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Effect of enzymatic treatment of microbial transglutaminase and addition of gelatin and carrageenan on some physicochemical properties of low-fat set yogurt

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ABSTRACT

Obesity increases the risk of many serious medical problems, such as type 2 diabetes, cardiovascular problems, high blood pressure, and some cancers. Therefore, consumption of low-fat dairy products, including low-fat yogurt, has attracted the attention of consumers due to their lower calorie and saturated fat content. However, the most important problem in producing these products is their poorer flavor and texture than similar high-fat products. These problems can probably be overcome by adding hydrocolloids such as gelatin and carrageenan as well as microbial transglutaminase (TG) enzymatic treatment, allowing the production of healthier products without compromising consumer acceptance. In this study, the effect of TG enzymatic treatment (at two levels of 0 and 0.015%) and two hydrocolloids gelatin (levels of 0, 0.5 and 1%) and carrageenan (0, 0.01, 0.02 and 0.03%) on the physicochemical properties of low-fat set yogurt during storage was investigated. The findings indicated that all three variables significantly improved the physicochemical properties; while reducing the amount of syneresis in low-fat yogurt samples, increased the viscosity of the product. In addition, these properties also improved with the passage of storage time. Based on the results of this study, it was determined that the best sample was obtained using TG enzymatic treatment (0.015%) and the use of 0.5% gelatin and 0.02% carrageenan.

1- Introduction

Yogurt is a fermented dairy product produced by the action of two lactic acid bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* [1, 2]. Unlike rennet-coagulated cheeses, whose texture derives from enzymatic coagulation, the consistency of fermented milks such as yogurt results primarily from acidification by lactic acid bacteria. Yogurt is the most widely consumed fermented dairy product globally [3]. However, the most common defect affecting consumer acceptance is whey separation (syneresis) [4]. Other key textural attributes influencing sensory quality include firmness, viscosity, and creaminess [5]. Syneresis in yogurt is directly influenced by factors such as the quality of the raw milk and processing conditions; inadequate control of pH and incubation temperature can disrupt the protein-micelle network and increase whey separation [3]. The International Dairy Federation (IDF) defines fermented milks as products obtained by milk fermentation through lactic acid bacterial activity [6].

Hydrocolloids are high–water-retention substances that, even at low concentrations (typically <1%), exert strong effects on the rheology and texture of food systems. Their principal functional properties include thickening (viscosity increase), gelling, stabilization of colloidal systems, inhibition of oil uptake, emulsification, film and edible-coating formation, moisture retention, and reduction of water mobility [7]. Sources of hydrocolloids include microbial products (e.g., xanthan gum), seed gums (e.g., guar gum, locust bean gum), tree exudates (e.g., gum arabic), fruit-derived pectins, animal-derived gelatin, algal polysaccharides (e.g., carrageenan and alginate), cellulose derivatives, and modified starches [8]. Because hydrocolloids absorb and bind water and can mimic fat by forming gel-like networks, they are widely used as fat replacers in low-fat dairy and meat products to recreate a fat-like mouthfeel [9].

Gelatin, derived by partial hydrolysis/thermal denaturation of animal collagen (from Latin *gelatus*, “frozen” or “congealed”), is an important food hydrocolloid. Gelatin gels are polyelectrolytic and their electrostatic interactions—and thus gel properties—are sensitive to pH and salt composition, owing to the presence of both cationic and anionic protein groups [10]. Gelatin is a popular natural polymer in the food industry due to its functional properties and has been proposed as a suitable substitute for yogurt fat to improve texture and mouthfeel [11].

Carrageenan, an extract from red seaweeds, is another widely used edible hydrocolloid with emulsifying, gelling, foam-stabilizing, thickening, and texture-improving functions [12]. Three principal forms—kappa (κ), iota (ι), and lambda (λ) carrageenan—consist of repeating galactose and anhydrogalactose units whose degree of sulfation and sequence determine functional characteristics such as gel strength. Carrageenan forms thermoreversible gels whose melting and setting behavior depend on the type and concentration of cations present, particularly potassium and ammonium [13,14].

Enzymes are also used in the food industry to enhance quality, nutritional value, and process efficiency across diverse products [15–17]. Microbial transglutaminase (TG; EC 2.3.2.13) is a transferase produced by Streptomyces species; it is typically a ~38 kDa protein of approximately 331 amino acids and is commercially obtained from strains such as Streptomyces mubarensis [18]. TG catalyzes acyl-transfer reactions between the γ-carboxamide group of glutamine residues and the ε-amino group of lysine residues in proteins, forming ε-(γ-glutamyl)lysine cross-links [19]. Cross-linking and gelation of food proteins by TG can modify physicochemical and functional properties—such as adhesion, gel strength, emulsification, and shaping—thereby affecting product quality [20].

Numerous studies have evaluated the effects of transglutaminase (TG) and hydrocolloids, alone or in combination, on the physicochemical, rheological, and sensory properties of diverse dairy products. Reported improvements include dairy desserts [21,22], set yogurts [23,24], stirred yogurts [16,25], condensed or strained yogurts

[3,26], semi-dairy yogurts [27,28], ice cream [2,20], kefir [29,30], ultrafiltrated (UF) cheeses [31,32], and traditional cheeses [33,34]. Yademellat et al. investigated the effect of Persian gum and Shirazi balango seed incorporation on the physicochemical and sensory attributes of low-fat stirred yogurt [35]; yogurts were compared to a low-fat stirred control over storage (days 1, 11, and 21). Addition of both gums significantly increased titratable acidity ($p < 0.05$) without significantly affecting pH, and higher gum concentrations—particularly Persian gum—reduced syneresis over storage. Torabi et al. (2021) reported that processed cheese treated with TG exhibited superior textural scores during 60 days of storage compared with probiotic cheeses (without TG) and a commercial control (without TG and inulin) [36]. Aryamanesh et al. (1403) examined the improvement of low-fat stirred yogurt by combined treatment with microbial TG and Persian gum [37]; their findings showed that gum and enzyme addition significantly reduced syneresis and acidity, although storage time increased acidity and decreased syneresis. Specifically, application of TG (0.015%) with Persian gum (notably at 0.2%) produced low-fat stirred yogurt with acceptable physicochemical characteristics. Despite these studies, to our knowledge no research has addressed the combined use of TG, gelatin, and carrageenan to improve set yogurt properties. Therefore, the present study was undertaken to evaluate the effects of transglutaminase, gelatin, and carrageenan on the quality of set yogurt over 14 days of storage.

2- Materials and methods

2-1- Materials

Low-fat set yogurt samples were prepared using low-fat pasteurized milk with 1.5% fat (Arjan Company, Shiraz). In this study, microbial transglutaminase (TG) enzyme with an average activity of 100 units per gram extracted from *Streptoverticillium mubaranis* (Agintomoto Company, France), non-fat dry milk powder (Pegah Khorasan Dairy Company), gelatin (Merck, Germany), and kappa-carrageenan with the trade name Genogel (CPK, Denmark) were used. Also, yogurt starter (YoFlex-Express 1.0, Christian Hansen, Denmark) of DVS type and containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* bacteria was used. The chemicals used in this study, which had a high degree of purity, were purchased from Merck (Germany).

