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Effect of Inulin and Whey Protein Concentrate as Fat Replacer in Low-Fat Wheyless Cheese: Textural, Physicochemical, and Sensory Characteristic

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A B S T R A C T

Objective: Increasing concerns over saturated fat intake and related chronic diseases have driven the demand for reduced-fat dairy products. However, fat reduction in cheese often results in increased hardness, higher syneresis, and poorer sensory quality, necessitating the use of functional fat replacers. This study aimed to evaluate the effects of inulin and whey protein concentrate (WPC) as fat replacers in low-fat wheyless cheese and to optimize the formulation using response surface methodology (RSM).

Methods: A Box-Behnken design was applied with inulin (0-5%), WPC (0-2%), and fat (5-15%) as independent variables. The responses measured included texture (hardness), syneresis, protein content, dry matter, and sensory attributes.

Results: The results showed that inulin enhanced hardness and markedly decreased syneresis due to its gel-forming and water-binding capacity. WPC significantly increased protein content and contributed to improved water retention, although its effect on hardness and sensory attributes was not significant ($P > 0.05$). Sensory evaluation showed that formulations containing moderate inulin and adequate fat achieved high overall acceptability, particularly in mouth-feel and texture. Fat exerted the strongest effect on hardness, syneresis, and dry matter, significantly increasing matrix compactness and reducing whey separation. Dry matter ranged from 27.56% to 41.11%. The optimized formulation yielded a desirability value of 0.709, indicating a favorable balance between functional and sensory properties.

Conclusion: The synergistic incorporation of inulin and WPC effectively compensates for fat reduction by improving water retention, protein content, and sensory acceptance, enabling the development of nutritionally enhanced, low-fat wheyless cheese with desirable functional properties.

Keywords: Inulin, WPC, Fat replacer, Low-fat cheese, Optimization

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Introduction

Milk and dairy products play a crucial role in human nutrition due to their high content of proteins, lipids, minerals, and vitamins, and their consumption has increased steadily worldwide due to the growth in population [1]. However, alongside this growing consumption, concerns related to high dietary fat intake - particularly saturated fatty acids and cholesterol - have intensified, as these components are strongly associated with cardiovascular diseases, obesity, diabetes, and certain cancers [2]. Milk fat contains more than 70% saturated fatty acids, among which lauric, myristic, and palmitic acids exhibit pronounced atherogenic properties [3]. As a result, nutritional guidelines increasingly recommend limiting total fat intake to less than 30% of daily energy and saturated fat to less than 10% [4].

Cheese is one of the most widely consumed dairy products and represents a major source of dietary fat and saturated fatty acids. While cheese offers high nutritional value due to its rich protein and calcium content and favorable mineral bioavailability [5], its fat fraction remains a key nutritional concern. The demand for reduced-fat and low-fat cheeses has therefore increased substantially, particularly among health-conscious consumers and individuals with

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cardiovascular risk factors [6]. Nevertheless, fat plays a fundamental role in determining the sensory, rheological, and structural properties of cheese. Reduction or removal of fat often leads to undesirable changes, including firm and rubbery texture, weak flavor development, poor meltability, and increased syneresis, ultimately reducing consumer acceptance [7,8].

To address these challenges, extensive research has focused on improving the quality of reduced-fat cheeses through technological modifications and formulation strategies. These include alterations in cheesemaking processes, selection of starter and adjunct cultures, and, most importantly, the incorporation of fat replacers [9]. Fat replacers are designed to mimic the functional and sensory roles of fat while reducing caloric density and saturated fatty acid content. They are commonly classified into carbohydrate-based, protein-based, and fat-based substitutes, each exhibiting distinct functional behaviors in dairy matrices [10].

Among carbohydrate-based fat replacers, inulin has attracted considerable attention because of its favorable technological and functional properties. Inulin is a non-digestible fructan that can interact with water and form a gel-like structure, thereby contributing to a creamy mouthfeel and improving the texture of low-fat food products [11]. In dairy systems, its ability to bind water and modify the structural properties of the food matrix makes it a promising ingredient for partially replacing fat while maintaining desirable sensory and textural characteristics [12,13]. Therefore, the application of inulin in reduced-fat dairy products has been investigated primarily for its potential to improve product structure, mouthfeel, and overall acceptability. In the present study, the role of inulin was considered from a technological perspective, particularly in relation to the physicochemical, textural, and sensory characteristics of low-fat wheyless cheese. These properties make inulin particularly suitable for the development of functional and reduced-fat dairy products. Whey proteins, especially whey protein concentrate (WPC), represent another promising fat replacer option. Whey proteins possess high nutritional value, excellent water-binding capacity, and favorable gelation and emulsification properties [14,15]. In cheese systems, inulin and WPC can enhance moisture retention, modify the protein–water matrix, and improve structural stability, thereby influencing the texture, mouthfeel, and overall sensory acceptability of low-fat cheese [14,15].Their incorporation into cheese formulations has been shown to improve texture, reduce syneresis, and enhance overall product yield [16,17]. Numerous research studies have shown that inulin and WPC can effectively substitute for fat [18-23]. Onsri et al. (2022) investigated how different levels of inulin (20, 35, and 50% wt) and WPC (5, 10, and 15% wt) affect both the crust (CPC) and the filling (CPF) of cream-puffs [24]. Their findings indicated that a formulation containing 20% inulin resulted in a well-structured crust. Sensory panels rated the combination of 20% inulin with 10% WPC in the crust and 20% inulin with 5% WPC in the filling as the most preferred, scoring highest for appearance, taste, texture, and overall acceptance. In another work, Kant et al. (2024) assessed the use of inulin and WPC as fat replacers in low-fat paneer [17]. The low-fat paneer was made from standardized skim milk (0.5% fat) and was fortified with inulin (0.25 g/g), WPC (0.30 g/g), and a combination of both (0.27 g/g), compared to a control sample. They reported that the paneer containing only inulin showed the highest overall sensory acceptability. The samples fortified with WPC had the highest hardness values, while those containing inulin exhibited improved cohesiveness and chewiness.

Although both inulin and WPC have been investigated individually as fat replacers in various dairy products, limited information is available on their combined application in low-fat wheyless cheese. In particular, the simultaneous optimization of inulin, WPC, and fat levels based on multiple physicochemical, textural, and sensory responses has not been sufficiently explored. Therefore, this study aimed to investigate the combined effects of inulin and WPC on the quality characteristics of low-fat wheyless cheese and to determine the optimal formulation using response surface methodology (RSM).

Materials and methods

Materials

Fat milk and pasteurized cream were procured from Mahdasht Dairy Co. (Sari, Iran). Milk protein concentrate (MPC) and skim milk powder were supplied by Pegah Dairy Co. (Isfahan, Iran). Food-grade salt was obtained from Taban Co. (Semnan, Iran). WPC and inulin were provided by Lactoprot (Kaltenkirchen, Germany) and Sensus (Roosendaal, the Netherlands), respectively. The starter and rennet enzyme were purchased from Chr. Hansen (Hørsholm, Denmark). All analytical-grade chemicals used throughout the study were obtained from Merck (Darmstadt, Germany).

