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Synbiotic Yogurt Enriched with Lactobacillus acidophilus, Long-Chain Inulin, and Moringa oleifera Leaves Extract

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Abstract

Background and aims: In today's world, the development of functional foods has become of paramount importance. This research aimed to produce a synbiotic yogurt enriched with Lactobacillus acidophilus, long-chain inulin, and Moringa oleifera extract.

Materials and Methods: Two levels of *Moringa oleifera* Leaves Extract (1 and 2% w/w) and inulin (3 and 5% w/w) were tested for their effects on the properties of the synbiotic yogurt over 20 days of storage at 4°C.

Results and Conclusion: The level of the extract had a profound impact on both acidity and pH, increasing the former and decreasing the latter. The extract increased yogurt's antioxidant activity compared to the control. Inulin had the most notable effect on viscosity, syneresis, water-holding capacity, and dry matter content. The yogurt sample containing 1% extract and 5% inulin received the highest sensory scores. Inulin improved the viability of probiotic bacteria, while *Moringa oleifera* extract did not affect any aspect of them. Neither Inulin nor *Moringa oleifera* extract significantly affected the fat content. This new synbiotic product, incorporating *Lactobacillus acidophilus*, M. oleifera extract, and inulin, possesses the desired characteristics and could effectively deliver probiotic strains and bioactive compounds together, thereby providing potential health benefits.

What is "already known":	• Incorporation of prebiotics, specifically inulin and oligofructose, significantly enhances the viscosity, sensory attributes, and probiotic viability of the resulting yogurt • *Moringa oleifera* leaves have numerous pharmaceutical applications and contain protein, β-carotene, vitamins, phenolic compounds, and amino acids
What this article adds:	• Inulin improved the viability of probiotic bacteria, while M. oleifera extract did not affect any aspect of them • The yogurt sample containing 1% extract and 5% inulin received the highest sensory scores • The extract increased yogurt's antioxidant activity compared to the control

1. INTRODUCTION

Yogurt is one of the most important fermented milk products, produced through fermentation by the yogurt starter bacteria, namely *Lactobacillus delbrueckii* subsp. bulgaricus and *Streptococcus thermophilus* [1]. Yogurt is highly popular across the world and is consumed for its nutritional value, great flavour and aroma, and a longer shelf life compared to milk. Owing to its high mineral, vitamin, and protein content, yogurt is considered a rich source of phosphorus, calcium, magnesium, zinc, riboflavin, nicotinic acid, B5, and B12 vitamins [2,3]. In recent times, there has been a surge in the need for novel varieties of dairy products, including synbiotic yogurt that incorporates both probiotics and prebiotics [4,5]. Probiotics are defined as live microorganisms that, when taken in adequate amounts, can provide health benefits. They should be able to withstand all stages of food storage without compromising the food's physical, chemical, or sensory characteristics The most frequently used probiotic strains include species of Lactobacillus, such as *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum*, and *Levilactobacillus brevis* [6,7]. Many dairy products are being enhanced with indigestible oligosaccharides, such as inulin, to boost their functionality. These ingredients are known as prebiotics [8]. Prebiotics should be selectively fermented by beneficial bacteria, favourably changing the intestinal microflora, producing beneficial effects on the host, and should not be absorbed and hydrolysed in the upper digestive tract. Addition of inulin to fermented milk keeps the population of LAB high during the storage of the product. Also, prebiotics increase the resistance of probiotics to molecular oxygen and acid. When a product contains both probiotics and prebiotics, it is referred to as synbiotic. Synbiotic yogurt, considered a functional food that could positively influence human health, is gaining widespread acceptance [9]. It has been consistently shown that the incorporation of prebiotics, specifically inulin and oligofructose, significantly enhances the viscosity, sensory attributes, and probiotic viability of the resulting yogurt [10-12]. These findings underscore the synergistic benefits of combining probiotics and prebiotics in the formulation of synbiotic dairy products, offering a nutritious dietary option for consumers by increasing the growth and stability of LAB [13,14]. *Moringa oleifera* is a tropical tree belonging to the *Moringaceae* family comprising 13 genera. Among these, *Moringa oleifera* is the most widely cultivated and studied species. Nearly all parts of the MO tree possess various medicinal properties. The *Moringa oleifera* leaves (MOLs), in particular, are rich in essential nutrients, vitamins, and minerals, making them a valuable food source [15]. MOLs have numerous pharmaceutical applications and contain protein, β-carotene, vitamins, phenolic compounds, and amino acids [16-18]. It is also a rich source of quercetin, zeatin, β-sitosterol, kaempferol, and quinic acid. The tree's diverse applications range from nutrition and biogas production to medicinal uses, highlighting its potential as a sustainable resource. Different parts of MO may have antifungal, anti-ulcer, anti-epileptic, anti-swelling, anti-seizure, cholesterol-lowering, anti-diabetic, and antioxidant properties [19]. In light of the growing interest in functional foods that offer health benefits beyond basic nutrition, this study was carried out to evaluate the physicochemical and microbial properties of a synbiotic yogurt containing Moringa oleifera leaves extract (MOLE), long-chain inulin, and Lactobacillus acidophilus during the cold storage period.

