Journal of
Food Process Engineering

Journal of Food Process Engineering ISSN 1745–4530

CHARACTERIZATION OF GRAPE MOLASSES/SESAME PASTE/ HONEY BLENDS: MULTIPLE RESPONSE OPTIMIZATION OF SOME PHYSICOCHEMICAL, BIOACTIVE, VISCOELASTIC AND SENSORY PROPERTIES

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Received for Publication January 28, 2016
Accepted for Publication April 12, 2016

doi:10.1111/jfpe.12406

ABSTRACT

In this study, blends of grape molasses, sesame paste and honey were prepared at different ratios according to the mixture design and some physicochemical, rheological, bioactive and sensory properties of final blends were studied to create the optimum formulation for the final product. The results showed that the mixture components had significant change on the studied parameters ($P < 0.05$). Sugar composition of samples changed significantly by the addition of grape molasses and honey which resulted an increase in fructose and glucose. Grape molasses increase in the blend provided a significant increment in total phenolic content and antiradical activity. The blends had viscoelastic character and the mixtures containing higher honey showed more viscous behavior. The highest complex viscosity values were recorded for the blends of sesame paste and honey. Regression models having quite high determination of coefficients ($R^2 > 0.71$) were constructed for the studied parameters. Multiple response optimization results showed that the most preferred blend should contain 34.66% of grape molasses, 34.11% of sesame paste and 31.23% of honey.

PRACTICAL APPLICATIONS

Grape molasses, honey and sesame paste are commonly consumed food products which are rich in energy and many functional components. The people generally consume them in their daily diets especially in their breakfasts. To combine their functionality and sensory properties, an optimization procedure was applied and the mostly desired blend was determined. Blending affected the rheological, textural and bioactive properties of the sample significantly. The results of the study are very important for food industry.

INTRODUCTION

Grape molasses, sesame paste and honey are the foods consumed in daily diet especially in the breakfasts by many people in the world because of their special properties. Grape molasses is a concentrated grape juice obtained by boiling of crushed grapes up to 70–80% soluble dry matter[1-3]. Grape molasses is a good energy source because of its quite high sugar level and it is also a functional food due to its compositions like mineral, phenolics, organic acids, etc. (Sengül *et al.* 2005; Akbulut *et al.* 2008; Karaman and Kayacier 2011). Sesame paste is pressed and pasted sesame seeds which are ground, dehulled and dry roasted. It is a popular food product in Turkey and other Eastern countries and this product is rich in proteins (17–27%), carbohydrates (6.4–21%), lipids (54–65%) and dietary fiber (9.3%) in addition to many functional components. It was reported that it had high antioxidant activity because of its high polyunsaturated fatty acid and it showed ability to reduce the cholesterol (Abu-Jdayil *et al.* 2002; Abu-Jdayil 2004; Gharehyakheh *et al.* 2014). Honey is a natural sweet product produced by bees from the nectar of plants and it is very important energy source because it is a concentrated sugar solution containing organic acids and some amino acids, as well as certain macro and microelements, and many biologically active compounds (Juszczak and Fortuna 2006; Ahmed *et al.* 2007; Arslan *et al.* 2008; Karaman *et al.* 2011). In general, people prefer to consume these types of products as blended and molasses/sesame paste blends are very popular food mixture and it is started to produce industrially. Because of the increase in demand for the consumption of blends of these food products, many studies have been conducted by the researchers to characterize the many different properties of the blended samples. Alpaslan and Hayta (2002) investigated the rheological and sensory properties of molasses/sesame paste (tahin) blends containing different levels of molasses and reported that the addition of molasses to the tahin changed the physicochemical, rheological and sensory properties of final product significantly. Similarly, Arslan *et al.* (2008) investigated the rheological properties of tahin/molasses blends at different ratios and concluded that the blends showed pseudoplastic behavior. Akbulut *et al.* (2012) performed a study investigating the rheological, some physicochemical and sensory properties of sesame paste and honey blends and reported that the blends of sesame paste and honey showed non-Newtonian shear thinning behavior and affected the many parameters by the blending. As a result, several studies have carried out to see the effect of blending of molasses/sesame paste and sesame paste/honey, but there has been no report on bioactive, viscoelastic and sensory properties of grape molasses/sesame paste/honey blends (GSHB). The aim of the current study was to investigate the effect of GSHB on some physicochemical, rheological, bioactive and sensory properties of the final blended product by using mixture design modeling approach and optimize the most preferred formulation which could be produced using grape molasses, sesame paste and honey to combine their desired sensory and functional characteristics.

