

Development of functional yogurt using millet starch and *Saccharomyces boulardii* yeast and study of its rheological and physicochemical properties

Ammar hammed Hannoon¹ and Mohanad Abbood Alhamid²

^{1,2}Department of Food Science, Faculty of Agriculture, University of Kufa, Najaf, Iraq.

¹E-mail: ammarh.gharbawi@student.uokufa.edu.iq

²E-mail: mohanada.alhamid@uokufa.edu.iq

Abstract

This study aimed to develop functional yogurt using *Saccharomyces boulardii* yeast and resistant starch extracted from Proso millet, and to study its rheological and physicochemical properties. Treatments Y0, Y1, Y2, and Y3 were prepared by adding different percentages of resistant starch (0.0, 0.1, 0.2, and 0.3%) respectively, to skim milk, in combination with 3% traditional lactic acid culture and 5% *Saccharomyces boulardii* yeast. The results indicated that adding Proso millet starch in moderate proportions contributed to improving the texture, increasing stability and shelf life, and improving the sensory properties of the product. The functional yogurt treatment Y3 fortified with 0.3% Proso millet starch achieved the best rheological and physicochemical properties, as the percentage of Viscosity, synereses, WHC, pH and Acidity % on the 28th day of refrigerated storage reached 2206 cP, 7.73%, 36.67%, 4.49, and 1.02, respectively, compared to the control treatment Y0, which recorded 1903 cP, 11.03%, 33.73%, 4.33, and 1.13, respectively. Y3 also achieved the highest total sensory evaluation results after 28 days of refrigerated storage, reaching 68.75. It also contributed to increased growth and viability of *S. boulardii* yeast, as Y3 recorded the highest live count at the end of refrigerated storage, reaching 7.01 log CFU./ml, compared to Y0, which recorded a live count of 6.01 log CFU./ml, thus enhancing its functional properties. It was further noted that the biological synergy between Proso millet starch and *S. boulardii* yeast represents a promising option for developing fermented yogurt products with acceptable sensory properties and enhanced consumer health benefits.

Keywords: Functional yogurt; symbiotic; *Saccharomyces boulardii*; resistant starch Millet

Introduction.

Proso millet is an ancient and important crop in the human diet, particularly in Asia, Australia, Europe, and the United States. [23] It contains high amounts of calcium, dietary fiber, and protein. It is also a gluten-free grain and helps reduce malnutrition. [15-25] Millet starch is used in the food and non-food industries as a gelling agent, thickener, water binder, emulsion stabilizer, and prebiotic for functional products. This is due to its high amylose content, which is a source of resistant starches. It also stimulates lactic acid bacteria (LAB) to produce a range of short-chain fatty

acids and antimicrobial agents in the colon. [8] The starch found in Proso millet grains is classified into three types: waxy starch with an amylose content of 0-4%; low-amylose starch with an amylose content of 8-16%; and non-waxy starch with an amylose content of 17-34%, which absorbs a small percentage of water during the cooking process. [20] Fermented dairy products are among the best means of delivering probiotics into the host body, such as functional yogurt and other lactic ferments containing probiotic strains [2-3], and they are widely spread throughout the

world due to the health benefits they offer to consumers, such as lowering blood cholesterol, preventing cancer, improving mineral bioavailability, and preventing diabetes [21]. Many studies have proven the health benefits of *S. boulardii* and its therapeutic role in regulating the digestive tract. Therefore, it has been used in the production of functional foods. Fermented dairy products such as yogurt, kefir, acidophilic milk, and cheese are among the most famous foods in which *S. boulardii* is used as a probiotic [17]. *S. boulardii* is a single-celled eukaryotic microorganism with a saprophytic feeding pattern. It is round, oval, or flat in shape, with a sticky or creamy texture. Its cell diameter ranges from 2-8 μm and lengths from 3-25 μm . It belongs to the genus *Saccharomyces*, which includes more than 20 species [19].

S. boulardii has many positive properties as a probiotic that is consumed orally. Its optimal growth temperature is 37°C, which is similar to the human body temperature. It also has the ability to resist bile salts, low pH, and enzymatic decomposition. It does not penetrate the membranes of the digestive system and is safe for human use and does not cause side effects and growing in large numbers [24]. The positive interactions that occur as a result of integrating the probiotic and the biostimulant in fermented dairy products have a major role in enhancing the viability of beneficial organisms, as well as achieving desirable rheological and texture properties and acceptable sensory evaluation even at the end

of the product's shelf life [10]. Therefore, the aim of this research was to study the rheological, physicochemical and sensory properties and increase the shelf life of fermented dairy with the bio-synergy between the probiotic yeast *S. boulardii* and the prebiotic resistant starch extracted from Proso millet.

Materials and Methods.

Materials.

Proso millet grains were obtained from the Iraqi Ministry of Agriculture, Seed Inspection and Certification Department. The variety was certified according to document number 6272 on September 17, 2024. Regilait French-made skimmed milk was purchased, as was a yogurt starter consisting of strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* Subsp *bulgaricus* in a 1:1 ratio, produced by the Italian company SACCO, and *Saccharomyces Boulardii* yeast prepared by NOW FOOD USA in a lyophilized form.

Methods.

Preparation of Proso Millet Starch.