Preparation of low-fat wheyless cheese

Cheese samples were produced following the method of Omrani Khiabanian et al. (2020) [25], with slight modifications. First, fat milk, cream, and powdered ingredients were combined in a thermomix (Vorwerk, Germany) at 40 °C for 5 min to ensure they dissolved completely and blended well. Next, the mixture was heated to 80 °C and held at this temperature for about 3 min for pasteurization. The temperature was then lowered to 35 °C, and starter along with rennet enzyme were added while gently stirring. The mixture was immediately poured into polypropylene (PP) containers and sealed with aluminum foil. These containers were incubated at 35 °C for around 10 h until the pH dropped to roughly 4.9. After incubation, the samples were stored in a cold room. The control sample (CS) was prepared using 1000 g of milk (3.2% fat), 670 g of cream (40% fat), 160 g of skim milk powder, 140 g of milk protein powder (70% protein), and 24 g of salt.

Experimental design

The experimental design was derived using RSM with a Box-Behnken design (BBD). A total of 54 runs were generated from the design, which included three repetitions of the central point. Three independent variables (i.e., inulin, WPC, and fat) were assessed at various levels: 0-5% for inulin, 0-2% for WPC, and 5-15% for fat (Table 1). The dependent or response variables included, physicochemical attributes (hardness), syneresis, protein content, dry matter, and sensory properties (odor, taste, texture, appearance, and overall acceptability). Equation (1) illustrates the second-order polynomial model that was fitted to both the independent and dependent variables (Sadeghi et al., 2019) [26]:

$$\mathrm{Y} = b_0 + \sum_{j=1}^{k} b_j X_j + \sum_{j=1}^{k} b_{jj} X_j^2 + \sum_{i} \sum_{<j=2}^{k} b_{ij} X_i X_j \quad (1)$$

Where Y represents the predicted response, b_0 is the model intercept, b_j, b_{jj}, and b_{ij} are the coefficients for linear, quadratic, and interactive regressions, respectively. X_i and X_j denote independent variables, while k signifies the count of independent variables.

Table 1. Coded and real values of the independent variables used in the experimental design

Run no.	Real values (Coded values)			
	Inulin (%)	WPC (%)	Fat (%)	Formula
Control sample	0	0	15	CS
1	2.5 (0)	2 (+1)	15 (+1)	S1
2	5 (+1)	1 (0)	15 (+1)	S2
3	2.5 (0)	0 (-1)	15 (+1)	S3
4	0 (-1)	1 (0)	15 (+1)	S4
5	0 (-1)	0 (-1)	10 (0)	S5
6	2.5 (0)	1 (0)	10 (0)	S6
7	2.5 (0)	1 (0)	10 (0)	S7
8	2.5 (0)	1 (0)	10 (0)	S8
9	5 (+1)	2 (+1)	10 (0)	S9

10	5 (+1)	0 (-1)	10 (0)	S10
11	0 (-1)	2 (+1)	10 (0)	S11
12	5 (+1)	1 (0)	5 (-1)	S12
13	2.5 (0)	0 (-1)	5 (-1)	S13
14	2.5(0)	2 (+1)	5 (-1)	S14
15	0 (-1)	1 (0)	5 (-1)	S15

Note: *Runs 7, 8, and 9 represent replicated experimental trials.*

Textural analysis (hardness)

The sample hardness was assessed using a texture analyzer (CT3, Brookfield, USA) equipped with a 50-kg load cell. From each sample, three cubes of cheese ($2 \times 2 \times 2.5$ cm^3) were prepared and then immediately compressed at room temperature using a TA25/1000 probe at 1 mm/s to half their original height [25]. Hardness was measured from the force-time curves on the day of production.

Syneresis determination

The level of spontaneous whey separation in cheese was assessed using a siphon drainage technique [27]. In this procedure, a cup of cheese was removed from the cold storage (4 °C), weighed, and positioned at about a 45° angle to facilitate whey collection at the cup's edge. A syringe was utilized to extract the whey from the surface of the sample every 10 seconds, after which the weight of the cheese cup was measured again. Syneresis was calculated as the percentage of weight loss of whey compared to the initial weight of the cheese sample.

Protein content

The protein content was determined by assessing total nitrogen through the Kjeldahl method (AOAC, 2005) using a Kjeldahl nitrogen analyzer (Velp, Italy). All measurements were done in triplicate.

Dry matter content

The dry matter of the samples was determined according to standard No. 1753 (ISIRI, 2006). In this procedure, the plate is dried at 104 °C, then cooled in a desiccator containing silica gel. After weighing the empty plate, 3 g of the sample are added. The plate is placed in the oven at 104 °C for 3 h, cooled, and the dry matter is calculated as the weight percentage.

Sensory properties

Sensory evaluation was conducted using a five-point hedonic scale, where 1 represents "very poor" and 5 represents "very good" to assess taste, mouth-feel, odor, color, texture, and overall acceptability. A total of 10 trained sensory panelists, comprising 5 women and 5 men aged 30 to 50, were selected from the members of Amol Zago Dairy Co. based on their willingness to participate, with the assurance that the samples evaluated were safe. This approach ensured a representative sample size for the study. Participants with food allergies were excluded from participation. In this study, a random sampling technique was used to select samples for each experimental block. The samples were appropriately coded and subsequently presented to the panelists for evaluation. Panel members were asked to rinse their mouths with water between testing samples to ensure that the results of the previous sample test did not affect the next sample test. Results were reported as the average of ten determinations per replicate.

Statistical analysis

The effects of each variable and their interactions were examined using analysis of variance (ANOVA) with a 95% confidence level, utilizing Design Expert Software version 12 (Stat-Ease Inc, Minneapolis, USA). Response surface plots depicted how a response variable varied with two factors, with the third factor fixed at its medium value, as per the software's default setting. Additionally, this software was used for process optimization.

Results

The hardness data measured by the texture analyzer are shown in Table 2. ANOVA results indicated significant differences between treatments for inulin and fat parameters ($P < 0.05$). S1, S2, S3, S9, and S10 displayed higher hardness compared to the CS. As illustrated in Fig. 1a, increasing inulin from 0 to 5% raised hardness by about 20.95%, while increasing fat from 5% to 15% raised hardness by approximately 45.86%. The more effect of fat compared to the other parameters thoroughly complied with its F-value (52.14) in Table 3. The results showed a 3.7-fold reduction in hardness in cheese containing WPC compared to control cheese.