MATERIALS AND METHODS

Materials

Sesame paste (Koska Food Co, Bursa, Turkey), flower honey (Balparmak Food Co., İstanbul Turkey) and grape molasses (Koska Food Co, Bursa, Turkey) were commercially purchased from a local supermarket in Kayseri, Turkey.

TABLE 1. COMPONENTS OF GRAPE MOLASSES/SESAME PASTE/HONEY BLENDS ACCORDING TO SIMPLEX LATTICE MIXTURE DESIGN

	Coded values			Uncoded values (Ingredient proportions)		
Blends	X_1	X_2	X_3	Grape molasses (%)	Sesame paste (%)	Honey (%)
1	1.00	0.00	0.00	100	0	0
2	0.00	1.00	0.00	0	100	0
3	0.00	0.00	1.00	0	0	100
4	0.50	0.50	0.00	50	50	0
5	0.00	0.50	0.50	0	50	50
6	0.50	0.00	0.50	50	0	50
7	0.25	0.75	0.00	25	75	0
8	0.00	0.25	0.75	0	25	75
9	0.75	0.00	0.25	75	0	25
10	0.75	0.25	0.00	75	25	0
11	0.00	0.75	0.25	0	75	25
12	0.25	0.00	0.75	25	0	75
13	0.50	0.25	0.25	50	25	25
14	0.25	0.50	0.25	25	50	25
15	0.25	0.25	0.50	25	25	50

Preparation of Blends

To prepare the blends, the samples were mixed according to the design given in Table 1. For this purpose, the samples were weighed in a same beaker and mixed for 5 min using a stirrer at 600 rpm (IKA, RW 20DZM, IKA-works Inc., NC) at room temperature. After the preparation of the blends, the analyses were performed immediately and accordingly.

Physicochemical Analysis

An automatic colorimeter (Konica Minolta, model CM-5, Mississauga, ON, Canada) was used for the measurement of color parameters recorded as L, a and b. Water activity values were measured at 25°C using an Aqualab water activity (a_w) meter (Decagon, Pullman, WA).

The pH values of each blend solutions 10% (w/v) in distilled water at 25°C were measured with a pH meter (WTW-Inolab, Weilheim, Germany). An automatic refractometer (Reichert AR 700) was used for the determination of brix values at 20°C. Dry matter contents were determined by conventional drying method according to the described method in AOAC (2000). For the ash content, the samples were incinerated at 625°C in a muffle oven (Protherm, Ankara, Turkey). All analyses were performed as triplicate.

Determination of Sugar Composition

Major sugar amounts (fructose, glucose and saccharose) of the blends were determined using High Pressure Liquid Chromatography (HPLC) according to the procedure described by Jahanbin *et al.* (2012). One gram of sample was dissolved with 9 mL of distilled water and the mixture was shaked to be sure the all sugars dissolved effectively. Then, the samples were mixed with 1 mL of Carrez I and 1 mL of Carrez II and the samples were centrifuged at 5,500 × g for 5 min. Thereafter, the supernatant was filtered using a 0.45 μm syringe filters. The filtrate was injected to the HPLC (Agilent 1100) system equipped with a refractive index detector. An Agilent Zorbax carbohydrate analysis column (5 μm and 4.6 mm × 150 mm) was used and the analysis conditions were set as following: mobile phase, 80% acetonitrile and 20% water; flow rate, 1.4 mL/min; injection volume, 20 μL and the column temperature was set to be 25°C. Sugars were identified according to their retention times by comparing with sugar standards. The sugar concentrations of the samples were calculated using the prepared calibration curve of the each sugar.

