Egypt. J. Chem. **Vol. 65,** No. SI:13B pp. 629- 639 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Combination of Prebiotic Inulin and Probiotics intervention on the Physicochemical, Microbiological, and Sensory Properties of an Innovative Synbiotic Ras Cheese.

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Abstract

Inulin is classified as a prebiotic since it possesses functional health-promoting and industrial processing capabilities in dairy and non-dairy products. The study aims to incorporate inulin as a fat substitute with probiotics to improve the physicochemical, sensory, and microbiological properties of synbiotic Ras cheese during 90 days of ripening. Five Ras cheese formulations were investigated, including full-fat control 1 (FFCC) and low-fat control 2 (LFCC), as well as three formulas supplemented with 1 %, 3 %, and 5 % inulin (LFT_1, LFT_3, and LFT_5), respectively. As a result of the inulin addition, significant changes ($P<0.05$) occurred in the textural profile, cheese yield, probiotic population, and sensory assessment. The addition of probiotic culture produced a synbiotic low Ras cheese with excellent sensory properties. It was discovered that LFT_3 has the highest overall score of 76.64±2.49 points, while LFCC has the lowest overall score of 60.94±1.35 points. Surprisingly, Ras cheese supplemented with 3 % or 5 % inulin exhibited the equivalent mouth feel and texture characteristics as full-fat cheese. As recommended, combining probiotic culture and inulin is an innovative technique for improving low-fat cheese quality, health benefits, and low manufacturing costs.

Keywords: Low-fat Ras cheese, probiotic, inulin, prebiotic, symbiotic

INTRODUCTION

Functional foods are therapeutic substances or foods containing microorganisms that can improve human health and prevent certain diseases when combined with a substrate(s), particularly carbohydrates known as “prebiotics”, that stimulate and encourage the development of probiotics known as "synbiotics" [1]. Prebiotics are short-chain carbohydrates that resist being digested by human digestive enzymes and increase the activity of certain types of beneficial gut flora [2]. They are employed in the metabolism of probiotic bacteria to create a range of postbiotic metabolites [3]. Also, it has numerous extra therapeutic benefits for the large intestine and human health, such as lowering the risk of colon cancer, constipation, and diarrhea, as well as increasing calcium and magnesium absorption [4].

Because of its favorable effects in industrial applications, inulin is commonly utilized as a prebiotic in dairy and non-dairy products as a fat replacer, sugar replacer, texture enhancer, organoleptic enhancement, and functional foods development [5]. Furthermore, it helps in the development of low-fat cheese, synbiotic products, and the stability of foams, emulsions, and mouth feels in a variety of culinary applications such as dairy products and bread [6].

The majority of customers favored nutritional and functional foods that were low in calories and fat while maintaining providing health advantages [7]. As a result, inulin was used as a fat substitute in the production of several cheese types, including Mozzarella, and Cheddar cheese [8].

Some of the most common microorganisms used in the manufacturing of successful probiotic products include *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Bacillus* [9]. Several studies have shown that the production of various forms of "bio-cheeses" is a beneficial probiotic carrier due to its distinct physicochemical properties and high

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Receive Date: 29 June 2022, Revise Date: 05 September 2022, Accept Date: 14 September 2022
DOI: 10.21608/EJCHEM.2022.147657.6405

potential survivability of probiotics in the product and delivery into the gastrointestinal system [6].

Ras cheese is a low-moisture Egyptian hard cheese with a strong sharp flavor that is similar to the Greek type (Kefalotyri cheese) and is made mostly from cow's milk or a 1:1 mixture of cow and buffalo milk. It is made by enzymatically coagulating raw or pasteurized milk with rennet, either with or without the use of starter cultures [10]. Ras cheese must be kept in the ripening chambers for at least three months at a constant relative humidity of roughly 80 % and a temperature of 12 – 15°C to produce its characteristic flavor and texture [11]. Furthermore, it must have at least 60 % solids and 45 % fats in solids [12]. The current study investigated the influence of inulin as a fat substitute and probiotic bacteria on the physicochemical, microbiological, and organoleptic properties of synbiotic Ras cheese during ripening.

1. Materials and Methods

2.1. Materials

Standard whole cow milk used in cheese-making content (of 3.0 % fat, 4.6 % lactose, 0.8 % ash, 3.1 % protein, and 87.5 % water) and inulin (CAS, 9005-80-5, Sigma, USA).

2.2. Probiotic and starter inoculum preparation

Misr Food Additives (MIFAD, Egypt) purchased freeze-dried DVS (nu-trish ABY-1) from Chr. Hansen Inc. Laboratories, Denmark, which included *Streptococcus thermophilus*; *Lactobacillus delbrueckii* subsp. *bulgaricus*; *Lactobacillus acidophilus* LA-5, and *Bifidobacterium animalis* subsp. *lactis* BB-12. Freeze-dried culture inoculation (2 %) in 100 ml of autoclaved reconstituted skimmed milk (RSM, MIFAD, Egypt) was incubated anaerobically at 37°C until it began to gel.

2.3. Cheese manufacture

Ras cheese was prepared by the conventional method described by Hofi [11] in 250-L cheese vats. Pasteurized cow milk (72°C, 15 s) was chilled to 32°C, and calcium chloride (0.02 %), and bacterial cultures were added. The milk was separated into two parts, the first of which was standard (whole milk 3 % with 0 % inulin) control 1 (FFCC), and the second of which was skimmed (0.2 % fat) using milk cream separator machine (Pune - 411035, Maharashtra, India) then divided into four parts, the first of which was used as a control 2 (LFCC) (skimmed milk 0.2 % with 0 % inulin), and the other three were supplemented with 1 %, 3 %, and 5 % inulin (LFT_1, LFT_3, and LFT_5).

Cheese milk was acidified for one hour with bacterial starter cultures before adding Microbial rennet, (Marzyme MT 2200 Powder, DuPont Danisco, France) to complete coagulation in 30 – 40 min. The coagulum was divided into tiny chickpea-sized particles (about 1 cm^3) and moves gently to drain the whey, and the vat's temperature was raised to 45°C in 40-50 min.

