small intestine, fermented by gut microbiota led to gut microbial composition alteration with a notably reduction of probiotic bacteria.

After two months, the intervention with the prepared FB supplemented with 5% RBJ and microencapsulated probiotics resulted in a significant increase of beneficial bacteria (genera of Bifidobacterium ssp. and lactobacilli ssp.) in rats' feces (G3 to G6) compared to normal and positive controls. Meanwhile, lower coliform group counts were found in rats' feces, which were provided with FB and microencapsulated probiotics, either single or mixed strains (G4 to G6). Total bacterial counts were slightly higher in all groups compared with their counts at the beginning of the experiment. The results align with Mohamed *et al.*, (13) found a significant increase of *B. lactis* Bb12 + *L. acidophilus* CH-2 counts in rats fecal which fed stirred yoghurt fortified with beetroot powder.

The current results revealed that microencapsulated probiotics and RBJ improved gut health. Whereas *L. paracasei* B-4564 *and B. bifidum* B-41410 help balance gut microbiota and enhance gut barrier function due to the therapeutic effects of probiotics, either live cells or dead cells, as well as their metabolic products. This agrees with Melia *et al.* (67) who found a significant increment of probiotic counts in the feces of rats fed on fermented buttermilk with *Pediococcus acidilactici BKO1* and reduction of pathogenic bacteria. Furthermore, Calvani *et al.* (68) observed that consumption of RBJ for two weeks provided beneficial effects in microbial gut composition by increasing probiotic counts in the host gut.

