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Formulation optimization of low-fat emulsion stabilized by rocket seed *(Eruca Sativa Mill)* gum as novel natural fat replacer: effect on steady, dynamic and thixotropic behavior

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ABSTRACT. This study aims to investigate the use of Rocket seed *(Eruca Sativa Mill)* gum (RSG) as a novel fat replacer in the formulation of low-fat emulsion. For this purpose, formulation of the salad dressing was optimized by using response surface methodology (RSM) based on the rheological properties measured by steady shear (K), dynamic rheological analysis (K′, K″), and three interval time test (Deformation (%) and Recovery (%)). All samples showed shear-thinning character and viscoelastic solid character. Herschel Buckley model parameters, namely, τ_0, *K,* and *n* values, were changed between 0.36 and 12.58 Pa, 1.67, and 27. 12 Pa.s^n, 0.15 and 0.27 respectively. In all samples, the G′ value was higher than G″ in all frequency range. The samples containing high RSG showed a lower percentage of deformation (Def %) and higher recovery (Rec %) values. Optimization was performed based on the rheological properties of the control samples, and the optimum formulation of low salad dressing was 3.73 % RSG, 10 % oil, and 1 % EYP. Low-fat salad dressing samples stabilized by RSG and control samples showed similar characteristics in terms of particle size, PDI, zeta potential, and emulsion stability index values. This study suggested that RSG could be used as a natural fat replacer in low-fat salad dressing type emulsions.

Keywords: Low fat emulsion; gums; rheology; fat replacer.

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Introduction

Oil-in-water (O/W) emulsions such as mayonnaise and salad dressings are among the food groups that have been highly preferred by consumers in recent years due to their unique flavor, aroma, and usage patterns (Hedayati, Shahidi, Koocheki, Farahnaky, & Majzoobi, 2020). These products contain specific amounts of fat to obtain the desired quality and mouth-feel perception. However, in recent years, with the awareness of consumers in a healthy diet and the improvement in the quality of life, the trend in the consumption of low-fat products has been increased due to not only the high-calorie value of fat and but also its possible relation with certain diseases such obesity and cardiovascular diseases (Chung, Degner, & McClements, 2013). In these products, fat plays a significant role in determining the rheological properties, emulsion stability, and organoleptic quality (Drakos & Kiosseoglou, 2008; Ma & Boye, 2013). There is a need for fat substitutes and stabilizing agents to balance the loss in the rheological and textural properties resulting from the reduction of the oil content (Ma & Boye, 2013). Therefore, finding suitable fat replacers is one of the major challenges in the production of low-fat salad dressing and mayonnaise.

Carbohydrate-based fat replacers have been reported to have the effect of reducing the movement of oil droplets in the water phase by improving the rheological properties and thus providing emulsion stability. Plant-based carbohydrates have a wide range of use as a thickener, stabilizer, and gelling agent and fat replacer in O / W food emulsions. The gum used in salad dressing and mayonnaise should resist acidic conditions due to their strong acidic condition. (Ma & Boye, 2013). Although starch derivatives are commonly used as texture modifiers, their use is very limited as fat replacers in low-fat food emulsions, because starch granules are not soluble in cold water and can only thicken when overheated (Razavi & Behrouzian, 2018). To eliminate these defects, new fat replacers with emulsifying products should be investigated. The water-soluble seed gums as potential fat replacers have attracted interest from the researchers, because they are derived from renewable sources, abundant in nature, biocompatible and environmentally friendly, and relatively low-cost and non-toxic. Seed gums form a weak gel-like polymer network of the continuous phase (water) in O / W emulsions, leading to very high viscosities in the low stress range and can give viscoelastic properties to the entire system (Chung, Degner, & McClements, 2013). By using seed gums as a fat replacer, specific functions and new rheological properties can be provided in low-fat O / W emulsions. (Mirhosseini & Amid, 2012; Ahmad, Ahmad, Manzoor, Purwar, & Ikram, 2019). Recently, the emulsifying and potential oil substitution properties of the newly characterized gums from Alyssum homolocarpum seeds, fenugreek seeds, basil seeds, cress seeds, asafoetida seeds have been investigated (Anvari et al., 2016; Gadkari, Tu, Chiyarda, Reaney, & Ghosh, 2018; Naji-Tabasi & Razavi, 2017; Razmkhah, Razavi, & Mohammadifar, 2016; Saeidy et al., 2018). The effect of extraction parameters on some technological properties of *Eruca sativa* seed mucilage was studied (Koocheki, Razavi, & Hesarinejad, 2012). To the best of the authors' knowledge, there has been no study conducted for the use of Rocket seed gum as a potential fat replacer in an oil-water emulsion.

The rocket seed gum (RSG), is a cream-colored powder extracted from rocket seed. Koocheki et al. (2012) reported that rocket seed mucilage contained 67.97% carbohydrate, 9.75% protein, 12.28% moisture, 10% ash, and no fat content. The comprehensive rheological characterization of RSG was studied by Kutlu, Akcicek, Bozkurt, Karasu, & Tekin-Cakmak (2021). The carbohydrate, protein, moisture, and ash content of RSG were found to be 80.38%, 5.81%, 10.26%, and 3.55%, respectively. They also reported that the RSG has very high galactose's substitution level in with 1.52 mannose/galactose ratio. RSG showed high protein content than some natural gums (Bhushette & Annapure, 2018; Hamidabadi Sherahi, Fathi, Zhandari, Hashemi, & Rashidi, 2017). The protein content is one of the crucial parameters for emulsifying properties of the natural gums. For this reason, RSG has the new potential to provide high gelling capacity, improved viscoelastic properties, and shear thinning behavior in oil-in-water emulsions especially in low-fat salad dressing and mayonnaise. The effect of common instability mechanisms such as flocculation, sedimentation, creaming, or Ostwald ripening that may occur in low-fat O / W emulsions can, therefore, be reduced by the addition of RSG.

The primary purpose of this study is to investigate the potential use of RSG as a natural plant-based fat replacer in low-fat salad dressing and to provide similar flow behavior characteristics of control full-fat salad dressing sample. For this aim, formulation optimization of the low-fat salad dressing was conducted based on the rheological properties, namely, steady shear, viscoelastic, and 3 Interval Time Thixotropy Test (3-ITT). After formulation optimization, particle size distribution, polydispersity index, and zeta potential values were also characterized for the sample obtained from the optimum formulation point and control the full-fat salad dressing sample.

Material and methods

Material

Rocket seeds (Eruca. *Sativa* Mill) were obtained from the local manufacturers in Istanbul. Rocket seeds were stored in a plastic bag at temperature of 25°C until they were extracted. Sunflower oil, vinegar, and egg yolk powder (EYP) were purchased from the local market and stored until the preparation of the analysis of the sample. All chemicals were analytical grade and obtained from Merck (Darmstadt, Germany).

Methods

Production of RSG

RSG was obtained from Rocket seed according to a modified method described by Razavi et al. (2009). 1 L of distilled water is added to 50 g of ground Rocket seed and extracted for 2h at 80°C on a magnetic heating mixer. Then, the solution was filtered to remove the seeds; This solution was evaporated to remove half of the water and mixed with ethanol (96%) as the ratio of 1:2 (v v^{-1}). and the mixture left overnight at 4°C to improve the accumulation of the gum on the surface of the solution. Afterward, the gum on the surface was collected and dried at oven (50°C) for one day. After dried gums were ground using a laboratory mill (PX-MFC 90 D, Kinematica, Malters, Switzerland) and passed through sieveno.100 and stored in a falcon tubes.