The method described by [29-15] was followed. The grains were washed several times with distilled water to remove dust and dirt. The moisture content was then brought to 10%. The grains were then ground in a coffee grinder and passed through a sieve (mesh 212 μm). 100 grams of the resulting flour were soaked in a 0.15% sodium hydroxide solution at a ratio of 1:7 w/v. The mixture was mixed in a magnetic stirrer for 30 minutes and left at 4°C for 24 hours. The flour was then centrifuged at 22°C for 10 minutes at 5000 x g cycles. The precipitate was discarded, and the precipitate was suspended in the sodium hydroxide solution twice. The precipitate was discarded each time, and the surface layer, which was yellowish and gelatinous in texture, was scraped off. The precipitate was washed with distilled water at laboratory temperature. The washing process was repeated three times, with centrifugation after each time, and the precipitate was discarded. The surface layer is scraped off, and the pH is adjusted to 6.8-7 by adding drops of 0.1 M dilute hydrochloric acid. It is then centrifuged, followed by drying the sample at 40°C until the weight is constant. It is then crushed, passed through a 100 mesh sieve, and packaged in airtight plastic containers. Bacterial Culture Activation

S. thermophile and *L. bulgaricus* were grown in 10% recovered skimmed milk, inoculated with 3% (w/v) and incubated at 42°C until coagulation and refrigerated at 5°C. [14]

Probiotic Yeast Culture Activation.

S. Boulardii was grown in 10% recovered skimmed milk + 2% sucrose, inoculated with 3% (w/v) and refrigerated at 37°C for 24 hours. [13]

Functional Yogurt Fortified with Proso Millet Starch.

12% recovered skimmed milk was added to the culture, and 0.0%, 0.1%, 0.2%, and 0.3% of Proso millet starch were added, representing treatments Y0, Y1, Y2, and Y3, respectively. Then, it was heated at 90°C for 10 minutes and cooled to $2 \pm 40^\circ\text{C}$. A 3% (v/v) yogurt starter and a 5% (v/v) probiotic starter, *S. boulardii*, were added. After mixing well, the mixture was filled under aseptic conditions into sterile plastic containers. The mixture was then incubated at $2 \pm 40^\circ\text{C}$ until curdling occurred. The mixture was stored in a refrigerator at 5°C. Tests were performed after 1, 7, 14, 21, and 28 days.

Physico-chemical Analysis of Functional Yogurt.

Viscosity Estimation.

Apparent viscosity was estimated using a Brookfield DVII viscometer and calculated in cP units [12].

Whey Synereses Estimation(%)

50ml of functional yogurt was placed in a beaker at a 45-degree angle for 2 hours at 5°C. Then the whey that had seeped from the surface was withdrawn using a syringe, then the cup was weighed again and the equation below was used [7].

whey separation= (Weight of separated whey)/(Total sample weight before whey extraction (g)) $\times 100$

Estimating water holding capacity

10grams of functional yogurt was taken and centrifuged at 3000 Xg for 60 minutes at 10°C. The filtrate was then removed, the remaining wet precipitate was weighed, and the equation below was used [16] Estimating water holding capacity

Water holding capacity=% (Original sample weight - Precipitate weight)/(Original sample weight) $\times 100$

Estimation of pH and Total Acidity.

The pH of functional yogurt was determined by placing a pH meter sensor directly into the sample, and the percentage of total acidity was determined according to the standard method (A.O.A.C., 2008) [9].

Microbiological Analysis.

During refrigerated storage of functional yogurt, MRS agar, M17 agar, and SDA media were used to estimate the number of *St. Thermophiles* and *L. bulgaricus* were used, and the probiotic yeast *S. Boulardii* was prepared. The microbial count was determined using log CFU g-1 for three replicates [22].

Sensory evaluation.

Sensory tests were conducted in the Department of Food Sciences, College of Agriculture, University of Kufa, by a panel consisting of ten specialized professors, using a sensory evaluation form that included flavor, texture, acidity, live probiotic yeast counts, and appearance according to the evaluation from shown below [3].

Nam of Evaluator..... Evaluation date.....

Sample code	Flavor (35)	Texture (30)	Acidity (10)	Live probiotic yeast counts(15)	Appearance (10)	Total Score
1						
2						
3						
4						

Statistical Analysis.

The statistical analysis program GenStat V.12.1 (GenStat, 2009) was used, using a Completely Randomized Design (CRD). The sensory evaluation results were analyzed using a Randomized Completely Block Design (RCBD). The least significant difference (LSD) was used to determine significant differences between means at a probability level of ($P \leq 0.05$) the physical, chemical, micro biological and sensory properties using Analysis of variance (ANOVA).

Results and Discussion.

Physicochemical Properties of Functional Yogurt.