Determination of Bioactive Properties

Total Phenolic Content. A modified method described by Karaman *et al.* (2014) was used for the determination of total phenolic content (TPC). For this purpose, roughly one g of sample was weighed and 9 mL of distilled water was added to obtain the extract. One hour shaking was performed for the extraction and finally, the samples were centrifuged a 5,500 × g for 5 min. The supernatant was filtered using 0.45 μm syringe filter. Then, 0.2 mL of the extract was mixed with 1.8 mL of distilled water in a tube and 1 mL of diluted Folin–Ciocelteau's phenol reagent (1:10 with distilled water) was added into a tube followed by mixing with a vortex for a while. At the end, 2 mL of Na_2CO_3 (2%, w/v) was added to the tubes and they were incubated for 2 h in a dark place at room temperature. At the end of the incubation, the absorbance of the samples was recorded at 760 nm using a spectrophotometer (8453E UV-Vis, Spectroscopy System, Agilent). TPCs of the mixed samples were calculated as mg of gallic acid equivalents (GAE) per kg sample.

Antiradical Activity. Antiradical activity (AA) analysis was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to the method of Karaman *et al.* (2014). Three point nine milliliter of DPPH solution (0.1 mM in methanol) was incorporated into the tubes containing 0.1 mL of extract and then the tubes were mixed using vortex. Then, the samples were incubated for 30 min in a dark place at 25°C. Absorbance of the samples was recorded at 517 nm using a spectrophotometer (8453E UV-Vis, Spectroscopy System, Agilent) at the end of incubation. AA of the blend samples was expressed as % inhibition using the following equation:

$$\%\,\text{Inhibition} = [(\text{Absorbance of control} - \text{Absorbance of sample})/(\text{Absorbance of control})] \times 100 \quad (1)$$

Rheological Measurements

In order to determine viscoelastic properties of samples, a stress/strain controlled and peltier temperature control unit equipped rheometer (Mars III, Karlsruhe, HAAKE, Germany) was used. To perform the rheological measurements, plate-plate geometry was (plate diameter 35 mm, and gap size 0.5 mm) used. Prior to frequency sweep test application, the linear viscoelastic region (LVR) of the blend samples was determined using stress sweep test. In LVR test, variation of dynamic mechanical spectra of samples versus increased stress was characterized. LVR of the blend samples was determined over a stress range of 0.1–10 Pa at constant frequency (1 Hz). In oscillatory frequency sweep test, dynamic mechanical spectra of the mixed samples were evaluated in the frequency range of 0.1–10 Hz at constant stress (0.2 Pa, within the range of LVR) at constant temperature (25°C). A sinusoidal stress or strain with an increasing frequency was applied to the samples and the elastic modulus G', the viscous modulus G'', complex modulus G^* and complex viscosity η^* and loss tangent were calculated as a function of frequency.

Sensory Analysis

The sensory analysis of the blend samples was performed based on the protocols described before[16]. Basically, 50 g of each blend was presented and served at certain intervals in randomly coded glass beakers of 100-mL capacity. Sensory evaluation was performed in a room with appropriate temperature (25°C) in open sitting. Sensory analyses of the samples were carried out by fifteen selected staff and graduate students of Food Engineering Department at Erciyes University, comprised of 10 females and 5 males. Each panelist was trained before evaluation in order to familiarize with the sensory analysis, samples and methodology. All coded blend samples were evaluated for color, oiliness, spreadability, firmness, adhesiveness, mouth coating, taste and overall acceptance properties in a scale ranging from 1 to 9 points where 1 reflected a very low in terms of disliking and 9 a very high score in terms of liking. Panelists evaluated all (15) samples in three sessions (five at each session) consecutively in same day.

Experimental Design and Optimization

Simplex Lattice Mixture Design. In the present study, the simplex lattice mixture design (SLMD) was used to evaluate the effect of grape molasses (x_1), sesame paste (x_2) and honey (x_3) on some physicochemical, compositional, bioactive, viscoelastic and sensory properties of the blended samples. Component proportions in the blends were expressed as fractions of the mixture with a sum ($X_1 + X_2 + X_3$) of one. These three factors; namely, grape molasses, sesame paste and honey (processing components), levels and experimental design in terms of coded and uncoded as 15 combinations are presented in Table 1.