2. Materials and methods

2.1. Extract Preparation

Three hundred grams of MOLs (Sabzroyan Co., Iran) were placed in a percolator and covered with 96% ethanol, ensuring a liquid level 4–5 cm above the powder. The extraction process occurred in three stages, each lasting 24, 48, and 72 hours. The resulting extract was concentrated using a rotary evaporator and stored in airtight containers at 4°C until use [16].

2.2.Yogurt Production

Low-fat (1.5%) milk from Damdaran Dairy (Iran) was used to produce synbiotic yogurt. The milk was first heated to 90°C for 10 minutes. Then, long-chain inulin (3% and 5%) from Benco (Germany), non-fat milk powder (2.5%) from Pegah (Iran), and MOLE (1% and 2%) were added. The mixture was cooled to 44–45°C and inoculated with natural Freeze-Dried Direct Vat Set (FD-DVS CH-1) yogurt starter culture at 3% w/w, along with the probiotic strain *Lactobacillus acidophilus* (LA5) at 10^7 cfu/ml from Christian Hansen (Denmark). The prepared samples (Table 1) were incubated at 42°C until the pH reached 4.6 [18].

Table 1. Different treatments and codes used throughout the paper

Code	Inulin (%)	MOL† (%)
T_1	0	0
T_2	3	1
T_3	3	2
T_4	5	1
T_5	5	2

† *Moringa oleifera* leaves

2.3. Yogurt Characterisation

The pH of the samples was measured at 25°C using a digital pH meter (SevenEasy, Mettler Toledo, Switzerland), following AOAC23 International guidelines. Titratable acidity was determined by mixing a 9 g sample with distilled water and titrating it with 0.1 N sodium hydroxide (NaOH). To assess dry matter content, a portion of the sample was dried in an oven at 103°C until a constant weight was reached. Fat content was analysed using the Gerber method, with the percentage of fat directly read from a calibrated butyrometer [20].

2.4. Yogurt Syneresis and Water Holding Capacity (WHC)

A modified method based on Hess et al.[21] was used to evaluate syneresis. Samples were equilibrated at 10°C for 2 hours and then centrifuged at 238 × g for 10 minutes at 10°C using a Sigma 3-30KS centrifuge. The expelled serum was collected and weighed, and syneresis was expressed as the ratio of the expelled serum weight to the initial yogurt weight. Water-holding capacity (WHC) was determined using a modified method adapted from Harte et al. [22]. Yogurt samples were centrifuged at 15,000 × g for 15 minutes at room temperature (Sigma 3-30KS, Germany). The supernatant was collected, and WHC was calculated as the percentage of water lost relative to the initial yogurt weight.

2.5. Yogurt Viscosity and Flow Behaviour

The viscosity and flow behaviour of the yogurt samples were analysed using a rotational viscometer (AMETEK CT3, Brookfield, USA) equipped with a thermal circulator. The sample was poured into the viscometer's cylinder, and the temperature was maintained at 25°C using the circulator [19]. To evaluate viscosity as a function of shear rate and assess the flow behaviour of the samples, the shear rate was varied from 0 to 100 s^{-1}. The Power Law (Eq. 1), Herschel-Bulkley (Eq. 2), and Casson (Eq. 3) models were applied to the data to identify the most suitable model for describing the samples' flow behaviour.

$$\tau = K\gamma^n \quad \text{(Eq. 1)}$$
$$\sigma=\sigma_0+K_H\gamma^n \quad \text{(Eq. 2)}$$
$$\sigma^{0.5}=\sigma_0^{0.5}+K_C\gamma^{0.5} \quad \text{(Eq. 3)}$$

where τ is the shear stress, γ is the shear rate, K is the consistency coefficient, n is the flow behaviour index, and σ_0 is the yield stress.