The acidic whey (0.14 % lactic acid) is drained after the coagulation process stabilized then the curd was salted (1 %). Gauze-coated Ras cheese forms molds are manually filled and pressed to release part of the leftover whey. After 4 h of mild mechanical pressure, the cheese is inverted and kept at ambient temperature for 15 – 20 h under high pressure (15 Pa). Finally, the surfaces of each cheese treatment were sprinkled with a small amount of dried salt, wrapped with paraffin wax, and stored in a ripening chamber for three months at 12-15°C and 80 % of relative humidity.

2.4. Physicochemical properties

The Association of Official Analytical Chemists recommended procedures for assessing all physicochemical characteristics of milk and cheese (AOAC 2005) [13]. The DM % was determined using a Turbofan oven (Bakbar Versatile Bench Top Model E32, Germany) at 105°C for 3 h. Fat was estimated using Gerber procedures, whereas protein and water-soluble nitrogen (WSN) were calculated using Kjeldahl techniques. pH was measured using an electrode pH meter (Hanna, Germany). The acidity was expressed as a percentage of lactic acid and determined by titrating the endpoint with phenolphthalein of diluted cheese solution using a 0.9 *N* NaOH.

Vakaleris and Price [14] designed a method for measuring the soluble tyrosine and tryptophan levels in cheese. The carbohydrate % was calculated by subtracting the cheese's moisture, ash, protein, and fat percentages from 100. Jeon [15] described a technique for determining total volatile fatty acids.

2.5. The salt content determination

The salt content of cheese (AOAC 2005, Method No. 935.43; SMEDP 15.052) [13]. Briefly, in the presence of a known number of moles of silver nitrate, approximately 3 g of cheese was heated and digested with nitric acid and potassium permanganate. Acid digestion causes chloride to be released in the sample, where it reacts with silver to form AgCl. Titration of unreacted residual silver with potassium thiocyanate with ferric ammonium indicator determines salt content.

2.6. Total yield of synbiotic Ras cheese

Ras cheese yield is a mathematical equation for the amount of cheese produced from a given amount of raw materials [16].

$$\text{Yield of cheese }\% = \frac{\text{Weighed of cheese(Kg)}}{\text{Raw ingredients(Kg)}} \times 100$$

2.7. Microbiological evaluation

The potential microorganisms were counted using the usual plate count method [17]. For one minute, 10 g of Ras cheese was homogenized in 90 mL of sterile peptone water in an electromechanical homogenizer (Stomacher Lab-blender 400; Seward Medical, London, UK). *Streptococcus thermophilus* was counted using M17 (Oxoid, UK) supplemented with lactose 1 % (Sigma, USA) and 1.5 % bacteriological agar (Merck, UK) and then inoculated 1 % aerobically at 42 – 45°C for 72 h, whereas *Lactobacillus delbrueckii* subsp. *bulgaricus* was counted using MRS (Oxoid, UK) and incubated anaerobically at 42 – 45°C for 72 h. *Lactobacillus acidophilus* was incubated aerobically and *Bifidobacterium animalis* was incubated anaerobically and both were inoculated 1 % and incubated at 37 – 40°C for 48 h in MRS agar supplemented with 5 % of lactose. Also, (0.05 %) of L-cysteine-HCL (Win Lab, Gemini House, Middlesex, Hab 7ET, UK) was added as a reducing agent. The gas generating kits (Unipath Ltd, Basingstoke, Hants., UK, Anaerobic System BR 038B) were used to create anaerobic conditions. The viable counts of bacteria were determined, and the results were represented as colony-forming units CFU X 10^6.

2.8. Texture profile analysis

Texture profile analysis (TPA) of cheese samples was examined using a texture analyzer (CNS-Farnell, Boreham-wood, Hertfordshire, UK) as described by Zhang [18]. During 90 days, cheese samples were examined using cheese samples cubes with a side length of 20 mm and were kept at room temperature for one hour before beginning the assay under conditions P/0.5 probe, test speed 1.0 mm/sec, while the test speed before and after 5.0 mm/sec, the time between first and second pressed 5.0 sec, compression ratio 30 %, and two compression cycles. The texture characteristics of hardness, chewiness, and cohesiveness were determined using the force-deformation curve. At least three measurements were taken for each type of sample, and the mean values of the computed sample texture characteristics were recorded.

2.9. Sensory evaluations

As sensory assessment test participants, about 15 experts (8 males and 7 females, ages 30 to 55). 30 g samples of Ras cheese were tagged with three numerical numbers and distributed to committee members in random order in a 26 ± 2°C condition for comparison. All participants stated that they did not have any dairy allergies or other health issues. The sensory elements such as appearance (10), body and texture (30), flavor (60), and overall acceptability (100) were evaluated as a point deducted from the total score of each evaluation. There was 3 min between the evaluation of each sample to avoid interference between the sensory evaluation of each sample for the tastes of the panelist while providing drinking water to ensure no sensory disturbance [19].

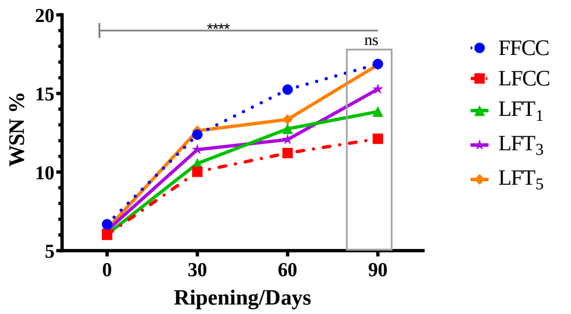
2.10. Statistical analysis

Samples were analyzed in three replicates for each test, and the results were provided as Mean±SE. GraphPad Prism was used to create graphs and calculate statistics (versions 8.1, GraphPad Inc., La Jolla, CA, USA). A two-way ANOVA is used for variance analysis, and a one-way ANOVA test was performed for the sensory analysis. For each storage duration, the significant value P<0.05 was used to compare the different groups (30, 60, and 90 days).