Multiple linear regression analysis approach was used in the modeling. The following second-order polynomial equation of function x_i was fitted for each factor assessed at each experimental point.

$$Y=\sum_{i=1}^{3}\beta_i x_i+\sum_{\substack{i=1\\ i<j}}^{3}\sum_{j=i+1}^{3}\beta_{ij}x_i x_j = \beta_1 x_1+\beta_2 x_2+\beta_3 x_3+\beta_{12}x_1 x_2+\beta_{13}x_1 x_3+\beta_{23}x_2 x_3 \quad (2)$$

where Y was the estimated mixture response; β_1, β_2, β_3, β_{12}, β_{13} and β_{23} were linear and interaction terms, respectively, produced for the prediction models of processing components. Predictive models were constructed to evaluate the effect of mixture components (grape molasses, sesame paste and honey) on the characterized properties of blended samples. The best fitting models were determined using multiple linear regressions with backward elimination regression (BER) wherewith insignificant factors and interactions were eliminated from the regression models and only the variables having significant effect at $P < 0.01$, $P < 0.05$ and $P < 0.1$ levels were selected for the model construction using BER procedure.

Multiple Response Optimization. In industrial applications, optimization should be synchronously performed for all the responses involved since all responses are correlatively changed. In other words, it is not possible to think a response would change alone; namely independent of other responses. Moreover, a competition occurs between these responses in many cases; namely, improving one response may lead another response to deteriorate, further complicating the situation. In order to overcome this problem, multiple responses are solved through use of a desirability function which combines all the responses into one measurement (Yilmaz *et al.* 2011). Therefore, in this study, multiple response optimization (MRO) procedure that is applied to find the operating conditions, x providing the "most desirable" response values was followed. After each response variable was calculated, desirability values were combined into a single desirability index, D. For this purpose, each response was transformed in a dimensionless function. This is called partial desirability function, d_i which reflects the desirable ranges for each response ranging from zero to one (least to most desirable, respectively). It is possible to calculate the weighted geometric mean of n individual desirability functions (all transformed responses) (Eq. (3)) by definition of the partial desirability functions (Eq. (3)). The simultaneous objective function is a geometric mean of all transformed responses (Lewis *et al.* 1949; Myers and Montgomery 1995):

$$D = \left(d_1^{p_1}\times d_2^{p_2}\times d_3^{p_3}\times\ldots\times d_n^{p_i}\right)^{1/\sum p_i} = \left[\prod_{i=1}^{n} d_i^{p_i}\right]^{1/\sum p_i} \quad (3)$$

where p_i was the weighting of the i_{th} term, and normalized in order that $\sum_{i=1}^{n} p_i = 1$. By weighting of partial desirability functions, it is possible to enable the optimization process to take the relative importance of each response into consideration. Allowing the examination of the form of the desirability function, it is permitted to find the region where the function is close to 1 and determine the compromised optimum conditions.

Statistical Analysis

Design-Expert version 7.0 (Stat-Ease Inc., Minneapolis) and JMP version 9.0.2 (SAS Institute, Inc., Cary, NC) were used for the computational work including designation of experimental points, randomization and fitting of the second-order polynomial models as well as optimization. Analysis of variance was performed using the JMP version 5.0.1 (SAS Institute, Inc.). Least Significant Differences test was used to determine the significant differences at ($P < 0.05$) between blends for each parameter.

