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BIOCHEMICAL, RHEOLOGICAL, and SENSORY CHARACTERISTICS of NON-FAT SET YOGURT SUPPLEMENTED with A MIXTURE of HYDROCOLLOIDS

Elham Khodadadi, Nasim Khorshidian, Morteza Mashayekh, Hedayat Hosseini, Amir Mohammad Mortazavian and Arezoo Ebrahimi**

Address(es):
Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

*Corresponding author: mortazvn@sbmu.ac.ir , aefood89@gmail.com

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ABSTRACT

Side effects of fat intake and consumers' awareness have forced the food industry to produce healthier food. In recent years, non-fat yogurt has increasingly been consumed all over the world. In this study, the effect of adding a mixture of inulin, whey protein isolate, modified starch with different levels (0.3, 0.5 and, 1%) and gelatin (0.2%) on biochemical (pH, titratable acidity (TA), and Redox potential), rheological, and sensory properties of non-fat set yogurt was investigated. The samples containing higher levels of whey protein were better treatments regarding biochemical, sensory and rheological properties. The highest syneresis was observed in control yogurts and the samples with 1% inulin, whereas no syneresis was observed in samples containing 1% starch or 1% whey protein. Overall, the yogurts containing 1% whey proteins, 0.5% modified starch, 0.3% inulin, and 0.2% gelatin showed the most desirable characteristics of non-fat yogurt.

Keywords: Fat replacer; Hydrocolloids; Rheological properties; Syneresis; Yogurt

INTRODUCTION

Consumers' awareness of how diet effects the health and their demand for healthy foods has encouraged the food industry to produce healthy food products **(Annunziata & Pascale, 2009)**. Nowadays, obesity is a major health problem in both Western and developing countries that may lead to chronic diseases **(Losasso et al., 2012; Pieniak, Pérez-Cueto, & Verbeke, 2009)**. Limiting the intake of fat through lowering the fat content of foods is a way to avoid overconsumption of this ingredient in the diet, thereby formulating low-calorie products and preventing obesity **(Modzelewska-kapitula & Klebukowska, 2009)**.
Yogurt is a highly-consumed dairy product with textural and rheological characteristics, which are substantial for consumer acceptability **(Paseephol, Small, & Sherkat, 2008; Vélez-Ruiz, 2019)**. The presence of fat in dairy products has a considerable effect on their physical, rheological, and textural properties. In addition, fat influences other characteristics; for instance, appearance, flavor, and mouth feel which affect overall acceptance of the product **(Rybak, 2016)**. The separation of whey protein (syneresis) and variations in viscosity has become a topic of great concern in yogurts, particularly in low fat yogurts. Accordingly, the characteristics of low and non-fat yogurt are impacted by reducing fat content **(Dai, Corke, & Shah, 2016)**. In other words, low-fat and non-fat yogurts have low total solids and exhibit several defects such as lack of flavor, weak body, poor texture and syneresis **(Aziznia, Khosrowshahi, Madadlou, Rahimi, & Abbasi, 2009; Nguyen, Kravchuk, Bhandari, & Prakash, 2017)**.
Several methods have been suggested to overcome these adverse effects of low and non-fat yogurt, including adding certain dairy ingredients and hydrocolloids, an appropriate choice of starter cultures, the addition of thickeners, enhanced total solids concentration, and modification of processing parameters. Carbohydrate-based fat replacers can mimic the functional properties of fat in the product while reducing the caloric value of foods **(Guven, Yasar, Karaca, & Hayaloglu, 2005)**. It has been reported that exopolysaccharide (EPS) produced by some starter cultures can affect the end product quality, including texture, sensory and water-holding capacity of yogurt **(Han *et al.*, 2016)**. The addition of thickeners (polysaccharides or gelatin) leads to new cross-links in the network and increases the rigidity of the gel and its water holding capacity. Also, adjusting the total solid and protein levels can increase apparent viscosity and viscoelasticity of yogurt up to two or three times. Different processing parameters, including heat treatment, homogenization, shearing and acidification can change the mechanical, texture attributes and microstructure of yogurt **(Tan, 2019)**. Shokrollahi Yancheshmeh et al. worked on *Vicia villosa*, as a good source of protein, fiber, and minerals. They reported that the good nutritional and functional properties of *V. villosa* protein isolate make it useful in various food formulations **(Yancheshmeh et al., 2022)**.
Various types of fat replacers have been applied to yogurt such as inulin **(Crispín-Isidro, Lobato-Calleros, Espinosa-Andrews, Alvarez-Ramirez, & Vernon-Carter, 2015; Paseephol et al., 2008; Rezaei, Khomeiri, Aalami, & Kashaninejad, 2014)**, starch **(Ares et al., 2007; Radi, Niakousari, & Amiri, 2009; Tavakolipour, Vahid-moghadam, & Jamdar, 2014)**, *β*-glucan **(Brennan & Tudorica, 2008; Gee, Vasanthan, & Temelli, 2007)**, and gelatin **(Ares et al., 2007)**. The objective of this study was to elucidate the effect of the application of a mixture of some fat replacers including inulin, whey protein isolate, starch, and gelatin at various levels on physicochemical, rheological, and sensory properties of non-fat set yogurt during storage.

METHOD AND MATERIALS

Materials

In this study, skim milk powder (Fontera, Netherlands), modified tapioca starch (Cargill, Saint-Nazaire, France), ultra-high temperature milk (Roozaneh, Tehran, Iran), yogurt starter culture (Chr. Hansen, Horsholm, Denmark), long-chain inulin (Sensus, Spain), whey protein powder (FLA, Germany), gelatin (Gelita, Italy) were used.

Study design and sample preparation

Six yogurt samples containing inulin, whey protein isolate, modified starch (0.3, 0.5, 1%), and gelatine (0.2%) in three replications were formulated using skim milk powder reconstituted in sterilized distilled water to obtain a solution of 12% (w/w) total solid non-fat (Table 1). The hydrocolloids were subjected to rehydration 24 h before adding to milk. Control samples with 12% (w/w) total solid, 3% milk solid not fat, and without the inclusion of stabilizers were prepared. The samples were exposed to heat treatment at 90°C for 15 min. After heat exposure, the samples cooled in an ice bath and inoculation of starter culture, according to the instruction of the manufacturer, at 42°C until pH 4.5±0.02 was performed. The ultimate samples were quickly cooled and kept at 5°C for 28 days. Biochemical parameters, including changes in pH, acidity, and redox potential were determined during fermentation. These parameters were recorded at 30-minute time intervals. Other features, including rheological properties, syneresis, and sensory characteristics were recorded every 7 h.

Table 1 Different treatments of yogurt.