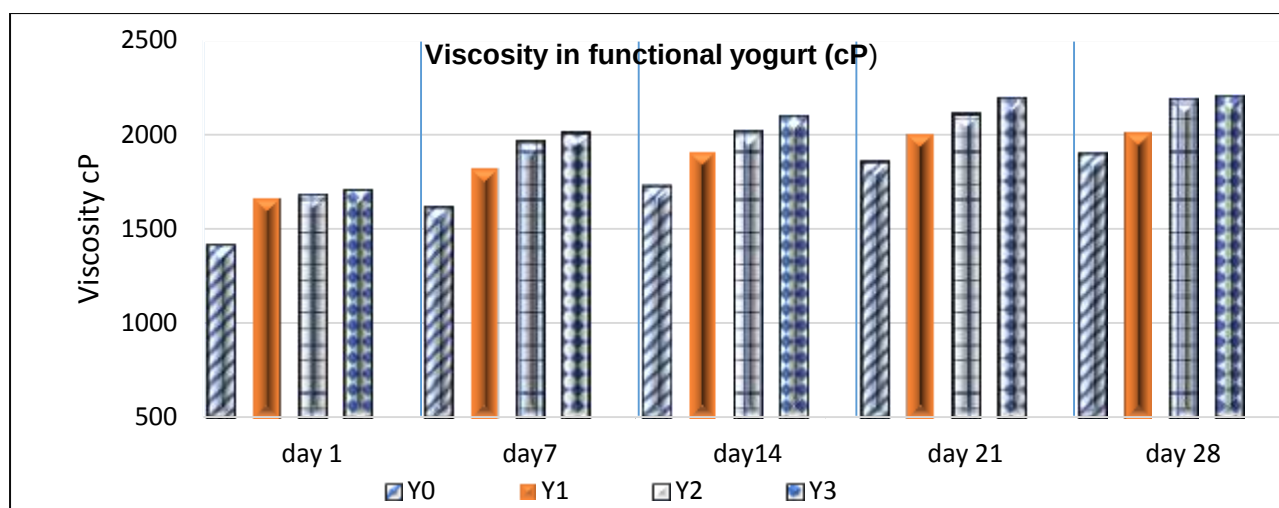
Viscosity.%

It is noted from Figure (1) that the viscosity values on the first day of refrigerated storage were higher for the functional yogurt treatments Y1, Y2, and Y3 to which Proso millet starch was added, which were 1670, 1683, and 1710 cP, respectively, compared to the control treatment Y0, which recorded the lowest viscosity of 1416 cP. The increased

viscosity values in the yogurt to which millet starch was added may be due to the higher carbohydrate content, which leads to increased growth and activity of the yogurt starter bacteria responsible for the production of extracellular Exopolysaccharides. This contributed to providing a strong texture to the

product and increasing its apparent viscosity after processing and during refrigerated storage. Viscosity is an important criterion in determining yogurt quality and is linked to product stability as well as taste. Viscosity stability is an important factor in determining its quality characteristics [28-3]. An increase in viscosity was observed as the refrigerated storage period progressed, as treatments Y1, Y2 and Y3 recorded viscosity of 2018, 2190

and 2206 cP respectively. The increase was significant compared to the control treatment Y0, which recorded the lowest viscosity of 1903 cP. The reason for the increase in viscosity during storage may be due to the decrease in pH values and the increase in EPS produced by starter bacteria, which increased the hardness of the product, in addition to the increase in viscosity with the increase in the percentage of addition of Proso millet starch [1-5.]



Y0, Y1, Y2, and Y3 represent the functional yogurt parameters added to Proso millet starch at concentrations of 0.0%, 0.1%, 0.2%, and 0.3%, respectively.

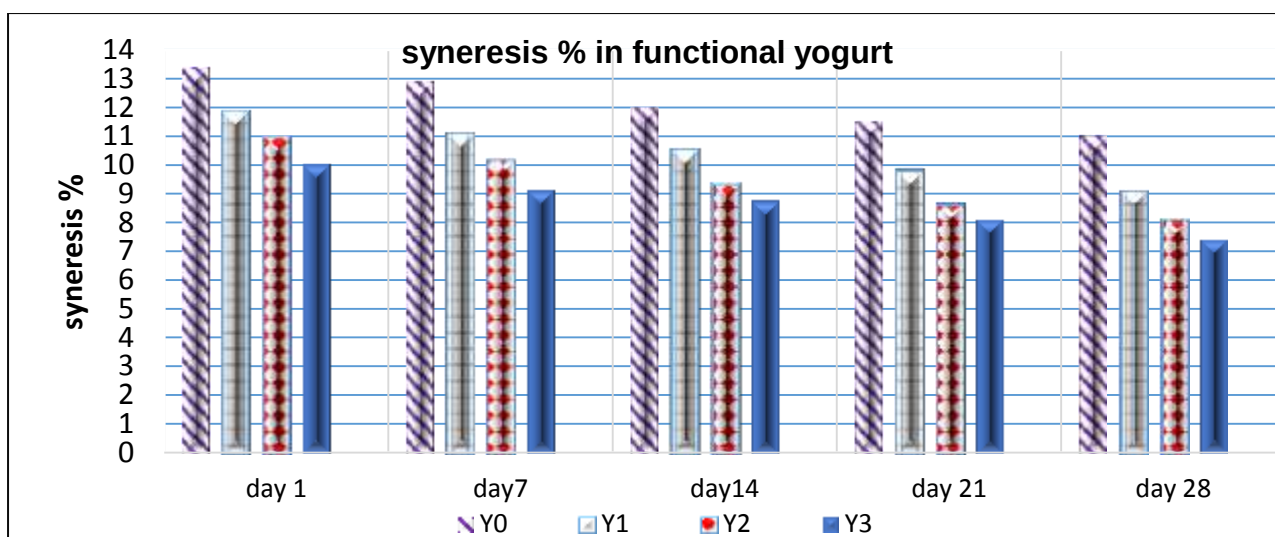
Figure 1. Viscosity (cP) of functional yogurt added to different proportions of Proso millet starch after processing and during refrigerated storage at 5°C for 28 days.

Estimation of spontaneous whey Synereses. %

It is noted from Figure (2) that the control treatment Y0 recorded the highest whey Synereses percentage on the first day after processing, reaching 13.43%. This significantly differed compared to all treatments to which Proso millet starch was added, in which a decrease in the percentage of spontaneous whey Synereses was observed with increasing starch concentration. Treatment Y3 also recorded the lowest percentage of spontaneous whey Synereses, reaching 10.05%, while the percentage of spontaneous whey Synereses for treatments Y1 and Y2 was 11.89% and 10.99%, respectively. It was noted that adding millet starch to yogurt reduced the spontaneous whey Synereses due to the increased water retention capacity of the curd [27.]