2-2- Method for preparing set yogurt samples

Low-fat set yogurt samples (1.5% fat) were produced following Jooyandeh et al. [23]. Production took place at Arjan Production Company (Shiraz Industrial City) using low-fat fresh milk with titratable acidity of 14.5 ± 0.2 °D. For formulations containing carrageenan (C), carrageenan was added to the milk prior to heat treatment at four concentrations: 0, 0.01, 0.02, and 0.03% (w/w). For formulations containing gelatin (G), gelatin powder was added to milk at 50 °C and hydrated in a hot-water bath at the same temperature; gelatin levels were 0, 0.5, and 1% (w/w). After hydrocolloid addition and hydration, samples were homogenized using a laboratory homogenizer at ~150 bar. Homogenized milks were heat treated at 90 °C for 10 min, then cooled to the inoculation temperature (45 °C). In enzyme-treated samples, microbial transglutaminase (TG) was added and mixed immediately prior to starter inoculation; the yogurt starter was then added at 0.05% (w/w). Following inoculation, milk was promptly aliquoted into 100 mL containers and incubated at 43 °C for approximately 4 h, until pH reached 4.5. After incubation, yogurts were stored at 7 °C. Physicochemical and sensory analyses were conducted on days 1, 7, and 14 of refrigerated storage. All sample productions and analyses were performed in triplicate. A sample without TG and without hydrocolloids served as the control. Selected enzyme and hydrocolloid levels were determined from preliminary experiments.

2-3- Physicochemical evaluation of yogurt samples

2-3-1- pH and acidity measurement

The pH and acidity of yogurt samples were measured using the AOAC method [38]. To

perform this test, the pH meter was first calibrated with buffers 7 and 4, and then the probe of the device was completely inserted into the sample, and after the device number stabilized, the pH value was read and noted. The titration method was used to measure acidity. In this method, a 1:1 ratio of the sample and distilled water was poured into a glass beaker, and then 2 to 3 drops of phenolphthalein reagent were added as an indicator. The mixture was titrated with 0.1 N sodium hydroxide solution until a pale pink color appeared. The volume of sodium hydroxide used was noted, and equation (1) was used to calculate the percentage of acidity in terms of lactic acid.

$$Acidity\ (\%) = \frac{N \times 0.09 \times 100}{M}$$ Equation (1)

2-3-2- Syneresis measurement

To measure the amount of syneresis, the method of Momenzadeh et al. [4] was used with some modifications. 30 to 40 g of yogurt sample was weighed into centrifuge tubes and then the tubes were centrifuged in a refrigerated centrifuge (Eppendorf, model R-5804, Germany) at 222 x g for 10 minutes at 10°C. After centrifugation, the released serum, which was located at the top of the test tube (a special centrifuge tube), was poured into an Erlenmeyer flask and the weight of the clear liquid was measured. Using equation (2) and dividing the amount of released serum by the amount of initial yogurt, the amount of syneresis was calculated as a percentage.

$$Syneresis\ (\%) = \frac{Amount\ of\ seperated\ whey}{Amount\ of\ yogurt\ sample} \times 100$$ Equation (2)

2-4- Measurement of apparent viscosity

The viscosity of the yogurt samples was measured by a Brookfield viscometer (model DVII+, USA) based on the method of Beirami et al. [29] with some modifications. In this test, considering the viscosity of the product and the viscosity range in different samples, the L4 spindle was selected as the appropriate spindle. The viscosity of the samples was measured during 14 days of storage under the same conditions with a temperature of 4°C and a speed of 30 rpm after 30 seconds of spindle rotation.

2-5- Sensory evaluation

The overall acceptance of the yogurt samples was evaluated by a panel of 20 evaluators based on sensory characteristics of color, aroma, flavor, and texture, and the samples were compared using a 9-point hedonic test. In this test, the samples were randomly given to the evaluators along with a sensory evaluation questionnaire, and they expressed their opinions on the samples on a scale of 0 to 9. Before evaluation, the samples were removed from the refrigerator for 30 minutes and kept at room temperature so that the temperature of all samples was the same during evaluation and did not affect the sensory results.

2-6- Statistical analysis

In this study, 24 low-fat set yogurt treatments were produced with 3 variables: carrageenan gum (4 levels), gelatin (3 levels), and TG enzyme (2 levels). All tests were performed in 3 storage periods of 1, 7, and 14 days in 3 replicates. The mean results were analyzed by SPSS version 20 software using a completely randomized design in a factorial format and Duncan's multiple range test at the 5% level. One-way analysis of variance was used to compare the means of the 24 treatments in this study without considering the effect of storage time.

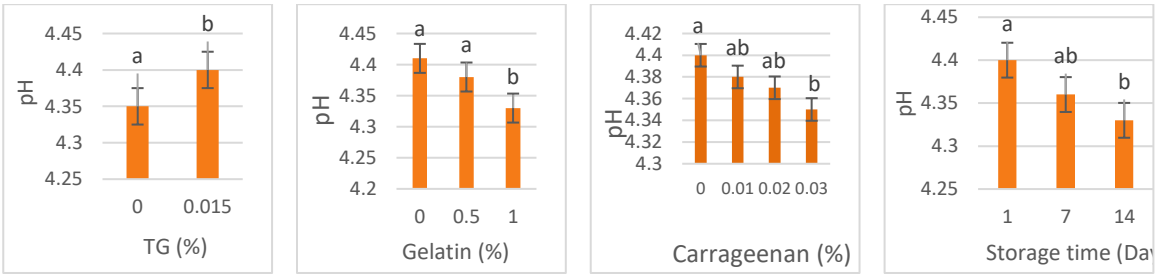
3- Results and discussion

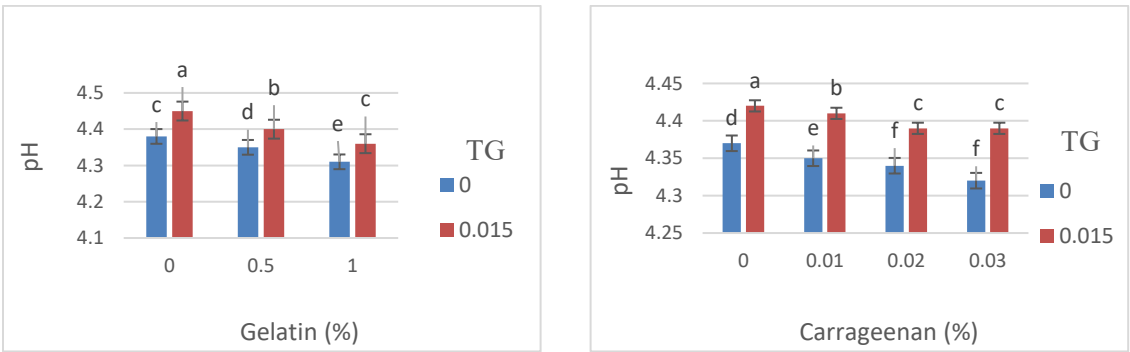
3-1- pH and acidity of yogurt

The results of this study showed that all the variables studied had a significant effect on the pH and acidity of the yogurt samples. As can be seen in Figure 1, with enzyme treatment, the pH of the yogurt samples increased significantly compared to the control sample ($p<0.001$). Also, the changes in the acidity of the samples (Figure 2) showed that with increasing the percentage of enzyme, the acidity decreased significantly from 0.88% lactic acid (control sample) to 0.82%. The reason for the increase in pH and decrease in acidity of the yogurt samples is probably related to the establishment of