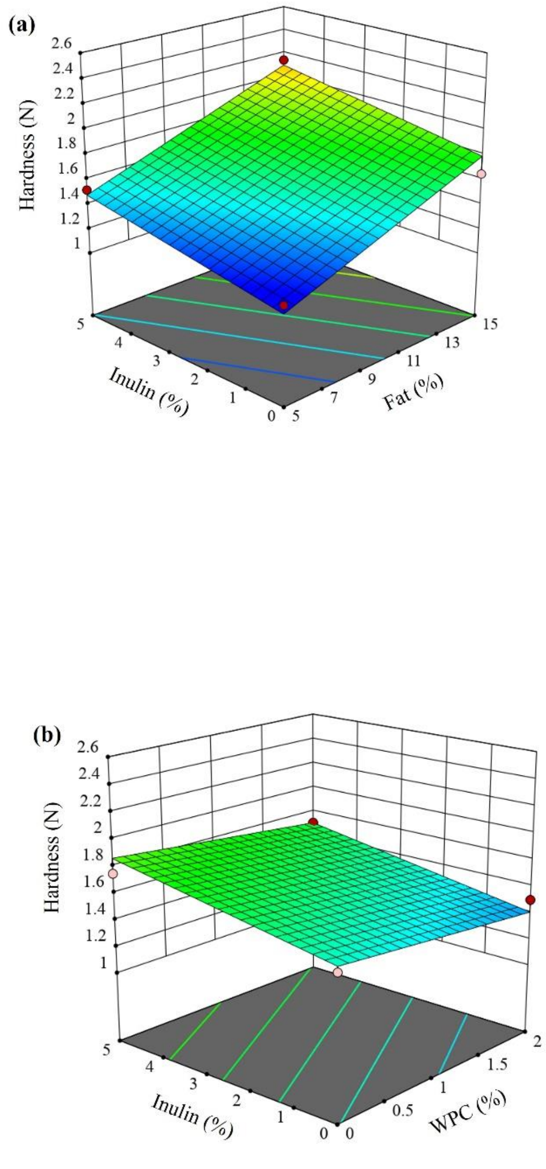


Figure 1: Response surface plots of the effects of inulin, whey protein concentrate (WPC), and fat on texture (hardness) of low-fat wheyless cheese

From a matrix interaction standpoint, the coexistence of inulin, fat, and WPC creates a competitive environment for structure formation. Inulin and fat work together to increase hardness by raising total solids, making the matrix more compact, and strengthening the overall structure. Conversely, WPC opposes this by improving hydration, breaking up dense protein clumps, and forming a more cohesive yet less rigid network. As a result, the observed texture in our study depends on the balance between the reinforcing effects of inulin and fat and the plasticizing and hydrating effects of WPC. This dynamic is especially relevant in formulated, wheyless cheese systems, where texture depends not only on casein interactions but also on the complex interactions among hydrocolloids, fat globules, and whey proteins. Our findings suggest that hardness mainly results from the degree of matrix density and water retention, both of which increase with added inulin and fat, while WPC softens the product by boosting moisture and creating a more flexible protein network.

Table 2. *Obtained results for responses under different formulations*

Run no.	Independent variables			Responses				
	Inulin (%)	WPC (%)	Fat (%)	Syneresis (%)	Texture (N)	Protein (%)	Dry matter (%)	Overall acceptability
CS	*0*	*0*	*15*	0.57	1.7	9.68	35.89	*4.96*
S1	*2.5*	*2*	*15*	0.51	1.85	11.15	39.81	*4.84*
S2	*5*	*1*	*15*	0.49	2.14	10.30	41.11	*4.38*
S3	*2.5*	*0*	*15*	0.54	2.3	9.61	38.07	*4.98*
S4	*0*	*1*	*15*	0.51	1.65	10.45	36.76	*4.82*
S5	*0*	*0*	*10*	0.71	1.52	9.83	31.29	*3.7*
S6	*2.5*	*1*	*10*	0.64	1.6	10.50	34.15	*4.16*
S7	*2.5*	*1*	*10*	0.66	1.54	10.58	34.74	*4.16*
S8	*2.5*	*1*	*10*	0.63	1.50	10.62	34.34	*4.16*
S9	*5*	*2*	*10*	0.52	1.71	11.22	37.38	*4.24*
S10	*5*	*0*	*10*	0.60	1.75	9.68	35.64	*4.1*
S11	*0*	*2*	*10*	0.57	1.50	11.37	33.03	*3.9*
S12	*5*	*1*	*5*	0.77	1.52	10.60	31.91	*4*
S13	*2.5*	*0*	*5*	0.86	1.41	9.91	28.87	*3.1*
S14	*2.5*	*2*	*5*	0.80	1.3	11.45	30.61	*3.42*

S15	*0*	*1*	*5*	0.89	1.24	10.75	27.56	*2.5*

**CS: control sample.*

Table 3: Summarized results of ANOVA for responses

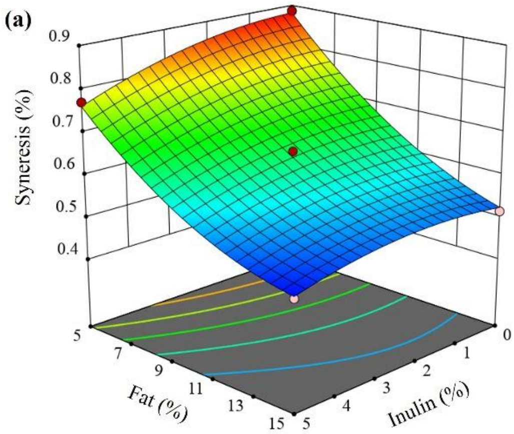
Response	Source	Predictive models	Equation terms								
			X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2
Texture		**Linear** ($R^2 = 0.8606$)									
	df	3	1	1	1						
	MS	0.3312	0.1830	0.0480	0.7626						
	F-value	22.64	12.51	3.28	52.14						
	P-value	< 0.0001	0.0047	0.0973^{ns}	< 0.0001						
	LoF	0.1341									
Syneresis		**Quadratic** ($R^2 = 0.9872$)									
	df	9	1	1	1	1	1	1	1	1	1
	MS	0.0264	0.0116	0.0117	0.1975	0.0011	0.0019	0.0002	0.0028	0.0006	0.0089
	F-value	42.69	18.83	18.95	319.75	1.76	3.06	0.3173	4.51	1.04	14.38
	P-value	0.0003	0.0074	0.0073	< 0.0001	0.2416^{ns}	0.1405^{ns}	0.5975^{ns}	0.0872^{ns}	0.3540^{ns}	0.0127
	LoF	0.1845									
Protein		**Linear** ($R^2 = 0.9977$)									
	df	3	1	1	1						
	MS	1.66	0.0450	4.7400	0.1800						
	F-value	1601.71	43.52	4587.50	174.09						
	P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001						
	LoF	0.9918									
Dry matter		**Linear** ($R^2 = 0.9991$)									
	df	3	1	1	1						
	MS	71.06	37.85	6.06	169.28						
	F-value	4021.44	2141.73	342.68	9579.91						
	P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001						
	LoF	1.0000									
Overall acceptability		**2FI** ($R^2 = 0.9712$)									
	df	6	1	1	1	1	1	1			
	MS	0.9889	0.4050	0.0338	4.50	0.0009	0.9409	0.0529			
	F-value	44.95	18.41	1.54	204.55	0.0409	42.77	2.40			
	P-value	< 0.0001	0.0026	0.2503^{ns}	< 0.0001	0.8448^{ns}	0.0002	0.1596^{ns}			

LoF	-	

Note: X_1, X_2, and X_3 *are inulin, WPC, and fat, respectively. df is degree of freedom. MS is Mean square. LoF is lack of fit. ns is not significant.*

Among the studied factors, fat exhibited the most pronounced effect on syneresis, as reflected by its substantially higher F-value (319.75, Table 3) compared with inulin and WPC. The magnitude of this effect indicates that fat played a dominant role in controlling moisture release within the matrix.