A gradual decrease in the percentage of spontaneous whey leaching was observed with the progression of the storage period for all treatments. The lowest percentage after 28 days of refrigerated storage was for treatment Y3, which reached 7.73%, followed by treatments Y1 and Y2, which recorded 9.11% and 8.12%, respectively. All treatments with Proso millet starch added significantly differed in the percentage of whey leaching compared to the control treatment Y0, which recorded the highest percentage of whey leaching at 11.03%. The decrease in spontaneous whey leaching in functional yogurt with Proso millet

Starch added may be due to the decrease in whey leaching. Proso millet starch as a result of increased metabolic activity of starter bacteria, their production of larger quantities of Exopolysaccharides, and decreased pressure in the protein matrix [22.]



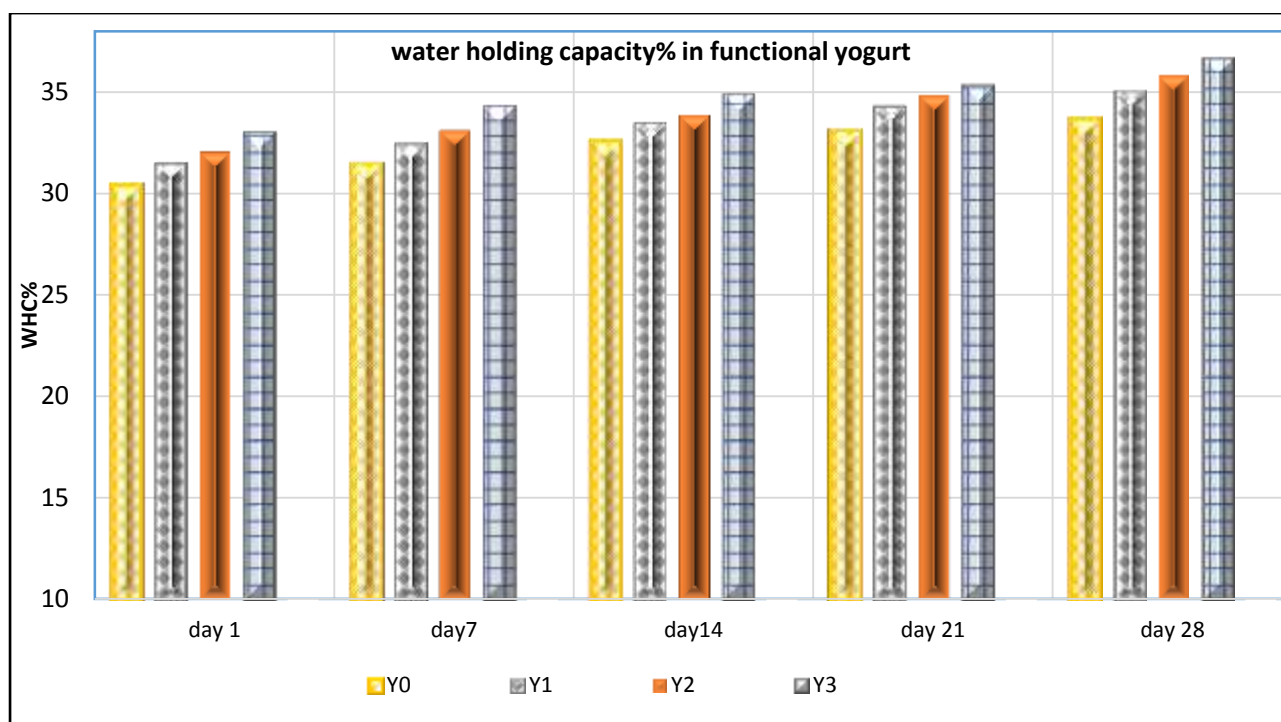
Y0, Y1, Y2, and Y3 represent functional yogurt treatments with Proso millet starch added at concentrations of 0.0%, 0.1%, 0.2%, and 0.3%, respectively.

Figure 2. Spontaneous whey leaching (WW) in functional yogurt with different levels of Proso millet starch added after processing and during refrigerated storage at 5°C for 28 days.

Water holding capacity.

The water holding capacity of the treatments containing Proso millet starch Y1, Y2, and Y3, shown in Figure (3), reached 31.53%, 32.07%, and 33%, respectively, one day after processing. The increase was significantly superior compared to the control treatment Y0, which reached 30.47%. Increased water holding capacity was observed with the increase in the concentration of added starch, which enhanced the protein network's ability to retain water. Furthermore, increasing the carbon source of the starter bacteria, which increased their growth and ability to produce

EPS intertwined with milk proteins, led to the production of a strong, consistent network capable of holding a greater amount of water [27]. It is also noted that there is an increase in the percentage of water retention with the advancement of the refrigerated storage period for all treatments. The values reached after 28 days for the control treatment 33.73% and the treatments to which Proso millet starch was added 35.07%, 35.87% and 36.67% respectively. Treatment Y3 recorded the highest percentage of water retention, followed by treatments Y1 and Y2, which differed significantly compared to the control treatment Y0, which recorded the lowest percentage of water retention. This result is consistent with what was found by [1.]



Y0, Y1, Y2, and Y3 represent the functional yogurt parameters added to Proso millet starch at concentrations of 0.0%, 0.1%, 0.2%, and 0.3%, respectively.