extra- and intramolecular covalent bonds between proteins by the TG enzyme. By establishing cross-links, peptides and amino acids required for the growth of microorganisms are trapped by the TG enzyme and become inaccessible to them. This slows down microbial activity and somewhat increases the pH and decreases the acidity [37 and 39]. Aprodu et al. [40], in a study, stated that the pH of enzyme-treated samples was higher than the control sample. As a result, the fermentation time in enzyme-containing samples to reach the final pH was higher and this time was directly related to the enzyme concentration. According to the pH, their acidity was also affected. These results were also consistent with the results of Ozer et al. [41]. However, the results of the research of Wróblewska et al. showed that the TG enzyme did not affect the pH of the produced product and did not cause any significant change in the rate of acid production [42].

According to Figure 1, with increasing gelatin percentage, the pH of yogurt samples decreased significantly ($p<0.001$); so that the control sample had the highest pH value of 4.41 and the sample with 1% gelatin had the lowest pH value of 4.33. Also, according to Figure 2, the changes in the acidity of the samples showed that with increasing gelatin percentage, the acidity increased significantly from 0.82% lactic acid (control sample) to 0.88%. The decrease in pH is probably due to the better activity of starter bacteria in the presence of gelatin (as a carbon source or dry matter) and as a result, more acid production. Supavititpatana et al. [43] reported that with increasing gelatin concentration, the acidity of the product increased significantly. In addition, with increasing carrageenan, the pH of yogurt samples decreased significantly ($p<0.001$). So that the control sample with 4.40 had the highest pH and the sample with 0.3% carrageenan had the lowest pH with 4.35. Also, the changes in the acidity of the samples showed that with increasing gelatin percentage, the acidity increased significantly from 0.84% lactic acid (control sample) to 0.87%. The results of Milani and Koocheki [44], similar to the present results, showed that adding guar gum to frozen yogurt causes an increase in acidity and a decrease in pH.





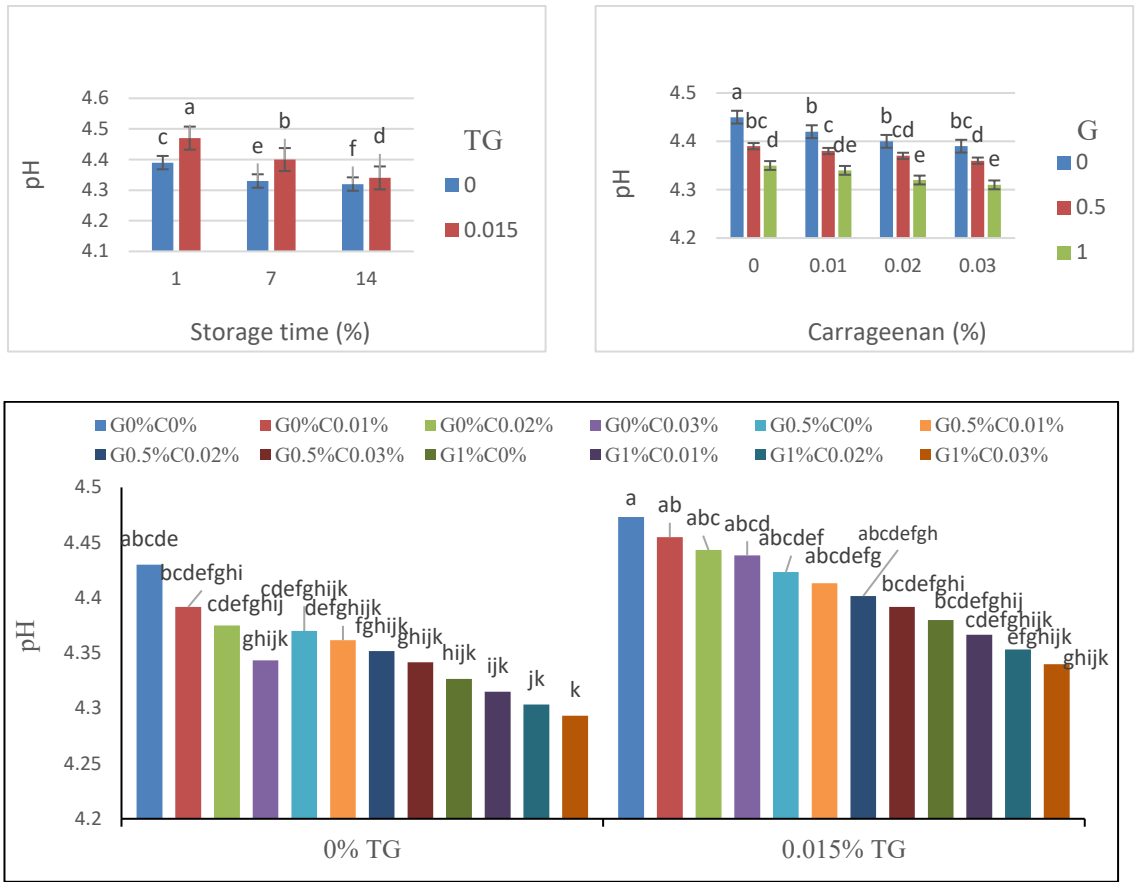
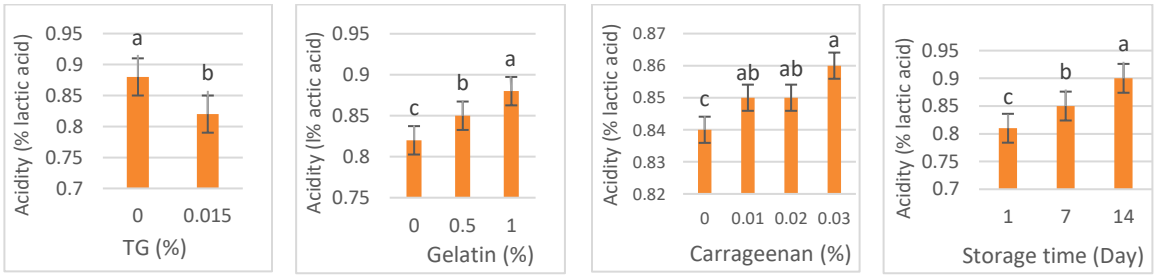


Figure 1. Main and interaction effects of transglutaminase enzyme (TG), gelatin (G) and carrageenan (C) on pH of manufactured yogurt samples during the storage period. Different lowercase letters indicate significant differences at the 5% level ($p<0.05$).