2.6. Antioxidant Activity

One gram of the sample was mixed with 10 mL of ethanol and stirred with a magnetic stirrer for 30 min. The mixture was then left to stand for 2 h before being filtered through filter paper. Next, 1 mL of the resulting extract was mixed with 4 mL of 96% ethanol and 1 mL of 0.004% 2,2-diphenyl-1-picrylhydrazyl (DPPH). The mixture was kept in the dark for 30 minutes. Finally, the absorbance was measured at 517 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan), and antioxidant activity was calculated using Eq 4. [16]:

$$\text{DPPH free radical scavenging activity} = \frac{A_C - A_S}{A_C} * 100 \quad \text{(Eq. 4)}$$

where Ac and As are the absorbances of the control and sample, respectively.

2.7.Probiotic Viability

The De Man–Rogosa–Sharpe (MRS) bile agar from Merck (Darmstadt, Germany) was used to enumerate *L. acidophilus*. The plates were incubated at 37°C for 3-5 days, and the bacterial colonies were counted using a colony counter on day 0 (production day), 10, and 20 [23].

2.8. Sensory Evaluation

The sensory attributes of the yogurt samples, including appearance, colour, flavour, mouthfeel, and overall acceptance, were assessed using 15 untrained panellists on a 5-point (1= not acceptable, 2= relatively acceptable, 3= moderate, 4= good, and 5= excellent) hedonic scale [24,25].

2.9. Statistical Analysis

A two-factor full-factorial, completely randomised design was used to examine the effects of the independent variables, inulin (3% and 5%) and MOLE (1% and 2%), on the dependent variables (Table 1). Mean comparisons were conducted using Duncan's multiple range test at a 95% confidence level. All statistical was used to examine the effects of the independent variables, inulin (3% and 5%) and MOLE (1% and 2%), on the dependent variables. Mean comparisons were conducted using Duncan's multiple range test at a 95% confidence level. All statistical analyses were performed using SPSS version 26, and graphs were created using Excel version 2021.

3. RESULTS AND DISCUSSION

3.1. Yogurt Characterisation

As the Lactobacillus species proliferates and converts lactose into lactic acid, the yogurt environment becomes more acidic, leading to a decrease in the pH level and an increase in acidity. The pH and acidity measurements are presented in Table 2. The pH of yogurt samples containing MOLE and inulin showed a significant downward trend from the first day to the 20th day. A significant decrease in pH was observed during storage ($p<0.05$). Additionally, there were notable differences in the pH values between the treated samples and the control sample ($p<0.05$). This could be attributed to the fermentation activity of the probiotic bacteria, which promotes increased lactic acid production. Higher concentrations of MOLE significantly lowered the pH ($p<0.05$). Increasing the inulin concentration did not result in significant changes in pH. Rinaldoni et al. [26] examined the effects of inulin on the physicochemical and sensory properties of soy milk yogurt and found that the pH decreased and the acidity increased during storage. Moreover, different inulin levels did not significantly alter the pH and acidity of yogurt samples. The acidity of the yogurt samples significantly increased during storage ($p<0.05$). Additionally, the acidity values of the treated samples were significantly different from those of the control ($p<0.05$). As the MOLE level increased, the yogurt acidity rose, whereas the increase in the inulin concentration did not have a significant effect. The highest acidity was observed in samples T5 (2% MOLE and 5% inulin) and T3 (2% MOLE and 3% inulin), whereas the control sample had the lowest acidity. These findings align with previous studies that have shown that the addition of inulin as a fat substitute did not significantly affect the pH or acidity of yogurt [12]. Similarly, it has been observed that probiotic yogurt containing green tea extract exhibited a higher acidity level compared to regular yogurt. This increase in acidity was attributed to the enhanced metabolic activity of the yogurt and/or probiotic bacteria in milk fermented with herbal extracts [27]. The results comparing dry matter and the fat contents of the synbiotic yogurt samples are shown in Table 3. Significant differences were observed between the treated and control samples in this regard ($p<0.05$). Furthermore, increasing the inulin concentration led to a significant increase in the yogurt's dry matter content ($p<0.05$), while an increase in the MOLE level did not significantly affect this response.