Fig. 2a depicts that an increase in inulin from 0 to 2.5% resulted in an approximate 1.53% reduction in syneresis, whereas a further increase from 2.5% to 5% led to a more pronounced decrease of about 10.11%.



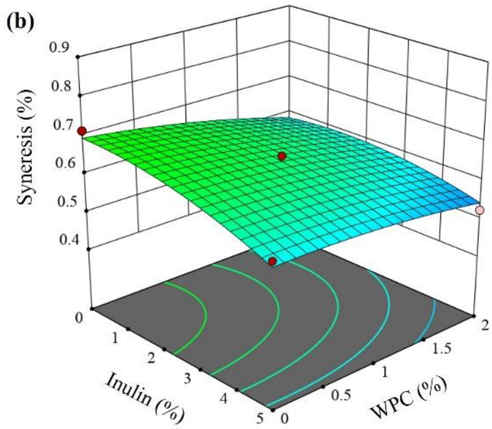


Figure 2: Response surface plots of the effects of inulin, whey protein concentrate (WPC), and fat on syneresis of low-fat wheyless cheese

Furthermore, S1, S2, S3, S4, and S9 exhibited lower syneresis values than the CS (S2 < S1 = S4 < S9 < S3 < CS). This observation is consistent with the individual and combined effectiveness of inulin, WPC, and fat in enhancing water retention. The lower syneresis of these samples can be attributed to their improved structural integrity and higher capacity to immobilize water within the protein-polysaccharide-fat network. Overall, the observed ranking of samples was in line with the expected behavior based on the significant contributions of the formulation variables, particularly the dominant and nonlinear effect of fat on reducing syneresis.

Cheese samples contained protein levels ranging from 9.61% to 11.45%. All three parameters significantly influenced these levels ($P < 0.05$). The lowest protein content was found in the sample with 2.5% inulin, no WPC, and 15% fat, while the highest was in the sample with 2.5% inulin, 2% WPC, and 5% fat, which is in line with the effectiveness priority of formulation variables. Also, other samples except S3 exhibited a higher protein content in comparison to the CS.

In contrast, based on Fig. 3b, increasing fat to 15% led to a reduction in protein content by approximately 2.81%. Additionally, increasing inulin to 5% slightly decreased protein content (by about 1.41%), likely due to the replacement of protein-rich components with a non-protein polysaccharide, thereby lowering the overall protein percentage on a mass basis.

The dry matter content in the cheese samples ranged from 27.56% to 41.11%. Statistical analysis indicated that all three formulation variables - inulin, WPC, and fat - significantly affected dry matter content ($P < 0.05$). The highest dry matter was found in S2 (5% inulin, 1% WPC, and 15% fat), while the lowest was in S15 (no inulin, 1% WPC, and 5% fat). ANOVA results showed that fat had the strongest impact on dry matter (F-value = 9579.91), followed by inulin (F-value = 2141.73), with WPC having a smaller but still significant effect (F-value = 342.68). As shown in Fig. 4a, increasing fat to 15% led to a 31.87% rise in dry matter, mainly due to the contribution of milk fat to total solids and moisture displacement in the cheese matrix.

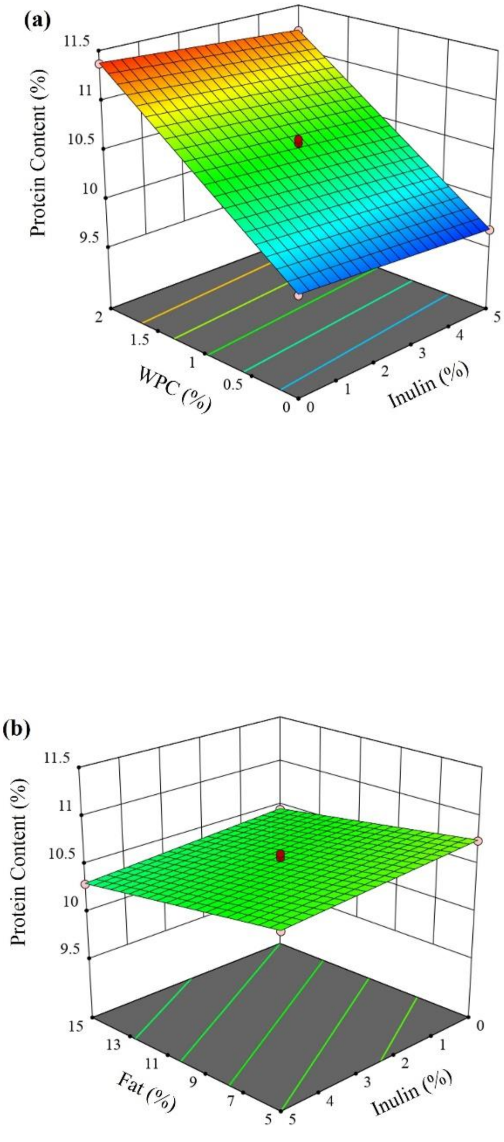


Figure 3: Response surface plots of the effects of inulin, whey protein concentrate (WPC), and fat on protein content of low-fat wheyless cheese