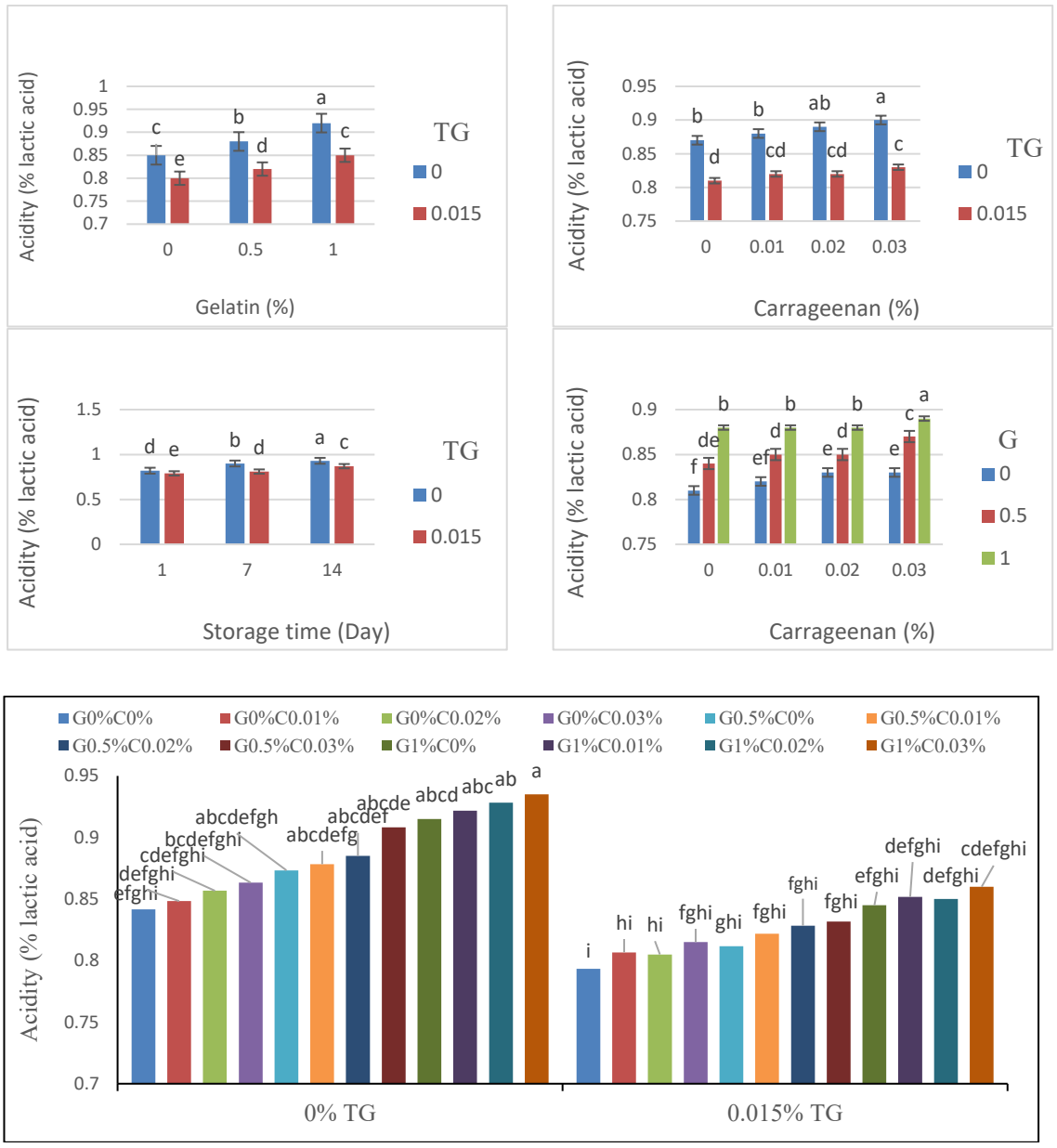


Figure 2. Main and interaction effects of transglutaminase enzyme (TG), gelatin (G) and carrageenan (C) on acidity of manufactured yogurt samples during the storage period. Different lowercase letters indicate significant differences at the 5% level ($p<0.05$).

Figures 1 and 2 show that with increasing storage time, the pH decreased and the acidity increased significantly ($p<0.001$). Considering the passage of storage time and the natural process of yogurt acidification due to the activity of lactic acid bacteria and the production of lactic acid, a decrease in pH is not far from expected. During storage, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are active even at refrigerator temperature and produce lactic acid by fermenting lactose, increasing acidity and

decreasing pH. The reason for the decrease in pH of the samples with time is probably the hydrolysis and conversion of lactose to lactic acid by starter bacteria [45].

According to the results obtained, there was a significant interaction ($p<0.01$) between the two enzyme variables and storage time on the pH and acidity values of the yogurt samples. The highest pH was 4.47 and the lowest acidity was 0.79% lactic acid for the treatment containing the highest enzyme level (0.15%) on the first day of storage; and the lowest pH was 4.32 and the highest acidity was 0.93% lactic acid for the treatment without enzyme on the 14th day of storage. Also, the results of the analysis of variance of the data showed that there was a significant interaction ($p<0.001$) between the two variables enzyme and gelatin on the changes in pH and acidity of the yogurt samples. As can be seen in Figures 1 and 2. The highest pH was 4.45 and the lowest acidity was 0.8% lactic acid for the gelatin-free sample with 0.15% enzyme. The lowest pH was 4.31 and the highest acidity was 0.92% lactic acid for the enzyme-free treatment with 1% gelatin. Also, a significant interaction effect was observed between the two variables of enzyme percentage and carrageenan on the pH and acidity values of yogurt samples ($p<0.05$). With the increase of both variables, the pH of the samples decreased and their acidity increased, but these changes were more significant with the addition of carrageenan. The highest pH with 4.42 and the lowest acidity with 0.81% lactic acid was assigned to the treatment without carrageenan with 0.15% enzyme, and the lowest pH with 4.32 and the highest acidity with 0.9% lactic acid was assigned to the treatment containing 0.3% carrageenan and without enzyme (results not shown). Regarding the interaction effect between the two variables of gelatin percentage and carrageenan on the pH and acidity values of yogurt samples (according to Figures 1 and 2), a significant interaction effect was observed between these two variables on the pH and acidity values of yogurt samples ($p<0.05$). The highest pH was 4.45 and the lowest acidity was 0.81% lactic acid for the control treatment, while the lowest pH was 4.32 and the highest acidity was 0.89% lactic acid for the 1% gelatin and 0.3% carrageenan treatments.

Based on the results of the mean data analysis, the interaction between the two variables gelatin percentage and storage time on the pH and acidity values of the yogurt samples was significant ($p<0.05$). The highest pH was 4.46 and the lowest acidity was 0.78% lactic acid for the control treatment on the first day of storage, while the lowest pH was 4.29 and the highest acidity was 0.9% lactic acid for the 1% gelatin treatment on the 14th day of storage. Also, according to the obtained results, a significant interaction effect ($p<0.05$) between the two variables carrageenan and storage time on changes in pH and acidity of yogurt samples was determined. The highest pH with 4.47 and the lowest acidity with 0.8% lactic acid was obtained for the control sample on the first day of storage. Also, the lowest pH with 4.32 at the end of the storage period and the highest acidity with 0.91% lactic acid was observed for the treatment containing 0.3% carrageenan (Figures 1 and 2).