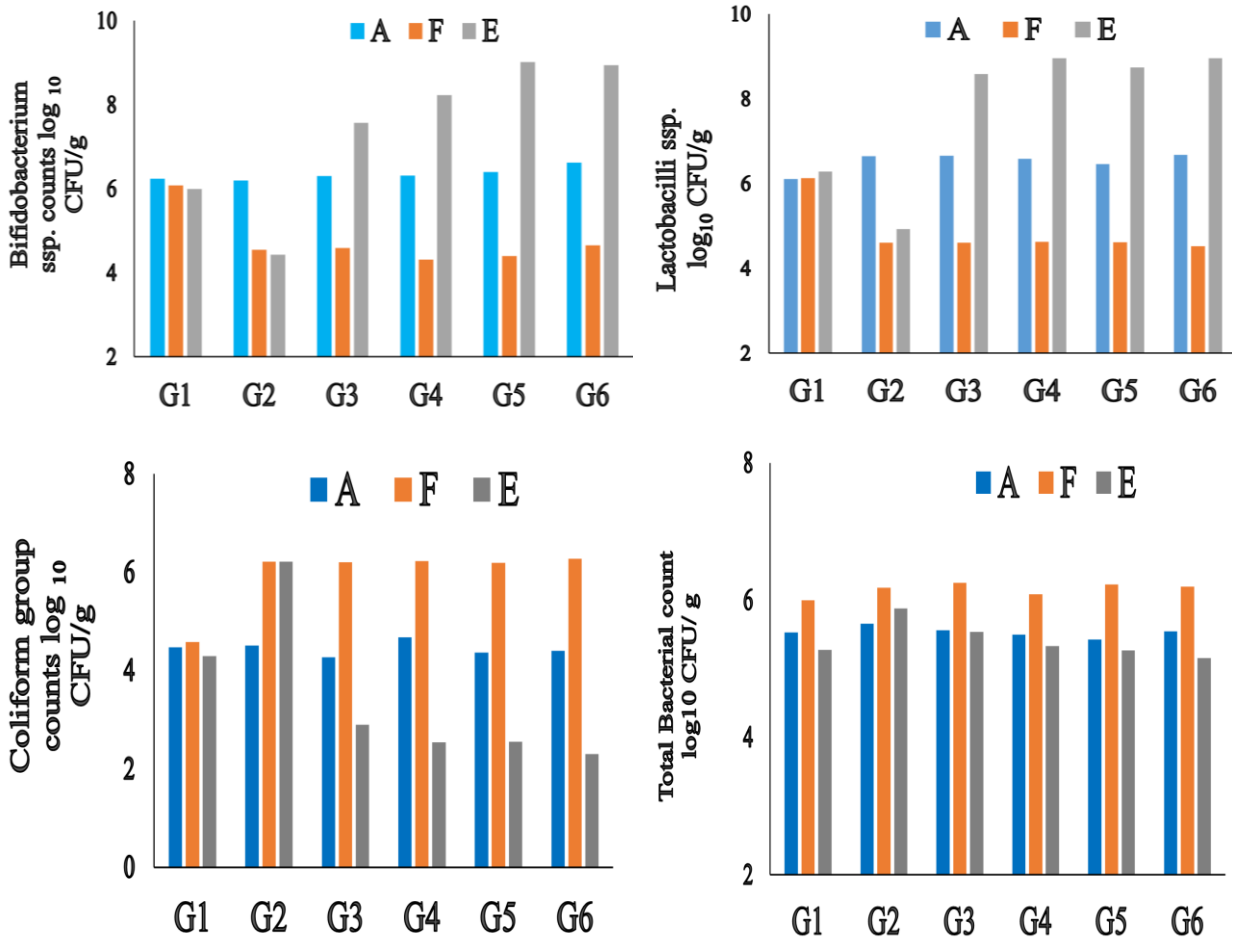


Figure 8. Microbiological Counts of Rats' Feces. A. refers to: The beginning of the experiment, F. refers to: After HFCS consumption, E. refers to: The end of the experiment.

Histopathological Examination of Liver and Intestinal Tissues

Figures 9 & 10 exhibited the effect of daily administration of FB supplemented with 5% RBJ with different microencapsulated probiotics on liver and intestinal tissues in rats that consumed 30% high-fructose corn syrup for 3 months, as well as controls.

Liver histopathology:

As presented in Figure 9, histological examination of liver sections revealed significant differences across the experimental groups. G1 (normal control) exhibited a normal histological structure, with well-defined central veins, portal areas, and healthy hepatic parenchymal cells. Meanwhile, G2 (control-positive, HFCS) showed severe pathological changes, including congestion of central veins and hepatic sinusoids, hepatocellular swelling, degeneration, necrosis, and scattered apoptotic cells, indicating significant liver damage due to HFCS consumption. G3 (FBR) displayed moderate protection of hepatic cells, with mild congestion,

swelling, and degeneration of the hepatic cells, and a few cells with pyknotic nuclei, indicating some cellular damage but less severe than G2. G4 (FBRP) demonstrated good restoration of hepatic cells, with only mild sinusoidal dilatation, indicating less liver damage and a better recovery compared to G3. While G5 (FBRB) showed good protection of hepatic parenchymal cells, with minimal damage, suggesting effective liver protection. In contrast, G6 (FBRM) exhibited the best protection, with healthy hepatic cells and intact hepatic parenchyma, indicating the most effective intervention among all groups.

Intestinal histopathology

As shown in Figure 10, the intestinal histological examination also revealed significant differences between the groups. Normal control, G1 displayed normal histology of the intestinal villi and epithelium, indicating healthy gut tissue. Whereas G2 (control-positive, HFCS) showed severe inflammatory reactions, including marked necrosis of the intestinal mucosal linings, desquamation of the epithelium, and degeneration of the intestinal epithelium, with scattered apoptotic bodies, reflecting significant intestinal damage due to HFCS intake. G3 (FBR) showed mild inflammatory cell infiltration and mild degenerative changes in the mucosal epithelial cells, indicating partial protection of the intestinal tissues. While G4 (FBRP) demonstrated significant protection of the intestinal mucosa, with very few mononuclear inflammatory cells, suggesting effective recovery and less damage. G5 (FBRB) showed significant recovery of the intestinal mucosa, though epithelial lifting suggested incomplete repair processes. On the other hand, G6 (FBRM) displayed strong protection of the intestinal mucosa, with only mild changes in the intestinal epithelium, indicating the best protective effect on gut health.

Microscopic examination of liver and intestinal tissues from G2 revealed marked tissue alterations, indicating significant damage caused by HFCS consumption. In contrast, tissue sections from treated groups showed varying degrees of protection, with G6 demonstrating the most effective protection of both hepatic and intestinal tissues. G4 and G5 also exhibited significant improvements, while G3 showed the least improvement effect.

These findings suggest that the bioactive and biological components within functional buttermilk (FB), along with red beetroot juice (RBJ), contributed to enhancing the growth of beneficial microorganisms in the gut, which helped maintain a healthy microbial community, reducing the growth of harmful bacteria and their metabolic byproducts, which could otherwise contribute to liver and intestinal damage.