Figure 3. Water retention capacity (%) of functional yogurt with different percentages of Proso millet starch added after processing and during refrigerated storage at 5°C for 28 days.

pH and total acidity

The results shown in Table (1) show the pH and acidity% values of functional yogurt with different percentages of Proso millet starch added during refrigerated storage at 5°C for 28 days. The control treatment Y0 recorded a significant decrease in pH during the first day, reaching 4.51 compared to treatments Y1, Y2, and Y3, which were 4.55, 4.56, and 4.60, respectively. This was accompanied by a significant increase in acidity, reaching 0.96%, compared to treatments Y1, Y2, and Y3, which were 0.92, 0.90, and 0.89%, respectively. The addition of millet starch to skimmed milk contributed to maintaining the acidity of the product by taking longer to coagulate [1]. A decrease in pH values and an increase in

acidity were observed with the progression of the refrigerated storage period for all treatments. After 28 days, the values for the control treatment Y0 were 4.33 and 1.13%, respectively. These values differed significantly compared to treatments Y1, Y2, and Y3, which recorded pH values of 4.42, 4.47, and 4.49, respectively. Total acidity was lower, reaching 1.06%, 1.03%, and 1.02%, respectively. It is noted that the increase in acidity during the progression of the storage period is due to the production of larger quantities of lactic acid by the yogurt starter bacteria. The addition of Proso millet starch to the functional yogurt reduced the acidity of the product and increased the pH values, maintaining the balance and growth of the starter bacteria and increasing the clotting time in the first days of refrigerated storage in the product [4].

Table 1. pH and Acidity% values in functional yogurt to which different percentages of millet starch were added. Proso during refrigerated storage at 5°C, for 28 days

treatments type	Acidity% in functional yogurt					pH in functional yogurt				
	1day	7day	14day	21day	28day	1day	7day	14day	21day	28day
Y0	c 0.96	d 0.98	c 1.04	d 1.08	c 1.13	a 4.51	a 4.47	a 4.46	a 4.40	a 4.33
Y1	b 0.92	c 0.94	b 0.98	c 1.03	b 1.06	b 4.55	ab 4.53	b 4.52	b 4.49	b 4.42
Y2	a 0.90	b 0.92	b 0.97	b 1.0	ab 1.03	b 4.56	bc 4.54	b 4.53	b 4.50	bc 4.47
Y3	a 0.89	a 0.90	a 0.93	a 0.98	a 1.02	c 4.60	c 4.59	c 4.56	b 4.52	c 4.49
LSD	0.015	0.02	0.028	0.018	0.026	0.03	0.057	0.033	0.028	0.065

-Values are expressed as the arithmetic mean of three replicates. - Means followed by different lowercase letters within a column indicate a significant difference ($P \leq 0.05$).

-Y0, Y1, Y2, and Y3 represent functional yogurt treatments supplemented with 0%, 0.1%, 0.2%, and 0.3% Proso proteomic starch.

Microbiological Analysis of Functional Yogurt.

Table (2) and Table (3) shows the live counts of *St. thermophiles*, *L. bulgaricus*, and the probiotic *S. boulardii* yeast in functional yogurt supplemented with proso proteomic starch during 28-day storage.

Treatment Y3 had the highest logarithm of live counts of *St. thermophiles*. *Thermophiles* reached 10.01 log CFU./ml on the first day of production. Treatments Y1 and Y2 significantly outperformed the live counts of these bacteria, reaching 9.92 and 9.98 log CFU./ml, respectively, compared to the

control treatment Y0, which recorded the lowest live count rate of 9.38 log CFU./ml.

The results obtained for the first day were higher than those found by [6]. A gradual decline in the live count rate was then observed with the aging of the product, with treatments Y3, Y2, and Y1 maintaining their significant superiority, achieving the highest live count rate of 7.86, 7.42, and 6.46 log CFU./ml, respectively, compared to the control treatment Y0, which recorded the lowest live count rate of 6.32 log CFU./ml on the 28th day of storage at 5°C. A significant increase in the live numbers of *L. bulgaricus* bacteria was also observed for treatments Y1 and Y3 since the first day, as the logarithm of the live numbers reached 10.02 and 10.03 log CFU./ml, respectively, compared to treatment Y2 and the control treatment Y0, as the live numbers reached 9.69 and 9.96 log CFU./ml, respectively, and the results of the first day of cold storage were higher than what was

found[6]. A gradual decrease in the logarithm of the live counts of *L. bulgaricus* was observed for all treatments, continuing until the end of the 28-day storage period. Treatment Y3 showed a significant superiority in the live counts of these bacteria, achieving the highest live count rate of 7.76 log CFU./ml, followed by treatment Y1, which was 7.1 log CFU./ml, and then treatment Y2, which was 6.87 log CFU./ml. Meanwhile, the control treatment Y0 recorded the lowest live count value of 6.3 log CFU./ml on the 28th day of refrigerated storage. Despite the decrease in bacterial counts during the refrigerated storage period, the treatments containing Proso millet starch were still above the minimum required level of 10⁶ CFU./ml [12-3.]

From the results shown in Table (3), treatment Y3 achieved the highest logarithm of live numbers of *S. boulardii* yeast, which reached 9.19 log CFU/ml compared to the other treatments. A significant superiority was observed for treatments Y1 and Y2, as their live numbers reached 9.04 and 9.08 log CFU /ml, respectively, compared to the control

treatment Y0, which was 8.92 log CFU/ml. As the product aged, a gradual decrease was observed in the logarithm of live numbers of *S. boulardii* yeast. S for all treatments until the end of the 28-day storage period, while treatment Y3 continued its significant superiority compared to the rest of the treatments, recording the highest live count values of 7.0 log CFU./ml on the 28th day of refrigerated storage, followed by treatments Y1 and Y2, which were 6.48 and 6.50 log CFU./ml, respectively, as they were significantly superior compared to the control treatment Y0, which was 6.01 log CFU./ml on the 28th day of refrigerated storage. Treatment Y3 maintained viable yeast counts for more than 7 logarithmic cycles until the end of the storage period. This is consistent with the minimum acceptable level for probiotics in dairy products to provide consumer health benefits, which is 10⁷ CFU /ml [6]. The addition of Proso millet starch to skimmed milk had a positive role in increasing the viability of *S. boulardii* yeast and its survival during storage. This may be due to starch being a good carbon source for yeast and lactic acid bacteria [18 .]