2. Results

3.1 The chemical composition of synbiotic low-fat Ras cheese.

The results presented in (Table 1) showed the chemical composition of low-fat Ras cheese during 90 days of storage. There were no significant differences p<0.05 across all treatments. DM % in fresh cheese ranged from 56.21±1.02 to 62.9±1.57 %, whereas it ranged from 60.15±1.53 to 67.14±2.96 % across all treatments after 90 days of ripening. The fat % rose with storage time. FFCC had the highest fat content of the fresh cheese treatments, whereas LFCC had the lowest values. Furthermore, after 90 days of ripening, all cheese treated with inulin had a greater fat level than LFCC. Notably, inulin treatments resulted in a decrease in salt %, while it increased as the ripening process continued. On the contrary, carbohydrate % declined even with inulin addition or as ripening time continued. In general, no significant changes in dm, fat, protein, ash, salt, or carbohydrate content (p<0.05) were seen in any of the treatments. However, its impact may be seen in its increase as ripening continues. Furthermore, the addition of inulin did not affect Ras cheese's overall nitrogen concentration. Figure 1 showed significant changes (p<0.05) in the WSN of LFCC and LFT_5 in fresh cheese (6.69±0.63 and 6.38±0.55 %), however no significant changes (p<0.05) after 90 days of ripening. It was also comparable to FFCC 16.88±1.14 and LFCC 12.12± 0.23 %.



Full-fat control cheese (FFCC), Low-Fat control cheese (LFCC), Low-Fat cheese (LFT_1; 1% inulin), Low-Fat cheese (LFT_3; 3% inulin), Low-fat cheese (LFT_5; 5% inulin). It was highly significant between treatments at p<0.0001, however, non-significant after 90 days of storage

Fig 1. Changes in water-soluble nitrogen (WSN %) of Ras cheese supplemented with inulin during ripening.

3.2. The influence of inulin, probiotic bacteria, and ripening on pH and the acidity of functional Ras cheese.

Figure 2 demonstrated that inulin-treated cheese had higher acidity and lower pH in zero time than FFCC and LFCC. It was developed in the same context, with 90 days of ripening. The titratable acidity of all Ras cheese treatments increased progressively ($p<0.05$), peaking at the end of the ripening.

Table 1. Chemical composition (Mean±SE) of Ras cheese fortified with inulin.

Storage/days	FFCC	LFCC	LFT_1	LFT_3	LFT_5
DM %					
0	62.9±1.57 ^{a}	61.69±1.92 ^{a}	60.5±1.52 ^{a}	58.03±4.24 ^{a}	56.21±1.02 ^{a}
30	63.67±1.80 ^{a}	62.98±1.15 ^{a}	60.99±3.38 ^{a}	58.4±2.21 ^{a}	57.09±2.56 ^{a}
60	64.97±3.44 ^{a}	64.02±3.44 ^{a}	62.0±3.51 ^{a}	60.9±3.01 ^{a}	58.79±3.99 ^{a}
90	67.14±2.96 ^{a}	66.3±1.03 ^{a}	63.2±2.73 ^{a}	62.1±1.11 ^{a}	60.15±1.53 ^{a}
Fat %					
0	25.56±0.63 ^{b}	2.33±0.03 ^{a}	2.17±0.07 ^{d}	1.93±0.14 ^{b}	1.79±0.11 ^{d}
30	27.00±0.12 ^{b}	2.95±0.38 ^{a}	2.41±0.07 ^{c}	2.20±0.00 ^{b}	2.08±0.04 ^{c}
60	29.73±1.13 ^{a}	3.18±0.59 ^{a}	2.77±0.08 ^{b}	2.58±0.37 ^{ab}	2.39±0.04 ^{b}
90	30.21±0.01 ^{a}	3.68±0.50 ^{a}	3.30±0.06 ^{a}	3.11±0.07 ^{a}	3.05±0.10 ^{a}
Protein %					
0	20.67±1.13 ^{b}	25.77±2.95 ^{a}	24.3±2.32 ^{b}	23.98±0.20 ^{c}	22.71±0.34 ^{b}
30	21.81±0.35 ^{b}	26.81±0.69 ^{a}	25.0±0.82 ^{ab}	24.43±0.04 ^{c}	23.86±0.59 ^{ab}
60	23.5±0.63 ^{ab}	28.31±0.17 ^{a}	27.8±0.73 ^{ab}	27.37±0.44 ^{b}	25.92±1.68 ^{ab}
90	25.03±1.27 ^{a}	30.49±0.59 ^{a}	29.22±0.61 ^{b}	28.78±0.02 ^{a}	27.66±0.53 ^{a}
Ash %					
0	7.00±1.15 ^{a}	7.37±1.10 ^{a}	7.20±1.27 ^{a}	7.14±1.24 ^{a}	7.00±1.15 ^{a}
30	7.10±1.16 ^{a}	7.45±1.13 ^{a}	7.33±1.01 ^{a}	7.25±1.13 ^{a}	7.17±1.27 ^{a}
60	7.27±0.58 ^{a}	7.63±0.55 ^{a}	7.49±0.46 ^{a}	7.38±0.59 ^{a}	7.35±0.55 ^{a}
90	7.55±0.62 ^{a}	7.75±0.24 ^{a}	7.55±0.26 ^{a}	7.64±0.32 ^{a}	7.51±0.58 ^{a}
Salt %					
0	2.22±0.56 ^{b}	2.31±0.41^{b}	2.45±0.43 ^{b}	2.37±0.46 ^{b}	2.32±0.59 ^{b}
30	3.15±0.61 ^{ab}	3.30±0.17 ^{ab}	3.07±0.18 ^{ab}	2.80±0.03 ^{b}	2.65±0.09 ^{b}
60	3.35±0.11 ^{ab}	3.59±0.03 ^{ab}	3.37±0.23 ^{ab}	3.10±0.35 ^{ab}	2.93±0.50 ^{b}
90	4.08±1.35 ^{a}	5.02±1.20 ^{a}	4.06±1.16 ^{a}	3.59±0.82 ^{a}	3.27±1.20 ^{a}
Carbohydrate %					
0	7.45±0.41^{b}	23.91±4.61 ^{a}	24.38±4.21 ^{a}	22.61±3.43 ^{a}	22.39±3.41 ^{b}
30	4.61±0.21^{b}	22.47±3.41 ^{a}	23.18±6.40 ^{a}	21.72±3.41 ^{a}	21.37±4.43 ^{ab}
60	1.12±0.02 ^{b}	21.31±2.53 ^{a}	20.61±2.31 ^{a}	20.47±2.41 ^{a}	20.30±1.78 ^{a}
90	0.27±0.13 ^{ab}	19.36±1.64 ^{a}	19.07±0.46 ^{a}	18.98±0.15 ^{a}	18.66±0.41 ^{a}