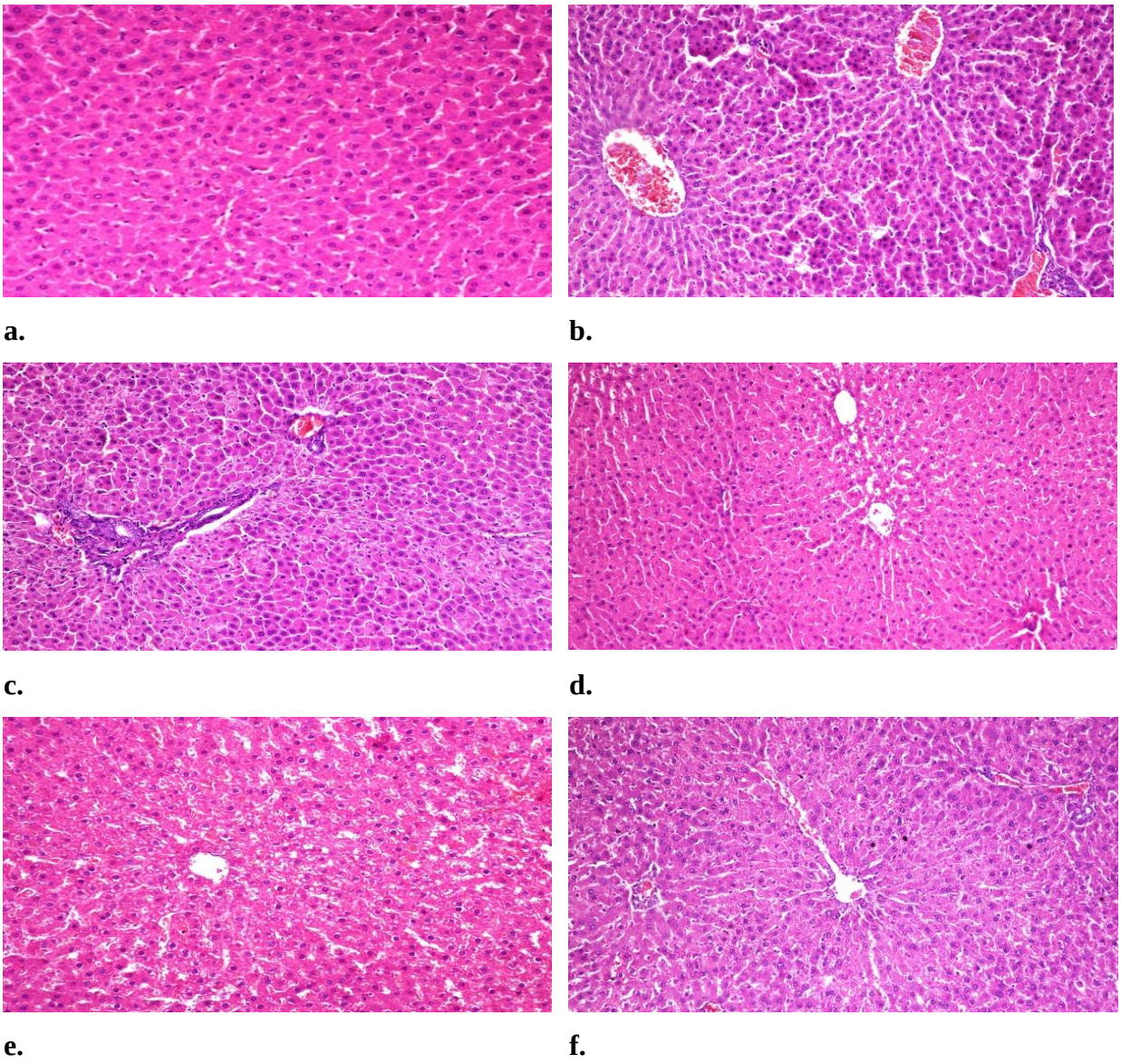
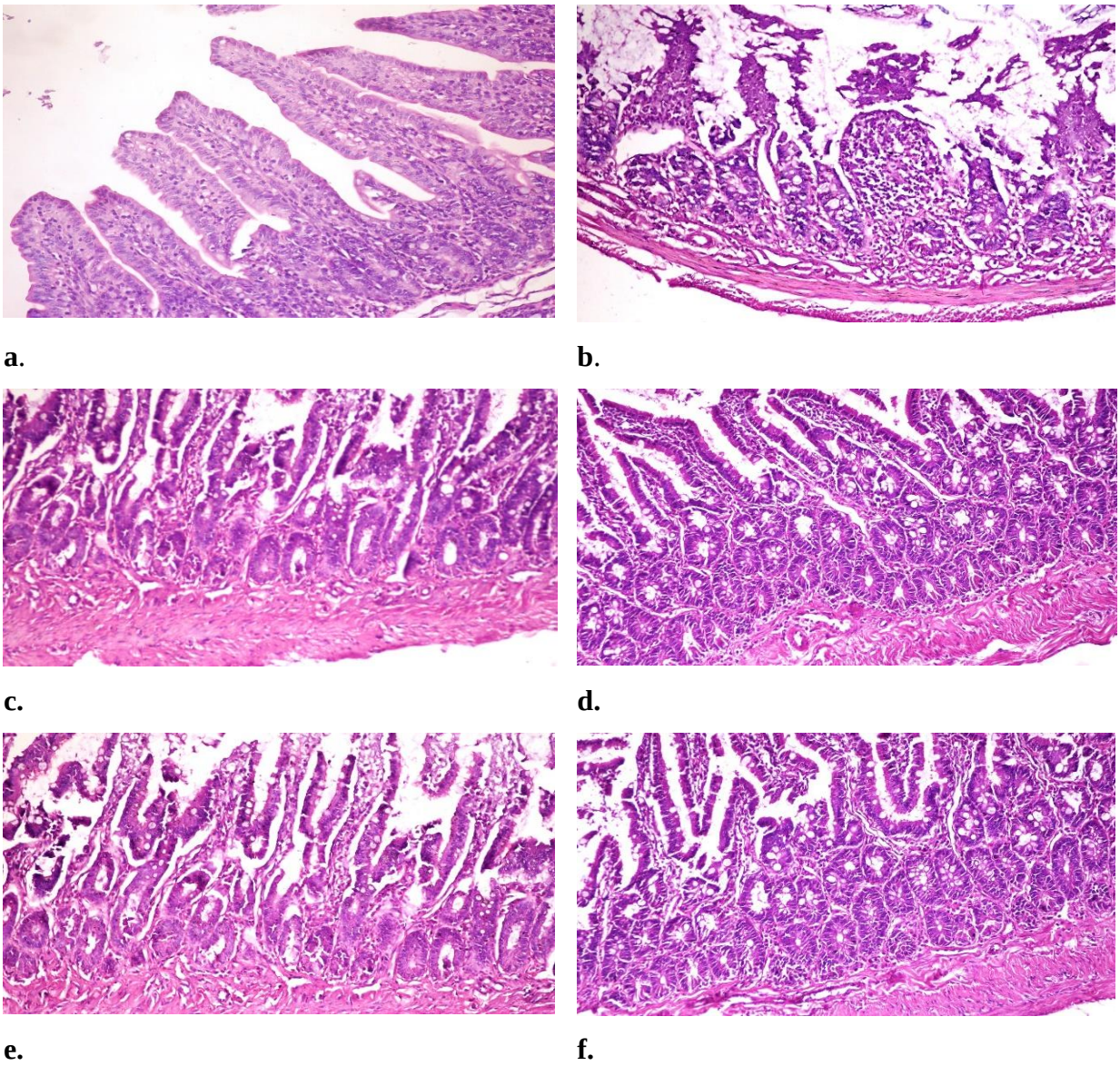