Non-fat yogurt code*	Inulin	Modified starch	Whey protein isolate	Gelatin
I_1-$S_{0.5}$-$W_{0.3}$-$G_{0.2}$ (T_1)	1	0.5	0.3	0.2
I_1-$S_{0.3}$-$W_{0.5}$-$G_{0.2}$ (T_2)	1	0.3	0.5	0.2
S_1- $I_{0.3}$-$W_{0.5}$-$G_{0.2}$ (T_3)	0.3	1	0.5	0.2
S_1- $I_{0.5}$-$W_{0.3}$-$G_{0.2}$ (T_4)	0.5	1	0.3	0.2
W_1- $I_{0.5}$- $S_{0.3}$-$G_{0.2}$ (T_5)	0.5	0.3	1	0.2
W_1- $I_{0.3}$- $S_{0.5}$-$G_{0.2}$ (T_6)	0.3	0.5	1	0.2

*B= control samples without hydrocolloids
T1= 1% inulin, 0.5% starch, 0.3% whey protein and 0.2% gelatin
T2= 1% inulin, 0.3% starch, 0.5% whey protein and 0.2% gelatin
T3= 1% starch, 0.5% whey protein, 0.3% inulin and 0.2% gelatin
T4= 1% starch, 0.5% inulin, 0.3% whey protein and 0.2% gelatin
T5= 1% whey protein, 0.5% inulin, 0.3% starch and 0.2% gelatin
T6= 1% whey protein, 0.5% starch, 0.3% inulin and 0.2% gelatin.

Chemical analysis

Titratable acidity (TA) (as % lactic acid) was measured every half hour during fermentation and every 7 days during refrigerated storage and determined according to the method adopted by the Association of Official Analytical Chemists (AOAC) 947.05 using 0.1 M NaOH (AOAC 1999). pH values and redox potential of the samples were determined every half hour during fermentation and every 7 days during refrigerated storage by a pH meter (MA235, Mettler, Toledo, Switzerland).
Various biochemical parameters were specified and determined as follows:
Titratable acidity increase rate = (final acidity value – initial acidity value) / incubation time [Dornic degree/ min or day] **(Mortazavian *et al.*, 2010)**.
pH drop rate = (final pH value – initial pH value) / incubation time [pH value/ min or day] **(Mortazavian *et al.*, 2010)**.
Redox potential increase rate = (final value – initial value) / incubation time [mV/ min or day] **(Mortazavian *et al.*, 2010)**.

Synersis measurement

Inoculated milk samples, prepared using the procedure described above, were fermented in test tubes with the same geometry and height at 42°C. The initial height of yogurt in the test tube and the height of the drained liquid were recorded during refrigerated storage. The degree of syneresis was represented as a percentage.
% Syneresis= [height of separated serum/initial height of yogurt in tubes] × 100

Rheological measurements

To monitor the rheological characteristics of yogurts, dynamic oscillatory shear testing was performed using a rheometer (Anton Paar, MCR 301, Graz, Austria). The temperature was set to 4±0.01°C before running rheological experiments. For each sample, a frequency sweep was executed with a frequency range between 0.01 and 100 Hz at a constant strain of 0.5%. The rheological parameters measured were elastic modulus (G´), viscous modulus (G″), loss tangent ($\tan \delta = G''/G'$), complex modulus (G*) and crossover point calculated using the Rheoplus/32 software (version V3.21).
Triplicate measurements were performed for each sample and the power law model satisfactory fitted the experimental data for each sample with a correlation coefficient (R^2) of at least 0.95. The strain sweep with strain varied from 0.01 to 1000% at a constant frequency of 1 Hz was done for each sample to define the linear viscoelastic range (LVE) and to determine above-mentioned moduli **(Guggisberg, Cuthbert-Steven, Piccinali, Bütikofer, & Eberhard, 2009; Staffolo, Bertola, & Martino, 2004)**.

Sensory evaluation

Sensory analysis was carried out by a panel of 30 assessors, all with previous experience in dairy products evaluation. The sensory properties included flavor, oral texture, appearance, non-oral texture (texture smoothness and scoopability), and overall acceptability. Each of these characteristics was scored on a five-point scale: 0= inconsumable; 1= unacceptable; 2= acceptable; 3= pleasant or satisfactory and 4= excellent. The samples were randomly numbered by three-digit coding and the sensory panel evaluated the coded yogurts. All sessions were carried out in a sensory laboratory with separate booths.

Statistical analysis

Analysis of variance (ANOVA) was conducted on the resulting data using Duncan's multiple range test to compare treatment means. The SPSS V 17 was used and the significance was defined at $P < 0.05$. The experiments were executed in triplicates.

RESULT AND DISCUSSION

Biochemical characteristics

As seen in Table 2, control samples presented the lowest pH during refrigerated storage. This can be ascribed to the greater level of lactose due to the additional amount of skim milk compared to other treatments. Addition of 1% inulin or 1% modified starch and 1% inulin had no significant effect on pH change at the end of fermentation. Guven *et al*. (2005) announced that the incorporation of inulin at different levels into low-fat set yogurt did not influence on pH of yogurts **(Guven *et al.*, 2005)**. Similarly, Radi *et al*. (2009) reported that the pH of low-fat yogurt was not affected by modified starch addition. The highest pH was recorded in treatments 5 and 6 on day 28 **(Radi *et al.*, 2009)**. Contrarily, Zhang *et al*. (2015) reported that the addition of whey protein concentrate to goat's milk non-fat yogurt had no impact on the pH of the samples **(Zhang, McCarthy, Wang, Liu, & Guo, 2015)**.

Table 2 Mean values of pH at the end of fermentation and during refrigerated storage (28 d, 4°C)*.

*Treatments***		*Refrigerated storage (days)*			
	End of fermentation	*7*	*14*	*21*	*28*
B	4.50^{aA}	4.36^{bB}	4.24^{cBC}	4.18^{dD}	4.11^{cE}
T1	4.52^{aA}	4.40^{abB}	4.32^{abC}	4.26^{bD}	4.21^{bE}
T2	4.50^{aA}	4.41^{abB}	4.33^{abC}	4.28^{abD}	4.23^{abE}
T3	4.54^{aA}	4.38^{abB}	4.30^{bBC}	4.22^{cD}	4.17^{bE}
T4	4.52^{aA}	4.37^{abB}	4.29^{bBC}	4.21^{cD}	4.18^{bE}
T5	4.50^{aA}	4.43^{aB}	4.38^{aC}	4.30^{aCD}	4.25^{aD}
T6	4.50^{aA}	4.45^{aB}	4.40^{aBC}	4.31^{aCD}	4.26^{aD}

*Means shown with different small and capital letters represent significant differences (p <0.05) in the same columns (among the treatments) and rows (between the two day in each treatment), respectively.
**B= control samples without hydrocolloids
T1= 1% inulin, 0.5% starch, 0.3% whey protein and 0.2% gelatin
T2= 1% inulin, 0.3% starch, 0.5% whey protein and 0.2% gelatin
T3= 1% starch, 0.5% whey protein, 0.3% inulin and 0.2% gelatin
T4= 1% starch, 0.5% inulin, 0.3% whey protein and 0.2% gelatin
T5= 1% whey protein, 0.5% inulin, 0.3% starch and 0.2% gelatin
T6= 1% whey protein, 0.5% starch, 0.3% inulin and 0.2% gelatin.

Control yogurts had the highest titratable acidity at the end of fermentation and during refrigerated storage due to the higher content of lactose and the generation of a higher amount of acid during fermentation. The lowest titratable acidity was observed in T5 and T6 during storage (Table 3). Paseephol *et al*. (2008) assessed the effect of inulin with different chain lengths on non-fat set yogurt and declared that the level and chain length of inulin had no effect on the titratable acidity of yogurt samples **(Paseephol *et al.*, 2008)**.

