Optimization of viscoelastic properties of low-fat stirred yogurt using mixture-process variable experiments

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A B S T R A C T

In this study, the effect of different amounts of konjac gum (KG) (0 to 0.2%) and sage seed gum (SSG) (0 to 0.2%) as experimental variables of the mixture design and fat content (0 to 3%) and homogenization rate (0 to 24000 rpm) as experimental variables of the process design on the viscoelastic characteristics of low-fat stirred yogurt were investigated. Based on the results of stress sweep test for different samples, the storage modulus (G') had a value more than the loss modulus (G''), explaining a solid-like behavior of the samples. Also, according to the stress sweep test, G' LVE (G' at linear viscoelastic region), G'' LVE (G'' at linear viscoelastic region), γc (critical strain), τy (yield stress), τf (yield stress at flowing point), and Gf (elastic modulus at flowing point) of the samples were significantly increased, especially for samples containing higher sage seed gum, with increasing fat content and homogenization rate. To optimize the properties in this study, G'' LVE were considered to be minimum and G' LVE, γc, τy and τf were considered to be maximum. According to the mentioned properties, the optimized amount of KG was 0.19%, the amount of SSG was 0.01%, the fat content was 0.59% and the homogenization rate was 12075 rpm.

Keywords: Homogenization rate; Konjac gum; Low-fat stirred yogurt; Sage seed gum; Viscoelastic properties

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1. Introduction

Over the past decade, the consumption of non-fat and low-fat food products has grown rapidly because of the direct association between fat consumption and many diseases, including obesity, cardiovascular diseases such as atherosclerosis and cancer. Therefore, many studies have been conducted to produce low-fat diet products, especially low-fat yogurt. Yogurt is a fermented dairy product with specific textures and rheological properties that are of great importance for consumer acceptance. Yogurt texture is influenced by various factors such as quality and composition of milk, fat, total solids, milk heat process, composition of the lactic acid-producing bacteria used, amount of acid produced, and shelf life of milk (Purwandari et al., 2007). However, the products produced are partial without texture and have good physicochemical properties compared to whole-fat yogurt samples. Therefore, various methods have been developed to replace fat in dairy products such as milk homogenization (Merrill et al., 1994), changing production methods (Perry et al., 1997), and the addition of fat emulator alternatives such as hydrocolloids (Rudan et al., 1998). Hydrocolloids are compounds that create consistency and texture, increase stability, act as an emulsifier, form gels, and improve oral sensation. In fact, hydrocolloids compensate for the low amount of fat by their ability to absorb and bind water and through their texture-giving properties (Guven et al., 2005). Meanwhile, the konjac gum is a neutral polysaccharide originated from the perennial plant belonging to the Araceae family and the Amorphophallus Konjac species and is famous in East Asian countries (Chua et al., 2012). This gum has a dual function, on the one hand, due to its lack of digestibility, it considers a dietary fiber and due to its essential role in weight control, modifying intestinal microbial metabolism, removing free radicals, and inhibiting the growth of occult and advanced rare tumors, it is highly regarded and, on the other hand, due to its high water absorption capacity, it...
is highly regarded as a gel-forming and firming agent in traditional Asian foods (Zhang et al., 2015). Sage is also one of the indigenous seeds of Iran that its gum can be introduced as a new nutritional hydrocolloid because it has extraordinary functional properties such as stabilizing, concentrating and gel-forming agent (Razavi et al., 2013). Combined use of hydrocolloids, due to their synergistic properties, is also widespread in food industry (Kayacier & Dogan, 2007). Because the use of a combination of gums can improve the quality of the product, on the one hand, and reduce the concentration of gum in the formulation, on the other hand. Such reduction will be more cost-effective as well (Walkenstrom et al., 2003). Accordingly, there have been numerous researches focused on the synergistic influence of different gums on model food systems (Demirkesen et al., 2010; Dolz et al., 2007; Mandala et al., 2008).

One of the suggested methods for modifying the characteristics of low-fat yogurt is utilizing the homogenization technique. This technique significantly decreases the size of the fat globules and causes changes in their membrane. In high-fat products, fat globules bind to the protein network and act as binding agents for proteins (Sandoval-Castilla et al., 2003). Therefore, proper homogenization can improve the adverse physicochemical and texture effects of yogurt fat reduction (Aguirre-Mandujano et al., 2009). Various homogenization conditions such as pressure (rotation speed) result in changes in the characteristics of yogurt including consistency, oral feel, color, taste, and its rheological properties. Therefore, it is highly important to select the right pressure (proper rotation speed) to produce a quality product, especially a product that uses a fat replacement.