Table 2. pH values and Acidity of synbiotic Yogurt samples during storage time†

Sample	**Storage time (day)**					
	pH			**Acidity** (Dornic degrees)		
	0	**10**	**20**	**0**	**10**	**20**
T1	4.68±0.03^{Aa}	4.47±0.73^{Ba}	4.17±0.73^{Ca}	81.35±0.12^{Ca}	84.35±0.27^{Ba}	89.36±0.27^{Aa}
T2	4.53±0.41^{Ab}	4.21±0.28^{Bb}	3.93±0.98^{Cb}	83.51±0.14^{Cb}	87.31±0.36^{Bb}	92.46±0.68^{Ab}
T3	4.26±0.36^{Ac}	4.03±0.01^{Bc}	3.74±0.52^{Cc}	85.43±0.36^{Cc}	89.43±0.26^{Bc}	94.52±0.93^{Ac}
T4	4.52±0.28^{Ab}	4.25±0.68^{Bb}	3.91±0.66^{Cb}	83.53±0.28^{Cb}	87.42±0.78^{Bb}	92.62±0.48^{Ab}
T5	4.27±0.03^{Ac}	4.01±0.51^{Bc}	3.76±0.54^{Cc}	85.39±0.43^{Cc}	89.44±0.35^{Bc}	94.47±0.53^{Ac}

†Different small letters in the same column indicate significant differences ($p<0.05$); Different capital letters in the same row indicate significant differences ($p<0.05$)

The highest dry matter content was observed in T5 (2% MOLE and 5% inulin) and T4 (1% MOLE and 5% inulin), while the control sample had the lowest dry matter content due to the formation of a gel network and the bonds between the protein molecules. Consistent with our findings, other studies control. The added ingredients (inulin and MOLE) were free of fat, and inulin itself is a dietary fibre that have reported that adding inulin to yogurt increases its dry matter content, likely due to the formation of a gel network and protein-protein interactions [9,10]. The results presented in Table 3 indicate that the fat content of the synbiotic yogurt samples was not significantly different from the control. The added ingredients (inulin and MOLE) were free of fat, and inulin itself is a dietary fibre that is used as a fat substitute in food products. Therefore, the yogurt's fat content remained unchanged. These findings align with previous studies that have demonstrated the addition of MOLE at different concentrations did not significantly impact the fat content of yogurt. However, a slight increase in dry matter was observed at higher concentrations [17,19].

Table 3. Dry matter and fat contents of synbiotic yogurt samples†

Sample	**Fat content (%)**	**Dry matter (%)**
T1	1.48±0.13^{a}	12.63±0.03^{c}
T2	1.53±0.23^{a}	15.84±0.41^{b}
T3	1.50±0.37^{a}	15.59±0.36^{b}
T4	1.54±0.42^{a}	17.44±0.21^{a}
T5	1.52±0.23^{a}	17.46±0.03^{a}

†Different small letters in the same column indicate significant differences ($p<0.05$)

3.2. Syneresis and WHC.

The results of yogurt syneresis quantification and WHC determination are summarised in Table 4. Syneresis in the inulin-containing samples significantly decreased during storage, whereas the control group showed an opposite trend ($p<0.05$). Additionally, increasing the concentration of MOLE led to a rise in syneresis ($p<0.05$).. The syneresis values of the treated samples differed significantly from those of the control samples. Notably, higher levels of the extract increased syneresis, whereas higher levels of inulin reduced it ($p<0.05$). During storage, the lowest syneresis value was observed in T4 (1% MOLE and 5% inulin), and the highest in the control sample. Syneresis in yogurt occurs due to the shrinkage of the three-dimensional structure of the protein network, which reduces the binding capacity of whey proteins [28].

This process is closely related to the amount of caseins in milk or the addition of stabilisers [29]. Inulin, known for its high-water retention capacity, can significantly affect yogurt's consistency and texture. This capacity can be measured by the amount of water removed from the inulin gel within the yogurt without applying external force. It has been reported that adding inulin up to 3% does not affect the degree of syneresis. Conversely, the level of MOLE significantly impacts syneresis. The MOLE showed higher acidity, calcium, and fibre content. These factors, coupled with MOLE's ability to reinforce protein-protein interactions over protein-water interactions, may contribute to increased syneresis. Additionally, potential thermodynamic mismatches between MOLE components and milk proteins, along with the weakening of the casein network and disruption of osmotic balance due to casein micelle flocculation in the presence of non-adsorbing polymers like dietary fibre, could further exacerbate whey separation [28,29]. The results of yogurt WHC determination are shown in Table 4. The WHC values of all treated samples significantly increased during storage ($p<0.05$), while the WHC of the control significantly declined ($p<0.05$). Whey separation during storage occurs due to protein rearrangement, which weakens the robustness of the casein network [29].