According to the results of the analysis of variance, the interaction effects of the three variables enzyme × gelatin × carrageenan on the changes in pH and acidity of the yogurt samples were significant ($p<0.05$). As can be seen in Figure 1, the highest pH was observed with a value of 4.47 for the control treatment and the lowest with a value of 4.29 for the sample treated with TG enzyme and containing 1% gelatin and 0.3% carrageenan. On the other hand, the lowest acidity was observed with a value of 0.79% lactic acid for the control treatment and the highest with a value of 0.94% lactic acid for the sample treated with TG enzyme and containing 1% gelatin and 0.3% carrageenan (Figure 2).

3-2- Syneresis (whey separation) of yogurt

One factor strongly affecting consumer acceptance of yogurt is whey separation (syneresis), defined as the appearance of whey on the surface of a gel (e.g., set yogurt) caused by contraction of the gel matrix. Common contributors to syneresis include excessively high incubation temperatures, high whey-protein-to-casein ratios, low total solids, and mechanical damage during storage or distribution [46]. Strategies to reduce syneresis include

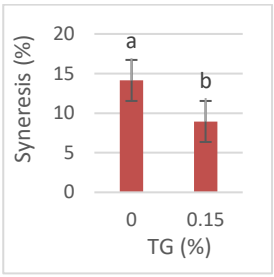
enrichment of dry matter or protein content and incorporation of hydrocolloids such as gelatin, starches, and various gums; protein cross-linking to stabilize the three-dimensional acid gel network produces a similar stabilizing effect [47]. Figure 3 presents the main and interaction effects of the tested variables on yogurt viscosity. Increasing transglutaminase (TG) concentration significantly reduced syneresis ($p < 0.001$). TG catalyzes formation of permanent ε-(γ-glutamyl)-lysine cross-links between milk proteins, which decreases gel permeability and reduces pore size in the yogurt network [48]. The resulting denser, finer-pored structure traps more free water and improves water-holding capacity, thereby reducing whey separation [27,37]. Several earlier studies have likewise reported improved texture and decreased syneresis after TG treatment [23,39]. Gelatin addition also produced a significant reduction in syneresis ($p < 0.001$); mean syneresis decreased markedly with increasing gelatin concentration (from 14.13% to 8.95%). This effect is largely attributable to gelatin's interaction with casein: gelatin can bind to the casein matrix and associate with milk protein aggregates, producing an interconnected binary network that more effectively retains the aqueous phase and limits whey release [49].

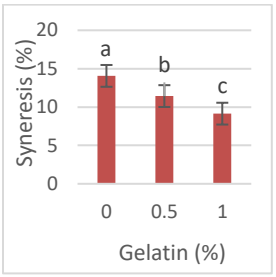
According to Figure 3, increasing gelatin significantly reduced the syneresis of yogurt samples ($p<0.001$), so that the average syneresis of the samples decreased from 12.33% to 10.69%. The reason for the reduction in syneresis with the addition of carrageenan hydrocolloid to yogurt can be attributed to the absorption of water by the polysaccharides forming the hydrocolloid, which leads to the creation of a denser gel network and, as a result, the reduction in the water content of yogurt. Increasing the concentration of hydrocolloid and its increased binding to protein molecules may create larger protein aggregates, which ultimately leads to an increase in bound water and a decrease in syneresis [25, 34]. Also, the results of data analysis showed that the storage time has a significant effect on the syneresis of yogurt samples ($p<0.001$). As can be seen in Figure 3, the average syneresis of the samples decreased from 18.50% on the first day to 5.12% on the fourteenth day. Storage time was evaluated as an effective factor on syneresis. It is possible that the change in the arrangement of proteins and the increase in the side connections of proteins lead to stronger bonding with hydrocolloids and consequently reduce syneresis. Also, during fermentation, due to the rapid decrease in pH, the protein network is formed irregularly and non-uniformly, as a result of which hydrophobic bonds are located on the surface of the gel network and ultimately cause an increase in syneresis at the end of fermentation. However, during the storage period, there will be enough time to rearrange the gel network of yogurt and increase the water retention capacity. This could be one of the reasons for the decrease in syneresis of yogurt during the storage period [50]. A decrease in syneresis in yogurt during storage has been reported by researchers [41 and 47]. The decrease in water loss during shelf life could be due to the increase in total dry matter of yogurt due to the gradual evaporation of moisture from the product [46]. However, contrary to the results of this study, a significant increase in syneresis in yogurt during storage has also been reported [45].

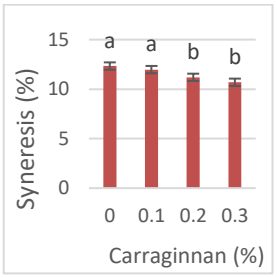
The results of this study showed that there was a significant interaction between each of the two tested variables in terms of synergism. In the case of enzyme and gelatin, it was determined that the highest synergism (17.12%) was related to the control sample and the lowest (6.95%) was related to the sample treated with enzyme and containing 1% gelatin ($p<0.001$). In the case of the two variables enzyme and carrageenan, a significant interaction was also determined on the amount of water loss of the yogurt samples ($p<0.05$). With an increase in both variables, the amount of water loss of the samples decreased, so that the highest water loss with a value of 15.11% was allocated to the control treatment and the lowest water loss with a value of 16.8% was allocated to the treatment containing 0.3% carrageenan and 0.15 enzyme. The results of the interaction between enzyme and storage time also showed a significant interaction between these two variables on the amount of water loss of the yogurt samples ($p<0.01$). The highest synergism was obtained with a value of 19.7% for the control treatment on the first day of

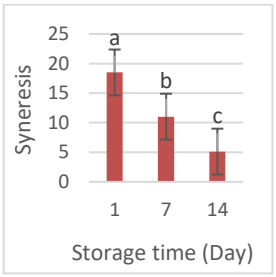
storage and the lowest with a value of 16.3% for the sample treated with 0.015 enzyme on the 14th day of storage. Also, the interaction effect of the two variables gelatin and carrageenan on the water-holding of yogurt samples was significant ($p<0.05$) and the highest synergism was obtained with a value of 14.91% for the control treatment and the lowest with a value of 33.8% for the treatment containing 1% gelatin and 0.3% carrageenan.

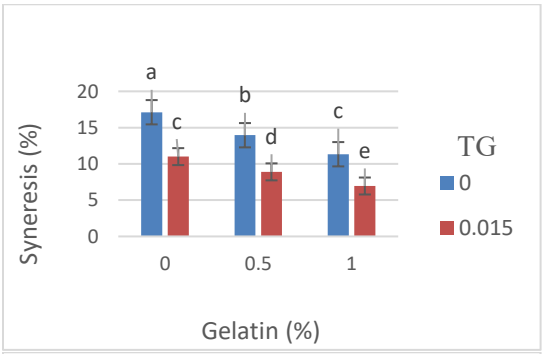
Interactions between hydrocolloid level and storage time were also significant ($p < 0.05$). The gelatin × storage-time interaction showed the highest syneresis (21.62%) for the control on day 1 and the lowest (4.00%) for the 1% gelatin treatment on day 14. For the carrageenan × storage-time interaction, the greatest syneresis (19.53%) occurred for the control on day 1 and the lowest (4.66%) for the treatment containing 0.03% carrageenan at the end of storage. Analysis of variance indicated a significant three-way interaction among TG enzyme, gelatin, and carrageenan on syneresis changes ($p < 0.05$). As illustrated in Figure 3, the highest syneresis (18.17%) was observed for the control treatment, whereas the lowest syneresis (6.33%) occurred in the treatment combining 0.15% TG, 1% gelatin, and 0.03% carrageenan.