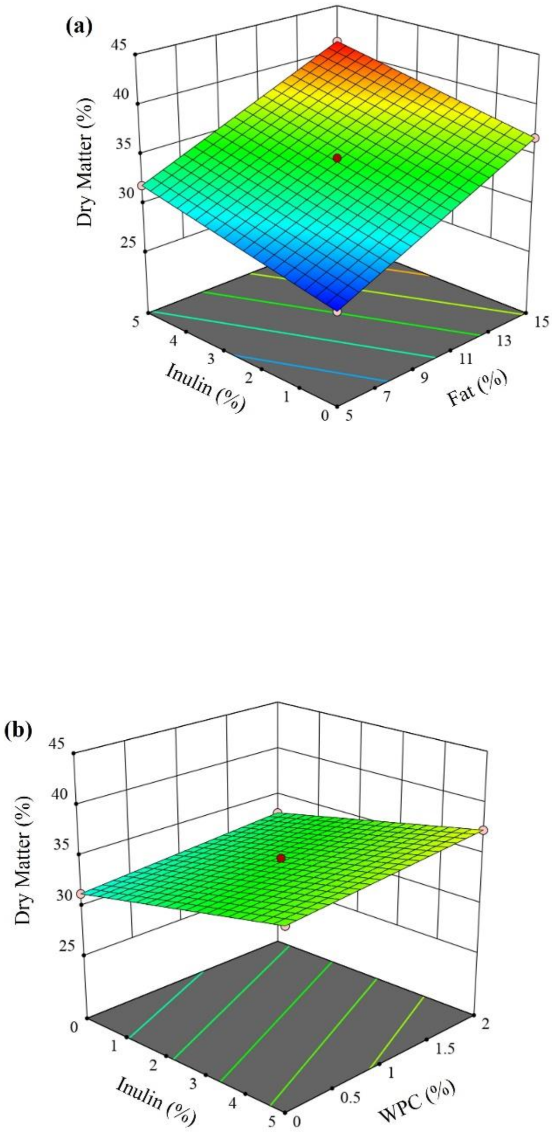


Figure 4. Response surface plots of the effects of inulin, whey protein concentrate (WPC), and fat on dry matter of low-fat wheyless cheese

Sensory characteristics are a key factor in potential consumer acceptance and market success. Table 4 displays the mean scores for taste, mouth-feel, odor, color, texture, and overall acceptability.

Results showed that, except for the CS, formulations S1 and S3 - which contain high fat (15%) and moderate inulin (2.5%) - received mean scores above 4.5 across all assessed attributes, indicating strong panelist approval. Among all samples, S3 scored highest for mouth-feel (5.0), surpassing the CS. This enhanced mouth-feel likely results from the combined effect of fat and inulin ($P < 0.05$) in improving creaminess and lubricity within the cheese matrix. Conversely, S15 - characterized by low fat (5%) and no inulin - scored lowest (< 3.0) for overall acceptability, especially in taste, mouth-feel, and texture. This underscores the importance of fat and functional ingredients in offsetting quality reductions linked to fat reduction. Statistical analysis showed that increasing fat and inulin significantly improved taste and overall scores ($P < 0.05$).

Table 4: Sensory properties of low-fat wheyless cheese

Samples	Formulation			Taste	Mouth-feel	Odor	Color	Texture	Overall acceptability
	Inulin (%)	WPC (%)	Fat (%)						
CS	0	0	15	5.0 ± 0.000	4.9 ± 0.316	5.0 ± 0.000	5.0 ± 0.000	4.9 ± 0.316	4.96 ± 0.126
S1	2.5	2	15	4.7 ± 0.483	4.5 ± 0.527	5.0 ± 0.000	5.0 ± 0.000	5.0 ± 0.000	4.84 ± 0.126
S2	5	1	15	4.2 ± 0.422	3.7 ± 0.483	5.0 ± 0.000	4.0 ± 0.000	5.0 ± 0.000	4.38 ± 0.114
S3	2.5	0	15	5 ± 0.000	5.0 ± 0.000	5.0 ± 0.000	5.0 ± 0.000	4.9 ± 0.316	4.98 ± 0.063
S4	0	1	15	4.3 ± 0.483	5.0 ± 0.000	4.9 ± 0.316	5.0 ± 0.000	4.9 ± 0.316	4.82 ± 0.175
S5	0	0	10	3.6 ± 0.516	3.3 ± 0.483	4.0 ± 0.000	4.0 ± 0.000	3.6 ± 0.516	3.70 ± 0.216
S6	2.5	1	10	4.5 ± 0.527	4.3 ± 0.483	4.0 ± 0.000	4.0 ± 0.000	4.0 ± 0.471	4.16 ± 0.126
S7	2.5	1	10	4.5 ± 0.527	4.3 ± 0.483	4.0 ± 0.000	4.0 ± 0.000	4.0 ± 0.471	4.16 ± 0.126
S8	2.5	1	10	4.5 ± 0.527	4.3 ± 0.483	4.0 ± 0.000	4.0 ± 0.000	4.0 ± 0.471	4.16 ± 0.126
S9	5	2	10	4.7 ± 0.483	3.9 ± 0.316	4.0 ± 0.000	4.0 ± 0.000	4.6 ± 0.516	4.24 ± 0.158
S10	5	0	10	4.5 ± 0.527	4.0 ± 0.000	4.0 ± 0.000	4.0 ± 0.000	4.0 ± 0.000	4.10 ± 0.105
S11	0	2	10	3.9 ± 0.316	3.6 ± 0.516	4.0 ± 0.000	4.0 ± 0.000	4.0 ± 0.000	3.90 ± 0.105
S12	5	1	5	3.9 ± 0.316	3.8 ± 0.422	4.0 ± 0.000	4.0 ± 0.000	4.3 ± 0.483	4.00 ± 0.094
S13	2.5	0	5	2.6 ± 0.516	2.5 ± 0.527	3.9 ± 0.316	3.0 ± 0.000	3.5 ± 0.527	3.10 ± 0.287
S14	2.5	2	5	3.0 ± 0.000	2.5 ± 0.527	3.9 ± 0.568	4.0 ± 0.000	3.7 ± 0.483	3.42 ± 0.114
S15	0	1	5	1.3 ± 0.483	2.2 ± 0.632	3.6 ± 0.516	4.0 ± 0.000	1.4 ± 0.516	2.50 ± 0.236

To produce low-fat wheyless cheese, multi-objective optimization was utilized. Responses such as texture (hardness) and syneresis were selected as the minimum value, and protein, dry matter, and overall acceptability scores at the maximum level. Furthermore, the control factors, including inulin and WPC were chosen to remain in the predefined range and fat was set as the minimum level, according to the work goal. Corresponding importance of goals were selected as 4 for texture (hardness), syneresis, protein, and dry matter, and 5 for overall acceptability in multi-objective optimization. After analyzing the data from 54 experiments with design expert software and achieving valid prediction models for each response, the software suggested a solution. The results of the combined optimization, yield a combined desirability of 0.709, as displayed in Fig. 5. The optimum conditions were detected at 4.99% inulin, 2% WPC, 7.63% fat, and under these optimized parameters, texture (hardness), syneresis, protein, dry matter, and overall acceptability were predicted to be 1.56 N, 0.61%, 11.30%, 35.21%, and 4.23, respectively. To assess the RSM model's prediction accuracy, a single test was performed under determined optimal parameters, resulting in response values of 1.62 N, 0.58%, 11.18%, 35.61%, and 4.31, for texture (hardness), syneresis, protein, dry matter, and overall acceptability, respectively, which showed that the expected values are consistent with the experimental values.