Table 3 Mean values of titratable acidity at the end of fermentation and during refrigerated storage (28 d, 4°C)*.

*Treatments***	*Refrigerated storage (days)*				
	End of fermentation	*7*	*14*	*21*	*28*
B	0.92^{aE}	1.00^{aD}	1.08^{aC}	1.15^{aB}	1.21^{aA}
T1	0.90^{aD}	0.96^{bC}	1.02^{bB}	1.07^{abAB}	1.11^{bA}
T2	0.91^{aD}	0.94^{bCD}	1.00^{bC}	1.06^{abB}	1.10^{bA}
T3	0.90^{aD}	0.95^{bCD}	1.01^{bC}	1.06^{abAB}	1.11^{bA}
T4	0.92^{aD}	0.96^{bCD}	1.03^{bC}	1.08^{abB}	1.12^{bA}
T5	0.89^{abC}	0.94^{bBC}	0.97^{cB}	1.01^{cAB}	1.05^{cA}
T6	0.90^{aC}	0.94^{bBC}	0.98^{cB}	1.03^{cAB}	1.06^{cA}

**Means shown with different small and capital letters represent significant differences (p <0.05) in the same columns (among the treatments) and rows (between the two day in each treatment), respectively.
**B= control samples without hydrocolloids
T1= 1% inulin, 0.5% starch, 0.3% whey protein and 0.2% gelatin
T2= 1% inulin, 0.3% starch, 0.5% whey protein and 0.2% gelatin
T3= 1% starch, 0.5% whey protein, 0.3% inulin and 0.2% gelatin
T4= 1% starch, 0.5% inulin, 0.3% whey protein and 0.2% gelatin
T5= 1% whey protein, 0.5% inulin, 0.3% starch and 0.2% gelatin
T6= 1% whey protein, 0.5% starch, 0.3% inulin and 0.2% gelatin.

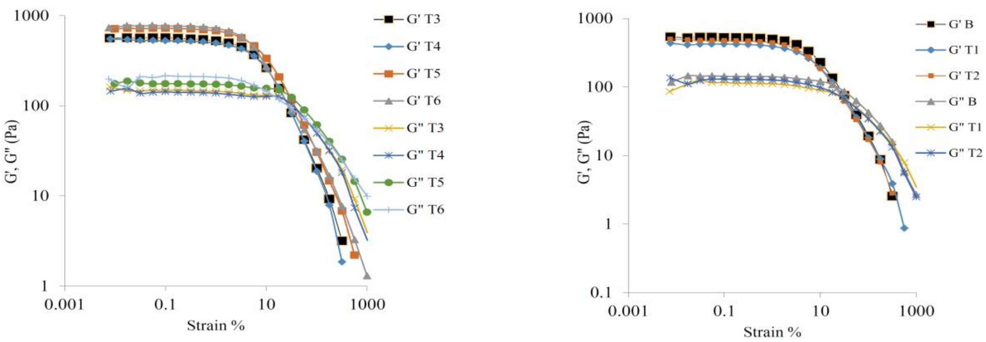

Figure 1 Storage and loss moduli in yogurts as a function of strain on day 0.

As shown in Table 4, at the end of fermentation, no significant difference regarding redox potential was observed among samples. The redox potential was increased in all of the samples during refrigerated storage. On day 28 of storage, control samples had the highest redox potential, while samples containing 1% whey protein showed the lowest values. There was no significant difference between the samples containing 1% inulin (T1and T2) and the ones containing 1% modified starch (T3 and T4).

Table 4 Mean values of redox potential at the end of fermentation and during refrigerated storage (28 d, 4°C)*.

*Treatments***	*Refrigerated storage (days)*				
	End of fermentation	*7*	*14*	*21*	*28*
B	127.8^{aEF}	130.2^{aDE}	136.6^{aCD}	142.9^{aBC}	149.4^{aA}
T1	126.9^{aEF}	127.7^{aDE}	133.4^{aCD}	139.1^{aBC}	144.8^{abA}
T2	126.7^{aDE}	127.2^{aD}	132.2^{aCD}	138.4^{aBC}	144.2^{abA}
T3	126.3^{aE}	129.1^{aD}	134.0^{aCD}	140.1^{aB}	145.3^{abA}
T4	127.5^{aE}	131.7^{aD}	135.2^{aCD}	141.4^{aB}	145.6^{abA}
T5	126.6^{aD}	125.4^{abCD}	131.1^{abBC}	134.6^{abAB}	140.1^{bA}
T6	127.1^{aDE}	126.7^{aC}	131.4^{abBC}	134.2^{abAB}	140.3^{bA}

*Means shown with different small and capital letters represent significant differences (p <0.05) in the same columns (among the treatments) and rows (between the two day in each treatment), respectively.
**B= control samples without hydrocolloids
T1= 1% inulin, 0.5% starch, 0.3% whey protein and 0.2% gelatin
T2= 1% inulin, 0.3% starch, 0.5% whey protein and 0.2% gelatin
T3= 1% starch, 0.5% whey protein, 0.3% inulin and 0.2% gelatin
T4= 1% starch, 0.5% inulin, 0.3% whey protein and 0.2% gelatin
T5= 1% whey protein, 0.5% inulin, 0.3% starch and 0.2% gelatin
T6= 1% whey protein, 0.5% starch, 0.3% inulin and 0.2% gelatin.

Table 5 Mean pH drop, mean titratable acidity and redox potential increase rates during refrigerated storage*

*Treatments***	*Parameters*		
	pH drop rate (pH/ day)	*Titratable acidity increase rate (°D/day)*	*Redox potential increase rate (mV/day)*
B	0.013^{a}	0.010^{a}	0.77^{a}
T1	0.011^{ab}	0.007^{ab}	0.63^{ab}
T2	0.009^{ab}	0.006^{ab}	0.62^{ab}
T3	0.011^{ab}	0.007^{ab}	0.67^{ab}
T4	0.011^{ab}	0.007^{ab}	0.64^{ab}
T5	0.008^{b}	0.005^{b}	0.48^{b}
T6	0.008^{b}	0.005^{b}	0.47^{b}

*Means shown with different small and capital letters represent significant differences (p <0.05) in the same columns (among the treatments) and rows (between the two day in each treatment), respectively.
**B= control samples without hydrocolloids
T1= 1% inulin, 0.5% starch, 0.3% whey protein and 0.2% gelatin
T2= 1% inulin, 0.3% starch, 0.5% whey protein and 0.2% gelatin
T3= 1% starch, 0.5% whey protein, 0.3% inulin and 0.2% gelatin
T4= 1% starch, 0.5% inulin, 0.3% whey protein and 0.2% gelatin
T5= 1% whey protein, 0.5% inulin, 0.3% starch and 0.2% gelatin
T6= 1% whey protein, 0.5% starch, 0.3% inulin and 0.2% gelatin.

The highest pH drop rate during the storage was obtained in control yogurts, while the lowest was reported in T5 and T6. pH drop rate did not differ significantly in T1, T2, T3, and T4 which indicates that the addition of the hydrocolloids at these levels was not effective on the fermentation process during storage (Table 5). In a similar trend, Radi *et al*. (2009) reported that the incorporation of wheat-modified starch into low-fat yogurts had no impact on biochemical parameters in comparison with control samples **(Radi *et al.*, 2009)**.
The highest titratable acidity increase rate, as well as redox potential increase rate, was observed in control yogurts. Whereas the lowest values were obtained in T5 and T6 which can be imputed to the buffering effect of whey protein in these treatments (Table 5).