In addition, the WHC of the treated samples was significantly different from that of the control. As the MOLE concentration increased, yogurt WHC decreased ($p<0.05$), whereas an increase in inulin level significantly enhanced WHC ($p<0.05$). As previously stated, the increase in whey expulsion from the yogurt could be attributed to the weakening of the casein network due to its interaction with the MOLE's active groups. This could also be due to the thermodynamic incompatibility between the extract's components and milk proteins, as well as the unbalanced osmotic potential caused by the depletion flocculation of casein micelles in the presence of non-adsorbing polymers like dietary fibre.

By the end of the storage period, the highest WHC was observed in T4 (1% MOLE and 5% inulin), while the lowest WHC was seen in T3 (2% MOLE and 3% inulin) and the control. The increased WHC in samples with higher inulin concentrations may be attributed to inulin's hygroscopic properties. Inulin's ability to absorb water and form a gel-like structure could explain this observation [10]. Syneresis and WHC are interconnected properties influencing yogurt's texture and stability. Inulin, with its water-binding capacity, can mitigate syneresis and enhance WHC. However, excessive MOLE can disrupt the protein network, leading to increased syneresis and potentially affecting WHC. By carefully balancing the addition of inulin and MOLE, it is possible to optimise the syneresis and WHC of yogurt, resulting in a product with improved texture, stability, and overall quality.

3.3. Viscosity and Flow Behaviour

The shear stress–shear rate and viscosity–shear rate relationships of the synbiotic yogurt samples are illustrated in Figs. 1. Our findings showed that viscosity significantly increased during storage ($p<0.05$). There were also significant differences between the viscosity of the treated and control samples ($p<0.05$). An increase in MOLE levels led to a notable decrease in viscosity, whereas higher inulin levels resulted in a significant increase. This is evident as the highest viscosity was observed in T4 (with 1% MOLE and 5% inulin), while the lowest was seen in the control group. Viscosity was affected by both inulin concentration and storage time. With

Fig. 1. Shear rate-shear stress curve of yogurt samples on the 0th day (a) and 20th day (b) and Viscosity-shear rate curve on the 0th day (c) and 20th day (d)