RESULTS AND DISCUSSION

Physico-chemical Properties of Blends

Physico-chemical properties of GSHB are presented in Table 2. *L*, *a* and *b* values were found to be 7.85, −0.008 and 1.168 for grape molasses; 45.11, 3.12 and 13.88 for sesame paste and 85.09, 1.308 and 39.79 for honey samples, respectively. Slight differences were observed between the results reported by Toker *et al.* (2013) and our findings, which might have resulted from concentration and types of pigment compounds such as anthocyanins present in molasses. *L*, *a* and *b* values of the blends changed between 12.41 and 41.74, 2.678 and 7.514 and 5.400 and 15.98, respectively, highlighting that as expected concentration of the mixture components significantly influenced the color values of the blends ($P < 0.05$). Effect of concentration of each mixture component and their interactions on color values of GSHB was indicated by the predicted model equations presented in Table 3. As is seen R^2 values calculated for *L*, *a* and *b* values were 0.93, 0.85 and 0.89, respectively, indicating that the

TABLE 2. MEAN VALUES FOR PHYSICO-CHEMICAL PROPERTIES OF GRAPE MOLASSES/SESAME PASTE/HONEY BLENDS

	Physical properties				Chemical properties				Sugar composition		
Blends	*L*	*a*	*b*	pH	a_w	Ash (%)	Dry matter (%)	Brix	Fructose content (%)	Glucose content (%)	Sucrose content (%)
1	7.852m	−0.008l	1.168k	5.73j	0.700a	1.447fg	73.5k	72.4h	22.1f	26.8c	0.37fg
2	45.11b	3.120i	13.88f	6.50a	0.446g	3.282a	99.6a	68.0fg	0.03o	1.86i	0.38fg
3	85.09a	1.308k	39.79a	4.22n	0.440g	0.136k	85.7g	81.7a	30.3b	27.9b	1.64b
4	23.60k	6.056d	10.27i	6.04f	0.632cd	2.321cd	82.3h	77.3c	12.3l	15.7g	0.30g
5	40.30d	5.298f	15.98b	6.23d	0.531f	1.760e	92.6c	73.8fg	15.9j	15.1g	0.87e
6	23.74k	7.404a	12.01h	5.31l	0.630d	0.747ij	79.7i	77.4c	31.1a	32.1a	1.52c
7	31.00h	5.406f	13.82f	6.25c	0.567e	2.874b	92. 8c	73.2gh	7.04n	10.0h	0.41f
8	37.75e	2.678j	13.67g	6.05f	0.537f	0.973hi	91.1d	81.9a	23.1e	22.2e	1.70b
9	12.41l	3.880g	5.400j	5.53k	0.670b	1.135h	75.3j	74.5e	25.6d	28.2b	0.90e
10	27.76j	7.514a	13.59g	5.99g	0.660bc	1.824e	79.8i	74.2f	19.1i	23.3d	0.37fg
11	41.74c	3.414h	13.92f	6.46b	0.364h	2.575c	95.7b	73.6fg	9.40m	9.26h	0.39fg
12	29.95i	6.630c	14.57d	4.96m	0.583e	0.511j	82.7h	78.7b	27.6c	27.7b	2.40a
13	31.47g	7.166b	15.17c	5.88h	0.630d	1.565ef	82.8h	76.4d	19.8h	23.2d	1.26d
14	33.95f	5.328f	14.36e	6.09e	0.584e	2.122d	89.7e	78.5b	14.2k	16.9f	0.13h
15	33.95f	5.796e	15.28c	5.78i	0.593e	1.214gh	86.7f	77.9bc	21.8g	23.0de	1.15d

[a–p]Different superscript lowercase letters show differences between the rows (mixtures) ($P < 0.05$).

generated models could be used to predict the color values of the blends depending on grape molasses, sesame paste and honey concentration. As understood from the equations, the linear terms in all predicted models were found to be significant ($P < 0.05$). Similar results attributed to concentration of mixture components and color values were reported by Akbulut *et al.* (2012) who determined that addition of honey in sesame paste/honey blends increased the *L*, *a* and *b* values. In addition, Alpaslan and Hayta (2002) determined that the brightness value decreased with addition of grape molasses in sesame paste/grape molasses blends. When conceiving the importance of color in preferability of the products, color of the blend could be adjusted depending on the consumer group by using predicted model equations.