Rheological analysis

Strain sweep test

Storage modulus (G′) and loss modulus (G″) on day 0 have been shown inFig. (1). According to the viscoelastic properties, all samples indicated weak gel behavior with storage modulus (G′) higher than loss modulus (G″). The highest values of G′ were obtained in T5 and T6, whereas the lowest were recorded in T1 and T2. A higher protein level would cause a higher degree of cross-linkage of the gel network, resulting in a much denser and more rigid gel structure and higher G′ values **(Robinson & Itsaranuwat, 2006)**.
Accordingly, Bikker and Anema (2003) stated that increasing the content of whey protein concentrate up to 15 g/L would enhance the G′ value which was attributed to the increasing the disulphide interactions between the denatured whey protein and the casein micelles **(Graveland-Bikker & Anema, 2003)**. Moreover, in samples with higher inulin content, inulin molecules would disperse among the casein micelles and interfere with protein matrix formation, leading to a softer yogurt gel formation and lower G′ and G″ **(Paseephol *et al.*, 2008)**.

Table 6 Mean values of rheological properties (strain sweep test)*.

*Treatment***	**parameters**					
	τ_f		*LVE*		*G'=G" (Pa)*	
	d0	*d28*	*d0*	*d28*	*d0*	*d28*
B	10.19^{bB}	14.2^{bA}	1.62^{dA}	1.65^{eA}	96.2^{bB}	115.3^{bA}
T1	7.12^{dB}	10.4^{eA}	1.64^{dB}	2.13^{dA}	67.2^{dB}	71.1^{deA}
T2	7.22^{dB}	11.5^{dA}	1.67^{dB}	2.15^{dA}	67.9^{dB}	84.4^{dA}
T3	11.7^{aB}	15.1^{aA}	1.94^{cB}	2.41^{bA}	114.5^{aB}	127.3^{aA}
T4	11.2^{abB}	14.3^{bA}	1.89^{cB}	2.34^{cA}	113.5^{abB}	125.1^{aA}
T5	10.3^{bB}	12.5^{cA}	2.06^{bB}	2.41^{bA}	93.4^{bB}	118.2^{bA}
T6	10.4^{bB}	12.7^{cA}	2.18^{aB}	2.51^{aA}	94.6^{bB}	117.6^{bA}

*Means shown with different small and capital letters represent significant differences (p <0.05) in the same columns (among the treatments) and rows (between the two day in each treatment), respectively.
**B= control samples without hydrocolloids
T1= 1% inulin, 0.5% starch, 0.3% whey protein and 0.2% gelatin
T2= 1% inulin, 0.3% starch, 0.5% whey protein and 0.2% gelatin
T3= 1% starch, 0.5% whey protein, 0.3% inulin and 0.2% gelatin
T4= 1% starch, 0.5% inulin, 0.3% whey protein and 0.2% gelatin
T5= 1% whey protein, 0.5% inulin, 0.3% starch and 0.2% gelatin
T6= 1% whey protein, 0.5% starch, 0.3% inulin and 0.2% gelatin

As can be seen in Table 6, treatments are significantly different regarding yield stress (τ_f). Samples containing 1% modified starch showed the highest values followed by T5, T6, and control samples. The lowest τ_f was reported in yogurts containing 1% inulin. According to Heydari *et al*. (2009), who evaluated the effect of prebiotics addition on probiotic yogurt, samples with 3% starch showed the highest τ_f value, which is in consistency with our finding. By increasing the modified starch level, a part of starch would be adsorbed on the casein micelle surface through electrostatic repulsion and the hydrophilic chains of polymer that forms a thick adsorption layer resulting in the decline of zeta potential and increasing the stability of the yogurt system **(Cui, Lu, Tan, Wang, & Li, 2014)**.
Assessing crossover point (G′=G″) indicated that T3 had the highest value, followed by T5, T6, control, T2, and T1. Monitoring viscoelastic range (LVE) shows that the greatest value belongs to T6, followed by T5, T3, T4, control, T2, and T1 (Table 6). It is worth noting that G′ and G″ on day 28 and during refrigerated storage were enhanced in all treatments compared to day 0 (Fig. 2).