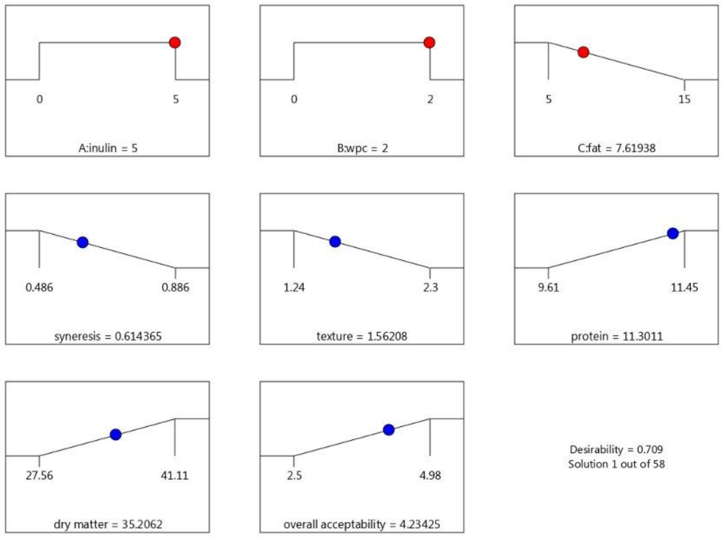


Figure 5: Ramps of the numerical optimization.

Discussion

The findings of this study demonstrated that the incorporation of inulin, whey protein concentrate (WPC), and fat can differentially influence the textural, physicochemical, and sensory properties of the product. Among these formulation variables, WPC exerted the strongest effect on protein content, whereas inulin and fat played important roles in improving water retention, structural characteristics, and sensory attributes. The effects of the main formulation variables and their interactions on hardness, syneresis, chemical composition, and sensory acceptance are discussed below in comparison with previous studies.

Junaid et al. (2024) reported that increasing fat levels significantly increased hardness, cohesiveness, and gel strength due to higher total solids and a more compact microstructure in cheese analogues [28]. Although WPC did not have a significant effect ($P > 0.05$) on hardness in our research, increasing WPC from 0 to 2% caused a decrease of around 9.20% (Fig. 1b). The increase in hardness is likely due to inulin's gel-forming ability, which reinforces the protein matrix, whereas WPC helps create a more cohesive network that prevents excessive firmness [29]. The results of the present study align with the findings of Moghiseh et al. (2021) [30], who observed that the textural properties of low-fat mozzarella cheese were significantly influenced by the incorporation of inulin. Specifically, they noted that as the ratio of inulin to kefiran mixtures changed from 8:1 to

12:1, the hardness increased from 21.29 N to 30.69 N. Bielska and Cais-Sokolińska (2023) analyzed the impact of adding whey proteins in the production of white cheese from buttermilk [31].

Syneresis is viewed as a drawback that can be minimized by raising the quantity of milk solids, particularly protein [25]. Changes in syneresis are presented in Table 2. All three formulation variables - inulin, WPC, and fat - significantly influenced syneresis ($P < 0.05$). An increase in the levels of each of these components led to a consistent reduction in syneresis, indicating an overall improvement in the water-holding capacity and structural stability of the cheese matrix. This trend suggests that the incorporation of functional ingredients and higher total solids contributed to enhanced serum retention and reduced whey separation [32].

Notably, the second-order term of fat was nearly comparable in significance to the linear effects of inulin and WPC (ANOVA, Table 3), demonstrating a nonlinear relationship between fat content and syneresis. This finding implies that at higher fat levels, the reduction in syneresis becomes more pronounced (Fig. 2a), likely due to increased matrix compactness and reduced mobility of entrapped moisture [33-35].

This behavior can be attributed to the high water-binding capacity of inulin, which inhibits syneresis and thereby improves the functional properties of the cheese system [36]. Arango et al. (2013) examined the effect of inulin as a fat replacer in milk gels. They showed that increasing the concentration of inulin to 6% reduced syneresis from 0.033 to 0.025 min^{-1} [37]. In another work, Żbikowska et al. (2020) examined how varying amounts of inulin (3%, 6%, 9%, 12%, or 15% w/w) affected the physicochemical properties of natural yogurt [38]. The findings indicated a statistically significant linear decrease in syneresis as the level of inulin increased. Specifically, higher levels of inulin led to a further reduction in syneresis; samples containing 9% and 12% inulin demonstrated reductions of 17.6% and 25%, respectively. Also, the results demonstrated that increasing the WPC level to 2% led to an approximate 11.53% reduction in syneresis (Fig. 2b). Similar findings were obtained in the thermosonicated milk-WPC system for producing reduced-fat Panela cheese; water holding capacity (syneresis index) values for blends containing WPC were higher than those of the control [39]. The mechanism was attributed to whey-protein aggregates forming complexes with casein and attaching to the milk-fat globule membrane, creating additional hydrophilic sites that bind water.

As indicated by the ANOVA results (Table 3), WPC exerted the most dominant influence on protein content (F-value = 4587.5), followed by fat (174.09) and inulin (43.52). The pronounced effect of WPC is expected, as whey protein concentrate directly increases the total protein fraction of the formulation and enhances protein retention within the matrix. Consequently, Fig. 3a shows that increasing WPC to 2% resulted in a substantial rise in protein content (approximately 15.78%), reflecting its role as a concentrated protein source and a structural component of the cheese system. These findings are consistent with the work of Pires et al. (2022) [40], who noted a direct correlation between the quantity of WPC added and the protein percentage in ice cream.

Likewise, raising inulin to 5% caused a 13.57% increase in dry matter. Sedaghati and Kavand (2026) noted that higher inulin levels increased solid content and significantly decreased protein in ricotta-like synbiotic cheese ($P < 0.05$). Conversely, increasing WPC to 2% resulted in a moderate dry matter increase of 5.22% (Fig. 4b). These findings align with Hanafy et al. (2016)'s report that higher WPC levels increase dry matter in cheese [41]. Consistent with these results, S1, S2, S3, S4, and S9 showed higher dry matter content compared to the CS.

The positive effect of inulin on sensory perception aligns with prior research; for instance, Sedaghati and Kavand (2026) found that adding inulin significantly boosted acceptance scores ($P < 0.05$) in ricotta-like synbiotic cheese [42]. Likewise, more fat raised taste, mouth-feel, and overall acceptability, while fat reduction decreased odor, color, and texture scores ($P < 0.05$). Meanwhile, WPC did not significantly influence any sensory attribute ($P > 0.05$), which matches findings by Akalın et al. (2012) [43], who observed no significant differences in appearance, aroma, flavor, or overall acceptability among yogurts with varying WPC levels during storage ($P > 0.05$). However, other research suggests that combining inulin and WPC can improve sensory qualities in reduced-fat dairy products, such as low-fat paneer [17].

Overall, increasing the levels of inulin and WPC improved water-holding capacity, reduced syneresis, and increased total solids, while WPC markedly increased the protein content. Increasing fat also reduced syneresis and improved several sensory attributes, although its effect on texture appeared to be more complex, particularly in combination with the other formulation components. Therefore, an appropriate balance of inulin, WPC, and fat may provide an effective strategy for achieving desirable structural, nutritional, and sensory properties in the studied product.