Regarding the other physico-chemical properties; namely, pH, a_w, ash, dry matter and brix values, they were observed to be affected by addition of each component since their properties were found to be different from each other. pH and a_w values were determined as 5.73 and 0.70 for grape molasses, 6.50 and 0.45 for sesame paste and 4.22 and 0.44 for honey, respectively. From magnitudes of the regression coefficients of each component (Table 3), it is clear that sesame paste and grape molasses had the greatest effect to increase pH and a_w values of the blends, respectively. As known pH and a_w values play an important role in microbial stability of the product; therefore, these values could be standardized regarding storage conditions of the blends by changing concentration of mixture compounds. Ash, dry matter content and brix values of grape molasses, sesame paste, honey and their mixtures were found to between 0.136 and 3.282%, 73.5 and 95.7% and 68.0 and 81.9, respectively. Model equations established for those values and their R^2 values are also presented in Table 3. R^2 values were found as 0.99, 0.98 and 0.86 for ash, dry matter content and brix value, respectively. All linear terms were significant in the models. Regression coefficients in the models asserted that as sesame paste had the greatest increasing effect on ash and dry matter content and regarding brix value, honey had the greatest effect, which was expected when those properties of the grape molasses, sesame paste and honey samples as mentioned above were taken into consideration. As it is well known that determination of calorie value of such products is substantial during product development step; therefore, those established models can assist calorie calculations of the blends.

Fructose, glucose and sucrose contents of the grape molasses, sesame paste and honey and their blends prepared according to formulation presented in Table 1 also tabulated in Table 2. Sugar contents of the sesame paste were found to be very low. Fructose, glucose and sucrose concentrations of the grape molasses were determined as 22.1, 26.8 and 0.37%, and those for honey was 30.3, 27.9 and 1.64, respectively. Blend formulation markedly influenced the sugar composition as expected and fructose, glucose and sucrose concentrations of the blends were found to between 7.04 and 31.1, 9.26 and 32.1 and 0.13 and 2.40%, respectively (Table 2). Model equations were established for sugar contents and R^2 values were stated in Table 3. The sugar levels of the samples prepared according to the mixture levels could be calculated using mass balance. In this study, all mixtures were exposed to sugar analysis to understand the interactive reactions levels between reducing sugars in grape molasses or honey and proteins in sesame paste. For that reason, regression models were constructed to predict the sugar levels of the sample

TABLE 3. EFFECT OF EACH MIXTURE COMPONENT[a] AND THEIR INTERACTIONS ON PARAMETERS OF GRAPE MOLASSES/SESAME PASTE/HONEY BLENDS AS PREDICTED BY MODEL EQUATIONS