Salad Dressing Preparation

The salad dressing samples was prepared following procedure: Firstly, RSG (2-4% (w w^{-1}) and EYP (1-5 % (w w^{-1}) were dispersed in a water at temperature of 25°C with sugar. Then the dispersion heated to 80°C and stirred for 20 min. Then the dispersion was cooled to 25°C and stirred at 1,000 rpm in a magnetic stirrer for a period of 6h to complete the hydration of RSG. The dispersion was mixed by sunflower oil (10-30 % (w w^{-1}) and homogenized by using Ultra Turrax (Daihan, HG-15D) at 10,000 rpm for 3 min. Finally, salad dressing was obtained and pasteurized at 65°C for 10 min. After the homogenization process, salad dressing samples were poured into brown bottles and cooled to at temperature of 25°C. All material (beakers, brown bottles, and probe) in this experiment was sterilized at 121°C for 15 min. (Mantzouridou, Karousioti, & Kiosseoglou, 2013). The control full fat salad dressing sample was also prepared by same procedures. Control sample was formulated with 0.35 % XG, 30 % sun flower oil and 3 % EYP.

Experimental Design

Response surface methodology (RSM) was conducted for the study of three factors, namely oil concentration (g 100 g^{-1}) (X_1), RSG concentration (g 100 g^{-1}) (X_2) and EYP concentration (g 100 g^{-1}) (X_3), using an unblocked full factorial central composite design (CCD). The coded and actual values of the factors at various levels were presented in Table 1. The levels of the Xi factor are coded as − 1, + 1 and 0 correspond to the low, high and mid-level of Xi. 17 different experimental points were obtained by using Design Expert Software (Version 7; Stat-Easy Co., Minneapolis, MN) to determine the optimum amount of RSG, EYP and oil content. For the estimation of the error, the design consist of three of the factorial points. A quadratic model was fitted to the experimental data for each response. Model applicability was evaluated based on the R^2, R^2-adj, lack of fit, F, and P- values obtained from ANOVA. The optimization was carried out based on the highest desirability value. The formulation with the lowest oil content with a desirability value of 1 was chosen as the optimum formulation. Three central points were used. Analysis of all points was conducted in triplicate, and the results were reported as mean value and standard deviations.

Table 1. Levels of factors in actual and coded values used in the experimental design.

Factor	Name	Levels		
		-1	0	1
		Actual values		
X1	Oil	10	20	30
X2	RSG	2	3	4
X3	EYP	1	3	5

Rheological Analyzes

The stress and temperature-controlled rheometer (MCR 302; Anton Paar, Austria) was used to conduct flow behavior, dynamic rheological, and 3-ITT rheological properties of salad dressings. All rheological analyses were performed in triplicate at temperature of 25°C. The rheological properties of control salad dressing were taken into consideration in the determination of optimum formulation of RSG, EYP, and oil ratio. The steady (K), dynamic (K', K'') and 3-ITT rheological parameters (the percentage deformation (Def (%)) the percentage recovery (Rec (%)) were response variables, and RSG, EYP, and oil were process factors.

Steady shear rheological properties

The flow behavior analysis was carried out in the range of 0–100 shear rate (1/s) using a parallel plate configuration. The gap between the rheometer probe and the sample plate was adjusted as 0.5 mm. The analysis was started after the temperature reached to the temperature 25°C. The shear stress values corresponding to the shear rate were recorded. The parameters of steady shear rheological properties namely K, $\boldsymbol{\tau}_0$, and n value were calculated using the Herschel Buckley model by applying nonlinear regression;

$$\tau = \tau_0 + K\gamma^n, \quad (1)$$

where $\boldsymbol{\tau}$ was shear stress (Pa), $\boldsymbol{\tau}_0$ was yield stress (Pa), K was consistency index (Pa.s^n), $\boldsymbol{\gamma}$ was shear rate (1/s), and n was the flow behavior index.

Dynamic rheological properties

The frequency sweep test was performed to determine the dynamic rheological analysis by a parallel plate configuration. First, the amplitude sweep test performed to determine the linear viscoelastic region (LVR) at a strain value of 0.1%. The frequency sweep test was applied in the range of 0.1−64 (ω) angular velocity range in LVR. The storage (G′) and the loss modulus (G′′) values were recorded in response to the angular velocity. The dynamic rheological parameters were determined using the Power Law model and nonlinear regression (Yoo & Rao, 1996);

$$G' = K'(\omega)^{n'} \tag{2}$$

$$G'' = K''(\omega)^{n''} \tag{3}$$

where G′ (Pa) is the storage modulus, G′′ (Pa) is the loss modulus, **ω** is the angular velocity value (1/s), and K′ ($Pa.s^{n'}$), K′′ ($Pa.s^{n''}$) refers to consistency index values, and n′, n′′ represents the flow behavior index values.

Three Time Interval Time Test (3-ITT)

The 3-ITT rheological analysis were performed at the constant shear rate of 0.5 1/s and variable shear rate 150 1/s. The linear viscoelastic region was considered to determine shear rate value applied at second time interval. The linear viscoelastic region of the samples ends at 55 1/s. The samples were subjected to 100s at a very low shear rate (0.5/s) in the first time interval. In the second time interval, 150 1/s was subjected to the specified shear force for 40 s. In the third time interval, the dynamic rheological behavior in the second time interval was tested by exposing the samples to a low shear rate in the first time interval. For this purpose, the change in viscoelastic solid structure (G′) of salad dressing samples was observed. The behavior of the salad dressing samples was modeled using a second-order structural kinetic model in the third time interval:

$$\left[\frac{G' - G_e}{G_0 - G_e}\right]^{1-n} = (n-1)kt + 1 \tag{4}$$

where G′ represents the storage module in (Pa), k is the thixotropic rate constant, G_0 is an initial storage modulus (Pa) in the third time interval, and G_e is the equilibrium storage modulus (Toker, Karasu, Yilmaz, & Karaman, 2015).

Zeta potential (ʒ)

The zeta potential value of the samples was measured by a zeta sizer (Zetasizer; Malvern Instruments, Worcestershire, UK). All the samples were diluted 500 times with ultrapure water and homogenized by mixing in an ultrasonic bath for 5 min. before the analysis. A single parallel measurement was made in the zeta potential measurement, and values were recorded (Akcicek & Karasu, 2018).

Emulsion stability index

The emulsion stability of the emulsion was determined by visual analysis for 28 days of storage periods. After 28 days, phase separation was observed, and the height of the oil was measured.

For this aim, 20 mL of each emulsion was poured into a cylindrical glass container (int. diameter, 16 mm; height, 160 mm), stored at room temperature and the height of the visible serum separation level was recorded

For this Emulsion stability index (ESI) was calculated by the following equation:

$$ESI = \frac{O_H}{E_H} \times 100\,, \tag{5}$$

where OH, EH represents the height of the oil phase and height total emulsion in cm, respectively (Mantzouridou, Spanou, & Kiosseoglou, 2012).