Table 2: Total live counts of functional yogurt starter bacteria (*St. thermophiles* and *L. bulgaricus*) in skimmed milk supplemented with different proportions of Proso millet starch during refrigerated storage at 5°C for 28 days.

treatments type	st. thermophilus (log CFU/ml) in functional yogurt					L. bulgaricus (log CFU/ml) in functional yogurt				
	1day	7day	14day	21day	28day	1day	7day	14day	21day	28day
Y0	a 9.38	a 9.36	a 8.57	a 7.81	a 6.23	a 9.96	a 9.50	a 9.02	a 7.90	a 6.3
Y1	b 9.92	b 9.88	b 9.23	b 8.21	b 6.46	b 10.02	c 9.96	b 9.42	b 8.19	c 7.1
Y2	c 9.98	c 9.94	c 9.41	c 8.51	c 7.42	a 9.96	b 9.71	c 9.24	c 8.38	b 6.87
Y3	d 10.01	d 9.98	d 9.72	d 8.75	d 7.86	b 10.03	c 9.98	d 9.86	d 8.73	d 7.76
LSD	0.0326	0.0292	0.0321	0.0527	0.0739	0.0391	0.0467	0.0580	0.184	0.0217

Values are expressed as the arithmetic mean of three replicates. Means followed by different lowercase letters within a column indicate a significant difference at $P \leq 0.05$. Y0, Y1, Y2, and Y3 represent the coefficients of functional yogurt supplemented with 0%, 0.1%, 0.2%, and 0.3% Proso millet starch.

Table 3. Total live counts of *S. boulardii* yeast in functional yogurt supplemented with different proportions of Proso millet starch during refrigerated storage at 5°C for 28 days.

probiotic	treatments	1day	7day	14day	21day	28day
S. boulardii (log CFU/ml) in functional yogurt	Y0	a 8.92	a 8.82	a 8.03	b 7.3	a 6.01
	Y1	b 9.04	b 8.98	b 8.71	a 8.04	b 6.48
	Y2	b 9.08	b 9.01	c 8.93	d 8.27	b 6.50
	Y3	c 9.19	c 9.1	c 9.0	c 8.66	c 7.01
	LSD	0.067	0.024	0.087	0.124	0.203

-Values are expressed as the arithmetic mean of three replicates. - Means followed by different lowercase letters within a single column indicate a significant difference at a probability level of $P \leq 0.05$. - Y0, Y1, Y2, and Y3 represent functional yogurt treatments with 0%, 0.1%, 0.2%, and 0.3% Proso millet starch added using the basic method.

Sensory evaluation of functional yogurt.

Table (4) displays the sensory evaluation results of the functional yogurt product after processing and during refrigerated storage for 28 days. Treatments Y3, Y2, and Y1 outperformed in the flavor scores one day after processing, scoring 34.2, 34.3, and 33.5, respectively, out of 35 points, compared to the control treatment Y0, which scored a lower flavor score of 32.7. When observing the scores assigned to the texture and consistency traits, it was found that treatments Y3 and Y2 significantly outperformed this trait, with scores of 28.7 for each out of 30, compared to the control treatment Y0, which scored 26.5. Meanwhile, treatment Y1 scored 27.7, which did not significantly differ from the control treatment. Regarding the scores assigned to the appearance trait, treatments Y2 and Y3 scored the highest, with scores of 10 for both, compared to the control treatment Y0 and treatment Y1, which scored 9.7 and 9.8, respectively. This result is consistent with what was found by [27]. Through the scores given to the trait of live numbers of the probiotic yeast *S. boulardii* and the acidity trait, it was found that the treatments to which Proso millet starch was added scored 15 out of 15 scores given to live numbers and differed significantly compared to the control treatment Y0, which scored 14.75, while all treatments scored close to 9, 9.4, 9.8 and 9.5, respectively, out of 10 scores given to the acidity trait. This result is consistent with [26]. The results of the statistical analysis indicate

that the treatments with different percentages of Proso millet starch added were superior at the probability level ($P \leq 0.05$) compared to the control treatment in the total sensory evaluation scores one day after manufacturing. Treatments Y2 and Y3 obtained the highest total sensory evaluation scores of 97.80 for both, followed by treatment Y1, which reached 96, while the control treatment Y0 obtained an average of 93.45 of the total sensory evaluation scores one day after manufacturing. This result is consistent with the findings of [11]. During the progress of the refrigerated storage period, it was noted that there was a decrease in all the scores given to the flavor, texture, consistency, acidity, live numbers of the probiotic yeast *S. boulardii* and appearance for all treatments until the end of the 28-day storage period. It was noted that the treatments to which Proso millet starch was added were superior in the total scores given for the sensory evaluation compared to the control treatment Y0. Treatment Y3 obtained the highest total score of 68.75, followed by treatments Y1 and Y2, which were 61.95 and 65.65, while the control treatment Y0 obtained the lowest total sensory evaluation score of 59.13 at the end of the 28-day storage period. The addition of Proso millet starch to functional yogurt improved the sensory properties of the final product even as the product aged during refrigerated storage. It also had a positive effect on increasing the growth and viability of *S. boulardii* yeast and keeping it alive. This is consistent with what was found by [1

[

Table 4: Sensory evaluation results of functional yogurt with different proportions of Proso millet starch added during refrigerated storage at 5°C for 28 days.