Parameters *(Y)*	Predicted model equations[b]	R^2
Physicochemical properties		
L	$Log_{10}(Y) = 0.88X_1+1.68X_2+1.82X_3+0.81X_1X_2-0.61X_2X_3+1.87X_1X_2(X_1-X_2)$	0.934
a	$Y = -0.10X_1+4.03X_2+2.40X_3+19.50X_1X_2+22.50X_1X_3+22.40X_1X_2(X_1-X_2)$	0.853
b †	$Log_{10}(Y) = 0.15X_1+1.09X_2+1.38X_3+2.16X_1X_2+1.03X_1X_3+2.53X_1X_2(X_1-X_2)$	0.893
pH	$Y = 5.71X_1+6.47X_2+4.31X_3+1.01X_1X_3+3.79X_2X_3-3.48X_2X_3(X_2-X_3)$	0.982
a_w	$Y = 0.68X_1+0.44X_2+0.44X_3+0.27X_1X_2+0.28X_1X_3+0.19X_2X_3-0.84X_2X_3(X_2-X_3)$	0.949
Ash †	$Y = 1.45X_1+3.30X_2+0.14X_3-0.14X_1X_2+0.23X_2X_3-0.73X_1X_2(X_1-X_2)$	0.993
Dry matter	$Log_{10}(Y) = 1.86X_1+2.00X_2+1.94X_3$	0.981
Brix	$Log_{10}(Y) = 1.86X_1+1.84X_2+1.92X_3+0.14X_1X_2$	0.861
Sugar composition		
Fructose content †	$Log_{10}(Y) = 1.36X_1-1.41X_2+1.49X_3+5.13X_1X_2+0.19X_1X_3+5.35X_2X_3-10.55X_1X_2X_3-4.68X_1X_2(X_1-X_2)+5.22X_2X_3(X_2-X_3)$	0.982
Glucose content †	$Log_{10}(Y) = 1.45X_1+0.30X_2+1.46X_3+1.40X_1X_2+1.27X_2X_3-1.06X_1X_2(X_1-X_2)+1.01X_2X_3(X_2-X_3)$	0.990
Sucrose content †	$Y = 0.34X_1+0.16X_2+1.84X_3+2.21X_1X_3$	0.820
Bioactive properties		
Total phenolic content	$Log_{10}(Y) = 3.25X_1+2.61X_2+2.49X_3+0.60X_1X_2+0.74X_1X_3+0.56X_2X_3-0.39X_1X_2(X_1-X_2)-0.39X_1X_3(X_1-X_3)+0.31\ X2X3\ (X2-X3)$	0.993
Antiradical activity †	$Log_{10}(Y) = 1.38X_1+0.67X_2+0.78X_3-0.16X_1X_2-1.05X_1X_3-2.51562X_2X_3+12.50X_1X_2X_3+5.98X_2X_3(X_2-X_3)$	0.839
Viscoelastic properties		
G' †	$Log_{10}(Y) = 2.88X_1+2.22X_2+1.53X_3+3.60X_1X_2+6.63X_2X_3-10.6X_1X_2(X_1-X_2)+9.66X_2X_3(X_2-X_3)$	0.826
G'' †	$Log_{10}(Y) = 2.51X_1+2.02X_2+2.02X_3+5.18X_1X_2+0.17X_1X_3+6.68X_2X_3-19.71X_1X_2X_3-12.63X_1X_2(X_1-X_2)+7.52X_2X_3(X_2-X_3)$	0.912
η^* †	$Log_{10}(Y) = 1.93X_1+1.41X_2+1.25X_3+5.34X_1X_2+0.94X_1X_3+7.17X_2X_3-21.77X_1X_2X_3-11.21X_1X_2(X_1-X_2)+9.35X_2X_3(X_2-X_3)$	0.903
G^* †	$Log_{10}(Y) = 2.72X_1+2.20X_2+2.05X_3+5.34X_1X_2+0.94X_1X_3+7.17X_2X_3-21.77X_1X_2X_3-11.20X_1X_2(X_1-X_2)+9.35X_2X_3(X_2-X_3)$	0.902
$\tan\delta$ †	$Y = 0.59X_1+1.15X_2+8.70X_3-0.40X_1X_2-18.32X_1X_3-17.06X_2X_3+34.22X_1X_2X_3+19.60X_1X_3(X_1-X_3)+8.85X_2X_3(X_2-X_3)$	0.958
Sensory properties		
Color	$Y = 8.05X_1+7.80X_2+7.93X_3-10.60X_1X_2-6.38X_1X_3-8.36X_2X_3+74.0X_1X_2X_3+8.70X_1X_2(X_1-X_2)$	0.893
Oiliness	$Y = 8.23X_1+7.80X_2+6.47X_3-8.28X_1X_2-10.2X_1X_3-4.25X_2X_3+56.4X_1X_2X_3-8.53X_1X_3(X_1-X_3)$	0.901
Spreadability	$Y = 5.03X_1+6.45X_2+7.58X_3-2.28X_1X_2-5.85X_1X_3-7.78X_2X_3+81.2X_1X_2X_3+17.7X_1X_3(X_1-X_3)$	0.822
Firmness	$Y = 6.55X_1+6.32X_2+7.72X_3+4.56X_1X_2-9.70X_1X_3$	0.804
Adhesiveness	$Y = 5.71X_1+4.40X_2+7.58X_3+2.51X_1X_2-4.86X_1X_3-2.54X_2X_3+54.8X_1X_2X_3$	0.851
Mouth coating	$Y = 5.88X_1+6.29X_2+7.44X_3+0.66X_1X_2-5.97X_1X_3-2.25X_2X_3+50.73X_1X_2X_3$	0.712
Taste	$Y = 8.30X_1+6.73X_2+8.43X_3-4.07X_1X_2-8.27X_1X_3-5.18X_2X_3+67.3X_1X_2X_3$	0.728
Overall acceptance	$Y = 7.67X_1+6.82X_2+8.28X_3-2.41X_1X_2-8.54X_1X_3-6.47X_2X_3+41.3X_1X_2X_3+10.13X_1X_2(X_1-X_2)$	0.812

[a] X_1, X_2 and X_3 were the mixture components; grape molasses, sesame paste and honey, respectively.