Statistical Analysis

The fabrication of emulsion samples and all analysis was carried out in triplicate and values expressed as mean. Design expert software, v7 (Stat-Ease, Minneapolis, MN), was used to determined regression and variance analysis (ANOVA) of experimental points. The effects of the dependent variables on the responses were evaluated by quadratic models and response surface plots. The model practically was determined by the coefficient of determination (R2), the lack of fit test, and model p-value ($p < .05$). The steady shear and frequency sweep rheological analysis parameters were respectively calculated by using Herchel Bulkley and Power Law model and nonlinear regression analysis using the Statistica software program (StatSoft, Inc., Tulsa, OK).

Result and discussion

Steady shear rheological behavior

Figure 1 showed steady shear rheological properties of salad dressing samples. As can be seen from the figure, the slope of the shear rate versus shear stress graphs decreased for all samples, indicating that the viscosity of samples decreased with increasing shear rate. In other words, all samples showed shear-thinning flow behavior, which is a typical rheological behavior expected from oil/water emulsions such as salad dressing and mayonnaise. The decrease in the viscosity can be explained by breaking of the weak bonds and decrease in the intermolecular interaction by applying shear force. (Bortnowska, Krzemińska, & Mojka, 2013; Chatsisvili, Amvrosiadis, & Kiosseoglou, 2012; Fernandez, Palazolo, Bosisio, Martínez, & Wagner, 2012).

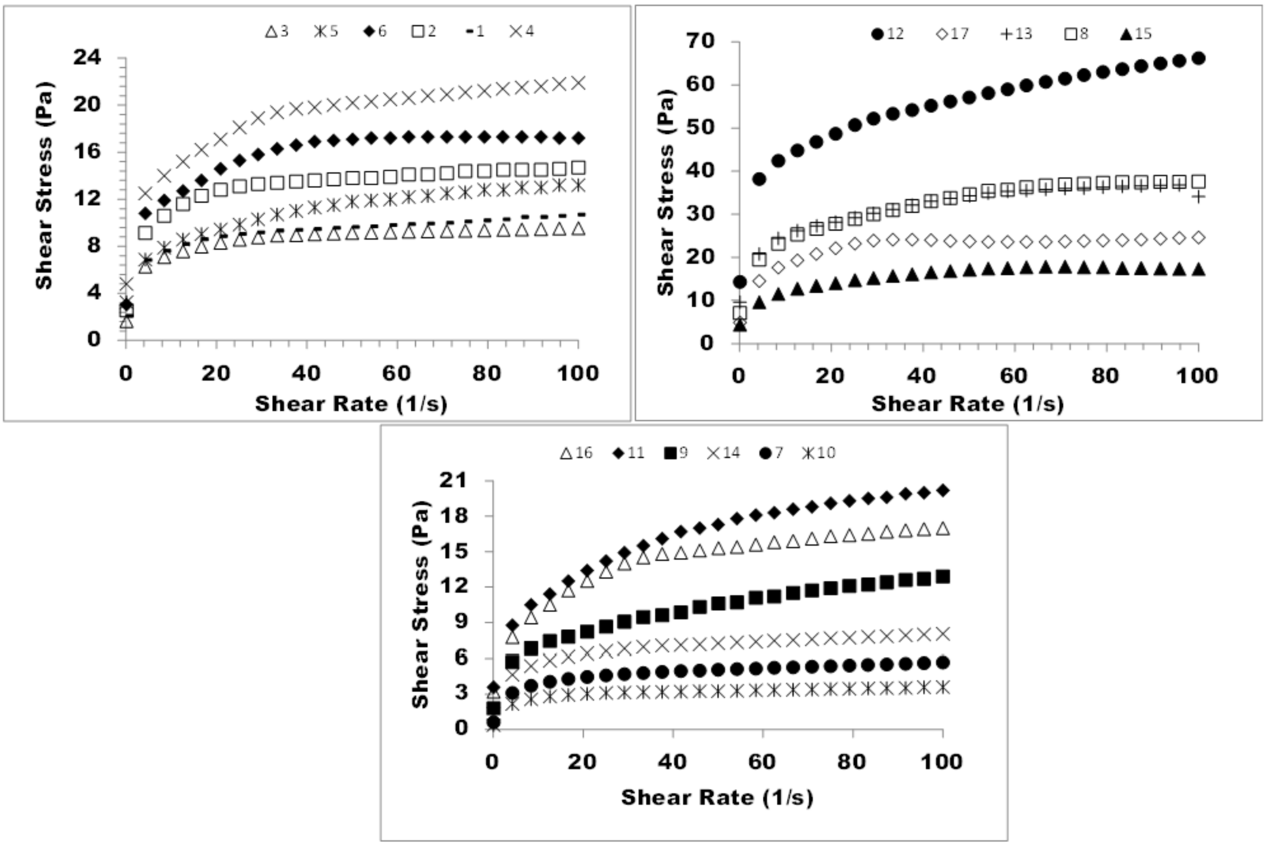

Figure 1. Steady shear rheological properties of the salad dressing samples.

The Herschel Buckley model modeled steady shear rheological behavior data, and the corresponding model parameters, namely, τ0, *K, n*, and R2 value, were presented in Table 2. The R2 value was higher than 0.97, indicating that the Power-law model successfully described steady shear rheological properties of salad dressing samples. Salad dressings should have a high degree of K with a moderate yield stress value (n), and low n value (Ma & Boye, 2013). The τ0, *K* and *n* values were 0.36-12.58 Pa, 1.67-27. 12 Pa.sn and 0.15 - 0.30 and, respectively. The *n* value of less than 1 indicates that all samples exhibited shear thinning behavior. According to Table 1, RSG and oil content improved the τ0 and K value while they decreased *n* value, indicated that RSG and oil strengthened the Pseudo-plastic characters. Approaching the value of n towards 0 shows the desired flow behavior in salad dressing type emulsions. As it was seen, n value was below 0.2 for the samples containing 3-4% of RSG.

Table 2. Herchel Bulkley model parameters obtained from steady shear rheological data.

Run	RSG (%)	EYP (%)	Oil (%)	τ_0 (Pa)	K (Pa.s^n)	n	R^2
				$\tau=\tau_0+K\times\gamma^n$			
1	3.00	3.00	20.00	1.75	5.21	0.16	0.97
2	4.00	5.00	10.00	1.97	7.33	0.16	0.96
3	3.00	3.00	10.00	1.39	4.91	0.15	0.96
4	3.00	3.00	20.00	4.21	9.69	0.18	0.99
5	2.00	1.00	30.00	2.90	5.08	0.21	0.99
6	4.00	1.00	10.00	2.61	8.34	0.17	0.96
7	2.00	3.00	20.00	0.59	2.33	0.20	0.98
8	4.00	1.00	30.00	6.69	14.47	0.22	0.99
9	2.00	5.00	30.00	1.70	3.65	0.27	0.99
10	2.00	1.00	10.00	0.36	1.67	0.17	0.96
11	3.00	3.00	30.00	3.30	6.08	0.26	0.99
12	4.00	5.00	30.00	12.58	27.12	0.19	0.98
13	4.00	3.00	20.00	8.94	16.46	0.18	0.99
14	2.00	5.00	10.00	1.26	3.54	0.18	0.98
15	3.00	1.00	20.00	3.94	8.00	0.18	0.98
16	3.00	3.00	20.00	2.95	6.18	0.23	0.99
17	3.00	5.00	20.00	7.55	12.82	0.15	0.93
C	0.4 XG	5	30.00	4.05	6.12	0.21	0.99

RSG: Rocket seed gum, EYP: Egg yolk powder, C: control samples containing 30 % oil content, XG: Xanthan gum.