Therefore, a careful review of the references reveals a separate consideration of the impacts of fat substitutes or process variations, such as the homogenization process in the majority of the published researches on low-fat yogurt. Nevertheless, process variations, particularly homogenization pressure variations, as well as having an impact on yogurt, affect fat substitutes, including hydrocolloids and their functional properties. Hence, such variations are to be simultaneously examined for the proper understanding of the requirements of industrial production in research. Therefore, in this study, the effect of KG, sage seed gum, fat content, and homogenization rate on the viscoelastic properties of low-fat yogurt was evaluated via an optimal mixture-process variable design. The reason for this is that low-fat yogurt production with appropriate rheological properties is of great importance.

## 2. Material and Methods

### 2.1. Materials

Cow milk (10.5% total solid, 0.05% fat, 3.75% protein, 5.63% lactose and 0.86% ash) was purchased from Iran Dairy Industries Co, Iran. Dried skim milk (3.6% moisture content, 0.5% fat, 32.5% protein, 45% lactose and 7.6% ash), milk protein concentrate (41.1% moisture content, 0.5% fat, 69.5% protein, 17% lactose and 8.5% ash) and commercial starter provided from Khorasan Razavi Pegah company (Optiferm Type: Joferm DD) and KG purchased from Food Chem. (China).

### 2.2. Low-fat stirred yogurt preparation

According to the formula, 1.5% non-fat dry milk, 1.2% milk protein concentrate (MPC), and 0.35% edible salt were added. The resulting mixture was slowly stirred using a thermoxim at 4-6 °C until the powders were completely dissolved. The fat content of the mixture was calculated by the Pearson’s square method according to Table 1 and added using 40% fat cream. The amounts of SSG and KG were also calculated according to Table 1 and added as well. Samples were then homogenized using a homogenizer (IKA, made in German) according to Table 1 and kept in the refrigerator for 4-5 °C overnight for complete dehydration. The next day, the samples were first heated at 90 °C for 5 min using a thermoxim. They were then cooled to inoculation temperature (45 °C). Yogurt starter was added according to the manufacturer’s instructions and the samples remained in the oven until pH = 4.6. The samples were then cooled to 25 °C and stirred for 30 s, and packed in 100 g polyethylene glasses for testing.

### Table 1. Actual level of process and mixture variable in mixture-process variable analyses.

<table>
<thead>
<tr>
<th>Sample codes</th>
<th>Mixture variables</th>
<th>Process variable</th>
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<tbody>
<tr>
<td></td>
<td>Sage seed gum (%)</td>
<td>Konjac gum (%)</td>
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2.3. Viscoelastic properties (Stress sweep test)

A Physica MCR301 controlled stress/strain rheometer (Anton Paar GmbH, Germany) equipped with the parallel plate system (50 mm diameter, and 1.000 mm gap) was utilized for the small amplitude oscillation shear (SAOS) analyses. For the measurement of elastic modulus (G'), loss modulus (G'') and η*, Rheo plus/32 version V3.40 software was adopted. Pelteir system was utilized for controlling the temperature of the plates, and for maintaining the temperature throughout the analysis, a hood was also applied. Stress sweep test was conducted at shear stress range of 1-100 Pa and at 1 Hz frequency and 20 °C to determine G' at linear viscoelastic region (G'\text{LVE}), G'' at linear viscoelastic region (G''\text{LVE}), critical strain (γ\text{LVR}), yield stress (τ\text{y}), loss tangent at linear viscoelastic region (Tan ∆\text{LR}), yield stress at flowing point (τ\text{f}) and elastic modulus at flowing point (G\text{f}; G'\text{f}=G'').