Functional yogurt	treatment s	Flavor °35	texture and consistency °30	live numbers °15	acidity 10°	Appearance °10	total °100
Day1	Y0	a 32.7	a 26.5	a 14.75	a 9	a 9.7	a 93.45
	Y1	b 33.5	ab 27.7	b 15	ab 9.4	ab 9.8	b 96
	Y2	c 34.3	b 28.7	b 15	b 9.8	b 10	c 97.80
	Y3	bc 34.2	b 28.7	b 15	ab 9.5	ab 10	c 97.80
	LSD	0.701	1.34	0.094	0.48	0.283	1.57
Day7	Y0	a 32.62	a 26.5	a 14.14	a 8.37	a 9	a 90.67
	Y1	ab 33.12	b 27.0	b 14.70	a 8.62	a 9.37	b 93.4
	Y2	bc 33.50	b 27.38	c 14.97	b 9.25	ab 9.62	b 94.75
	Y3	c 34.0	c 28.12	c 15	c 9.87	b 9.87	c 96.62
	LSD	0.501	0.467	0.096	0.448	0.596	1.47
14Day	Y0	a 28	a 22.17	a 11.8	a 7	a 6.83	a 75.98
	Y1	ab 28.5	ab 22.83	b 12	a 7.33	ab 7.33	b 78
	Y2	bc 28.8	bc 23.5	c 13	b 8	b 7.66	c 81
	Y3	c 29.5	c 24	d 14	b 8	b 7.83	d 83.33
	LSD	0.786	0.858	0.119	0.449	0.594	1.55
Day21	Y0	a 24.33	a 20.83	a 8.46	a 6.16	a 6.33	a 66.13
	Y1	b 25.67	a 21.33	b 9.33	a 6.83	ab 6.66	b 69.83
	Y2	b 26.17	b 22.17	c 9.88	b 7.5	b 7.33	c 73.22
	Y3	b 26.33	b 22.50	d 10.7	b 7.66	b 7.66	d 74.72
	LSD	0.838	0.576	0.389	0.576	0.430	1.012
	Y0	a 21	a 19.67	a 6.13	a 6.16	a 6.16	a 59.13

Day28	Y1	ab 22.17	a 20.17	b 6.66	a 6.5	a 6.5	b 61.95
	Y2	bc 23.33	b 21.33	b 6.81	b 7.33	bc 7	c 65.65
	Y3	c 24	b 21.67	c 8.08	b 7.34	c 7.5	d 68.75
	LSD	1.26	0.933	0.265	0.343	0.858	0.858

Conclusions

Finally, we conclude from this study that adding Proso millet starch to skimmed milk in the production of functional yogurt improved the physicochemical and sensory properties and extended the shelf life of the final product during storage. It also served as a bioprecursor to enhance the growth and activity of *S. boulardii*, which has numerous health benefits

for consumers through biosynthesis. However, further studies are needed on the potential of resistant starch extracted from Proso millet grains as a bioprecursor to fortify functional foods, especially dairy products, which are among the most important carriers of probiotics

References

Amrutha, U. A., Sharon, C. L., Panjikaran, S. T., Lakshmy, P. S., & Beena, A. K. (2023). Standardisation and quality evaluation of barnyard millet incorporated probiotic yoghurt.

[2] Alfatlawi, M. A. M., Al-Shaikh, S. A. H., & Alhamid, M. A. (2025, April). Study of the Chemical and Biological Properties of the Aqueous and Alcoholic Extract of *Phyllanthus* (Amla) Fruits and Its Effect on the Microbial Load of Soft Cheese Sold in Local Markets. In IOP Conference Series: Earth and Environmental Science (Vol. 1487, No. 1, p. 012133). IOP Publishing.

[3] Alhamid, M. A. M., & Mousawi, A. A. (2024). PHYSICO-CHEMICAL PROPERTIES OF ALOE VERA GEL AND ITS UTILIZATION IN PREPARING SYNBIOTIC FERMENTED MILK WITH

Bifidobacterium Lactis BB-12. Iraqi Journal of Agricultural Sciences, 55(2), 823-835.

[4] Alneamah, S. J. A., Al-Jannah, S. M., Aldhalemi, A. A. J., Gazal, M. M. A., & Abd, N. S. (2024, July). Production of Yoghurt Fortified With Many Aqueous Extracts as a New Functional Dairy Product. In IOP Conference Series: Earth and Environmental Science (Vol. 1371, No. 6, p. 062032). IOP Publishing.

[5] Al-Shaikh, S. A. H., Alhamid, M. A., & Aldhalemi, A. A. (2020). Study of the Effect of Fortified Milk by Zinc Salts in Different Concentrations on the Sensory and Physicochemical Properties of the Processed Yoghurt. *Plant Archives*, 20(2), 81-90.

[6] Alwohaibi, A. A., Ali, A. A., Sakr, S. S., Mohamed Ahmed, I. A., Alhomaid, R. M., Alsaleem, K. A., ... & Hassan, M. F. (2023).

[1]

Valorization of different dairy by-products to produce a functional Dairy–Millet Beverage Fermented with *Lactobacillus paracasei* as an adjunct culture. *Fermentation*, 9(11), 927 .

starter cultures and levels of solids. *Int. J. Dairy Tech.* 59. 216–221.

[8]Anitha, S., Kane-Potaka, J., Botha, R., Givens, D. I., Sulaiman, N. L. B., Upadhyay, S., ... & Bhandari, R. K. (2021). Millets can have a major impact on improving iron status, hemoglobin level, and in reducing iron deficiency anemia—a systematic review and meta-analysis. *Frontiers in nutrition*, 8, 725529.