The primary purpose of this study was to investigate whether RSG compensates for a less compact structure resulting from fat reduction. A control salad dressing sample with high oil content and with xhantan gum (commercially used gum for salad dressings) was additionally produced at the same conditions and each of the measured rheological properties was determined and compared with the data obtained from the emulsions samples.

When the K values of sample 1 and sample 2 are compared, it can be inferred that RSG can exhibit the desired consistency value in low fat emulsions. Although sample 2 contains 10% less oil than sample 2, the 1% increase in RSG in sample 2 provided more consistency than sample 1. The K value of sample 2 also showed higher K value than the that control sample, which formulated with 30% oil content. The same result was obtained from between sample 5 and sample 6. However, when RSG was used at 2%, it showed very low K value in low-fat samples. The samples containing 30% oil and 4% RSG showed a very high K value (27.12 Pa.s^n). These results indicated that RSG could be used successfully in a low percentage of standard salad dressing samples.

A quadratic model was used to statistically explain the effect of linear and interaction between RSG, oil, and EYP ratio on the K value of salad dressing samples. R^2 and Adj-R^2 values higher than 0.89 and 0.75, respectively, indicate that the quadratic model can be used successfully. A p-value of less than 0.1 indicates that the model used was significant. The model parameters showed that the linear effects of oil and RSG content were significant, and whereas the linear effect of EYP concentration was insignificant ($p > 0.05$) (Table 3). The interaction was only significant between RSG and oil content ($p < 0.05$). This result can be explained by decreasing the ratio of water/oil by increasing oil content and RSG fraction in the water phase (Ye, Hemar, & Singh, 2004). A higher ratio of RSG and an increased amount of oil droplets in the structure would cause a compact network and higher K value. The linear effect of RSG % has the lowest p-value which indicated that it was the most significant parameter affecting the K value.

A dramatic increase in K value was observed, while the RSG concentration was higher than 2.5%. From the 3D graph counterplots given in Figure 2, the interaction between RSG and oil content could be observed. As the RSG content increased when the oil content was low, a rapid increase in K value was observed. Besides, the increase in K value was more pronounced as it increased in both fractions. The increase in the content of EYP also increased the K value. However, this increase was not at a significant level. The interaction between RSG and oil content was significant, and RSG content was the most important parameter affecting K value. Therefore, these results indicate that RSG can be used as a stabilizing agent in desirable fat-reduced salad dressing samples.

Table 3. Quadratic model parameters for K value.

Regression coefficients	df	K		
		Mean square	F-Value	p-Value
Model	9	64.10	6.42 0.0114	0.0114
Linear			Significant	
A	1	330.05	33.05	0.0007
B	1	28.56	2.86	0.1347
C	1	93.70	9.38	0.0182
Cross product				
AB	1	15.68	1.57	0.2504
AC	1	62.72	6.28	0.0406
BC	1	13.42	1.34	0.2844
Quadratic				
A2	1	5.58	0.56	0.4792
B2	1	16.19	1.62	0.2436
C2	1	16.18	1.62	0.2438
Lack of fit	5	11.76	2.12	0.3513
			Not significant	
R^2		0.8919		
Adj-R^2		0.7529		

A: RSG: Rocket Seed Gum, B: EYP (Egg yolk Powder), C: Oil, Values of *"p"* less than 0.1000 indicate model terms are significant.

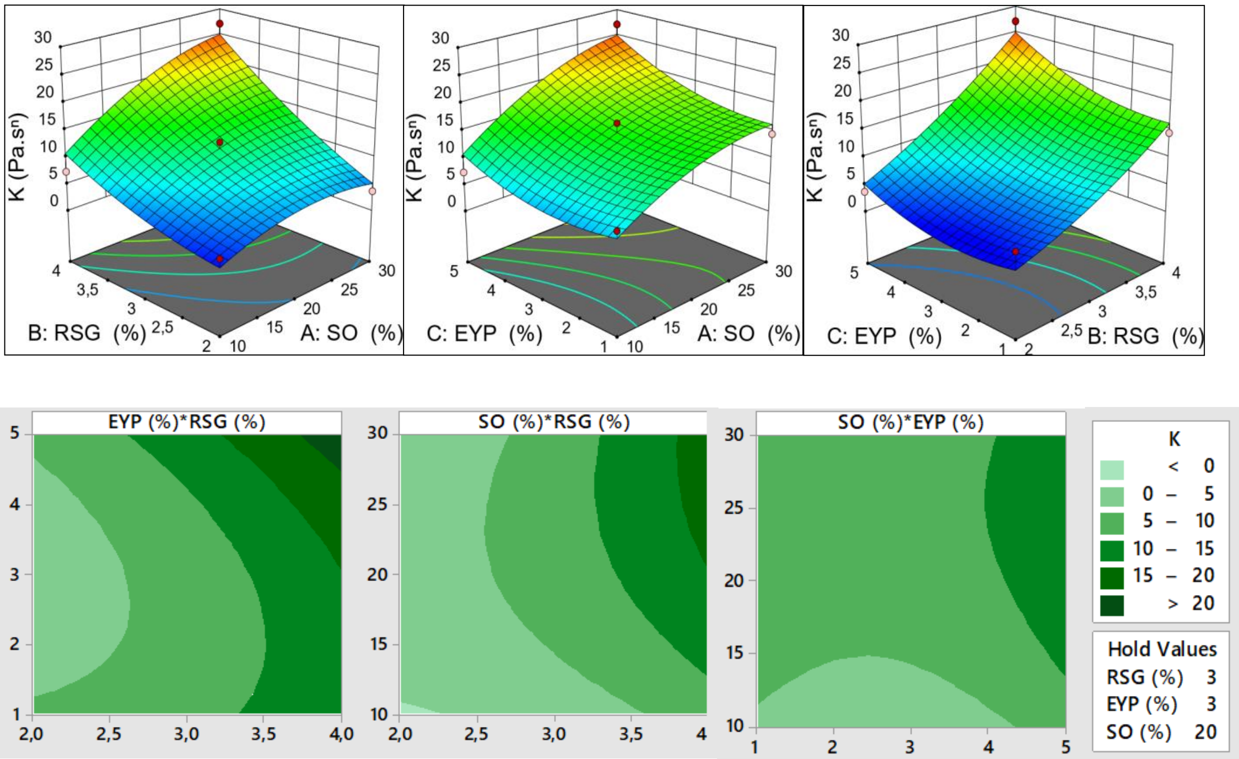

Figure 2. The effect of RSG, oil and EYP on the K value of the samples (Counter and 3D graph).