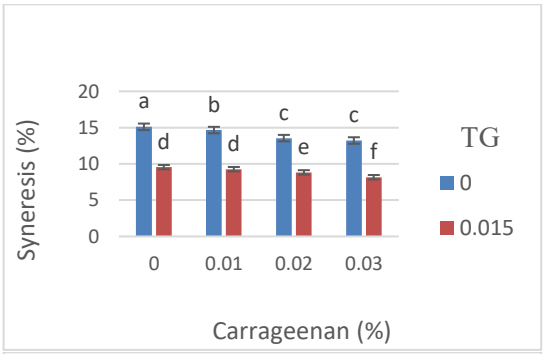


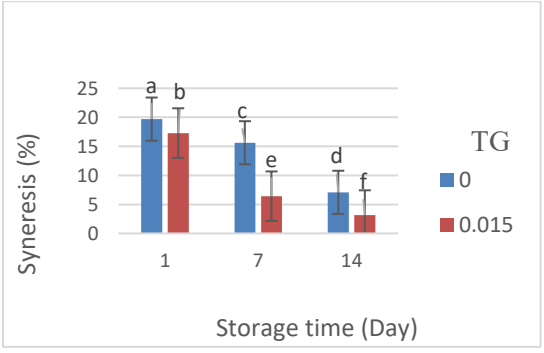


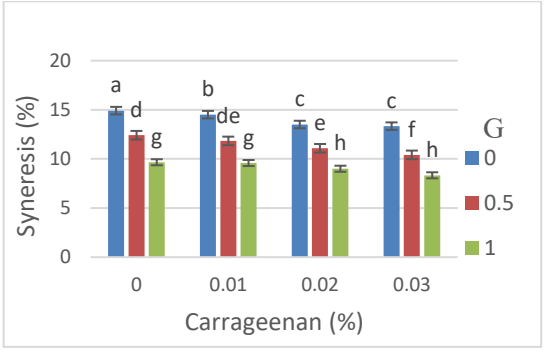












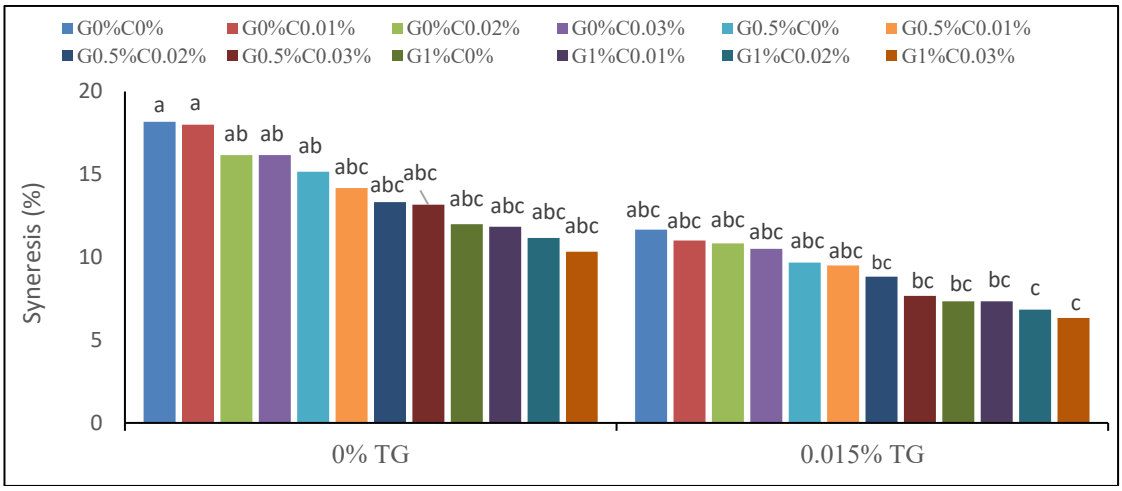


Figure 3. Main and interaction effects of transglutaminase enzyme (TG), gelatin (G) and carrageenan (C) on syneresis of manufactured yogurt samples during the storage period. Different lowercase letters indicate significant differences at the 5% level ($p<0.05$).

According to the results of the analysis of variance, the interaction effects of the three variables enzyme × gelatin × carrageenan on the changes in synergism of yogurt samples were significant ($p<0.05$). As can be seen in Figure 3, the highest synergism was observed with a value of 18.17% for the control treatment and the lowest with a value of 6.33% for the sample treated with TG enzyme and containing 1% gelatin and 0.3% carrageenan.