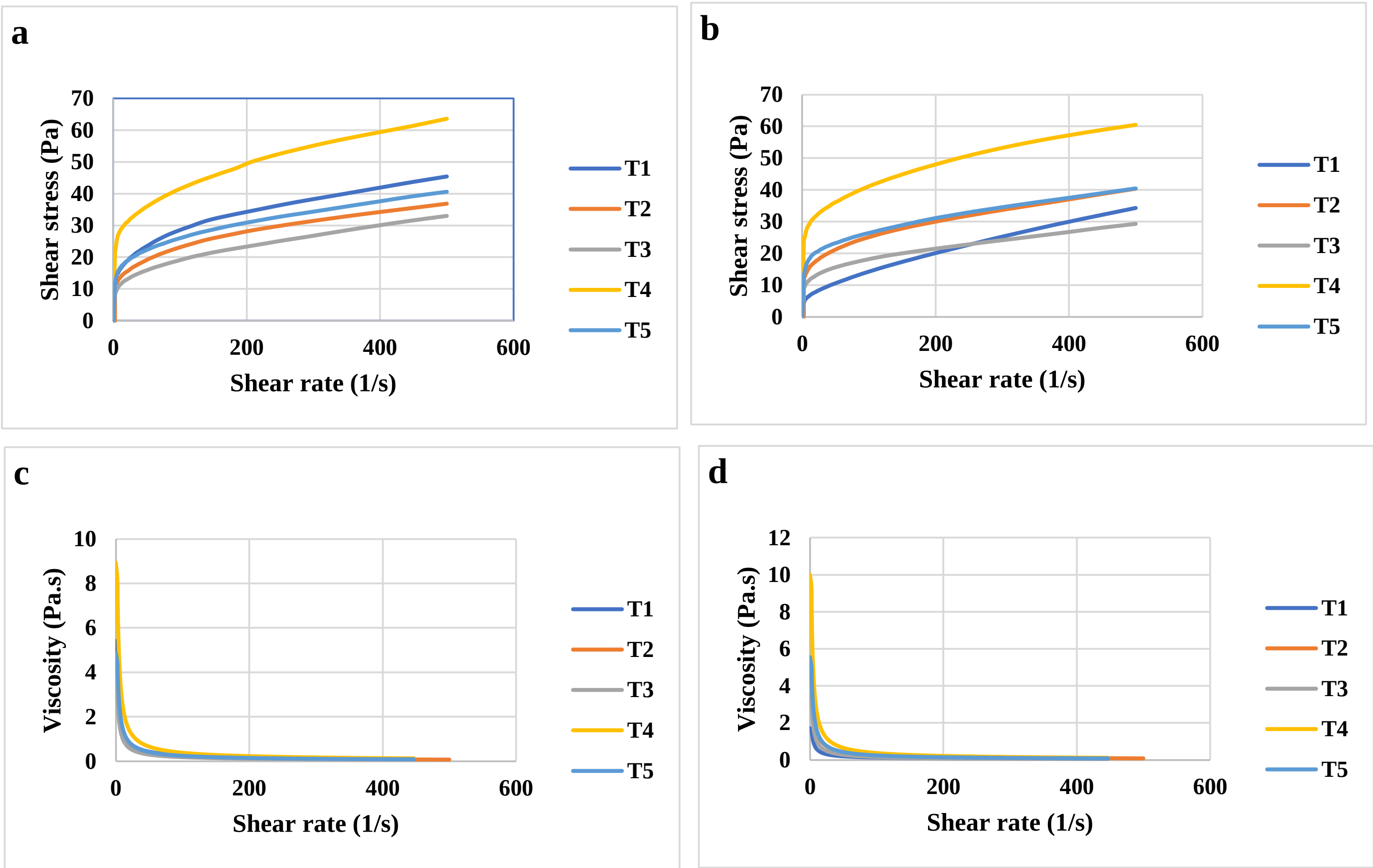

Table 4. **Syneresis values and WHC of synbiotic yogurt samples during storage time**

Sample	Storage time (day)					
	Syneresis			WHC		
	0	10	20	0	10	20
T1	17.08±0.23Aa	17.94±0.44Ba	19.10±0.51Ca	54.50±0.21Aa	53.53±0.43Ba	52.10±0.42Ca
T2	17.63±0.43Aa	17.36±0.25Ba	16.83±0.38Cb	52.46±0.46Cb	53.45±0.48Ba	54.42±0.68Ab
T3	18.21±0.56Ab	17.82±0.42Ba	17.24±0.53Cc	50.21±0.56Cc	51.42±0.42Bb	52.30±0.63Aa
T4	13.55±0.26Ac	12.57±0.28Bb	11.26±0.38Cd	58.43±0.27Cd	59.48±0.48Bc	60.32±0.38Ac
T5	14.30±0.03Ad	13.30±0.73Bc	12.33±0.63Ce	56.61±0.42Ce	57.32±0.46Bd	58.33±0.23Ad

Different small letters in the same column indicate significant differences ($p<0.05$); Different capital letters in the same row indicate significant differences ($p<0.05$)

prolonged storage time, the yogurt viscosity also increased, likely due to the development of a more extensive gel network formed by inulin or changes in the interactions among the proteins within the three-dimensional protein network of the yogurt. Viscosity is a crucial parameter in yogurt quality and is affected by various factors such as incubation temperature, dry matter content, milk thermal processing, acidity, and starter culture type [13]. The results indicated that adding the MOLE significantly affected yogurt viscosity by stimulating the activity of starter bacteria. As the MOLE concentration increased, the yogurt viscosity decreased. yogurt is a protein gel in which denatured soluble proteins form connections with casein, creating a porous and network-like structure that retains water. Adding the MOLE appears to weaken this network, resulting in reduced viscosity. In addition, viscosity is affected by the activity of starter bacteria and acidity. The observed effects of MOLE on yogurt properties, such as those reported in previous studies [19,20], highlight the potential of this natural extract to influence the texture and reduce the viscosity of different yogurts. The data from treatments T1 to T5 were fitted with Newtonian, Power Law, Herschel-Bulkley, Bingham, and Casson models, with the results and coefficients presented in Table 5 along with their R^2 and RMSE values. According to the data analysis, the highest R^2 values were observed in the Herschel-Bulkley and Power Law models for treatments T1 to T5. These treatments can be considered pseudoplastic, as indicated by the flow behaviour index (n), which measures the fluid's closeness to Newtonian behaviour. The control sample had a higher flow behaviour index than the samples containing inulin. As the amount of inulin increased, the fluid's behaviour deviated from the Newtonian state. Since the flow behaviour index is less than one, the samples exhibited shear-thinning behaviour. The degree of deviation of the flow index from one indicates the degree of deviation of the fluid from Newtonian behaviour. The results showed that the sample also exhibited shear-thinning behaviour. The inulin gel structure in the mixture enhanced the shear-thinning characteristics. The K coefficient, also known as the consistency coefficient in both the Newtonian viscosity model and other models, indicates increased consistency in samples containing 5% inulin compared to the control sample and those with 3% inulin. This suggests that as the percentage of inulin increases, the consistency coefficient also rises. Conversely, increasing the percentage of MOLE from 1 to 2% results in a decreased consistency coefficient. Thus, the highest consistency coefficient is observed in T4 and the lowest is seen in the control treatment. Similarly, several studies demonstrated that hydrocolloids such as inulin and guar gum can significantly enhance the viscosity of yogurt mixes [11,12].