Figure 9. Liver section of different groups, a. normal control (G1), b. Positive control (G2), c. G3 rats treated with FBR, d. G4 rats treated with FBRP, e. G5 rats treated with FBRB, f. G6 rats treated with FBRM. (H&E, X200)



a. **b**. **c.** **d.** **e.** **f.**

Figure 10. Intestinal tissues of different groups, a. normal control (G1), b. Positive control (G2), c. G3 rats treated with FBR, d. G4 rats treated with FBRP, e. G5 rats treated with FBRB, f. G6 rats treated with FBRM. (H&E, X200)

CONCLUSION

The current study has highlighted the potential use of functional buttermilk (FB) supplemented with 5% red beetroot juice (RBJ) and microencapsulated probiotics (*B. bifidum* B-41410 and *L. paracasei* B-4564) in single or combination form as a dietary intervention for managing metabolic syndrome induced after high-fructose corn syrup consumption. Microencapsulation improved the efficacy of probiotics in managing metabolic disorders by enhancing their viability and stability during processing, storage of FB, and through gastrointestinal transit. Incorporation of RBJ and microencapsulated probiotics to produce FB enhanced probiotics stability that promotes metabolic health, gastrointestinal function, and sensory properties. Results of the biological experiment concluded that FB supplemented with 5% RBJ and combined microencapsulated probiotics showed the most significant improvements in glucose metabolism, lipid profile, and liver functions in the HFCS rat model.