Viscoelastic properties

Figure 3 shows the dynamic mechanical spectra of the salad dressing samples. As can be seen, G′ and G″ values increased with the increasing frequency value for all samples. Besides, G′ value was higher than G″ value throughout the all frequency range in all samples except samples 7 and 10, which formulated with 2% RSG and 10 % oil. In sample 7, G′ and G″ values are close to each other at the initial frequency values. The fact that the G′ value was higher than the G″ value over the whole frequency range showed that the salad dressing samples exhibited a solid-like viscoelastic structure. This structure is the desired rheological character for oil/water emulsions, such as salad dressing and mayonnaise. For the oil/water emulsion, the solid-like structure has a very close relationship with the oil content. With the reduction of the oil content in oil/water emulsion, compact packing of oil droplets in the continuous phase is disturbed, and the solid structure turns into a liquid structure, which is not desirable viscoelastic behavior (Ma & Boye, 2013).

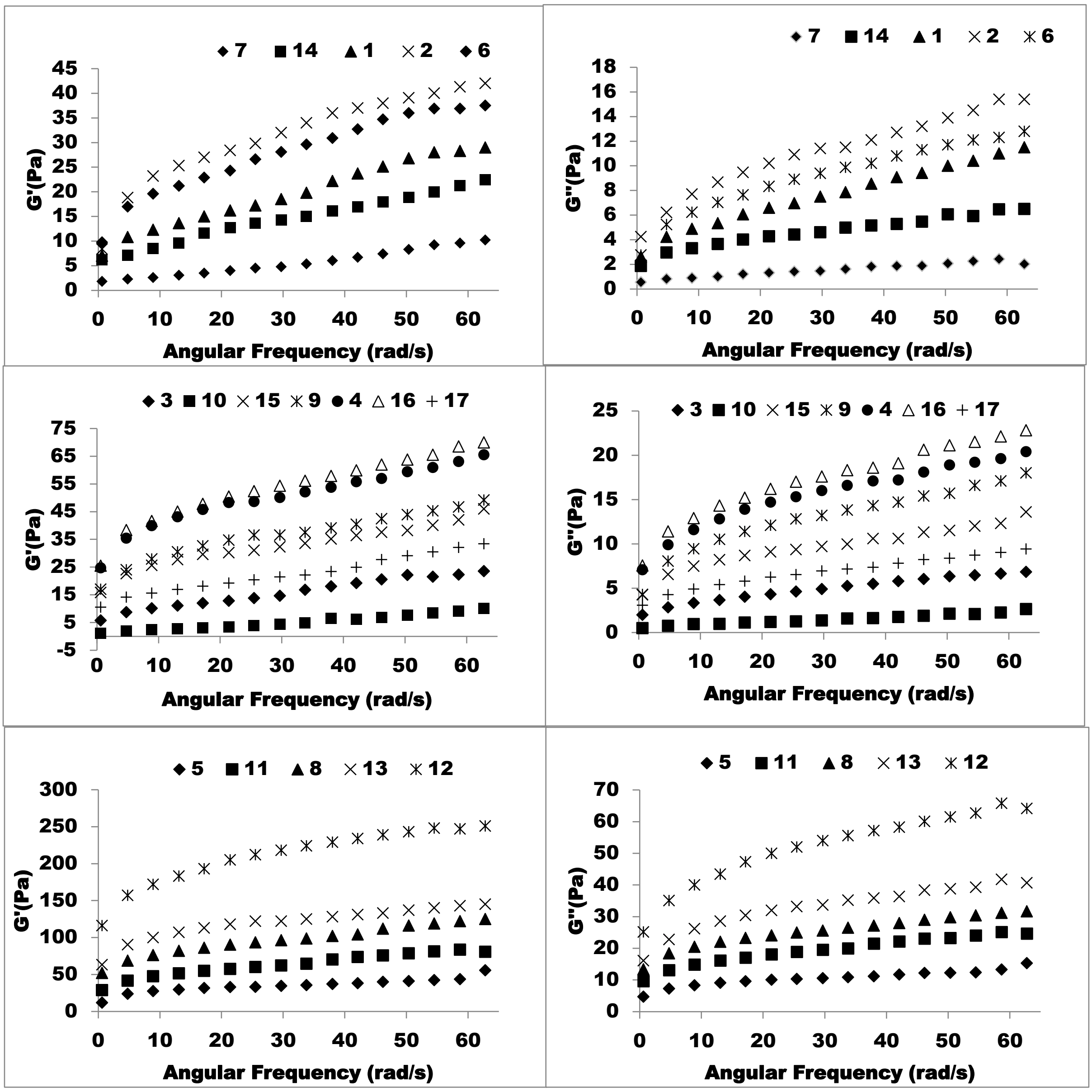

Figure 3. Viscoelastic properties of the salad dressing samples.

As a result, the physical stability of emulsions is decreased, and phase separation is observed. G′ was higher than G′′ for the samples contained more 2% RGS and the desired gel-like structure is obtained. These results can be explained by the interaction of the RSG with water and reducing the mobility of the continuous phase. This interaction could lead to a packed form of an oil droplet in a water phase. Similar results have been achieved with the use of different polysaccharides and gums in oil-reduced emulsions (Diftis, Biliaderis, & Kiosseoglou, 2005; Hedayati et al., 2020; Ma, Boye, & Simpson, 2016; Mantzouridou et al., 2013).

The Power law model was used to numerically express the effect of RSG, EYP, and oil on viscoelastic behavior and K', K'' n' and n'' values were calculated (Table 4). As can be seen, K' value was higher than K'' value in all samples. K' represents the magnitude of solid-like behavior and it ranged from 0.24 to 98.13 Pas^n. The samples containing 30 % oil and 4 % RSG showed a very high K' value (98.13 Pas^n) compared to that of the control samples (21.25 $Pa.s^n$). Besides, K' and K'' values of the samples containing 10% fat and 3-4% RSG were found close to the control salad dressing samples, indicating that RSG leads to a desirable solid-like structure in the low-fat salad dressing in this concentration. The K' value of the containing 2 % RSG with 10-20 % oil showed lower compared to other samples. Linear effect of the RSG and oil on K' and K'' was significant ($p < 0.05$). The effect of oil and RSG interaction was significant in similar to steady shear behavior. The quadratic effect of RSG was also found to be significant ($p < 0.05$) (Table 5). The 3D and counterplot was shown in Figure 4. As can be seen, higher RSG and oil content dramatically increased in K' and K'' values. These results may suggest that RSG was able to substitute oil.

Table 4. Dynamic rheological power law parameters of the emulsion samples.