Conclusion

This study demonstrated that the incorporation of inulin and WPC, together with controlled fat reduction, can be used to formulate low-fat wheyless cheese with acceptable physicochemical, textural, and sensory characteristics under the conditions investigated. Among the formulation variables, fat had the strongest effect on hardness, syneresis, and dry matter, highlighting its important role in determining the structural and moisture-retention properties of the cheese matrix. Inulin contributed to hardness and moisture retention, whereas WPC primarily increased protein content and was associated with reduced syneresis. The results suggest that appropriate combinations of inulin and WPC may help mitigate some of the textural and physicochemical changes associated with fat reduction. The response surface optimization identified a formulation with an overall desirability of 0.709, indicating that the selected combination of formulation factors provided a satisfactory balance among the measured responses within the experimental range. However, the findings should be interpreted within the scope of the present study. The sensory evaluation involved a relatively small panel, and shelf-life, microbiological safety, rheological or microstructural properties, and the actual fat content of the final products were not evaluated. In addition, the optimized formulation requires further experimental validation under independent conditions. Therefore, the present findings provide preliminary evidence for the technological potential of inulin and WPC in low-fat wheyless cheese rather than definitive evidence of industrial applicability. Further studies involving larger sensory panels, storage and microbiological evaluations, direct determination of final fat content, rheological and microstructural analyses, and independent validation of the optimized formulation are warranted to confirm product quality and assess its potential for broader dairy applications.

Statements and Declarations

Conflict of Interest

The authors declare that they have no conflict of interest.

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Ethics Approval

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Consent to Participate

Informed consent was obtained from all participants involved in the sensory evaluation.

Author Contributions

Leila Rostami, Mehdi Naeini, and Ebrahim Sadeghi contributed to the conception and design of the study, experimental work, data analysis, interpretation of the results, and preparation and revision of the manuscript. All authors read and approved the final version of the manuscript.

Declaration of AI Use

The authors declare that no artificial intelligence (AI) tools were used in the preparation, writing, editing, data analysis, interpretation, or presentation of this manuscript. The manuscript was prepared entirely by the authors, who take full responsibility for its content, accuracy, and integrity.

References

1. Joodi Attar S, Hakimzadeh V, Rashidi H (2018) Optimization of formulation of UF white cheese with flaxseed oil as milk fat replacer by using of response surface methodology. Iran Food Sci Technol Res J 14:645-655. https://doi.org/10.22067/ifstrj.v1396i0.64148
2. Umayangani S, De Silva I, Munasinghe D, Deshika C, Chandrapala J, Silva M (2025) Elimination of Trans Fatty Acids from Foods Using Novel Technologies. Food Rev Int 1-29. https://doi.org/10.1080/87559129.2025.2502439
3. Rashidimehr A, Mohammadi-Nasrabadi F, Alhouei B, Khoshtinat K, Esfarjani F (2025) Fatty acid composition of dairy products and their impact on atherogenicity and thrombogenicity. Sci Rep 15:43613. https://doi.org/10.1038/s41598-025-27445-4
4. Sampaio GR, Castellucci CM, e Silva MEMP, Torres EA (2004) Effect of fat replacers on the nutritive value and acceptability of beef frankfurters. J Food Compos Anal 17:469-474. https://doi.org/10.1016/j.jfca.2004.03.016
5. Keyvani Bostanabad M, Bolandi M, Mohammadi Nafchi A, Baghaei H (2022) Wheyless cheese: an alternative method to reduce the environmental hazards of Lighvan cheese production. J Food Bioprocess Eng 5:93-98. https://doi.org/10.22059/jfabe.2022.345145.1122
6. Murtaza MS, Sameen A, Rafique S, Shahbaz M, Gulzar N, Murtaza MA, . . . & Hafiz I (2023) Impact of dietary fiber (inulin and resistant starch) on the quality parameters of low fat cheddar cheese from buffalo milk. Pak J Zool 55:855-862. https://dx.doi.org/10.17582/journal.pjz/20200630120620
7. Bourouis I, Pang Z, Liu X (2023) Recent advances on uses of protein and/or polysaccharide as fat replacers: Textural and tribological perspectives: A review. J Agricu Food Res 11:100519. https://doi.org/10.1016/j.jafr.2023.100519