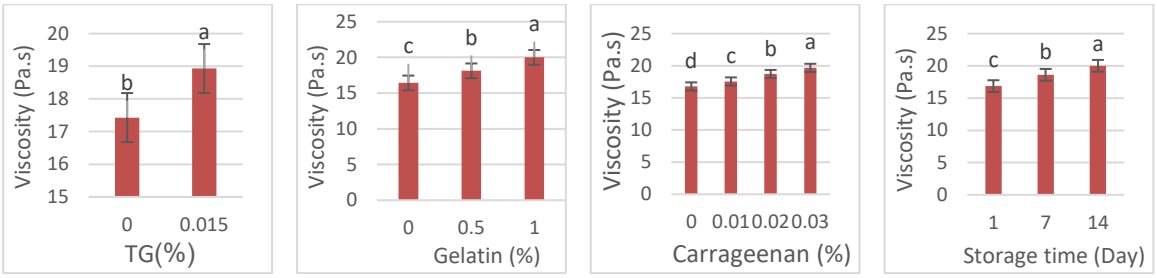
3-3- Viscosity of yogurt

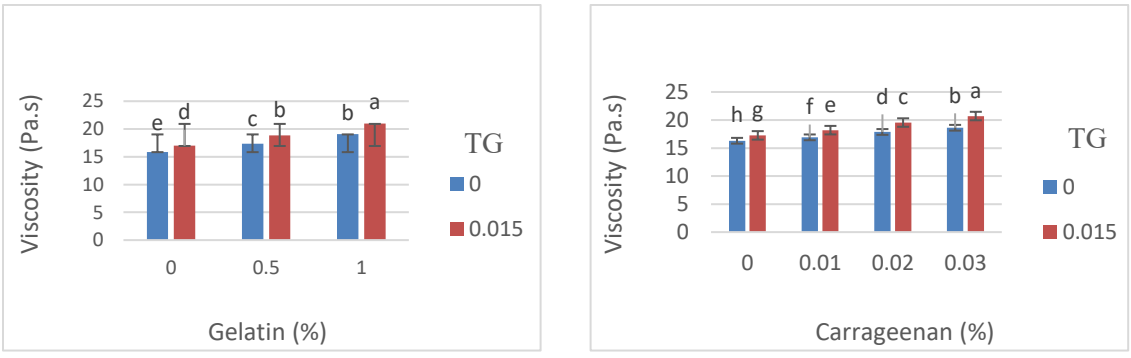
One of the most important factors affecting product quality is viscosity and gel structure, which depend on factors such as milk composition (ratio of casein to whey protein), incubation temperature, type of starter used, and ability to produce exopolysaccharides [42]. Figure 4 shows the effect of different enzyme percentages on the viscosity of yogurt samples. As can be seen, increasing the enzyme significantly increased the viscosity of yogurt samples ($p<0.001$). The ability of the enzyme to form high molecular weight polymers from protein monomers without changing the chemical properties of yogurt increases the viscosity [48]. Lorenzen et al. [47] reported that the increase in gel strength in molded yogurt is due to the modification of milk proteins by transglutaminase. Gauche et al. [51] showed that yogurt samples treated with enzyme before fermentation had higher apparent viscosity. They stated that because there was no significant difference in the amount of solid matter (protein and fat) between the yogurt samples produced under different treatments, the enzyme trans-glutaminase increased the viscosity. Abd-Rabo et al. [52] reported that the enzyme has the ability to form covalent bonds between protein molecules. The addition of enzyme caused faster gel formation compared to enzyme-treated samples, in other words, as a result of the formation of cross-links between glutamine and lysine in enzyme-treated samples, high molecular weight polymers were formed. In addition, as can be seen in Figure 4, the addition of gelatin significantly increased the viscosity of the yogurt samples ($p<0.001$), so that the average viscosity of the samples increased from 16.42 Pa.s to 20.00 Pa.s.

Figure 4 shows the effect of different amounts of carrageenan on the viscosity of yogurt samples. As can be seen, increasing carrageenan significantly increased the viscosity of yogurt samples ($p<0.001$), such that the average viscosity of the samples increased from 16.78 Pa.s to 19.67 Pa.s. Most gums increase viscosity due to their water-absorbing properties, and by increasing their water-binding capacity and stabilizing the physical state of the mixture, they increase the resistance of

the samples to flow [46]. Therefore, it is obvious that increasing the gum concentration also increases the viscosity. Gomez-Diaz and Navaza [53] attributed the synergistic effect of hydrocolloids in changing the behavior of mixtures not to strong chemical bonds between them, but to weak hydrophobic and electrostatic interactions. Also, Milani and Koocheki [44] reported in a study that investigated the effect of date honey and guar gum on the physicochemical, rheological and sensory properties of low-fat frozen yogurt dessert that the viscosity of the mixture increased with increasing the concentration of these two substances. Whey proteins are another important additive used to improve the viscosity of low-fat dairy products, which are used today as fat substitutes [54].

According to Figure 4, storage time had a significant effect on the viscosity of the yogurt samples ($p<0.001$), such that the average viscosity of the samples increased from 16.86 Pa.s to 20.01 Pa.s at the end of the storage period. The increase in viscosity with time during storage in yogurt has been previously reported and the reason for this has been attributed to the rearrangement of proteins and protein linkages over time [39]. As can be seen in Figure 4, a significant interaction was observed between the variables tested. Regarding the interaction of enzyme, gelatin and carrageenan (Figure 4), it was determined that by increasing the amount of both carrageenan and gelatin hydrocolloids and performing transglutaminase enzymatic treatment, the viscosity of the yogurt samples increased significantly, so that the highest viscosity (23.11 Pa.s) was assigned to it. In contrast, the control yogurt sample (without enzyme and hydrocolloid) had the lowest viscosity (14.54 Pa.s).





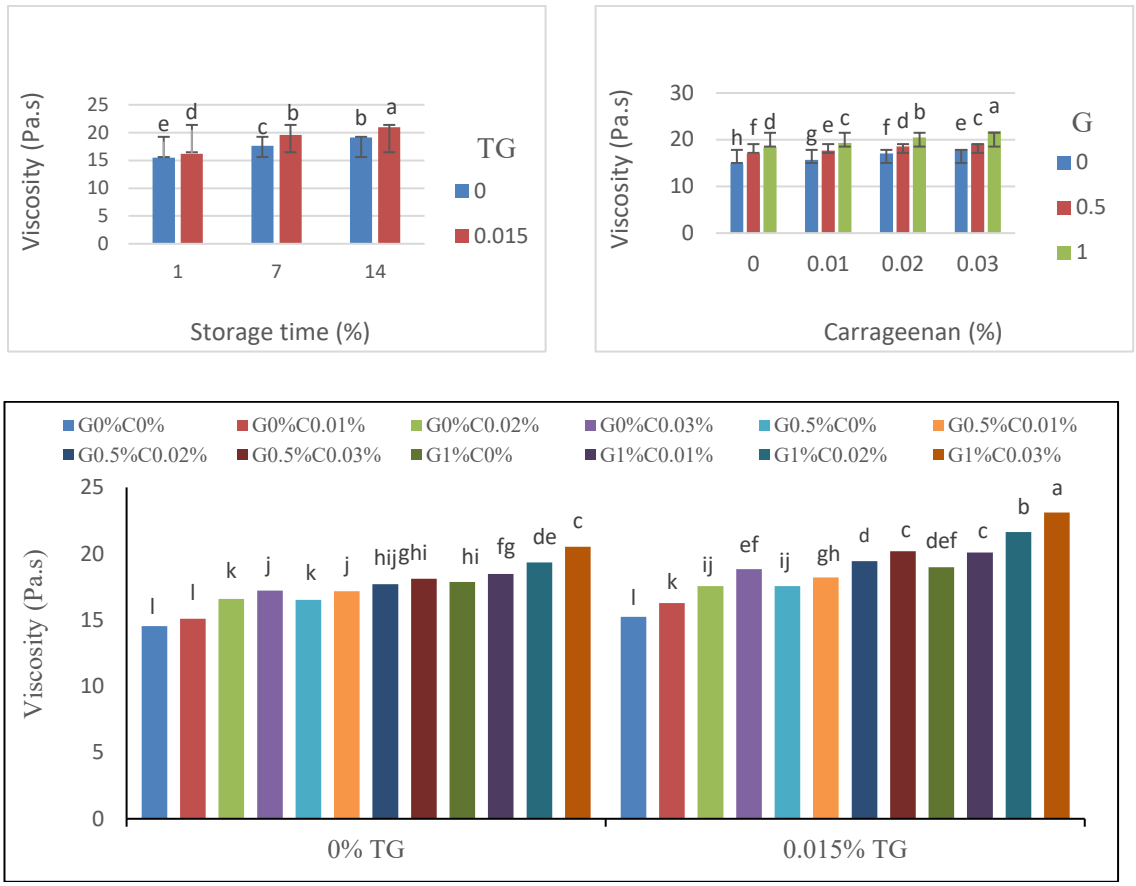


Figure 4. Main and interaction effects of transglutaminase enzyme (TG), gelatin (G) and carrageenan (C) on viscosity of manufactured yogurt samples during the storage period. Different lowercase letters indicate significant differences at the 5% level ($p<0.05$).