Run	RSG (%)	EYP (%)	Oil (%)	K' (Pa.s^n)	n'	R^2	K'' (Pa.s^n)	n''	R^2
					$G'=K'\times(\omega)^{n'}$		$G''=K''\times(\omega)^{n''}$		
1	3.00	3.00	20.00	4.37	0.45	0.98	1.87	0.43	0.98
2	4.00	5.00	10.00	11.15	0.30	0.99	3.67	0.34	0.99
3	3.00	3.00	10.00	3.65	0.44	0.98	1.55	0.35	0.99
4	3.00	3.00	20.00	24.36	0.23	0.99	6.66	0.26	0.99
5	2.00	1.00	30.00	13.48	0.29	0.95	4.68	0.25	0.97
6	4.00	1.00	10.00	9.53	0.32	0.99	2.80	0.36	0.99
7	2.00	3.00	20.00	0.38	0.79	0.98	0.34	0.45	0.97
8	4.00	1.00	30.00	47.46	0.22	0.98	13.15	0.20	0.99
9	2.00	5.00	30.00	16.00	0.26	0.99	4.70	0.31	0.99
10	2.00	1.00	10.00	0.24	0.88	0.98	0.23	0.55	0.96
11	3.00	3.00	30.00	25.98	0.27	0.98	8.72	0.25	0.99
12	4.00	5.00	30.00	98.13	0.25	0.95	24.67	0.23	0.99
13	4.00	3.00	20.00	67.56	0.18	0.99	16.30	0.22	0.99
14	2.00	5.00	10.00	4.65	0.36	0.97	1.64	0.32	0.98
15	3.00	1.00	20.00	13.63	0.27	0.92	4.17	0.26	0.98
16	3.00	3.00	20.00	23.91	0.25	0.97	7.30	0.27	0.99
17	3.00	5.00	20.00	6.91	0.36	0.95	2.58	0.30	0.98
C	0.4 XG	5	30.00	21.25	0.25	0.99	7.55	0.32	0.99

RSG: Rocket seed gum, EYP: Egg yolk powder, C: control samples containing 30 % oil content, XG: Xanthan gum

Table 5. Quadratic model parameters for K′ and K″ value.

Regression coefficients	df	K′			K″		
		Mean square	F-Value	p-Value	Mean square	F-Value	p-Value
Model	9	1087.14	5.73	0.0157	66.42	6.42	0.1114
Linear			Significant			Significant	
A	1	*3963.28*	20.87	0.0026	*240.10*	*23.22*	*0.0019*
B	1	*275.63*	1.45	0.2674	*14.96*	*1.45*	*0.2682*
C	1	*2952.55*	15.55	0.0056	*211.88*	*20.49*	*0.0027*
Cross product							
AB	1	*257.19*	1.35	0.2826	*15.02*	*1.45*	*0.2673*
AC	1	*1258.01*	6.63	0.0368	*71.04*	*6.87*	*0.0343*
BC	1	*278.01*	1.46	0.2655	*10.72*	*1.04*	*0.3425*
Quadratic							
A2	1	*762.00*	4.01	0.0852	*29.28*	*2.83*	*0.1363*
B2	1	*125.19*	0.66	0.4435	*7.20*	*0.70*	*0.4316*
C2	1	*14.06*	0.074	0.7934	*0.039*	0.0379	*0.9526*
Lack of fit	5	213.73	1.64	0.4204	*10.95*	1.24	0.50
			Not significant			Not significant	
R^2		0.8804			0.8920		
Adj-R^2		0.7266			0.7531		

A: RSG: Rocket Seed Gum, B: EYP (Egg yolk Powder), C: Oil, Values of *"p"* less than 0.1000 indicate model terms are significant.

3-ITT rheological behavior

Salad dressing type emulsions can be exposed to sudden and high shear deformations during and after the process. For example, sudden shaking during its use is an example of this. While emulsions with the desired structural properties provide structural recovery again after sudden deformation, emulsions with weak structure do not provide structural recovery and flow away from the foods (Toker et al., 2015). 3-ITT test was applied to test the structural recovery of the samples. Figure 5 shows the 3-ITT behavior of salad dressing samples. As can be seen, all of the samples are exposed to high and sudden deformation in the 2nd time interval and G' values decrease. In the third time interval, there was a structural recovery in all samples. In particular, thixotropic behaviors of samples with high fat and RSG content appear to be more evident, that is, faster recovery.

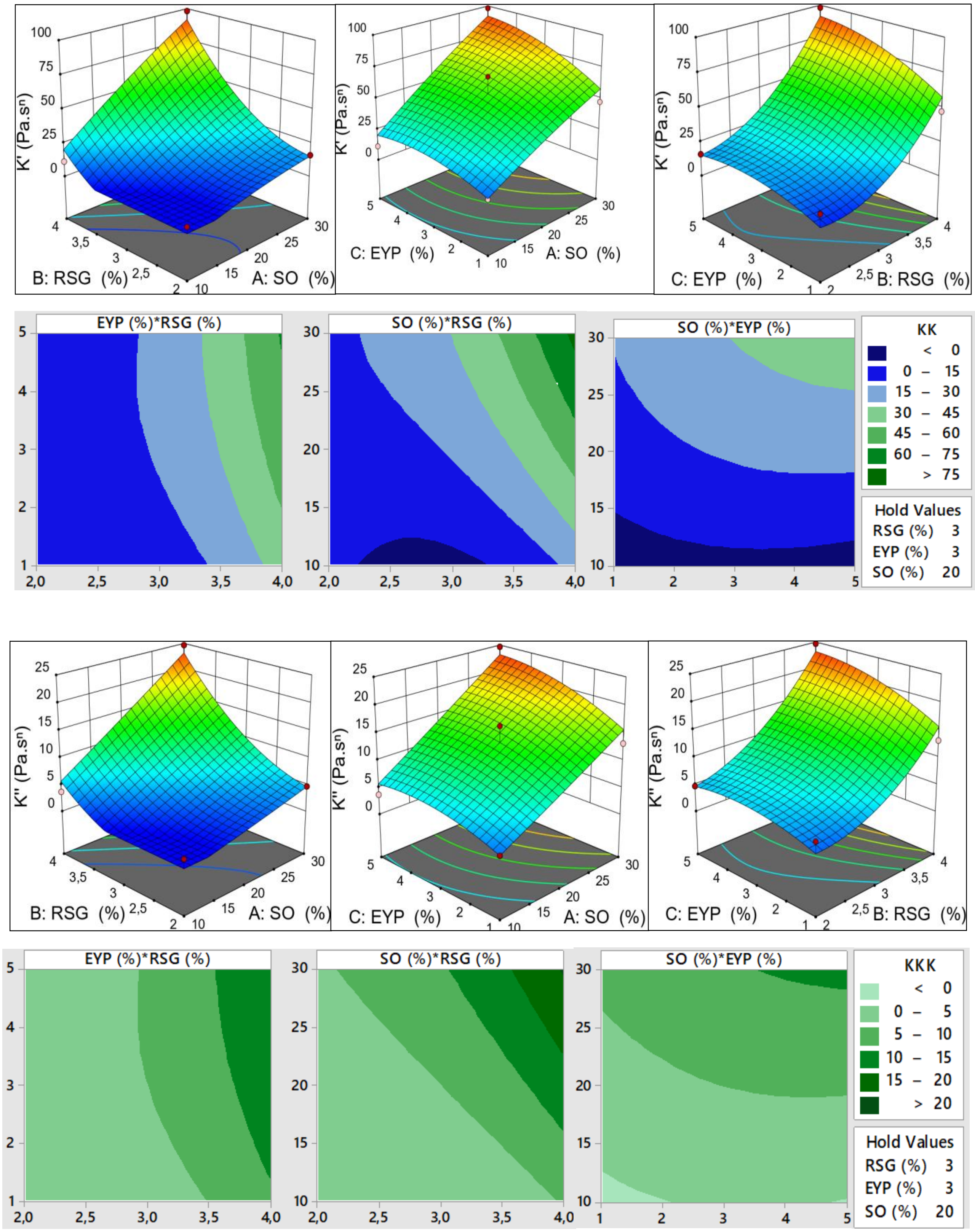

Figure 4. Effect of RSG, oil and EYP on the K' and K'' value (Counter and 3D graph).