2.4. Experimental design and statistical analysis

In this research, components of mixture design are consisting of 0 to 0.2% KG and 0 to 0.2% SSG in a way that they constituted 0.2% of total formulation and process factor was consist of 0 to 3% fat content and 0 to 24000 rpm homogenization rate. The total numbers of treatments were 20 (Table 1). Design-Expert software (version 11) was adopted for analyzing the findings. For the analysis, mixture-process variable experiments were used and combinatorial Steff regression in mixture-process experimental design for modeling each response as follows: (Dal Belloa & Vieirab, 2011).

\[
Y = \sum_{k=1}^{q} y_{k}x_{k} + \sum_{k=1}^{q-1} \sum_{i=k+1}^{q} y_{ki}x_{k}x_{i} + \sum_{k=1}^{m} \sum_{i=1}^{q-1} y_{ki}x_{k}x_{i}z_{i} + \sum_{k=1}^{m-1} \sum_{i=k+1}^{m} \sum_{j=1}^{q-1} y_{kij}x_{k}x_{i}x_{j}z_{i}z_{j} \tag{1}
\]

which \(\sum_{k=1}^{q} y_{k}x_{k}\) and \(\sum_{k=1}^{q-1} \sum_{i=k+1}^{q} y_{ki}x_{k}x_{i}\) refers to linear and non-linear component of mixture, \(\sum_{k=1}^{m} \sum_{i=1}^{q-1} y_{ki}x_{k}x_{i}z_{i}\) refers to linear and non-linear effect of process variables and \(\sum_{k=1}^{m-1} \sum_{i=k+1}^{m} \sum_{j=1}^{q-1} y_{kij}x_{k}x_{i}x_{j}z_{i}z_{j}\) refers to interaction effect of mixture and process variables. The significance of linear, quadratic and interaction coefficient of regression model was investigated using the table of analysis of variance (ANOVA) at the confidence of 0.001, 0.1 and 0.05.

3. Results and Discussion

3.1. Stress sweep test

Fig. 1 demonstrates the stress sweep test for the treatment of No. 11 (Table 1) at 20 °C. According to Fig. 1, the rheological performance of the stirred yogurt can be divided into 4 regions. 1. The first region, or the linear viscoelastic region (LVE), where the elastic/storage modulus (G') and the viscose/loss modulus (G'') are nearly constant, but the storage modulus is larger than the loss modulus, indicating a solid-like behavior. 2. The second region or the nonlinear viscoelastic region where G' and G'' begin to reduce with increasing strain, which indicates partial destruction of yogurt structural network. 3. The third region or the crossover point (flow point) where the loss modulus (G'') and the storage modulus (G') of the samples intersect at this point. 4. The fourth region, where the loss modulus (G'') is greater than the storage modulus (G') and the samples exhibit a liquid-like behavior (Saleh et al., 2020).

In this test, the stress or strain corresponding to the onset of the nonlinear behavior and the rapid decrease of the storage modulus (G') are called critical strain (γ\text{LVR}) or yield stress (τ\text{y}). In sample 11, as the stress increased, the bonds that hold the network together started to tear. It means that at the yield stress (τ\text{y}), the network structure collapses during which G' decreases with a steep slope (Pai & Khan, 2002).

Parameters of strain sweep test including storage modulus in the linear region (G'\text{LVE}), loss modulus in the linear region (G''\text{LVE}), critical strain (γ\text{LVR}), yield stress (τ\text{y}), loss tangent in the linear region(Tan ∆\text{LR}), yield stress at the flow point (τ\text{f}) and the storage modulus at the flow point (G\text{f}; G'\text{f}=G'') of the low-fat stirred yogurt samples at 20 °C and a frequency of 1 Hz were investigated.

\[
G'\text{LVE} = 5792.80A + 17567.41B - 51370.77AB - 0.005AC - 1590.14AD - 0.56BC - 630.17BD + 16.35ABC + 1245160.6BD
\]
Fig. 2 shows the effect of KG, SSG and fat content on the $G'_\text{LVE}$ of the samples under conditions where the homogenization rate was kept at the at 24,000 rpm with respect to the coefficients of Model 2.