3-4- Total acceptability

The results of sensory evaluation showed that the treatments studied, as well as the physicochemical properties, had a significant effect on the overall acceptance of low-fat molded yogurt samples. Although the enzymatic treatment significantly increased the sensory score, however, according to Figure 5, no significant difference was observed in this regard between the samples containing hydrocolloids, and among them, only the sample containing 0.5% gelatin and 0.02% carrageenan had a significant difference with the control sample. In addition, no interaction was observed between the variables studied. In accordance with these results, Danesh et al. reported no significant difference in the overall acceptance score of low-fat Iranian white cheese samples when whey protein isolate was added [55]. Yademellat et al. also reported similar results when producing low-fat stirred yogurt containing Persian gum [35]. The results of the present study also showed that the sensory quality of the product decreased significantly with the passage of storage time. However, all yogurt samples had acceptable sensory quality (score higher than 7) at the end of 14 days of refrigerated storage. Therefore, in a general conclusion of this study, the enzyme-treated sample containing 0.5% gelatin and 0.02% carrageenan was selected as the best yogurt sample.

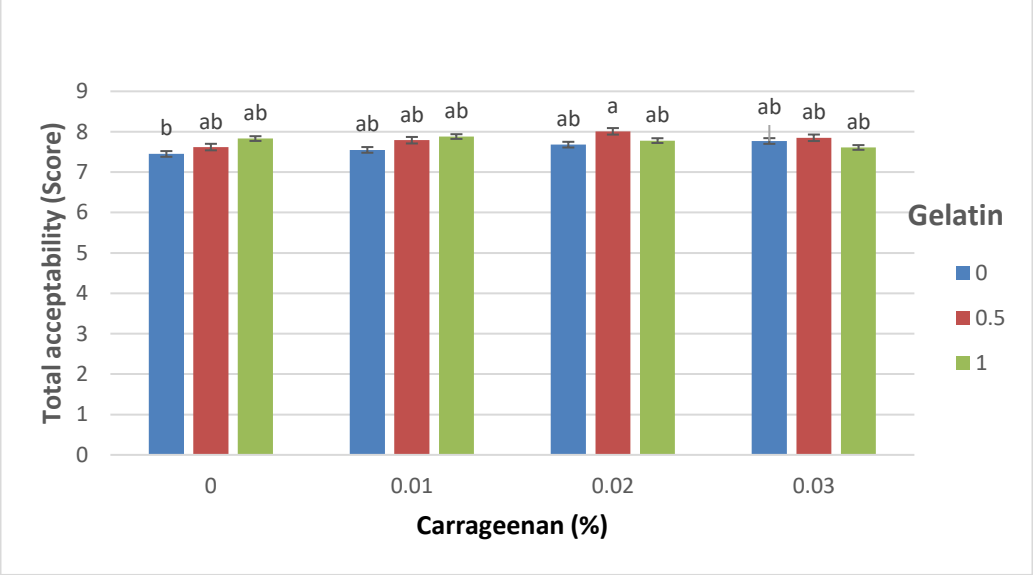


Figure 5. The effects of gelatin (G) and carrageenan (C) on total acceptability of low-fat set yogurt samples. The values are the mean scores of yogurts treated with or without transglutaminase and stored for 14 days. Different lowercase letters indicate significant differences at the 5% level ($p<0.05$).

4- Conclusion

Excessive dietary fat intake is associated with increased risk of diabetes and cardiovascular diseases such as atherosclerosis; consequently, reduction of fat intake and replacement of high-fat foods with low-fat alternatives are recommended. Fat, however, plays a critical role in food products by contributing to flavor and textural attributes. Partial replacement of fat with fat mimetics and increasing total solids—particularly protein—can mitigate the adverse effects of fat reduction. Incorporation of hydrocolloids (e.g., gelatin, starches, and various gums) is a common approach to reduce whey separation in yogurt, while transglutaminase (TG) treatment can stabilize the three-dimensional acid gel network through protein cross-linking, producing a similar effect. This study evaluated the combined effects of TG, gelatin, and carrageenan on the quality of low-fat molded yogurt during 14 days of refrigerated storage. Among hydrocolloid-containing formulations, the lowest syneresis and highest viscosity were observed in samples treated with TG and containing the highest hydrocolloid levels tested (1% gelatin + 0.03% carrageenan). However, these properties did not differ significantly from those of the TG-treated sample containing 0.5% gelatin + 0.02% carrageenan. Considering the significantly higher sensory scores of the lower-hydrocolloid sample versus the control and its economic advantage, the TG-treated formulation with 0.5% gelatin and 0.02% carrageenan was selected as the optimal low-fat molded yogurt.

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Data availability

Data will be made available on request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Author Contributions

Leila Bakhtiari: Data collection, Resources, Writing - original draft.

Hossein Jooyandeh: Conceptualization, Project administration, Investigation, Visualization, Methodology, Validation, Software, Formal analysis, Writing – review & editing.

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مقاله علمی_پژوهشی

بررسی تأثیر تیمار آنزیمی ترانس‌گلوتامیناز میکروبی و افزودن ژلاتین و کاراگینان بر برخی خصوصیات فیزیکوشیمیایی ماست قالبی کم‌چرب

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چکیده

چاقی احتمال ابتلا به بسیاری از بیماری‌های مهم همانند دیابت نوع ۲، مشکلات قلبی عروقی، فشار خون بالا و برخی سرطان‌ها را افزایش می‌دهد. بنابراین مصرف فراورده‌های لبنی کم‌چرب منجمله ماست کم‌چرب به‌دلیل کالری و چربی اشباع‌شدهٔ کمتر مورد توجه مصرف‌کنندگان قرار گرفته است. درهرحال، مهمترین مشکل تولید این محصولات عطر و طعم و بافت ضعیف‌تر نسبت به فراورده‌های مشابه پرچرب است که احتمالاً می‌توان با افزودن هیدروکلوئیدهایی مانند ژلاتین و کاراگینان و همچنین تیمار آنزیمی ترانس‌گلوتامیناز (TG) میکروبی این مشکلات را برطرف نمود و امکان تولید محصولات سالم‌تر را بدون به خطر انداختن مقبولیت مصرف‌کننده فراهم کرد. در این پژوهش، تأثیر تیمار آنزیمی TG (در دو سطح ۰ و ۰/۰۱۵ درصد) و دو هیدروکلوئید ژلاتین (سطوح ۰، ۰/۵ و ۱ درصد) و کاراگینان (۰، ۰/۰۱، ۰/۰۲ و ۰/۰۳ درصد) بر خواص فیزیکوشیمیایی ماست کم‌چرب قالبی طی مدت نگهداری بررسی شد. یافته‌ها حاکی از آن بود که هر ۳ متغیر مورد بررسی به شکل معنی‌داری سبب بهبود ویژگی‌های فیزیکوشیمیایی مورد بررسی گردیدند و ضمن کاهش مقدار سینرزیس در نمونه‌های ماست کم‌چرب، ویسکوزیتهٔ محصول را افزایش دادند. به‌علاوه، با گذشت مدت زمان نگهداری نیز این ویژگی‌ها بهبود یافت. براساس نتایج این تحقیق مشخص گردید که بهترین نمونه با استفاده از تیمار آنزیمی TG (۰/۰۱۵ درصد) و به‌کارگیری ۰/۵ درصد ژلاتین و ۰/۰۲ درصد کاراگینان به‌دست می‌آید.