3.4. Antioxidant Activity

The comparison of the antioxidant activities of the synbiotic yogurt samples containing inulin and MOLE is summarised in Table 6. The antioxidant activity significantly declined during storage ($p<0.05$). Moreover, there were significant differences between the treated samples and the control group. Increasing the concentration of MOLE led to a significant rise in antioxidant activity ($p<0.05$), whereas increasing the inulin level did not have a significant effect. The highest antioxidant activity was observed in T3 (2% MOLE and 3% inulin) and T5 (2% MOLE and 5% inulin), while the lowest one was observed in the control. yogurt is known as a rich source of bioactive peptides with potent antioxidant activity. This is due to the metabolic activity of LAB that produce peptides and amino acids with antioxidant properties during fermentation [6]. Also, the MOLE plays an important role in its antioxidant activity [17,18,30]. The antioxidant activity in yogurt is also attributed to the presence of catalase, superoxide dismutase, caseins, whey proteins, and LAB. These components collectively help prevent the oxidation of particles caused by free radicals. The antioxidant properties of MOLE, primarily attributed to compounds like ascorbic acid, β-carotene, quercetin, kaempferol, and phenolic acids, can contribute to the extended shelf life of dairy products by preventing oxidative damage caused by free radicals [14]. This can also have positive implications for human health, as oxidative stress is linked to various chronic diseases. Incorporating MOLE into dairy products can enhance their antioxidant capacity, as demonstrated by studies showing increased levels of phenolic compounds and antioxidant activity [30].

Table 5. Fitting the data with Newtonian, Power Law, Herschel Balkley, Bingham and Casson Models

Model	T1	T2	T3	T4	T5
Newtonian model					
k	0.08593	0.1227	0.08557	0.1876	0.1223
R^2	0.5713	-1.216	-1.66	-3.31	-2.426
RMSE	5.323	13.17	9.617	23.8	14.78
Power law					
k	2.002	8.651	6.689	18.82	10.96
n	0.4444	0.244	0.2247	0.1785	0.2002
R^2	0.9818	0.9917	0.9871	0.984	0.9903
RMSE	1.109	0.8078	0.6771	1.464	0.7872
Herschel-Bulkley					
k	0.6259	6.937	5.331	16.63	10.01
N	0.6234	0.2718	0.2525	0.192	0.2104
τ_0	3.477	2.201	1.678	2.491	1.11
R^2	0.9945	0.9931	0.9887	0.9849	0.9909
RMSE	0.6034	0.7483	0.641	1.437	0.779
Bingham plastic					
K	0.06222	0.06349	0.04214	0.07945	0.05557
τ_0	6.285	15.69	11.51	28.66	17.69
R^2	0.9634	0.847	0.8403	0.7903	0.7967
RMSE	1.571	3.469	2.381	5.304	3.638
Casson					
k	0.3251	1.599	1.362	2.635	2.175
τ_0	3.389	9.27	5.52	98.43	4.145
n	0.4105	0.1786	0.1698	0.1272	0.1336
R^2	0.9968	0.9953	0.9914	0.9875	0.9922
RMSE	0.472	0.6207	0.5578	1.311	0.7182