To express this image numerically, Def (%) and Rec (%) were calculated and ranged between 18.42 -64.60 and 45.61-102.50, respectively. The samples containing 3-4% RSG showed low deformation and a similar recovery trend to the control sample. The sample containing 4 % RSG and 30 oil showed a % 100 structural recovery. The samples containing 3-4 % RSG showed similar Def (%) and Rec (%) value compared to control samples (Table 6).

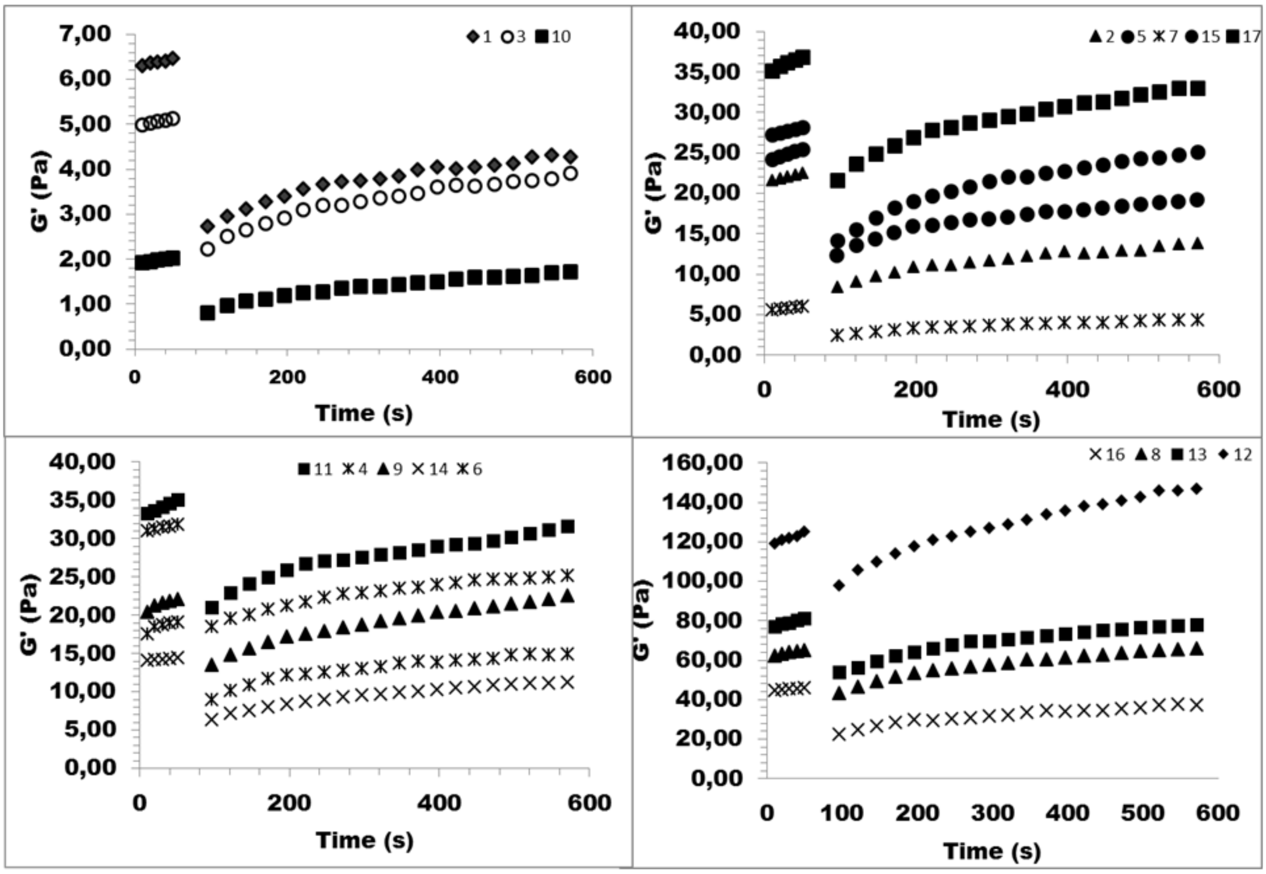

Figure 5. 3-ITT Rheological properties of the samples.

Table 6. Deformation and Recovery Percentage of The Emulsion Samples.

Run	RSG (%)	EYP (%)	Oil (%)	G'	
				Def (%)	Rec (%
1	3.00	3.00	20.00	57.81	52.55
2	4.00	5.00	10.00	64.60	45.61
3	3.00	3.00	10.00	56.53	56.92
4	3.00	3.00	20.00	42.01	66.77
5	2.00	1.00	30.00	53.41	62.50
6	4.00	1.00	10.00	52.67	63.87
7	2.00	3.00	20.00	61.22	53.53
8	4.00	1.00	30.00	33.23	82.62
9	2.00	5.00	30.00	38.91	81.90
10	2.00	1.00	10.00	59.90	58.62
11	3.00	3.00	30.00	42.15	74.10
12	4.00	5.00	30.00	18.42	102.50
13	4.00	3.00	20.00	35.36	76.46
14	2.00	5.00	10.00	56.64	57.47
15	3.00	1.00	20.00	49.65	67.73
16	3.00	3.00	20.00	51.30	65.00
17	3.00	5.00	20.00	41.19	75.61
C	0.4 XG	5.00	30.00		

Def (%): Percentage deformation, Rec. (%): Percentage recovery.

To determine statistical the effect of RSG, oil, and EYP on Def (%) and Rec (%), the quadratic model was applied, and model parameters were presented in Table 7. R^2 and adj-R^2 value showed that the quadratic model was successfully used to determine the effect of the model parameter on Def (%) and Rec (%). The model p-value for both Def (%) and Rec (%) was lower than 0.05, meaning that models were significant. Increasing either oil or RSG content reduced the percentage deformation values of emulsions, and the lowest deformation value (18.42%) observed was at 30% oil and 4% RSG content. In the model, the only linear term of oil and RSG content was found as significant, whereas their interaction was not (p = 0.059). Different than other responses, for Rec (%), the interaction of EYP and oil content was significant (p = 0.017). Other significant terms were in the model were oil (%), RSG (%), and their interaction. The highest recovery value (102.5%) obtained in the same formulation where minimum deformation observed. The recovery values higher than 70% were found at a high level of oil (30%) and a minimum of 3% RSG, whereas increasing the gum content similar recovery values can be obtained at lower oil content. When EYP and oil interaction was evaluated, it showed that only in a narrow region, higher recovery values were obtained when EYP content was low (<2%), and oil content was high. Increasing the EYP content at more moderate content of oil provide higher levels of recovery for the emulsion, which might be related to surface-active properties of EYP due to mainly its lecithin content. Figure 6 showed 3D and counterplot of the Def (%) and Rec (%). The Def (%) sharply decreases both oil and RSG increase, and Rec (%) increases as oil and RSG increase.

Table 7. Quadratic model parameters for Def (%)′ and Rec. (%) values.