Histopathological examination confirmed the therapeutic effects of FB with mixed microencapsulated probiotics and 5% RBJ to overcome pathological features contributed to HFCS in liver and intestinal tissues.

Conflict of interest: NIL

Funding: NIL

REFERENCES

1. Gauthier, E., Milagro, F. I., & Navas-Carretero, S. Effect of low-and non-calorie sweeteners on the gut microbiota: A review of clinical trials and cross-sectional studies. Nutrition. (2024), 117, 112237.
2. Jung S., Bae H., Song W., Jang C. Dietary Fructose and Fructose-Induced Pathologies. Annu Rev Nutr. (2022), 22; 42: 45–66.
3. Sánchez-Terrón, G., Martínez, R., Ruiz, J., Luna, C., & Estévez, M. Impact of sustained fructose consumption on gastrointestinal function and health in wistar rats: glycoxidative stress, impaired protein digestion, and shifted fecal microbiota. Journal of Agricultural and Food Chemistry. (2023), 71(43), 16270-16285.
4. Arenas-Jal, M., Suñé-Negre, J. M., & García-Montoya, E. An overview of microencapsulation in the food industry: Opportunities, challenges, and innovations. European Food Research and Technology. (2020), 246, 1371-1382.
5. Ali A. H. Current knowledge of buttermilk: Composition, applications in the food industry, nutritional and beneficial health characteristics. International Journal of Dairy Technology. (2019), Vol 72, No 2.
6. Baheti, R., Mahore, J., & Patole, V. Different formulation approaches to improve the survivability of probiotics in the digestive tract. *Novel Research in Microbiology Journal*. (2023), *7*(2), 1873-1896.
7. Yao, B., Wei, W., & Zhang, H. Efficacy of probiotics or synbiotics supplementation on chemotherapy-induced complications and gut microbiota dysbiosis in gastrointestinal cancer: a systematic review and meta-analysis. *European Journal of Clinical Nutrition*. (2024), 1-11.
8. Abdo E., El-Sohaimy S., Shaltout O., Abdalla A. and Zeitoun A. Nutritional Evaluation of Beetroots (Beta vulgaris L.) and Its Potential Application in a Functional Beverage. Plants. (2020), 9, 1752.
9. Milton-Laskibar, I., Martínez, J. A., & Portillo, M. P. Current knowledge on beetroot bioactive compounds: Role of nitrate and betalains in health and disease. *Foods*. (2021), *10*(6), 1314.
10. Zamani H., de Joode M. E. J. R., Hossein I. J., Henckens N. F. T., Guggeis M. A., Berends J. E., de Kok T. M. C. M., and van Breda S. G. J. The benefits and risks of beetroot juice consumption: a systematic review. Critical Reviews in Food Science and Nutrition. (2021), 61(5) 788–804.
11. Jakubczyk, K.; Melkis, K.; Janda-Milczarek, K.; Skonieczna Zydecka, K. Phenolic Compounds and Antioxidant Properties of Fermented Beetroot Juices Enriched with Different Additives. Foods. (2024), 13, 102.
12. El-Abd, M. M., Abdel-Hamid, M., Ahmed-Farid, O. A., El-demerdash, M. E., & Mohamed, Z. F. Protective Effect of Synbiotic Fermented Camel Milk on Non Alcoholic Fatty Liver Disease in Rats. *Curr. Sci. Int*. (2018), *7*(4), 466-480.
13. Mohamed, D. A., El-Sayed, H. S., Abd El-Gawad, M. A. M., Abdelgayed, S. S., Hamed, I. M., & Mohamed, R. S. Characterization of stirred yoghurt enriched with probiotics and beetroot and its therapeutic potential in experimental type 2 diabetes. *Acta Scientiarum Polonorum Technologia Alimentaria*. (2021), *20*(4), 429–448.
14. Salman, K. H., Mehany, T., Zaki, K. G., & Al-Doury, M. K. W. Development of functional probiotic yogurt from buffalo milk supplemented with red beetroot (Beta vulgaris L.) as an antioxidant, natural colorant, and

starter growth stimulant. *Food Chemistry Advances*. (2024), *5*.