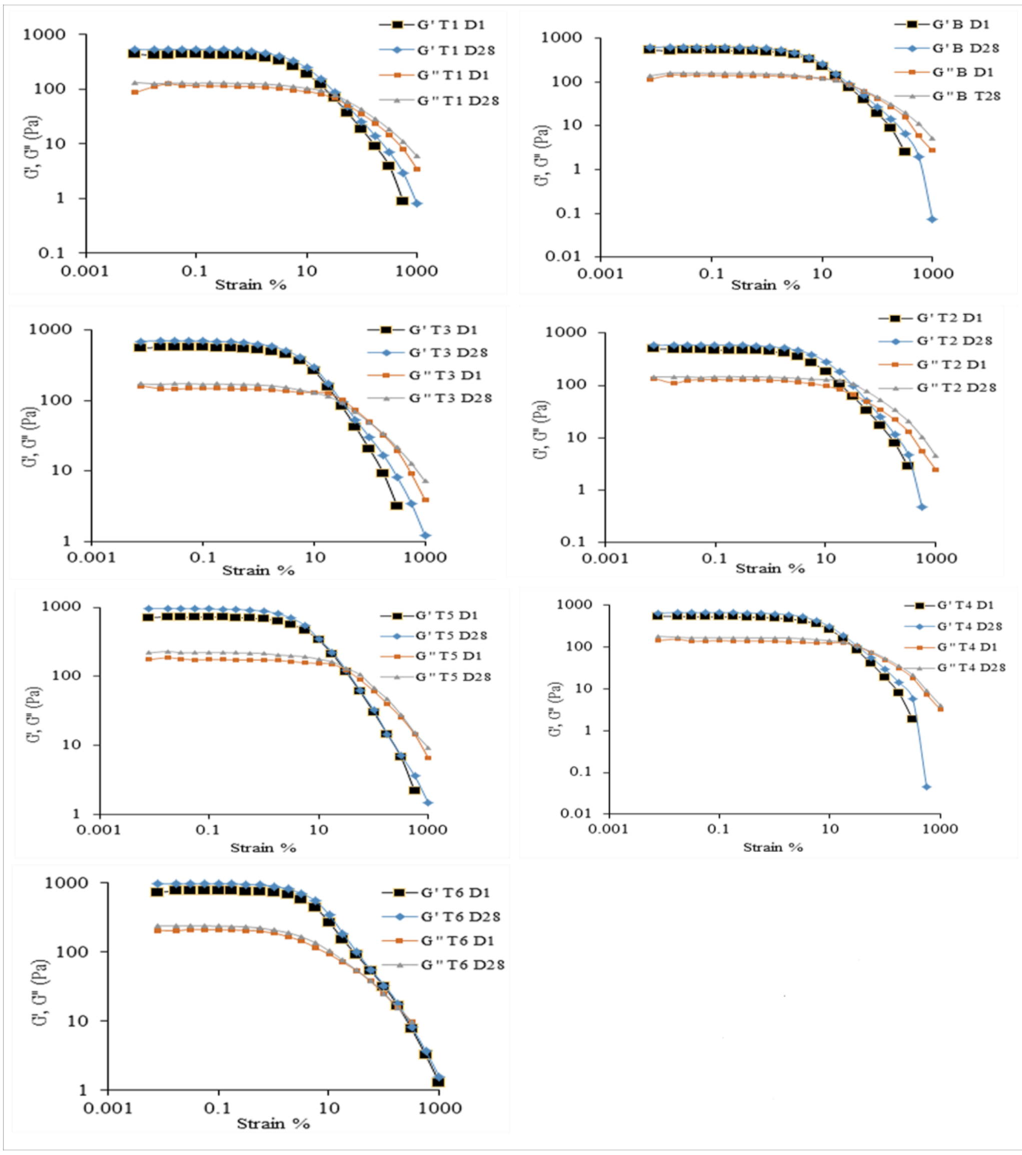

Figure 2 Storage and loss moduli in yogurts as a function of strain on days 0 and 28.

Frequency sweep test

Fig. 3 illustrates the changes of storage modulus (G′) and loss modulus (G″) in a frequency range of 0.628-314 rad/s. In all treatments, G′ was higher than G″, indicative of a more elastic feature of the samples than a viscous feature. Fig. 3 shows that T5 and T6 had the greatest G′ compared to other treatments, while T1 and T2 demonstrated the lowest values. Generally, n, b, and tan δ are altered with frequency (ω) according to the power law model ($G' = k\omega^n$).

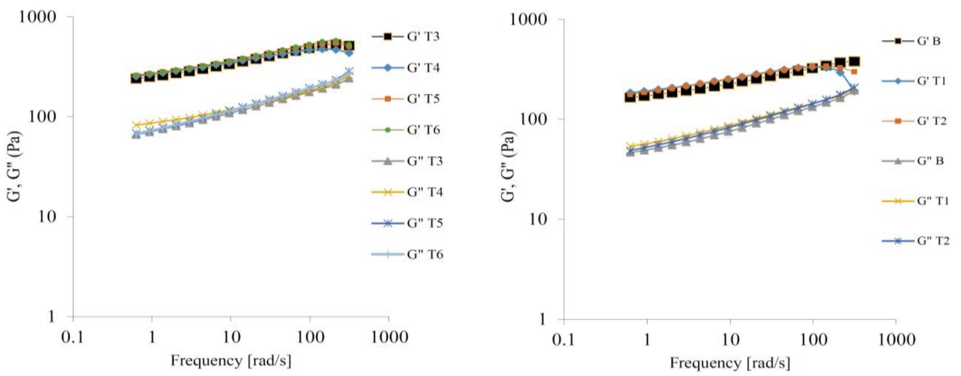

Figure 3 Storage and loss moduli in yogurts as a function of frequency on day 0.

According to Table 7, there is a significant difference among treatments in respect of the factors ‘n’ and ‘k’. The highest values were recorded in T5 and T6 while the lowest values were observed in T1 and T2. Furthermore, T5 and T6 had the highest ‘k’ followed by control yogurt, T3, T4, T2, and T1, respectively. The higher ‘k’ factor is an indication of a strong gel structure, whereas with increasing ‘n’ factor; samples exhibit characteristics of a gel with higher sensitivity to mechanical stresses **(Steffe, 1996)**.

As shown in Fig. 4, T2 and T5 indicated the highest and lowest tan δ, respectively. The higher value of tan δ is indicative of a weaker gel structure. Accordingly, Brennan and Tudorica (2008) implied that full and low-fat stirred yogurt samples containing inulin and guar gum showed higher tan δ compared to control samples **(Brennan & Tudorica, 2008)**.

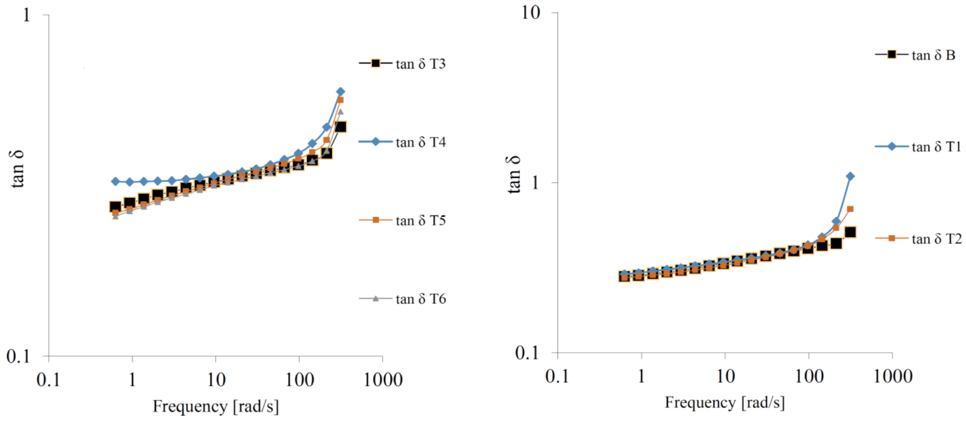

Figure 4 Tan δ in yogurts as a function of frequency on day 0.

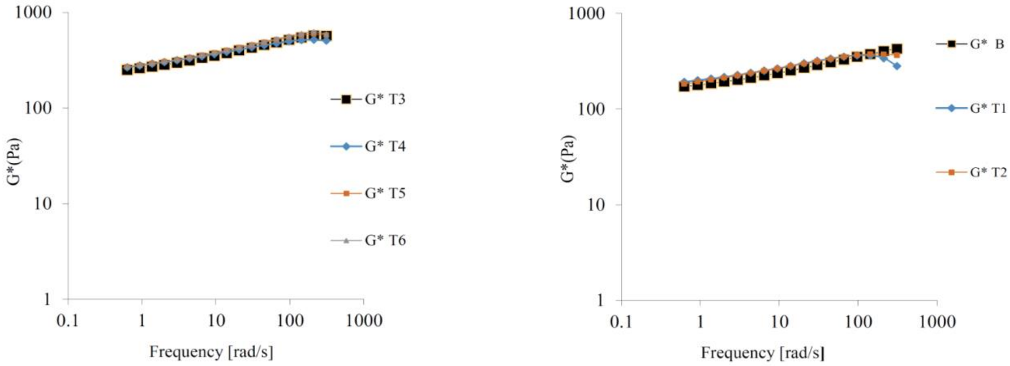

Figure 5 Complex modulus (G*) in yogurts as a function of frequency on day 0.

Table 7 Mean values of rheological properties (Power Law parameters, frequency sweep test)*.