As shown in Fig. 2, when the homogenization rate was constant at 24,000 rpm, with an increase in the amount of KG and a decrease in the amount of SSG under the conditions where the fat content was low, the storage modulus of the samples did not change significantly, indicating a similar performance of the two gums on the storage modulus of the samples under low-fat conditions. If the fat content increased under these conditions, the storage modulus of the samples would first increase and then decrease, which means that the highest amount of storage modulus was under the condition where both gums had the same amount, showing the synergistic impact of these two gums on the storage modulus of the samples. Sandoval-Castilla et al. (2003) stated that the carbohydrate molecules, due to their high water absorption capacity, can bind tightly to water molecules and trap them, so they increase the viscosity of the aqueous phase, thereby increasing the resistance against the force applied. Walstra et al. (1999) found that in the set yogurt, the homogenized fat particles act as cations surrounded by the casein micelles and submicelles, leading to the expansion and completion of the yogurt network. Prentice (1992) also stated that if the milk fat in the non-homogenized yogurt is high, the high percentage of fat may cause the loosening of network gel, because large fat globules having an average diameter of more than casein micelles prevent the fusion of micelles and give rise to the dissociation of gel network.

3.3. Loss modulus at linear viscoelastic region

The results showed that the loss modulus in the linear region of test samples varied between 53.5 to 611.5 Pascal. Since the lack of fit was not significant at 95% confidence level and due to a coefficient of determination of 0.99, the third-order polynomial

![Graph 1: Effect of KG, SSG and fat content on the G'_{LVE} of the samples (homogenization rate of 24,000 rpm).]

![Graph 2: Effect of KG, SSG and homogenization rate on the G'_{LVE} of the samples (fat content of 3%).]

![Graph 3: Effect of KG, SSG and fat content on the G''_{LVE} of the samples (homogenization rate of 24,000 rpm).]

![Graph 4: Effect of KG, SSG and fat content on the γc of the samples (A: homogenization rate of 24,000 rpm and B: homogenization rate of 0 rpm).]
model had the best fit to the $G'_\text{LVE}$ response data as well. In this model, A, B, C and D were KG, sage seed gum, homogenization rate, and fat content, respectively. Furthermore, the review findings revealed that in this model the effects of A, B, AB, AD, BC, ABC, ABD at 95% level on the $G'_\text{LVE}$ of the samples were significant.

\[ G'' \text{LVE} = 1525.78A + 3539.21B - 101952.69AB - 0.002AC \\
- 408.842AD - 0.108 BC - 160.39BD \\
+ 3.10ABC \\
+ 25237.54ABD \\
(3) \]

Fig. 3 shows the effect of KG, SSG and homogenization rate on the $G'_\text{LVE}$ of the samples under conditions where the fat content was kept at 3% concerning the coefficients of Model 3.

According to Fig. 3, under conditions where the fat content was 3% constant, with an increase in the amount of KG and an increase in the amount of SSG under conditions where the homogenization rate was low, the loss modulus of the samples did not change significantly, indicating a similar performance of the two gums on the loss modulus of the samples. If the homogenization rate increased under these conditions, the loss modulus of the samples would first increase and then decrease, which means that the highest amount of loss modulus was under condition where the amount of the both gums was identical, indicating a synergistic impact of the two gums on the loss modulus of the samples. Therefore, according to the results obtained from this research, the process of changes in KG, sage seed gum, fat content and homogenization rate were similar regarding the storage modulus and the loss modulus. In the oscillation tests, the modulus $G'$ relies heavily on Pro-Pro bonds, and the modulus $G''$ indicates weaker attractions and interactions. The strength of the gel network not only depends on the number of protein strands and the number of bonds, but also on the amount of water bonded and the network arrangement (Skriver et al., 1999). Therefore, as the homogenization of fat makes it easier to distribute the stabilizers uniformly, changes in KG, sage seed gum, fat content, and homogenization rate lead to changes in the arrangement of the gel network and the water bonded and eventually changes in $G'$ and $G''$ modules.