All data were determined in triplicate and presented as Mean±SE. Values with the same subscriptions in the same columns and rows are not significantly different ($p<0.05$). Full-fat control cheese (FFCC), Low-Fat control cheese (LFCC), Low-Fat cheese (LFT_1; 1% inulin), Low-Fat cheese (LFT_3; 3% inulin), Low-fat cheese (LFT_5; 5% inulin). a,b,c,d: Means of treatments in the same storage period with the same letter in the same character are not significantly different ($P<0.0001$).

3.3. Changes in soluble tyrosine and tryptophan mg/100 g during ripening.

Tyrosine and tryptophan were examined as a rapid method for measuring the degree of proteolysis in ripening cheese. Figure 3 showed that the tyrosine concentration varied considerably ($p<0.05$) throughout fresh cheese ripening. LFT_3 and LFT_5 detected 59.04±1.73 and 67.82±1.45 mg/100g, respectively, in comparison to FFCC, LFCC, and LFT_1, which were 58.43±2.42, 54.12±2.94, and 55.12±3.17 mg/100g, respectively. There was no significant difference $p<0.05$ in inulin treatments compared to the FFCC, and LFCC throughout 90 days of ripening. The tryptophan content didn't change substantially ($p<0.05$). Furthermore, there was no significant change between FFCC and LFCC or in inulin treatments after 90 days of ripening.

3.4. Total volatile fatty acids (TVFA) mg/100 g assessment

The total volatile fatty acid profile was shown to be a significant predictor of Ras cheese maturity, rising dramatically as the ripening progressed. Figure 4 showed that there was no significant change between treatments ($p<0.05$). LFT_1, LFT_3, and LFT_5 in fresh Ras cheese varied from 9.12±0.92 to 11.53±0.58 mg/100 g respectively, compared to 12.32±0.77 and 6.87±0.77 mg/100 g in FFCC and LFCC. Notably, TVFA developed significantly after 90 days of ripening.

3.5. Yield % of synbiotic low-fat Ras cheeses

Figure 5 showed that there was no significant change $p<0.05$ between treatments or during the ripening period. Notably, a rise in cheese yield was seen with an increase in inulin proportion, as observed in LFT_5, which had a considerable yield in fresh 15.26±1.03 % or after 90 days of storage 14.09±1.12 %

3.5. Microbiological assessment of a synbiotic low-fat Ras cheese

Figure 6 demonstrated that the count of *Streptococcus thermophiles* in fresh cheese did not change significantly across all treatments ($p<0.05$).

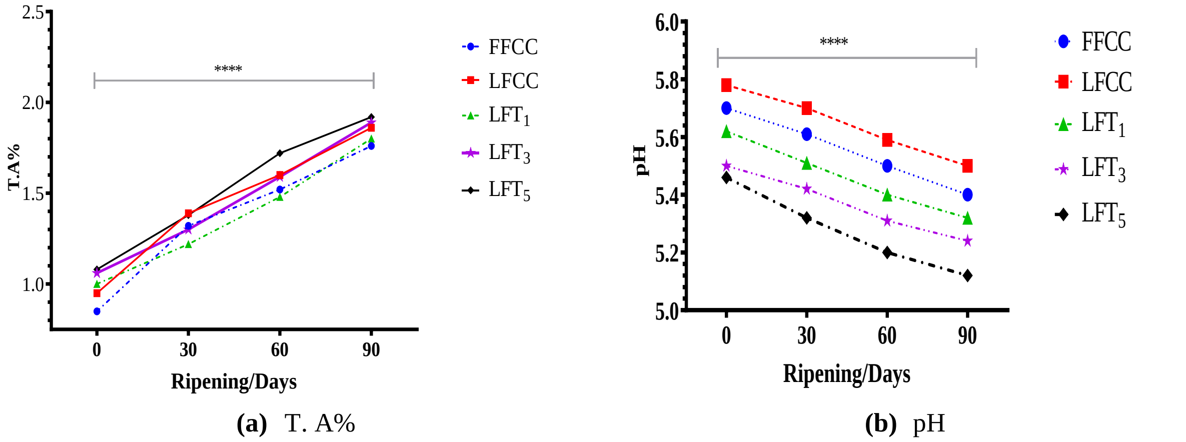
LFT_3 and LFT_5 had (10.33±0.23, and 9.53±0.02) CFU X10^6 respectively, whereas FFCC and LFCC had (10.38±0.26 and 10.7±0.09) CFU X 10^6. On the contrary, after 90 days of ripening, FFCC and LFCC exhibited a significant reduction in the count (7.21±0.52 and 8.08±0.61) CFU X 10^6 respectively, whereas LFT_1, LFT_3, and LFT_5 did not show a significant reduction in the count. While the count of *Lactobacillus delbrueckii* subsp. *bulgaricus* in fresh cheese or after 90 days of ripening did not differ significantly at $p<0.05$ within all treatments. Furthermore, the count of *Lactobacillus acidophilus* had no significant change $p<0.05$ in inulin treatments during fresh cheese ripening. However, after 90 days of ripening, it showed (6.51±0.28, 6.59±0.26, and 7.23±0.12) CFU X 10^6. Notably, the count of *Bifidobacterium animalis* did not change significantly at $p<0.05$ in fresh cheese. However, it decreased gradually with ripening progress among all groups. On the other hand, after 90 days of maturation, the effect of adding inulin was fully evident compared to the control groups. Inulin influences bacterial culture development rate since it promotes their growth during ripening. Furthermore, it was shown that ripening durations had a substantial influence on the lowering of growth rates of both starter and probiotic bacteria.