3.5. Viability of Probiotic Bacteria

The results comparing the mean probiotic counts of the yogurt samples are presented in Table 7. The probiotic counts significantly decreased during storage ($p<0.05$). Furthermore, the probiotic counts in the treated and control samples differed significantly ($p<0.05$). The highest counts were observed in treatment T5 (2% MOLE and 5% inulin) and the lowest in the control. Numerous studies have indicated that the minimum viable probiotic count should be 10^7 cfu/ml or cfu/g when consuming probiotic products [23]. Prebiotic compounds could play a crucial role in the survival of probiotics by stimulating their growth and activity [8,9]. The significant decrease in the probiotic count of the control sample can be attributed to the high acid production by yogurt starter bacteria and the absence of growth-stimulating prebiotics. These findings align with previous research demonstrating that the addition of MOLE can stimulate the growth of yogurt starter cultures [30]. The impact of MOLE on bacterial growth is influenced by several factors, including the extraction process, inoculation volume, the growth phase, chosen growth environment, environmental pH, and the greenhouse's duration and temperature. As a result, the reported outcomes show considerable variation.

3.6. Sensory Evaluation

The sensory attributes of the samples, including appearance, colour, flavour, texture, mouthfeel and overall acceptance, were assessed one day after production by a panel of 15 male and female participants (aged 25-45). The sensory scores of the treated and control samples showed significant differences ($p<0.05$) (Fig. 2). An increase in MOLE resulted in lower sensory scores, due to discoloration and syneresis. In contrast, higher inulin content significantly improved the scores for appearance, mouthfeel, and overall acceptance ($p<0.05$), while colour and flavour scores remained unchanged. The lowest sensory scores were observed in treatment T3 (2% MOLE and 3% inulin), while the highest scores were in the control. Notably, MOLE significantly enhanced the yogurt flavour as its concentration increased. The incorporation of plant-based extracts, such as green tea and MOLE, can enhance the functional properties of yogurt [19,27].

However, challenges in sensory acceptance may arise, particularly with MOLE. While green tea extract can improve sensory attributes of yogurt like appearance, colour, and odour, it is essential to balance desired functional benefits with potential impacts on texture and flavour. Encapsulation technologies offer a promising approach to address these challenges by protecting bioactive compounds, controlling their release, and improving the overall sensory profile of yogurt [31].

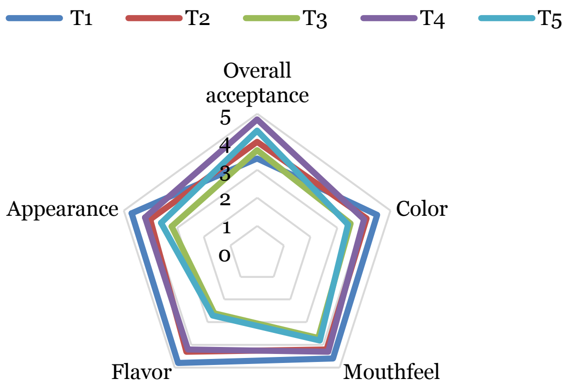

Fig 2. Sensory scores of yogurt samples

4. CONCLUSIONS

Based on the results of the present study, it can be concluded that inulin and MOLE have both positive and negative effects on the physicochemical properties of synbiotic yogurt. The addition of inulin resulted in decreased yogurt syneresis, while WHC, dry matter content, and viscosity increased. Conversely, the MOLE exhibited opposite effects. The acidity and pH of yogurt were not affected by inulin, whereas MOLE increased acidity and lowered pH. Owing to its antioxidant properties, MOLE enhanced the antioxidant activity of the yogurt. Inulin, with its prebiotic properties, promoted probiotic growth, whereas MOLE did not affect the probiotic count. Additionally, the extract did not positively impact the yogurt's sensory properties, while inulin gave rise to higher sensory scores. Encapsulating MOLE could enhance the sensory acceptance of the final synbiotic yogurt. In conclusion, the findings of this study suggest that the combined use of MOLE, inulin, and probiotic bacteria in yogurt could be a promising strategy for creating a functional food product that enhances the health of consumers.

5. DECLARATIONS

5.1. Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

5.2. ACKNOWLEDGMENTS

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5.3. Funding

No funding was received for conducting this study.

5.4. AUTHOR CONTRIBUTIONS

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Soroush Orouji and Zahra BeigMohammadi. The first draft of the manuscript was written by Soroush Orouji and Zahra BeigMohammadi, and Hamid B. Ghoddusi commented on previous versions of the manuscript. All authors read and approved the final manuscript.

5.5. Ethical Considerations

All ethical principles were adhered to in conducting and writing this article.

5.6. Data availability

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

5.7. Using Artificial Intelligence chatbots

No AI chatbot has been used in this study.

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