15. Brand-Williams, W., Cuvelier, M. E., & Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*. (1995), *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
16. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. (1999), *26*(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.
17. Stintzing, F. C., Schieber, A., & Carle, R. Evaluation of colour properties and chemical quality parameters of cactus juices. *European Food Research and Technology*. (2003), *216*, 303–311.
18. Krasaekoopt, W., Bhandari, B., & Deeth, H. C. Survival of probiotics encapsulated in chitosan-coated alginate beads in yoghurt from UHT-and conventionally treated milk during storage. *LWT-Food Science and Technology*. (2006), *39*(2), 177–183.
19. Ayama, H., Sumpavapol, P., & Chanthachum, S. Effect of encapsulation of selected probiotic cell on survival in simulated gastrointestinal tract condition. *Songklanakarin Journal of Science & Technology*. (2014), *36*(3).
20. Adebayo-Tayo, B., & Akpeji, S. Probiotic viability, physicochemical and sensory properties of probiotic pineapple juice. *Fermentation*. (2016), *2*(4), 20.
21. Terzaghi, B. E., & Sandine, W. Improved medium for lactic streptococci and their bacteriophages. *Applied Microbiology*. (1975), *29*(6), 807–813.
22. De MAN, J. C., ROGOSA, M., & SHARPE, M. E. A MEDIUM FOR THE CULTIVATION OF LACTOBACILLI. *Journal of Applied Bacteriology*. (1960), *23*(1), 130–135. https://doi.org/10.1111/j.1365-2672.1960.tb00188.x
23. Dave, R. I., & Shah, N. P. Evaluation of media for selective enumeration of Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, and bifidobacteria. *Journal of Dairy Science*. (1996), *79*(9), 1529–1536.
24. Marshall, R. T., Goff, H. D., & Hartel, R. W. *Ice cream*. Springer Science & Business Media. (2003).
25. APHA. Standard Methods for the Examination of Water and Wastewater. 18th Edition, American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Washington DC. (1992).
26. Mudgil, D., & Barak, S. Development of functional buttermilk by soluble fibre fortification. *Agro Food Industry Hi Tech*. (2016), *27*(2), 44-47.
27. Reeves, P. G., Nielsen, F. H., Fahey, G. C. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J. Nutr. (1993), 123, 1939–1951.
28. Wang, X., Zhu, L., Li, X., Wang, X., Hao, R., & Li, J. Effects of high fructose corn syrup on intestinal microbiota structure and obesity in mice. *NPJ Science of Food*. (2022), *6*(1), 17.
29. Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*. (1972), *18*(6), 499–502.
30. Guerin-Danan, C.; C. Chabanet; C. Pedone; F. Popot; P. Vaissade; C. Bouley; O. Szylit and C. Andrieux. Milk fermented with yogurt cultures and *Lactobacillus casei* compared with yogurt and gelled milk: influence on intestinal microflora in healthy infants. Am. J. Clin. Nutr. (1998), 67, 111-117.