3.6. Sensory evaluation

As demonstrated in Table 2, the sensory assessment was used to compare the impact of inulin concentrations and probiotic bacteria on the sensory properties of synbiotic low-fat Ras cheese. Except for LFT_3, which demonstrated a highly significant difference at $p<0.05$, the flavor was evaluated as 48.67±0.88 points in fresh Ras cheese. Meanwhile, there was no significant change at $p<0.05$ during 90 days of ripening. Also, LFT_3 earned a high taste score of 56.67±0.33 points. On the contrary, LFCC obtained the lowest flavor score of 48.00±1.15 points.

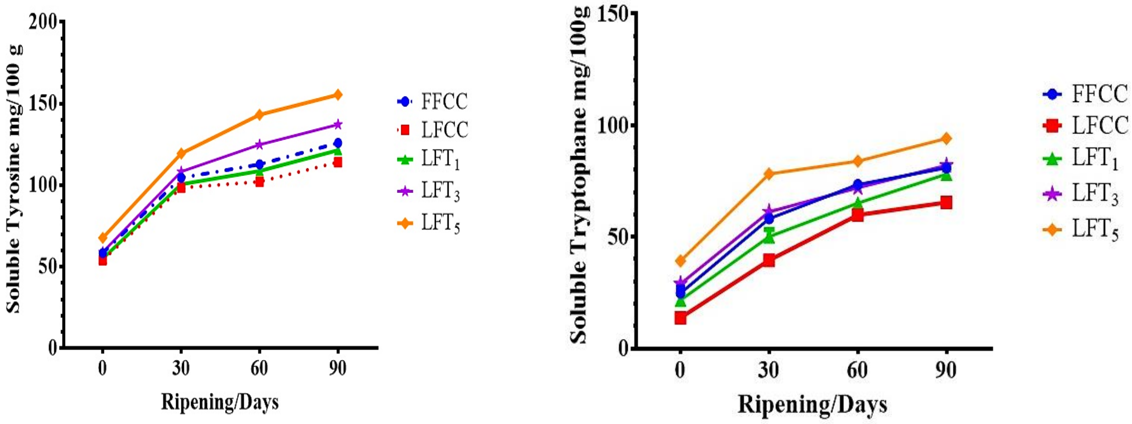
Likewise, the body and texture qualities were assigned 30 points each. Within groups, fresh cheese revealed a highly significant difference ($p<0.05$). LFT_3 received the highest body and texture score of 22.87±0.96 points. While the LFCC earned the lowest score of 14.00±1.15 points. On the other hand, after 90 days of ripening, there was no significant change $p<0.05$ within all groups. LFT_3 also showed the highest body and texture score 26.33±0.41 points. while LFCC showed the lowest score of 20.45±1.36 points.

In addition, the appearance was graded on a ten-point scale. Within groups, fresh cheese exhibited a $p<0.05$ significant difference. According to the observed results, FFCC and LFT_3 got the highest appearance score, with 8.58±0.39 and 8.21±0.50 points respectively of resemblance. Furthermore, there was no significant difference between groups after 90 days of ripening at $p<0.05$. FFCC and LFT_5 seemed similar and received scores of 8.03±0.12 and 8.06±0.15 points respectively, while LFT_3 received the highest score of 9.02±0.06 points. Finally, the total overall score is displayed as 100 points. In fresh Ras cheese, there was a significant variation within groups ($p<0.05$). LFT_3 earned the highest overall score of 76.64±2.49 points, followed by FFCC with 76.46±2.32 points, while LFCC obtained the lowest score of 60.94±1.35 points. Furthermore, after 90 days of maturation at $p<0.05$, there was no significant difference between the groups. Once again LFT_3 achieved the highest overall score of 94.28±0.55 points, followed by FFCC at 88.48±0.43 points, while LFCC got the lowest score of 74.680.32 points. In comparison to the control, the observed findings demonstrated that LFT_3 had excellent mouthfeel and textural features.



Full-fat control cheese (FFCC), Low-Fat control cheese (LFCC), Low-Fat cheese (LFT_1; 1% inulin), Low-Fat cheese (LFT_3; 3% inulin), Low-fat cheese (LFT_5; 5% inulin). There was a highly significant between treatments at $p<0.0001$

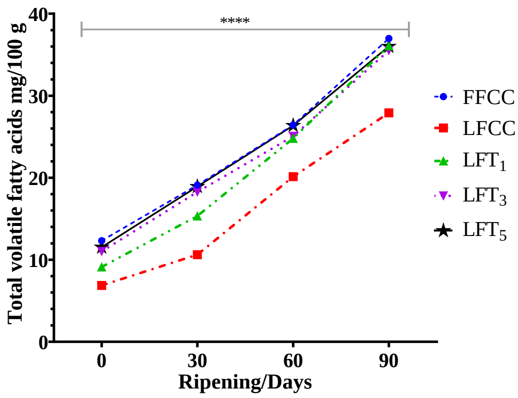
Fig 2. The influence of inulin concentration and ripening time on the acidity and pH of low-fat Ras cheese



(**a**) Soluble tyrosine mg/100g (**b**) Soluble tryptophan mg/100g

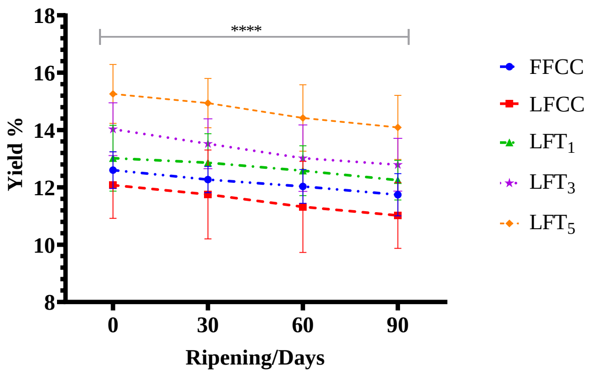
Full-fat control cheese (FFCC), Low-Fat control cheese (LFCC), Low-Fat cheese (LFT_1; 1% inulin), Low-Fat cheese (LFT_3; 3% inulin), Low-fat cheese (LFT_5; 5% inulin).