	Parameters			
	K		***n***	
Treatment**	***d0***	***d28***	***d0***	***d28***
B	247.1 bB	287.3 Ba	0.13 abA	0.13 cA
T1	158.4 dB	226.1 dA	0.12 bA	0.12 cdA
T2	168.3 dB	237.4 dA	0.12 bA	0.12 cdA
T3	191.5 cB	268 cA	0.14 aB	0.17 aA
T4	186.9 cB	261.9 cA	0.14 aB	0.16 abA
T5	265.3 aB	296.3 aA	0.13 abAB	0.14 bcA
T6	266.6 aB	302 aA	0.13 abB	0.15 bA

*Means shown with different small and capital letters represent significant differences (p <0.05) in the same columns (among the treatments) and rows (between the two day in each treatment), respectively.
**B= control samples without hydrocolloids
T1= 1% inulin, 0.5% starch, 0.3% whey protein and 0.2% gelatin
T2= 1% inulin, 0.3% starch, 0.5% whey protein and 0.2% gelatin
T3= 1% starch, 0.5% whey protein, 0.3% inulin and 0.2% gelatin
T4= 1% starch, 0.5% inulin, 0.3% whey protein and 0.2% gelatin
T5= 1% whey protein, 0.5% inulin, 0.3% starch and 0.2% gelatin
T6= 1% whey protein, 0.5% starch, 0.3% inulin and 0.2% gelatin.

Fig. 5 illustrates that the highest G* values are associated with T5 and T6, followed by control, T3, and T4. The lowest values were observed in T2 and T1.

Synersis characteristic

Whey separation is a major defect in the yogurt and can be described as the appearance of serum on the surface of set yogurt gels. Typically, syneresis happens due to the shrinkage of the gel **(Farnsworth, Li, Hendricks, & Guo, 2006)**. Table 8 demonstrates the syneresis data for all treatments at 0, 7, 14, 21, and 28 days of storage. Control samples showed the highest syneresis at the end of fermentation and during refrigerated storage. As mentioned before, these samples showed the highest acidity, and by increasing the concentration of hydrogen ions during acidification, the repulsive forces decrease, and the casein micelles begin to aggregate **(Karimi, Mortazavian, & Karami, 2012)**.

Table 8 Mean syneresis values (%) at the end of fermentation and during refrigerated storage*.

Treatments**	*Refrigerated storage (days)*				
	End of fermentation	***7***	***14***	***21***	***28***
B	1.7 aC	3.6 aBC	4.5 aB	6.7 aA	5.1 aAB
T1	1.2 bBC	2.4 abB	3.1 abAB	4.5 bA	2.6 bB
T2	1.0 bc	1.8 bBC	2.6 bAB	3.9 bCA	2.1 bcB
T3	0 cA	0 cA	0 dA	0 eA	0 dA
T4	0 cA	0 cA	0 dA	0 eA	0 dA
T5	0 cA	0 cA	0 dA	0 eA	0 dA
T6	0 cA	0 cA	0 dA	0 eA	0 dA

*Means shown with different small and capital letters represent significant differences (p <0.05) in the same columns (among the treatments) and rows (between the two day in each treatment), respectively.
**B= control samples without hydrocolloids
T1= 1% inulin, 0.5% starch, 0.3% whey protein and 0.2% gelatin
T2= 1% inulin, 0.3% starch, 0.5% whey protein and 0.2% gelatin
T3= 1% starch, 0.5% whey protein, 0.3% inulin and 0.2% gelatin
T4= 1% starch, 0.5% inulin, 0.3% whey protein and 0.2% gelatin
T5= 1% whey protein, 0.5% inulin, 0.3% starch and 0.2% gelatin
T6= 1% whey protein, 0.5% starch, 0.3% inulin and 0.2% gelatin.

In treatments of control, T1 and T2, the syneresis was increased till day 21 but declined from day 21 to 28. In treatments of T3, T4, T5, and T6, no syneresis was observed at the end of fermentation and during storage. In the samples containing 1% whey protein, syneresis decreased due to the reduction of casein to whey protein ratio. Accordingly, Remeuf *et al*. (2003) announced that by increasing the whey protein level in yogurt, gel strength would increase and subsequently, a decrease in syneresis would be observed **(Remeuf, Mohammed, Sodini, & Tissier, 2003)**. Puvanenthiran *et al*. (2002) reported that by reducing the ratio of the casein to whey protein, the protein network became finer, the size of the aggregates became smaller, the pores smaller, and the network of cross-links denser, which entraps water leading to lower whey drainage **(Puvanenthiran, Williams, & Augustin, 2002)**. It was pointed that starch can absorb water and reduce the whey separation in yogurt **(Radi *et al.*, 2009)**. In T1 and T2, syneresis was lower compared to control yogurts but was not reduced entirely. This is in agreement with the data obtained by Heydari *et al*. (2011) and Vasiljevic *et al*. (2007) that ascribed this phenomenon to the presence of long chain polysaccharides. These polysaccharides could interfere with the development of a three-dimensional casein structure, leading to the formation of a weaker gel with less water retention **(Heydari S, Mortazavian AM, Ehsani MR, Mohammadifar MA, & H., 2011; Vasiljevic, Kealy, & Mishra, 2007)**.

Sensory analysis

Sensory evaluation data for oral texture, non-oral texture (texture smoothness and scoopability), flavor, appearance, and overall acceptability are shown in Table 8. T5 and T6 received the highest score, and T2 obtained the lowest score regarding oral texture at the end of fermentation and there was no significant difference between these treatments and T4 as well as control samples. It can be elucidated that higher protein content improves the texture of non-fat yogurt, but greater concentrations of inulin or starch compared to whey protein have no effect on the oral texture of the samples. Likewise, Radi *et al*. (2009) implied that increasing the starch level from 1.6% to 3.2% improved the sensory characteristics of low-fat yogurt **(Radi *et al.*, 2009)**. On day 28, the highest and the lowest scores in this regard were attributed to T6 and T2. In a study, the effect of inulin and agav fructans addition on microstructural, rheological, and sensory characteristics of reduced-fat stirred yogurt was investigated **(Crispin-Isidro *et al.*, 2015)**. It was reported that inulin at the level of 4% could mimic the sensory perception of the full-fat yogurt; while in the present study utilization of 1% inulin in non-fat yogurt had no remarkable effect on product acceptability. In control samples, the oral texture acceptability were reduced on day 28 compared to day 0, which can be ascribed to the increment of acidity and low pH values in these products.

Table 9 Sensory analysis of the treatments at day 0 and 28 using hedonic methodology.

Treatment**	***Parameters***									
	Flavor		***Oral texture***		***Non-oral texture***		***Color and appearance***		***Overall acceptability***	
	d0	***d28***	***d0***	***d28***	***d0***	***d28***	***d0***	***d28***	***d0***	***d28***
B	3.4^{abA}	2.8^{bB}	3.1^{abA}	2.6^{cA}	3.6^{aA}	2.5^{bA}	3.2^{aA}	2.7^{bA}	3.4^{aA}	2.8^{bB}
T1	2.9^{bcA}	2.9^{bcA}	2.3^{cA}	2.0^{cdA}	2.2^{cdA}	2.0^{cAB}	1.7^{dA}	1.6^{dA}	2.4^{cA}	2.5^{cA}
T2	3.0^{bA}	3.1^{abA}	2.5^{bcA}	2.1^{cAB}	$2.0c^{dA}$	1.8^{cdAB}	1.9^{dA}	1.8^{dA}	2.5^{cA}	2.3^{cdA}
T3	1.9^{dA}	2.0^{dA}	2.9^{abA}	2.7^{bcA}	3.1^{bAB}	3.0^{abA}	3.5^{aA}	3.2^{aAB}	2.85^{bA}	2.5^{cAB}
T4	2.0^{dA}	2.2^{dA}	2.7^{bA}	2.6^{bcA}	3.0^{bA}	2.8^{bA}	3.1^{abA}	3.0^{aAB}	2.8^{bA}	2.6^{cA}
T5	3.6^{aA}	3.5^{aA}	3.1^{abA}	3.0^{bA}	3.2^{abAB}	3.4^{aA}	3.4^{aA}	3.2^{aAB}	3.3^{aA}	3.1^{aAB}
T6	3.8^{aA}	3.7^{aA}	3.5^{aA}	3.6^{aA}	3.0^{bA}	3.1^{aA}	3.5^{aA}	3.3^{aAB}	3.6^{aA}	3.5^{aA}