3.4. Critical strain and yield stress ($\gamma_c$ and $\tau_y$)

The research findings revealed that $\gamma_c$ and $\tau_y$ of test samples varied from 0.109 to 0.583% and 1.19 to 8.20 Pascal, respectively. Heldman and Lund reported that LVE for most soft solid foods ranged from 0.1-2% (Heldman & Lund, 2007). Clark & Ross-Murphy (1987) reported that the linear region in dilute solutions was lower than in concentrated solutions and the linear region in concentrated solutions was lower than in gels. While this value is rarely greater than 0.1% in colloidal gels, there is a larger linear region of 1% or more for natural biopolymer gels. Hence, given the critical strain of the samples, the low-fat stirred yogurt produced a relatively weak gel. Ozer et al. (1997) showed that the critical viscoelastic strain of the strained yogurt was 0.02%. Paulsson & Dejmek (1990) considered the strain amplitude of 0.01% at 1 Hz as the critical strain to investigate the formation of beta-lactoglobulin thermal gel. Since the lack of fit was not significant at 95% confidence level and due to a coefficient of determination of 0.99, the third-order polynomial model (4 and 5) had the best fit to the critical strain and yield stress responses as well. In these models, A, B, C and D were KG, sage seed gum, homogenization rate, and fat content, respectively. The review results also showed that in the model 4 the effects of A, B, AD, BC, ABC at 95% level on the $\gamma_c$ and in the model 5 the effects of A, B, AD, BC, ABC at 95% level on the $\tau_y$ of the samples were significant.

\[ \text{Strain value at the limit of the LVE region} = 2.07A + 3.11B + 5.54e - 0.5AC \\
+ 0.371AD - 5.95e - 0.5BC - 0.54BD \\
- 6.9047e - 0.5ACD + 2.242e \\
- 0.5BCD \]  

\[ \text{Yield stress at the limit of the LVE region} = 27.13A + 29.87B - 669.07AB \\
+ 0.001AC - 7.49AD - 0.001BD \\
+ 3.72BD 0.019ABC \\
+ 117.6ABCD \]  

Fig. 4 shows the effect of KG, SSG and fat content on the $\gamma_c$ of the samples under conditions where the homogenization rate was kept at the a: at 24,000 rpm and b: at 0 rpm with respect to the coefficients of Model 4.

As shown in Fig. 4a, under conditions where the homogenization rate was constant at 24,000 rpm, increasing the amount of KG and decreasing the amount of sage seed gum, when the fat content was low, increased the critical strain of the samples, but under the conditions where fat content was high, the critical strain of the samples decreased, indicating a different performance of the two gums in different fat contents on the critical strain of the samples. In other words, when the fat content was low, KG had a better performance on the critical strain of the samples, whereas SSG had a better performance on the critical strain of the samples when the fat content was low. Fig. 4b also showed that changes in the amounts of SSG and KG, in different fat contents, had little effect on the critical strain of the samples under conditions where the homogenization rate was constant at 0 rpm. Thus, by comparing Fig. 4a and 4b, one can find the significant effect of homogenization rate on the performance of gums and finally the effect on the critical strain of the samples. The critical strain (the strain whose increase results in a sharp decrease in $G'$) depends on the spatial structure of the food polymer (Heldman & Lund, 2007) and is related to the deformability of gel samples (Farahnavy et al., 2010). Therefore, in high-fat yogurt, using SSG and high homogenization rate reinforces the yogurt gel and consequently increases the critical strain that reflects the longer time of polymer structure interactions. As time passes, new bonds are replaced by bonds that have been broken by applying the external strain in the shear stress test with low oscillation amplitude. KG also had this effect in low-fat yogurt and at high homogenization rate. Staff reported that strong gels stayed in linear state longer than weak types (Steffe, 1996). Delorezi et al. (1995) observed that both low-fat and high-fat yogurt decreased in terms of linear viscoelastic region with a decrease in fat content. The results also showed that under conditions where the amounts of KG and gage seed gum were constant at 0.1%, the yield stress of the samples increased with increasing the homogenization rate and fat content, and changes in the amounts of KG and SSG did not have a significant
effect on this incremental process. As mentioned before, if the fat is homogenized, high fat percentage will strengthen the gel network due to the increased hydrophobic effect in the liquid phase of the network (Tamime & Robinson, 1999), and if the fat is not homogenized, the large fat globules will weaken the fusion of casein micelles resulting in the weakening of the yogurt gel network (Prentice, 1992) and eventually the yield stress of the samples changes as the gel network structure changes.

Fig. 5. Influence of fat content and homogenization rate on the loss tangent of the samples (KG and SSG of 0.1%).

3.5. Loss tangent (Tan (δ) LVE)

The results showed that the tangent loss of test samples ranged from 0.195 to 0.286. The loss tangent is a characteristic number to describe the viscoelastic behavior of the material, its smaller numbers (tan δ < 1) represent the dominant elastic behavior and the loss tangents greater than 1 represent the dominant viscous behavior. A loss tangent greater than 0.1 indicates that the sample is not a real gel and its structure is between the physical gel and the real gel (Kashaninejad & Razavi, 2019). Therefore, the loss tangent of low-fat stirred yogurt samples was less than one and greater than 0.1, indicating the existence of elastic structure in the weak biopolymer gel.