Fig 3. Changes in soluble tyrosine and tryptophan mg/100 g of Ras cheese enriched with inulin during ripening.



Full-fat control cheese (FFCC), Low-Fat control cheese (LFCC), Low-Fat cheese (LFT_1; 1% inulin), Low-Fat cheese (LFT_3; 3% inulin), Low-fat cheese (LFT_5; 5% inulin). It was highly significant between treatments at p<0.0001

Fig 4. Total volatile fatty acids (TVFA) mg/100 g of Ras cheese enriched with Inulin during ripening



Full-fat control cheese (FFCC), Low-Fat control cheese (LFCC), Low-Fat cheese (LFT_1; 1% inulin), Low-Fat cheese (LFT_3; 3% inulin), Low-fat cheese (LFT_5; 5% inulin). It was highly significant between treatments at p<0.0001

Fig 5. Yield % of Ras cheeses fortified with Inulin during ripening

3.7. Texture profile analysis (TPA) of synbiotic Ras cheeses

Texture profile analysis was used to evaluate low-fat Ras cheese's hardness (g), adhesiveness (gs), cohesiveness (%), springiness (mm), gumminess (N×mm), and chewiness (gmm).

Table 3 showed a significant change (P<0.05) in the textural profile examination of Ras cheeses from zero vs. 90 days of ripening at 4°C. LFT_5 hardness was the lowest compared to other inulin cheese treatments (11.54±0.53 vs. 44.51±0.52) g, as well as LFCC (28.46±1.16 vs. 64.89±0.28) g. Furthermore, the springiness of LFCC (6.51±0.01 vs. 2.53±0.02) mm was substantially lower (P<0.05) compared to LFT_5 (10.06±0.44 vs. 2.98±0.03) mm.

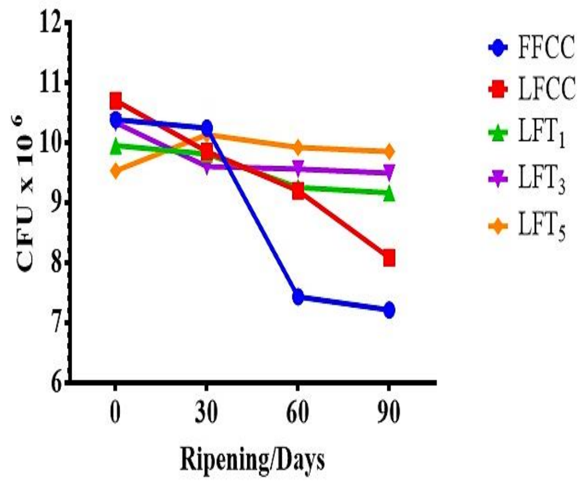
The chewiness was shown to be strongly linked to cheese hardness (gmm). At the end of the ripening, LFT_5 was 24.12±0.58 gmm and had a slight chewiness similarity to FFCC 25.32±0.18 gmm. FFCC and LFT_5 exhibit a substantial resemblance in terms of cohesiveness (%) due to elevated amounts of primary proteolysis. After 90 days of ripening, the adhesiveness of LFT_5 was 0.74±0.07 gs, which was much higher than FFCC 0.09±0.01 gs. Gumminess was also measured after 90 days of ripening for FFCC and LFT_5, yielding values of 6.02±0.57 and 5.92±0.05 g, respectively.

3. Discussion

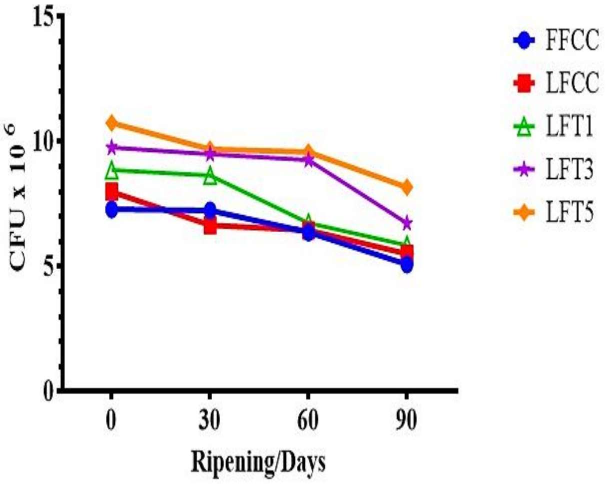
The combination of inulin and probiotic bacteria to produce a synbiotic low-fat Ras cheese is an innovative technique that has a great significant influence. The results were consistent with those obtained by Sahan [20] for kashar cheese, who suggested that increased fat substitution in cheese may be resulting in increased DM % retention in low-fat cheese. Furthermore, the findings were comparable to those of Khodear [21] who discovered that adding inulin resulted in a considerable increase in total solids, and the production of titrated acidity.

Sousa [22] ascribed the increase in WSN % to the hydrolysis of whole casein by residual chymosin and, to a minor extent, the indigenous milk proteinase, plasmin, and the proteolytic activity of the cheese bacterial culture. Coherently, the achieved results were consistent with those described by Abd-Elmonem [23] who indicated that the fat content of cheese is the most important component determining its flavor since it also determines the smoothness and richness of the body as well as the texture of the dairy product. There is a constant rise in the fat of Ras cheese content percent ($p<0.05$), which is owing to the incremental loss of moisture accumulated during ripening, but it might also be due to a decrease in other non-fat constituents due to the development and activity of microbes in all treatments. The pH values of all Ras cheese treatments decreased steadily ($p<0.05$) and reached their lowest levels at the end of the ripening phase than the control.