31. Fayed B, Abood A, El-Sayed HS, Hashem AM, Mehanna NSHA. Synbiotic multiparticulate microcapsule for enhancing inulin intestinal release and Bifidobacterium gastro-intestinal survivability. Carbohydr. Polym. (2018), 193: 137- 143.
32. Bancroft, J. D., & Gamble, M. *Theory and practice of histological techniques*. Elsevier health sciences. (2008).
33. Snedecor, G. W., & Cochran, W. G. *Statistical methods Oxford and J*. BH Publishing Com. (1980).
34. Farghaly, M. A., Soliman, A. H. S., & Abdellatif, A. S. A. Evaluation of Red Pigment Extracted from Beetroot. *Current Science International*. (2019), *8*(04), 930–941.
35. Skalicky, M., Kubes, J., Shokoofeh, H., Tahjib-Ul-Arif, M., Vachova, P., & Hejnak, V. Betacyanins and betaxanthins in cultivated varieties of Beta vulgaris L. compared to weed beets. *Molecules*. (2020), *25*(22), 5395.
36. Omer, T. A., Majeed, S. N., Farkha, T. K., & Abdullah, S. N. Determination of total phenol, antioxidant and antimicrobial activity of beetroot and strawberry in Sulaimani City-Kurdistan Region, Iraq. *Egyptian Journal of Chemistry*. (2022), *65*(11), 583–593.
37. Vasconcellos, J., Conte-Junior, C., Silva, D., Pierucci, A. P., Paschoalin, V., & Alvares, T. S. Comparison of total antioxidant potential, and total phenolic, nitrate, sugar, and organic acid contents in beetroot juice, chips, powder, and cooked beetroot. Food Science and Biotechnology. (2016), 25, 79-84.
38. Esteves, L. C., Machado, C. O., Gonçalves, L. C. P., Cavalcante, V. F., Obeid, G., Correra, T. C., & Bastos, E. L. Structural effects on the antioxidant properties of amino acid betaxanthins. *Antioxidants*. (2022), *11*(11), 2259.
39. Diasari, N. R., Nurrahman, N., & Yusuf, M. ANTIOXIDANT ACTIVITY AND PHYSICAL PROPERTIES SOYGHURT EDAMAME WITH RED BEET. *Edible: Jurnal Penelitian Ilmu-Ilmu Teknologi Pangan*. (2021), *10*(1), 6–16.
40. Vaithilingam, M., Chandrasekaran, S., Mehra, A., Prakash, S., Agarwal, A., Ethiraj, S., & Vaithiyanathan, S. Fermentation of beet juice using lactic acid bacteria and its cytotoxic activity against human liver cancer cell lines HepG2. *Current Bioactive Compounds*. (2016), *12*(4), 258–263.
41. Chen, H., Kou, J., Hu, M., & Shu, G. Optimization of nitrogen source for using response surface methodology. *Acta Universitatis Cibiniensis. Series E: Food Technology*. (2016), *20*(1), 53-64.
42. Etchepare, M. de A., Barin, J. S., Cichoski, A. J., Jacob-Lopes, E., Wagner, R., Fries, L. L. M., & Menezes, C. R. de. Microencapsulation of probiotics using sodium alginate. *Ciência Rural*. (2015), *45*, 1319–1326.
43. Pupa, P., Apiwatsiri, P., Sirichokchatchawan, W., Pirarat, N., Muangsin, N., Shah, A. A., & Prapasarakul, N. The efficacy of three double-microencapsulation methods for preservation of probiotic bacteria. *Scientific Reports*. (2021), *11*(1), 13753. https://doi.org/10.1038/s41598-021-93263-z
44. Shori, A. B. Microencapsulation Improved Probiotics Survival During Gastric Transit. *HAYATI J. of Biosci*.(2017),*24*(1),1–5. https://doi.org/https://doi.org/10.1016/j.hjb.2016.12.008
45. Peteán, M., Beccaría, A., Burns, P., Sihufe, G., Zacarías, M. F., Binetti, A. & Vinderola, G. Influence of technological variables on the functionality of the cell-free fraction of fermented buttermilk. International Journal of Dairy Technology. (2014), 67(1), 39-46.
46. El-Hameed, A., Mohamed, E., El-Sattar, A., Khalifa, S. A., & El-Neshawy, A. A. An attempt to produce synbiotic buttermilk beverages supplemented with barley and oat flour. *Zagazig Journal of Agricultural Research*. (2019),

46(6), 1985–1994.

47. Ortakci, F. A. T. İ. H., Broadbent, J. R., McManus, W. R., & McMahon, D. J. Survival of microencapsulated probiotic Lactobacillus paracasei LBC-1e during manufacture of Mozzarella cheese and simulated gastric digestion. *Journal of dairy science*. (2012), *95*(11), 6274-6281.
48. FAO/WHO Expert Committee on Food Additives. Meeting, & World Health Organization. Evaluation of certain mycotoxins in food: Fifty-Sixth report of the Joint FAO/WHO Expert Committee on Food Additives ((2002, Vol. 56). World Health Organization.
49. Raghunath, B. V, Kumaresan, G., Elango, A., Sureshkumar, S., & Chandrashekara, N. *Effect of different herbal infusions on texture of yoghurt on various storage periods*. (2020).
50. Supavititpatana, P., & Kongbangkerd, T. The effect of partial replacement of non-fat dry milk with sodium caseinate on qualities of yogurt ice cream from coconut milk. *International Food Research Journal*. (2011), *18*(1).
51. Naseem, Z., Mir, S. A., Wani, S. M., Rouf, M. A., Bashir, I., & Zehra, A. Probiotic-supplemented fruit juices: Health benefits, challenges, and future perspective. *Nutrition*. (2023), *115*, 112154.
52. Ahmad A. F., Ali A. O. Effect of Beta vulgaris root extracts in Rayeb milk on its microbiological, chemical and nutritional composition. Novel Research in Microbiology Journal. (2019), 3(2): 286-296
53. Abbaszadeh, S., Gandomi, H., Misaghi, A., Bokaei, S., & Noori, N. The effect of alginate and chitosan concentrations on some properties of chitosan-coated alginate beads and survivability of encapsulated Lactobacillus rhamnosus in simulated gastrointestinal conditions and during heat processing. *Journal of the Science of Food and Agriculture*. (2014), *94*(11), 2210–2216.
54. Sandeva R. V., Mihaylova S. M., Sandeva G. N., Trifonova K. Y., Popova-Katsarova R. D. Effect of high fructose solution on body weight, body fat, blood glucose and triglyceride levels in rats. J Biomed Clin Res. (2015), 8 (1).
55. El-Ghandour, H. M., & Ragheb, E. M. Effect of red beetroot juice on body weight status of obese anemic rats. *Egyptian Journal of Nutrition*. (2018), *33*(1), 1–36.
56. Sun, Y., Chen, S., Ren, F., & Li, Y. Lactobacillus paracaseiN1115 attenuates obesity in high-fat diet-induced obese mice. *Food Science & Nutrition*. (2023), *11*(1), 418–427.
57. Pan, Y., Yang, Y., Wu, J., Zhou, H., & Yang, C. Efficacy of probiotics, prebiotics, and synbiotics on liver enzymes, lipid profiles, and inflammation in patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis of randomized controlled trials. *BMC Gastroenterology*. (2024), *24*(1), 283. https://doi.org/10.1186/s12876-024-03356-y
58. Ban Q., Cheng J., Sun X., Jiang Y., Zhao S., Song X., and Guo M. Effects of a synbiotic yogurt using monk fruit extract as sweetener on glucose regulation and gut microbiota in rats with type 2 diabetes mellitus. J. Dairy Sci. (2020), 103:2956–2968
59. Ninfali, P., Antonini, E., Frati, A., Scarpa, E. S. C- -Glycosyl flavonoids from Beta vulgaris cicla and betalains from Beta vulgaris rubra: antioxidant, anticancer and antinflammatory activities – A review. Phytother. Res. (2017), 31(6), 871–884.
60. Singh, R. P., Shadan, A., & Ma, Y. Biotechnological Applications of Probiotics: A Multifarious Weapon to Disease and Metabolic Abnormality. *Probiotics and Antimicrobial Proteins*. (2022), *14*(6), 1184–1210. https://doi.org/10.1007/s12602-022-09992-8
61. Sahini, N., & Borlak, J. Recent insights into the molecular pathophysiology of lipid droplet formation in hepatocytes. *Progress in Lipid*