*Means shown with different small and capital letters represent significant differences (*p* <0.05) in the same columns (among the treatments) and rows (between the two day in each treatment), respectively.
**B= control samples without hydrocolloids
T1= 1% inulin, 0.5% starch, 0.3% whey protein and 0.2% gelatin
T2= 1% inulin, 0.3% starch, 0.5% whey protein and 0.2% gelatin
T3= 1% starch, 0.5% whey protein, 0.3% inulin and 0.2% gelatin
T4= 1% starch, 0.5% inulin, 0.3% whey protein and 0.2% gelatin
T5= 1% whey protein, 0.5% inulin, 0.3% starch and 0.2% gelatin
T6= 1% whey protein, 0.5% starch, 0.3% inulin and 0.2% gelatin.

Treatments T1 and T2 received the lowest score on day 0 concerning non-oral texture. These two treatments showed high syneresis values that justify their low acceptability by panelists. Control samples had the highest acceptability from this point of view. On day 28, T5 and T6 showed the highest acceptability in terms of non-oral texture, which was consistent with the higher values of rheological parameters (storage, loss, and complex moduli), while T1 and T2 received the lowest acceptability in this context.
The highest flavor acceptability on days 0 and 28 were recorded in T5 and T6 and the lowest was observed in T3 and T4. T1 and T2 were more favorable by the assessors on day 28 compared to day 0 because of the impact of inulin on masking the sour taste of acid **(Meyer & Blaauwhoed, 2009)**.
The least acceptable samples in respect of the appearance on day 0 were T1 and T2 because of higher syneresis values in these samples, while no significant difference was observed between other treatments, as well as control samples. On day 28, the highest scores were ascribed to T3, T4, T5, and T6 followed by control yogurts and the lowest ones were observed in T1 and T2. Regarding overall acceptability, the highest scores on day 0 were reported in T5 and T6 and the lowest scores were obtained in the case of T1 and T2. Pang *et al*. (2016) examined the effect of whey protein along with xanthan, starch, and carrageenan on rheological and sensory properties of yogurt and reported that yogurt samples containing whey protein were more acceptable in comparison with other treatments **(Pang, Deeth, Prakash, & Bansal, 2016)**. On day 28, T5 and T6 received the highest scores followed by control sample and T1, T2, T3, and T4 received the lowest ones.

CONCLUSIONS

In this study, the effect of the mixture of hydrocolloids on biochemical, rheological, and sensory characteristics of non-fat set yogurt was investigated. The results revealed that a higher concentration of whey protein compared to modified starch and inulin prevents the increase of acidity and pH decrease and subsequently defects of yogurt texture and sensory properties of non-fat yogurt during refrigerated storage. Moreover, 1% whey protein improved the rheological characteristics of yogurt samples. The highest values of G′ were obtained in T5 and T6 (maximum whey protein content) on day 0. G′ and G″ on day 28 and during refrigerated storage were enhanced in all treatments compared to day 0. Sensory evaluation data for oral texture, non-oral texture (texture smoothness and scoop ability), flavor, appearance, and overall acceptability revealed that T5 and T6 obtained the highest score and T2 received the lowest score regarding oral texture at the end of fermentation. T5 and T6 were the most acceptable on days 0 and 28 of storage. Treatments containing 1% whey protein and 1% modified starch showed no syneresis at the end of the fermentation and during the storage period, while 1% inulin could not inhibit the syneresis completely. It can be concluded that the best mixture of hydrocolloids as fat replacer regarding biochemical, rheological, and sensory properties is 1% whey protein, 0.5% starch, 0.3% inulin, and 0.2% gelatin.

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REFERENCES

Annunziata, A., & Pascale, P. (2009). Consumers' behaviours and attitudes toward healthy food products: The case of Organic and Functional foods. *The 113th EAAE Seminar "A resilient European food industry and food chain in a challenging world"*, Chania, Crete, Greece. https://doi.org/10.22004/ag.econ.57661
Ares, G., Gonçalvez, D., Pérez, C., Reolón, G., Segura, N., Lema, P., & Gámbaro, A. (2007). Influence of gelatin and starch on the instrumental and sensory texture of stirred yogurt. *International journal of dairy technology, 60*(4), 263-269. https://doi.org/10.1111/j.1471-0307.2007.00346.x
Aziznia, S., Khosrowshahi, A., Madadlou, A., Rahimi, J., & Abbasi, H. (2009). Texture of nonfat yoghurt as influenced by whey protein concentrate and Gum Tragacanth as fat replacers. *International journal of dairy technology, 62*(3), 405-410. https://doi.org/10.1111/j.1471-0307.2009.00507.x
Brennan, C. S., & Tudorica, C. M. (2008). Carbohydrate-based fat replacers in the modification of the rheological, textural and sensory quality of yoghurt: comparative study of the utilisation of barley beta-glucan, guar gum and inulin. *International journal of food science & technology, 43*(5), 824-833. https://doi.org/10.1111/j.1365-2621.2007.01522.x
Crispín-Isidro, G., Lobato-Calleros, C., Espinosa-Andrews, H., Alvarez-Ramirez, J., & Vernon-Carter, E. (2015). Effect of inulin and agave fructans addition on the rheological, microstructural and sensory properties of reduced-fat stirred yogurt. *LWT-Food Science and Technology, 62*(1), 438-444. https://doi.org/10.1016/j.lwt.2014.06.042
Cui, B., Lu, Y.-m., Tan, C.-p., Wang, G.-q., & Li, G.-H. (2014). Effect of cross-linked acetylated starch content on the structure and stability of set yoghurt. *Food Hydrocolloids, 35*, 576-582. https://doi.org/10.1016/j.foodhyd.2013.07.018
Dai, S., Corke, H., & Shah, N. P. (2016). Utilization of konjac glucomannan as a fat replacer in low-fat and skimmed yogurt. *Journal of dairy science, 99*(9), 7063-7074. https://doi.org/10.3168/jds.2016-11131
Farnsworth, J., Li, J., Hendricks, G., & Guo, M. (2006). Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. *Small Ruminant Research, 65*(1-2), 113-121. https://doi.org/10.1016/j.smallrumres.2005.05.036
Gee, V. L., Vasanthan, T., & Temelli, F. (2007). Viscosity of model yogurt systems enriched with barley β-glucan as influenced by starter cultures. *International dairy journal, 17*(9), 1083-1088. https://doi.org/10.1016/j.idairyj.2007.01.004
Graveland-Bikker, J. F., & Anema, S. G. (2003). Effect of individual whey proteins on the rheological properties of acid gels prepared from heated skim milk. *International dairy journal, 13*(5), 401-408. https://doi.org/10.1016/S0958-6946(02)00190-5