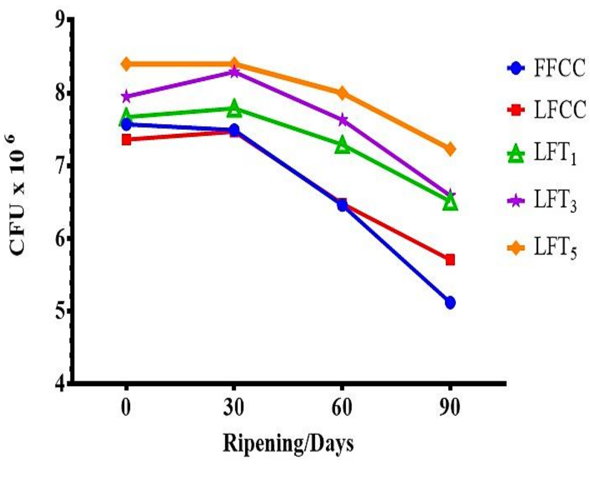
Leeuwendaal [24] attributed the overall acidity of cheese to it being naturally created by milk ingredients in addition to the acidity that develops during cheese ripening. The breakdown intermediates of protein and amino acids, as well as fatty acids emerging from fat hydrolysis, would significantly contribute to a rise in cheese acidity.



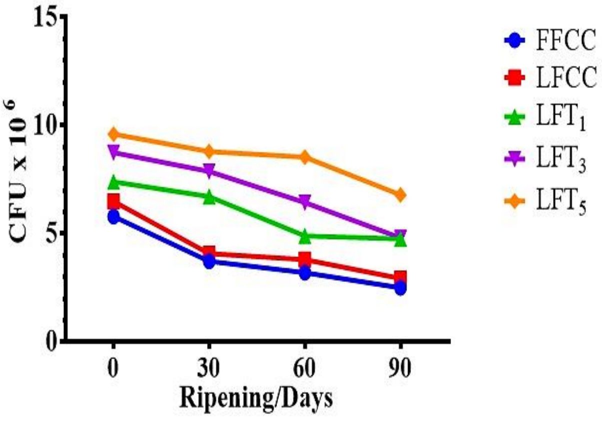
(**a**) *Streptococcus thermophiles* CFUX10^6



(**b**) *Lactobacillus delbrueckii* subsp *bulgaricus* CFUX10^6



(**c**) *Lactobacillus acidophilus* CFUX10^6



(**d**) *Bifidobacterium animalis* CFUX10^6

Full-fat control cheese (FFCC), Low-Fat control cheese (LFCC), Low-Fat cheese (LFT_1; 1% inulin), Low-Fat cheese (LFT_3; 3% inulin), Low-fat cheese (LFT_5; 5% inulin).

Fig 6. Microbiological assessment of a synbiotic low-fat Ras cheese enriched with inulin during ripening CFU X 10^6

Table 2. Sensory evaluation of synbiotic Ras cheeses

Storage/days	FFCC	LFCC	LFT_1	LFT_3	LFT_5
Flavor (60 points)					
0	47.00±0.58 c	40.33±2.03 c	42.00±1.15 c	48.67±0.88 b	42.33±1.20 c
30	49.67±0.88 b	42.67±1.45 bc	44.33±0.33 c	50.67±0.67 b	46.33±0.67 b
60	51.67±0.88 b	46.67±0.67 ab	47.67±0.33 b	55.00±0.58 a	50.67±0.67 a
90	54.67±0.33 a	48.00±1.15 a	51.00±1.00 a	56.67±0.33 a	52.67±0.33 a
Body and Texture (30)					
0	20.07±0.58 a	14.00±1.15 b	16.48±0.78 b	22.87±0.96 a	20.33±1.45 b
30	22.33±1.20 a	16.67±0.88 ab	16.67±1.45 b	24.00±1.00 a	23.00±1.00 ab
60	22.00±2.31 a	19.67±2.73 ab	20.67±1.20 a	24.00±1.53 a	25.00±0.58 a
90	24.67±0.88 a	20.45±1.36 a	22.47±0.87 a	26.33±0.41 a	25.07±0.69 a
Appearance (10 points)					
0	8.58±0.39 a	6.25±0.26 a	6.44±0.56 a	8.21±0.50 ab	7.12±0.18 ab
30	9.00±0.43 a	6.29±0.68 a	6.23±0.73 a	7.63±0.37 b	6.93±0.58 b
60	8.03±0.12 a	7.14±0.08 a	7.14±0.10 a	8.86±0.07 a	8.03±0.12 a
90	8.03±0.12 a	7.17±0.09 a	7.07±0.04 a	9.02±0.06 a	8.06±0.15 a
Total (100 points)					
0	76.64±2.49 c	60.94±1.35 b	64.28±0.49 b	77.94±0.76 d	70.28±3.57 c
30	79.42±0.84 bc	64.36±2.62 b	68.27±2.05 b	83.07±2.60 c	76.40±0.20 bc
60	83.18±0.72 b	71.52±0.82 a	74.39±0.37 a	89.14±0.65 b	82.43±0.43 ab
90	88.48±0.43 a	74.68±0.32 a	77.82±2.12 a	94.28±0.55 a	86.14±1.08 a

All data were determined in triplicate and presented as Mean±SE. Values with the same subscriptions in the same columns and rows are not significantly different (p<0.05). Full-fat control cheese (FFCC), Low-Fat control cheese (LFCC), Low-Fat cheese (LFT_1; 1% inulin), Low-Fat cheese (LFT_3; 3% inulin), Low-fat cheese (LFT_5; 5% inulin). a,b,c,d: Means of treatments in the same storage period with the same letter in the same character are not significantly different (P<0.0001).