8. Singh S, Chauha AK, Prakash R, Maiti P, Ranjan R (2023) Quarg Cheese: The Impact of Fat Content Change on its Microstructure, Characterization, Rheology, and Textural Properties. Curr Res Nutr Food Sci J 11:1061-1073. https://dx.doi.org/10.12944/CRNFSJ.11.3.12
9. Rahmati M, Hashemiravan M, Khani M (2018) Effect of hydrocolloids Kappa-Carrageenan and Maltodextrin as Fat substitutes on the production of Low-Fat Cream Cheese. Iran J FST (JFST) 73:120-131.
10. Shinde DR, Surendra Nath Battula SN (2025) Inulin and Polydextrose as Fat Replacers in Non-Fat Dahi: Physicochemical, Textural and Sensory Properties. Indian J Dairy Sci 78:313-319. https://doi.org/10.33785/IJDS.2025.v78i04.003
11. Akbari M, Eskandari MH, Davoudi Z (2019) Application and functions of fat replacers in low-fat ice cream: A review. Trends Food Sci. Technol. 86:34-40. https://doi.org/10.1016/j.tifs.2019.02.036
12. Alisawi SAM, Alneamah SJA (2025) Therapeutic benefit of dietary inulin. International J Environ Sci 11:247-255. https://doi.org/10.64252/pjbg4434
13. Anand A, Manjula SN, Fuloria NK, Sharma H, Mruthunjaya K (2025) Inulin as a prebiotic and its effect on gut microbiota. In: Akram W, Mishra N, Haider T (ed), Inulin for Pharmaceutical Applications: A Versatile Biopolymer. Springer, Singapore, pp. 113-135. https://doi.org/https://doi.org/10.1007/978-981-97-9056-2_6
14. Kusio K, Szafrańska JO, Radzki W, Sołowiej BG (2020) Effect of whey protein concentrate on physicochemical, sensory and antioxidative properties of high-protein fat-free dairy desserts. Appl Sci 10:7064. https://doi.org/10.3390/app10207064
15. Tang C, Xi T, Zheng J, Cui X (2025) Chemical Properties of Whey Protein in Protein Powders and Its Impact on Muscle Growth in Athletes: A Review. Nat Prod Commun 20:1934578X251326124. https://doi.org/10.1177/1934578X251326124
16. Hovjecki M, Radovanovic M, Miloradovic Z, Jurina IB, Mirkovic M, Ignjatovic IS, Miocinovic J (2023) Fortification of goat milk yogurt with goat whey protein concentrate–Effect on rheological, textural, sensory and microstructural properties. Food biosci 56:103393. https://doi.org/10.1016/j.fbio.2023.103393
17. Kant R, Chakraborty C, Rout P, Roy T, Dutta S (2024) A comprehensive evaluation of inulin and whey protein concentrate as fat replacers in low-fat paneer: Physicochemical, textural and sensory studies. Food Human 2:100240. https://doi.org/10.1016/j.foohum.2024.100240
18. Alzamili ASA-H, Al-Bedrani DIJ (2023) The role of inulin as a fat replacer in the low-fat Oshari-like cheese physicochemical, textural and sensory properties. J Hyg Eng Design 42.
19. Berizi E, Shekarforoush S, Mohammadinezhad S, Hosseinzadeh S, Farahnaki A (2017) The use of inulin as fat replacer and its effect on texture and sensory properties of emulsion type sausages. Iran J Vet Res 18:253-257. https://doi.org/10.22099/ijvr.2017.4622
20. Ghazal S, Rai DC, Rai S (2022) Process optimization for the manufacture of low-calorie yoghurt. Indian J Dairy Sci 75.
21. Krishna K, Ghosh B (2019) Development of Reduced Fat Cream Cheese by using inulin and WPC as a fat replacer. Indian J Dairy Sci 72:590-598.
22. Poursharif K, Fadaei V, Daneshi M (2013) Effects of the substitution of inulin for fat on textural characteristics and sensory evaluation of reduced-fat wheyless cream cheese. Int J Biol Biotech 10:67-71.
23. Sołowiej B, Glibowski P, Muszyński S, Wydrych J, Gawron A, Jeliński T (2015) The effect of fat replacement by inulin on the physicochemical properties and microstructure of acid casein processed cheese analogues with added whey protein polymers. Food Hydrocolloids 44:1-11. http://dx.doi.org/10.1016/j.foodhyd.2014.08.022
24. Onsri N, Hudthagosol C, Sanporkha P (2022) Effect of inulin and whey protein concentrate on the physicochemical properties and shelf life of reduced-fat cream puffs. J Food Process Preserv 46:e16955. https://doi.org/10.1111/jfpp.16955
25. Omrani Khiabanian N, Motamedzadegan A, Naghizadeh Raisi S, Alimi M (2020) Chemical, textural, rheological, and sensorial properties of wheyless feta cheese as influenced by replacement of milk protein concentrate with pea protein isolate. J text stud 51:488-500. 10.1111/jtxs.12508
26. Sadeghi E, Movagharnejad K, Asl AH (2019) Parameters optimization and quality evaluation of mechanical properties of infrared radiation thin layer drying of pumpkin samples. J Food Process Eng https://doi.org/10.1111/jfpe.13309
27. Saleh A, Mohamed AA, Alamri MS, Hussain S, Qasem AA, Ibraheem MA (2020) Effect of different starches on the rheological, sensory and storage attributes of non-fat set yogurt. Foods 9:61. https://doi.org/10.3390/foods9010061
28. Junaid M, Rehman T, Imran M, Inayat S, Rehman HU, Ullah E, . . . & Younas U (2024) Impact on physico-chemical characteristics of cream cheese analogue using blend of dairy and vegetable fat. Discov Appl Sci 6:580. https://doi.org/10.1007/s42452-024-05862-w
29. Guo M, Wang H, Wang C (2018) Interactions between whey protein and inulin in a model system. J. Food Sci. Technol. 55:4051-4058. https://doi.org/10.1007/s13197-018-3331-7
30. Moghiseh N, Arianfar A, Salehi EA, Rafe A (2021) Effect of inulin/kefiran mixture on the rheological and structural properties of mozzarella cheese. Int J Biol Macromol 191:1079-1086. https://doi.org/10.1016/j.ijbiomac.2021.09.154
31. Bielska P, Cais-Sokolińska D (2023) Fresh white cheeses from buttermilk with polymerized whey protein: Texture, color, gloss, cheese yield, and peptonization. Appl. Sci. 13:11692. https://doi.org/10.3390/app132111692
32. Brodziak A, Król J, Barłowska J, Teter A, Florek M (2020) Changes in the physicochemical parameters of yoghurts with added whey protein in relation to the starter bacteria strains and storage time. Animals 10:1350. https://doi.org/10.3390/ani10081350

33. Castaneda N, Lee Y (2019) Microstructure of a model fresh cheese and bioaccessibility of vitamin D3 using in vitro digestion. Gels 5:16. https://doi.org/10.3390/gels5010016
34. Cinar K, Gulec HA, Gunes G, Hicsasmaz Z (2024) Utilization of retentates for white brined cheese production: comparative evaluation of physico-chemical and sensory properties. J. Food Meas. Charact. 18:8397-8407. https://doi.org/10.1007/s11694-024-02808-z
35. Rako A, Kalit MT, Kalit S (2019) Effect of sheep's milk composition on strength and syneresis of rennet-induced milk gel during lactation. Food Technol Biotechnol 57:426. https://doi.org/10.17113/ftb.57.03.19.6218
36. Fadaei V, Poursharif K, Daneshi M, Honarvar M (2012) Chemical characteristics of low-fat wheyless cream cheese containing inulin as fat replacer. Eur J Exp Biol 2:690-694.
37. Arango O, Trujillo A, Castillo M (2013) Influence of fat replacement by inulin on rheological properties, kinetics of rennet milk coagulation, and syneresis of milk gels. J. Dairy Sci. 96:1984-1996. http://dx.doi.org/10.3168/jds.2012-5763
38. Żbikowska A, Szymańska I, Kowalska M (2020) Impact of inulin addition on properties of natural yogurt. Appl Sci 10:4317. https://doi.org/10.3390/app10124317
39. Amador-Espejo GG, Ruiz-Lopez II, Gibbens-Bandala PJ, Delgado-Macuil RJ, Ruiz-Espinosa H (2021) Thermosonicated whey protein concentrate blends on quality attributes of reduced fat Panela cheese. Ultrason Sonochem 76:105621. https://doi.org/10.1016/j.ultsonch.2021.105621
40. Pires A, Gomes D, Noronha J, Díaz O, Cobos A, Pereira CD (2022) Evaluation of the characteristics of sheep's and goat's ice cream, produced with UF concentrated second cheese whey and different starter cultures. Foods 11:4091. https://doi.org/10.3390/foods11244091
41. Hanafy N, Ghanimah M, Hassanein A, Hashim M (2016) The effect of using whey protein concentrate on the quality of nonfat fresh cheese. J. Biol. Chem. Environ. Sci 11:455-469.
42. Sedaghati M, Kavand R (2026) Impact of inulin and calcium chloride on the quality of ricotta-like synbiotic cheese. J Food Sci Technol https://doi.org/10.1007/s13197-026-06599-7
43. Akalın A, Unal G, Dinkci N, Hayaloglu A (2012) Microstructural, textural, and sensory characteristics of probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate. J. Dairy Sci. 95:3617-3628. https://doi.org/10.3168/jds.2011-5297