Research. (2014), *54*, 86–112.

62. Fadlilah, D. N., Izati, R., Al Faizah, B. N., Kavitarna, S. A., Ardiansyah, E., Sa'adah, N. A. M., Atho'illah, M. F., Arifah, S. N., Soewondo, A., & Jatmiko, Y. D. Green Tea Yoghurt with Encapsulated Lacticaseibacillus paracasei E1 Improves Hepatocyte Damage in High-Fat High-Fructose Diet Mice by Reducing MDA and Increasing SOD. *Molecular and Cellular Biomedical Sciences*. (2024), *8*(3), 185–193.
63. Rodrigues, F. J., Cedran, M. F., Bicas, J. L., & Sato, H. H. Encapsulated probiotic cells: Relevant techniques, natural sources as encapsulating materials and food applications–A narrative review. *Food Research International*. (2020), *137*, 109682.
64. Fateh, H. L., Rashid, S. A., Muhammad, S. S., Al-Jaf, S. H., & Ali, A. M. Comparing effects of beetroot juice and Mediterranean diet on liver enzymes and sonographic appearance in patients with non-alcoholic fatty liver disease: a randomized control trials. *Frontiers in Nutrition*. (2023), *10*, 1181706.
65. Busnatu, S.-S., Salmen, T., Pana, M.-A., Rizzo, M., Stallone, T., Papanas, N., Popovic, D., Tanasescu, D., Serban, D., & Stoian, A. P. The role of fructose as a cardiovascular risk factor: an update. *Metabolites*. (2022), *12*(1), 67.
66. Gungor, A., Balamtekin, N., Ozkececi, C. F., & Aydin, H. İ. The Relationship between Daily Fructose Consumption and Oxidized Low-Density Lipoprotein and Low-Density Lipoprotein Particle Size in Children with Obesity. *Pediatric Gastroenterology, Hepatology & Nutrition*. (2021), *24*(5), 483.
67. Melia S., Juliyarsi I., Kurnia Y.F., Fitria N., Pratama Y. E., Ramadhanti N. Probiotic effect of fermented buttermilk from *Pediococcus acidilacticiBKO1* in fecal wister rat microflora. IOP Conf. Series: Earth and Environmental Science (2021), 888:012050.
68. Calvani R., Giampaoli O., Marini F., Marzetti E., Landi F. Beetroot juice intake positively influenced gut microbiota and inflammation but failed to improve functional outcomes in adults with long COVID: A pilot randomized controlled trial. Clinical Nutrition, (2024), 34 (12).