Table 3. Texture profile analysis (TPA) of synbiotic Ras cheeses

Storage/days	FFCC	LFCC	LFT_1	LFT_3	LFT_5
Hardness (N)					
0	22.3±1.13 c	28.46±1.16 d	20.13±1.34 c	16.76±0.57 d	11.54±0.53 d
30	41.26±0.57 b	46.17±0.2 c	21.32±0.87 c	19.76±0.6 c	20.15±0.49 c
60	42.7±0.51 b	50.62±1.2 c	41.37±0.7 b	38.21±1.09 b	35.44±0.46 b
90	60.2±1.08 a	64.89±0.28 b	48.49±0.66 a	41.62±0.48 a	44.51±0.52 a
Adhesiveness (mJ)					
0	0.29±0.01 c	0.40±0.03 c	0.54±0.03 b	0.62±0.08 b	0.74±0.17 b
30	0.39±0.03 b	0.40±0.06 c	0.58±0.03 b	0.66±0.05 b	0.78±0.09 b
60	1.38±0.03 a	0.72±0.06 b	0.61±0.01 b	0.83±0.02 ab	0.91±0.07 b
90	1.44±0.03 a	1.40±0.02 a	0.87±0.09 b	1.03±0.16 a	1.39±0.01 a
Cohesiveness (%)					
0	0.28±0.01 b	0.25±0.01 b	0.26±0.03 a	0.24±0.00 a	0.27±0.04 a
30	0.29±0.05 b	0.41±0.01 b	0.28±0.05 a	0.35±0.01 a	0.38±0.06 a
60	0.31±0.05 b	0.52±0.03 b	0.49±0.03 a	0.53±0.03 b	0.58±0.01 b
90	0.69±0.06 a	0.72±0.04 a	0.78±0.01 b	0.82±0.02 c	0.89±0.03 c
Springiness (mm)					
0	1.13±0.57 b	1.175±0.02 b	1.123±0.6 b	1.556±0.18 c	2.09±0.03 c
30	1.339±0.55 b	2.371±0.63 b	3.574±0.3 b	1.98±0.57 b	2.547±0.60 b
60	1.461±0.08 b	2.212±0.27 a	2.226±0.35 a	1.544±0.25 a	2.063±0.53 a
90	3.53±0.59 a	3.747±0.01 a	3.510±0.44 a	1.78±0.22 a	3.059±0.44 a
Gumminess (N)					
0	6.02±0.57 d	6.70±0.52 c	6.61±0.51 c	3.99±0.59 b	5.92±0.05 b
30	11.21±1.20 c	11.21±1.20 b	8.14±0.61 c	5.13±0.62 b	6.08±0.57 b
60	15.09±0.59 b	25.33±1.18 b	15.64±0.51 b	8.11±1.21 a	11.74±0.47 a
90	29.03±1.00 a	35.20±0.05 a	20.80±0.58 a	10.04±0.57 a	12.17±1.24 a
Chewiness (mJ)					
0	25.32±0.18 a	23.65±0.58 b	22.61±0.48 ab	26.08±0.26 a	24.12±0.58 b
30	55.27±2.89 a	109.46±3.36 b	76.21±2.19 c	39.14±0.66 a	51.33±4.81 b
60	62.37±4.02 a	112.47±1.44 b	92.11±1.20 a	59.01±0.19 c	73.12±1.20 a
90	212.75±1.59 a	243.12±6.90 a	170.21±5.54 a	74.09±4.62 c	136.15±2.11 a

All data were determined in triplicate and presented as Mean±SE. Values with the same subscriptions in the same columns and rows are not significantly different (p<0.05). Full-fat control cheese (FFCC), Low-Fat control cheese (LFCC), Low-Fat cheese

(LFT_1; 1% inulin), Low-Fat cheese (LFT_3; 3% inulin), Low-fat cheese (LFT_5; 5% inulin). a,b,c,d: Means of treatments in the same storage period with the same letter in the same character are not significantly different (P<0.0001).

Tyrosine and tryptophan concentrations rose dramatically as the cheese aged, providing a rapid approach for assessing the degree of proteolysis in maturing cheese. It reflected the effect of inulin content, probiotic bacteria, and ripening duration on sensory evaluation. The results matched with those of [25] who found that adding inulin enhanced tyrosine and tryptophan solubility, acidity, WSN, probiotic bacteria survivability, and total volatile fatty acids. According to Fox [16] the increase in cheese production can be related to the high total solids content of used milk and inulin's capability to bind protein, which may explain the current study's cheese yield. Furthermore, EL-Dardiry [26] has shown that dietary fiber-enriched diets can give important functional characteristics such as texture, gelling, thickening, emulsification, stability, and total yield. The obtained findings were consistent with those of Toczek [27] who proved the usefulness of inulin as a prebiotic in enhancing the survival of probiotics, namely *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Bifidobacterium animalis*. Additionally, Goktas [28] concluded that adding inulin to probiotic ice cream enhanced the counts of *Saccharomyces boulardii* and *Lactobacillus rhamnosus*. Furthermore, the observed results were compared with those of Zhang [8] who investigated the effect of inulin on *Lactobacillus plantarum* survivability as well as the physicochemical and sensory characteristics and gave it the same mouthfeel and texture as full-fat cheese of cheddar cheese.

Inulin is especially suitable for substituting fat in low-fat cheese due to its interaction with whey protein and caseinates, as it may contribute to the strengthening of the texture of the cheese as well as its ability to maintain the aqueous phase structure, enhancing the creamy mouth texture [29]. On the other hand, Salvatore [30] discovered that adding inulin improved texture in low-fat cheese by reducing hardness and gumminess while keeping cohesiveness, adhesiveness, and springiness.

Conclusion

The combination of inulin and probiotic bacteria plays a vital role in dairy and non-dairy manufacturing technologies as functional foods. It demonstrated the enhancement of probiotics and increases human wellness. The study investigated the sensory properties and physicochemical, and microbiological assessments of low-fat Ras cheese during 90 days of ripening. The results demonstrated that there is no significant effect of inulin on the composition of low-fat cheese during ripening. However, it has a significant influence on cheese yield, the proliferation of probiotic bacteria, and sensory evaluation. Meanwhile, the results suggest that inulin at up to a 3 % inclusion rate might be used as a functional component for fat substitution in low-fat cheese to preserve bacterial viability, improve sensory attributes, and save manufacturing costs.

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