Regression coefficients	df	Def (%)			Rec. (%)		
		Mean square	F-Value	p-Value	Mean square	F-Value	p-Value
Model	9	228.51	5.41	0.0183	308.29	6.98	0.009
Linear							
A	1	432.96	432.96	0.0150	325.36	7.36	0.0301
B	1	84.68	84.68	0.1997	77.01	1.74	0.2284
C	1	1086.18	1086.18	0.0014	1467.25	33.20	0.0007
Cross product							
AB	1	27.68	27.68	0.4448	34.57	0.78	0.4058
AC	1	214.25	214.25	0.0590	280.02	6.34	0.0400
BC	1	180.31	180.31	0.0776	430.56	9.74	0.0168
Quadratic							
A2	1	0.055	0.055	0.9722	0.33	7.54×10^{-3}	0.9332
B2	1	24.33	24.33	0.4726	107.09	2.42	0.1635
C2	1	2.20	2.20	0.8259	0.071	1.59×10^{-3}	0.9692
Lack of fit		33.90	0.54	0.7511	37.85	0.63	0.7073
R^2		0.8743			0.8997		
Adj-R^2		0.7128			0.7707		

A: Rocket Seed Gum, B: EYP (Egg Yolk Powder), C: Oil, Def (%): Percentage deformation, Rec. (%): Percentage recovery
Values of *"p"* less than 0.1000 indicate model terms are significant.

Optimization of RSG, EYP and oil content for low-fat dressing

The optimization was performed based on the rheological parameters of the control sample. The sample with a desirability value of 1 and containing minimum oil and EYP was chosen as the optimum formulation. The predicted value of the optimum formulation was 3.73 % RSG, 10 % oil, and 1 % EYP. The K, K ', K'', Def (%) and Rec (%) values corresponding to this formulation were 6.05 Pa.sn, 6.55 Pa, 1.92 Pa, 54.4 and 61.09, respectively. The experimental values of the sample produced at these points were determined as 6.25 Pa.sn, 7.05 Pa, 52.55 Pa, 64.05. As can be seen, the predicted and actual value of the optimum samples was close to each other, indicating that optimization procedures could be successfully validated.

Particle size, PDI, zeta potential and emulsion stability index

The average particle size diameter, PDI, and zeta potential values of the low fat and full-fat salad dressing samples were respectively found as 350 and 523 nm, 0.200 and 0.150, and 24.75 and 27.50 (mV). To determine emulsion stability, full-fat control samples, and low-fat salad dressing samples were stored at a temperature of 25°C to determine phase separation. No phase separation was observed in both two samples. As can be seen, low-fat salad dressing samples stabilized by RSG showed similar characteristics in terms of particle size, PDI, zeta potential, and emulsion stability index.

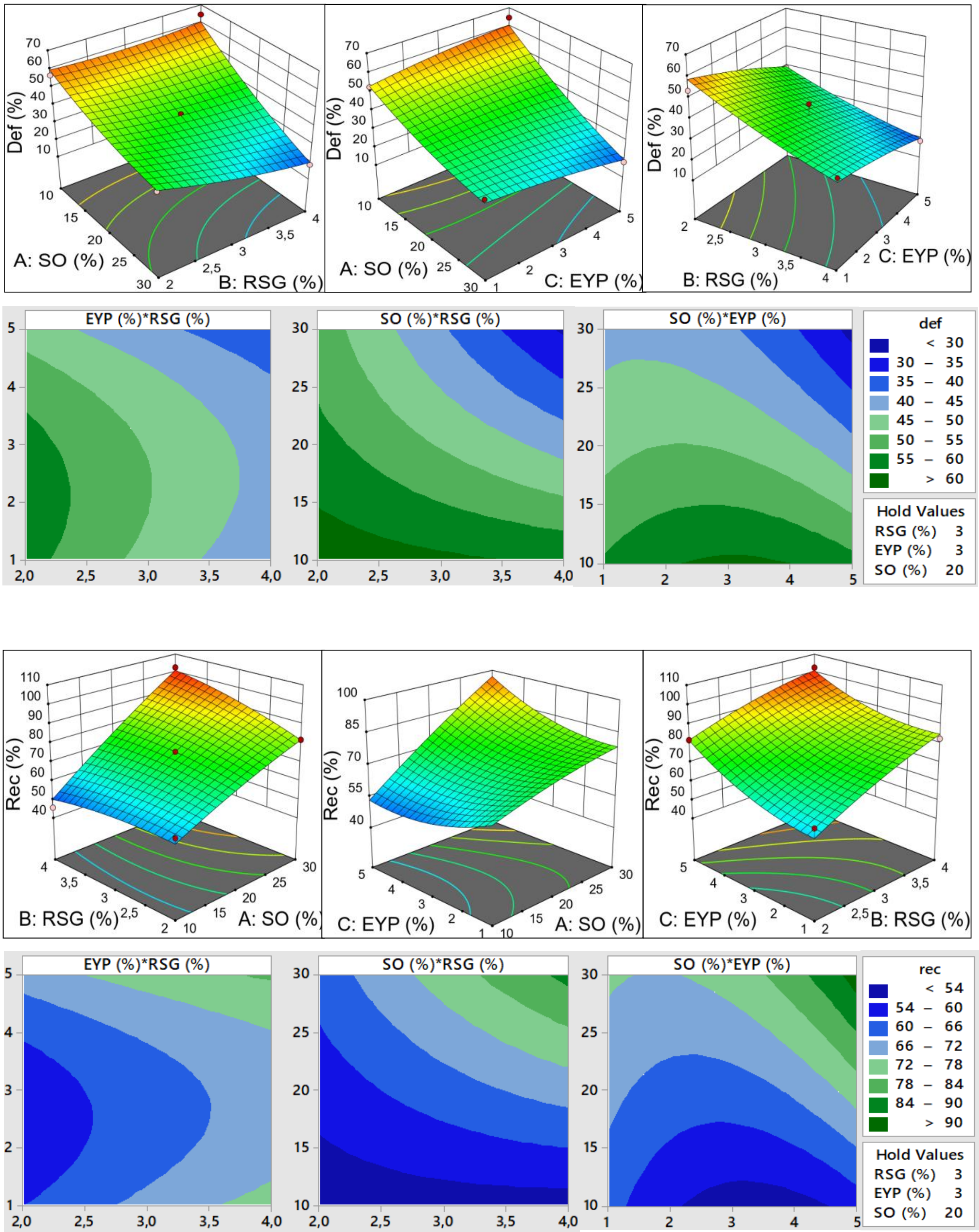

Figure 6. The effect of RSG, oil and EYP on the Def (%) and Rec (%) (Counter and 3D graph).

Conclusion

RSG and oil content significantly affected steady shear, viscoelastic, and 3-ITT rheological parameters. All samples prepared according to experimental design showed shear thing, viscoelastic behavior, and showed a certain amount of percentage recovery. The samples containing %3-4 RSG and low oil content (%10) showed higher τ_0, K, K', and % recovery compared to control samples. The samples prepared with moderate RSG and low oil contents showed desired rheological properties. The samples prepared according to optimum points showed similar particle size, PDI, and zeta potential value and showed no phase separation during the storage period. This study suggested that RSG could be used as natural fat replacers in low-fat salad dressing other emulsion formulation.

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