Guggisberg, D., Cuthbert-Steven, J., Piccinali, P., Bütikofer, U., & Eberhard, P. (2009). Rheological, microstructural and sensory characterization of low-fat and whole milk set yoghurt as influenced by inulin addition. *International dairy journal, 19*(2), 107-115. https://doi.org/10.1016/j.idairyj.2008.07.009

Guven, M., Yasar, K., Karaca, O., & Hayaloglu, A. (2005). The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture. *International journal of dairy technology, 58*(3), 180-184. https://doi.org/10.1111/j.1471-0307.2005.00210.x

Han, X., Yang, Z., Jing, X., Yu, P., Zhang, Y., Yi, H., & Zhang1, L. (2016). Improvement of the Texture of Yogurt by Use of Exopolysaccharide Producing Lactic Acid Bacteria. *BioMed Research International*. https://doi.org/10.1155/2016/7945675

Heydari S, Mortazavian AM, Ehsani MR, Mohammadifar MA, & H., E. (2011). Biochemical, microbiological and sensory characteristics of probiotic yogurt containing various prebiotic compounds. *Italian Journal of Food Science, 23*, 153–164.

Karimi, R., Mortazavian, A., & Karami, M. (2012). Incorporation of Lactobacillus casei in Iranian ultrafiltered Feta cheese made by partial replacement of NaCl with KCl. *Journal of dairy science, 95*(8), 4209-4222. https://doi.org/10.3168/jds.2011-4872

Losasso, C., Cibin, V., Cappa, V., Roccato, A., Vanzo, A., Andrighetto, I., & Ricci, A. (2012). Food safety and nutrition: Improving consumer behaviour. *Food Control, 26*(2), 252-258. https://doi.org/10.1016/j.foodcont.2012.01.038

Meyer, D., & Blaauwhoed, J.-P. (2009). Inulin. In *Handbook of hydrocolloids* (pp. 829-848): CRC Press, New York: Washington, DC.

Modzelewska-kapitula, M., & Klebukowska, L. (2009). Investigation of the potential for using inulin HPX as a fat replacer in yoghurt production. *International journal of dairy technology, 62*(2), 209-214. https://doi.org/10.1111/j.1471-0307.2009.00481.x

Mortazavian, A., Khosrokhavar, R., Rastegar, H., & Mortazaei, G. (2010). Effects of dry matter standardization order on biochemical and microbiological characteristics of freshly made probiotic Doogh (Iranian fermented milk drink). *Italian Journal of Food Science, 22*(1).

Nguyen, P. T. M., Kravchuk, O., Bhandari, B., & Prakash, S. (2017). Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. *Food Hydrocolloids, 72*, 90-104. https://doi.org/10.1016/j.foodhyd.2017.05.035

Pang, Z., Deeth, H., Prakash, S., & Bansal, N. (2016). Development of rheological and sensory properties of combinations of milk proteins and gelling polysaccharides as potential gelatin replacements in the manufacture of stirred acid milk gels and yogurt. *Journal of food engineering, 169*, 27-37. https://doi.org/10.1016/j.jfoodeng.2015.08.007

Paseephol, T., Small, D. M., & Sherkat, F. (2008). Rheology and texture of set yogurt as affected by inulin addition. *Journal of Texture Studies, 39*(6), 617-634. https://doi.org/10.1111/j.1745-4603.2008.00161.x

Pieniak, Z., Pérez-Cueto, F., & Verbeke, W. (2009). Association of overweight and obesity with interest in healthy eating, subjective health and perceived risk of chronic diseases in three European countries. *Appetite, 53*(3), 399-406. https://doi.org/10.1016/j.appet.2009.08.009

Puvanenthiran, A., Williams, R., & Augustin, M. (2002). Structure and visco-elastic properties of set yoghurt with altered casein to whey protein ratios. *International dairy journal, 12*(4), 383-391. https://doi.org/10.1016/S0958-6946(02)00033-X

Radi, M., Niakousari, M., & Amiri, S. (2009). Physicochemical, textural and sensory properties of low-fat yogurt produced by using modified wheat starch as a fat replacer. *Journal of Applied Sciences, 9*(11), 2194-2197.

Remeuf, F., Mohammed, S., Sodini, I., & Tissier, J. (2003). Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yogurt. *International dairy journal, 13*(9), 773-782. https://doi.org/10.1016/S0958-6946(03)00092-X

Rezaei, R., Khomeiri, M., Aalami, M., & Kashaninejad, M. (2014). Effect of inulin on the physicochemical properties, flow behavior and probiotic survival of frozen yogurt. *Journal of food science and technology, 51*(10), 2809-2814. https://doi.org/10.1007/s13197-012-0751-7

Robinson, R., & Itsaranuwat, P. (2006). Properties of yoghurt and their appraisal. In A. Tamime (Ed.), *Fermented milks* (pp. 76-94): Blackwell publishing, Oxford, UK.

Rybak, O. (2016). Milk fat in structure formation of dairy products: a review. *Ukrainian food journal*(5, 3), 499-514.

Staffolo, M. D., Bertola, N., & Martino, M. (2004). Influence of dietary fiber addition on sensory and rheological properties of yogurt. *International dairy journal, 14*(3), 263-268. https://doi.org/10.1016/j.idairyj.2003.08.004

Steffe, J. F. (1996). *Rheological methods in food process engineering*: Freeman press, East landing, USA.

Tan, J. (2019). Structuring Semisolid Foods. In H. S. Joyner (Ed.), *Rheology of Semisolid Foods* (pp. 167-201). Cham: Springer International Publishing, Switzerland AG.

Tavakolipour, H., Vahid-moghadam, F., & Jamdar, F. (2014). Textural and sensory properties of low fat concentrated flavored yogurt by using modified waxy corn starch and gelatine as a fat replacer. *International Journal of Biosciences, 5*(6), 61-67.

Vasiljevic, T., Kealy, T., & Mishra, V. (2007). Effects of β-glucan addition to a probiotic containing yogurt. *Journal of Food Science, 72*(7), C405-C411. https://doi.org/10.1111/j.1750-3841.2007.00454.x

Vélez-Ruiz, J. F. (2019). Rheological characterization and pipeline transport needs of two fluid dairy products (Flavored milk and yogurt). In *Milk-Based Beverage, Volume 9: The Science of Beverages* (pp. 427-472): Elsevier.

Yancheshmeh, B.S., Marvdashti, L.M., Emadi, A., Abdolshahi, A., Ebrahimi, A. Shariatifar, N. (2022). Evaluation of Physicochemical and Functional Properties of Vicia villosa Seed Protein. *Food Analytical Methods*, 15, 1187–1202. https://doi.org/10.1007/s12161-021-02185-z

Zhang, T., McCarthy, J., Wang, G., Liu, Y., & Guo, M. (2015). Physiochemical properties, microstructure, and probiotic survivability of nonfat goats' milk yogurt using heat-treated whey protein concentrate as fat replacer. *Journal of Food Science, 80*(4), M788-M794. https://doi.org/10.1111/1750-3841.12834