Since the lack of fit was not significant at 95% confidence level and due to a coefficient of determination of 0.88, the third-order polynomial model (6) had the best fit to the loss tangent response data as well. In this model, A, B, C and D were KG, sage seed gum, homogenization rate, and fat content, respectively. Furthermore, the review findings revealed that in this model the effects of A, B, BC, ABC at 95% level on the loss tangent of the samples were significant.

\[
\text{Loss tangent} = 1.29A + 1.58B + 5.33AB - 6.616e - 07AC + 0.03AD + 1.40e - 05BC - 0.18BD - 0.0004ABC - 0.92ABD
\] (6)

Fig. 5 shows the influence of fat content and homogenization rate on the loss tangent of the samples under conditions where the KG and SSG were kept at the 0.1% with respect to the coefficients of Model 6.

As shown in Fig. 5, the loss tangent of the samples decreased linearly with increasing the homogenization rate and fat content under conditions where the amounts of KG and SSG were constant at 0.1%. Changes in the amount of KG and SSG also had little effect on this decreasing trend, indicating the similarity of the nature and types of forces and the interaction between the samples (Ozer et al., 1999). Also, since none of the samples were real gels, the strand and macromolecule interactions were temporary and could be disrupted at high shear rates (Naji-Tabasi, & Razavi, 2017).

Fig. 6. Effect of fat content and homogenization rate on the A: τf and B: γc of the samples (KG and SSG of 0.1%).

3.6. Yield stress and elastic modulus at flowing point

According to the research findings, the τf and Gf of test samples varied from 5.39 to 23.11 and 8.09 to 84.64 Pascal, respectively. Since the lack of fit was not significant at 95% confidence level and due to a coefficient of determination of 0.99, the third-order polynomial model (7 and 8) showed the best fit to the yield stress and elastic/storage modulus responses data at flow point as well. In these models, A, B, C and D were KG, sage seed gum, homogenization rate, and fat content, respectively. The review results also showed that in the model 10 the effects of A, B, AB, AD, BC, ABC, AB at 95% level on the τf and in the model 11 the effects of A, B, AB, AD, BC, BD, ABDb at 95% level on the Gf of the samples were significant.
3.7. Numerical optimization

In the present study, the calculation of the optimal value of each independent variable (X) for the optimization is performed via the received models by software. In this case, if the importance of the properties is different, a weighted coefficient in its range can be assigned. In the present survey, the fat and $G''_{LVE}$ are to be minimized and the $G'_{LVE}$, $\gamma_c$, and $\tau_f$ are to be maximized for the optimization. The optimum point measured as 0.19% KG, 0.01% SSG, 0.59% fat content and 12,075 rpm homogenization rate, in this situation, the responses were: $G'_{LVE}=684.69$ pa, $G''_{LVE}= 193.46$ pa, $\gamma_c=0.49 \%$, $\tau_f=4.35$ pa, $\tan(\delta)=0.26$, $\tau_f=69.84$ pa and $G_f=638$pa (Fig. 7).

4. Conclusion

The rheological properties of products including yogurt have significant effects on the processing and marketability of the product. In yogurt, such properties are primarily affected by the production process, the fat content, and the type of gum used in them. Determining the optimal combination of gum, fat and homogenization rate for improving the rheological properties, especially for fat reduction in the formulation is very important.
economically and nutritionally. Therefore, in this study, the effect of different amounts of KG, sage seed gum, fat content and homogenization pressure on the viscoelastic characteristics of low-fat yogurt was investigated and these conditions were optimized such that a minimum fat content and a maximum hardness were considered. According to the above-mentioned properties, the amount of KG was 0.19 %. In general, the results of this study also showed that the konjac and sage seeds gums, as hydrocolloids with high nutritional value, in addition to having a synergistic impact on some characteristics of low-fat yogurt can be used as a suitable fat substitute in the stirred yogurt (KG in low-fat yogurt and SSG in high-fat yogurt) and can provide good rheological properties.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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