[9]Association of Official Analytical Chemists A.O.A.C. (2008). *Official Methods of Analysis* 16th ed. Association of Official

[10]Beitane, I., & Ciprovica, I. (2012). The study of synbiotic dairy products rheological properties during shelf-life. *International Journal of Nutrition and Food Engineering*, 6(7), 1889-1891. Analytical Chemists International Arlington, Virginia, U.S.A

[11]Chaudhary, J. K., & Mudgal, S. (2020). Antidiabetic and hypolipidaemic action of finger millet (*Eleusine coracana*)-enriched probiotic fermented milk: An in vivo rat study. *Food technology and biotechnology*, 58(2), 192

[12]Donkor, O.N., S.L.I. Nilmini., P. Stolic., T. Vasiljevic., and N.P. Shah. (2007). Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. *Int. Dairy J.* 17, 657-665.

[13]Eunice T.; Hector E.; Jose O.; Rafael Z.; Rafael S.; Rosa E. and Perez S. (2017). Survival rate of *Saccharomyces boulardii* adapted to a functional freeze-dried yogurt: experimental study related to processing, storage and digestion by Wistar rats. *Functional Foods in Health and Disease*. Universidad Michoacana de San Nicolas de

Hidalgo, Uruapan, Mich., Mexico 7(2): 98-114

[14]Gilliland, S. E. (1985). Concentrated starter cultures. *Bacterial starter cultures for foods*, 145-157.

[15]Hannoon, A. H. A & Al-Hamid, M. A. M. (2025, April). Study of the Chemical Composition of Millet Flour and Methods of Starch Extraction. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1487, No. 1, p. 012130). IOP Publishing.

[16]Hattem, I. H., & Jerro, D. I. (2020). Determination of chemical and rheological quality of yogurt in Babil governorate markets in Iraq.

[17]Ivanova, G., Momchilova, M., Rumyan, N., Atanasova, A., & Georgieva, N. (2012). Effect of *Saccharomyces boulardii* yeasts addition on the taste and aromatic properties of kefir. *Journal of the University of Chemical Technology and Metallurgy*, 47(1), 59-62.

[18]Joseph, B., Bhavadharani, M., Lavanya, M., Nivetha, S., Baskaran, N., & Vignesh, S. (2024). Comparative analysis of LAB and non-LAB fermented millet drinks fortified with *Chlorella* sp. *Food Bioengineering*, 3(3), 352-364.

[19]Khatr, I., Tomar, R., Ganesan, K., Prasad, G. S., & Subramanian, S. (2017). Complete genome sequence and comparative genomics of the probiotic yeast *Saccharomyces boulardii*. *Scientific reports*, 7(1), 371 .

[20]Mahajan, P., Bera, M. B., Panesar, P. S., & Chauhan, A. (2021). Millet starch: A review. *International Journal of Biological Macromolecules*, 180, 61-7

[21]Marco, M. L., Heeney, D., Binda, S., Cifelli, C. J., Cotter, P. D., Foligné, B., ... & Hutkins, R. (2017). Health benefits of fermented foods: microbiota and beyond. *Current opinion in biotechnology*, 44, 94-102..

[22]Mousa, A. H., Bakry, A. M., Wang, G., & Zhang, H. (2019). Efficacy of *Saccharomyces boulardii* metabolism during fermentation of milk fortified with wheat grain juice. *Food Science and Technology Research*, 25(5), 657-665.

[23]Mustac NC^ˆ, Novotni D, Habus^ˆ M, Drakula S, Nanjara L, Vouc^ˆko B, C^ˆuric^ˆ D (2020) Storage stability, micronisation, and application of nutrient-dense fraction of Proso millet bran in gluten-free bread. *J Cereal Sci* 91(10):1–7.

[24]Pais, P., Oliveira, J., Almeida, V., Yilmaz, M., Monteiro, P. T., & Teixeira, M. C. (2021). Transcriptome-wide differences between *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae* var. *boulardii*: Clues on host survival and probiotic activity based on promoter sequence variability. *Genomics*, 113(2), 530-539.

[25]Rao, B. D., Ananthan, R., Hariprasanna, K., Bhatt, V., Rajeswari, K., Sharma, S., & Tonapi, V. A. (2018). Nutritional and health benefits of nutri cereals. Rajendranagar, Hyderabad: Nutri hub TBI, ICAR_Indian Institute of Millets research (IIMR); 2018.

[26]Sharma, M., Yadav, D. N., Singh, A. K., Vishwakarma, R. K., & Sabikhi, L. (2018).

Impact of octenyl succinylated pearl millet (*Pennisetum typhoides*) starch addition as fat replacer on the rheological, textural and sensory characteristics of reduced-fat yoghurt. *International Journal of Dairy Technology*, 71(3), 723-733.

[27]Sharma, S., & Sharma, N. (2021). Preparation of probiotic enriched functional beverage of Kodo millet (*Paspalum scrobiculatum*) a nutritionally enriched absolute new product for commercialization. *Journal of Pharmacognosy and Phytochemistry*, 10(1), 752-758.

[28]Sudha, A., Devi, K. S., Sangeetha, V., & Sangeetha, A. (2016). Development of fermented millet sprout milk beverage based on physicochemical property studies and consumer acceptability data.

[29]Verma, V. C., Kumar, A., Zaidi, M. G. H., Verma, A. K., Jaiswal, J. P., Singh, D. K., ... & Agrawal, S. (2018). Starch isolation from different cereals with variable amylose/amylopectin ratio and its morphological study using SEM and FT-IR. *Int. J. Curr. Microbiol. App. Sci*, 7(10), 211-228