[b] By applying BER "backward elimination regression" procedure, non-significant interactions were removed from the equations. Only the variables significant at $P<0.01$, $P<0.05$ and $P<0.1$ levels were selected for the predicted model construction.

† These were parameters whose ratios of maximum response to minimum one were greater than 10, which means that a transformation was required. Therefore, a logarithmic transformation, $Y = \log_{10}(y+k)$ was performed for these parameters.

TABLE 4. MEAN VALUES FOR BIOACTIVE AND VISCOELASTIC PROPERTIES OF GRAPE MOLASSES/SESAME PASTE/HONEY BLENDS

	Bioactive properties		Viscoelastic properties				
Blends	Total phenolic content (mg GAE/kg sample)	Antiradical activity (% inhibition)	*G*′ (Pa)	*G*″ (Pa)	η* (Pa s)	*G** (Pa)	tan δ
1	1751a	18.91a	359.4cd	258.2c	70.55e	443.3e	0.747cd
2	410.2i	4.883g	74.87d	70.61c	16.42e	103.21e	1.023cd
3	310.3j	9.942d	12.49d	108.0c	17.31e	108.75e	8.965a
4	1232c	9.601d	1658cd	1159c	322.1d	2023d	0.703cd
5	528.7h	2.286h	2342cd	2786c	579.3c	3640c	1.190cd
6	1122d	8.974de	389.1cd	188.1c	68.85e	432.6e	0.488cd
7	815.1f	7.545ef	38870b	51820a	10375a	65195a	1.387bc
8	379.7i	0.306i	264.2cd	525.0c	97.47de	612.3de	2.225b
9	1462b	16.00b	406.8cd	294.9c	82.97de	521.2de	0.909cd
10	1436b	15.15b	545.0cd	271.1c	96.85de	608.7de	0.498cd
11	497.6h	4.516g	48460a	16899b	8390.0b	52715b	0.352d
12	725.3g	2.163h	147.4d	123.2c	30.72e	193.0e	0.910cd
13	1166d	11.98c	713.0cd	365.6c	127.5de	801.2de	0.514cd
14	886.3e	8.517def	3006c	2433c	616.6c	3874c	0.810cd
15	805.5f	6.745f	295.7cd	328.8c	70.68e	444.1e	1.144cd

[a–i]Different superscript lowercase letters show differences between the rows (mixtures) ($P < 0.05$).

without performing an analysis. R^2 values calculated for fructose, glucose and sucrose content were found to be 0.98, 0.99 and 0.82, respectively, implying that sugar contents of the GSHB could be satisfactorily predicted depending on grape molasses, sesame paste and honey concentration found in the formulation. All of the linear factors significantly affected the sugar contents as understood from equations. As expected sesame paste had the lowest effect on the change in concentration of all sugar types and honey had the greatest effect. Generally, sugar contents were increased by honey and grape molasses in GSHB (Table 2), sesame paste which contain less sugar than the others decreased fructose (Tables 2 and 3). Chemically, honey (80–85%) and molasses (%70–72) comprise sugar (Sengül *et al.* 2005; Akbulut *et al.* 2012; Tornuk *et al.* 2013). As is seen, generally sugar composition level increased with the addition of grape molasses and honey in blends, which should be taken into consideration during product optimization.

Bioactive Properties of Blends

Table 4 shows the TPC and AA values of both sole and blend samples. For the mixture components, grape molasses showed the highest TPC (1,751 mg GAE/kg sample) compared to sesame paste (410.2 mg GAE/kg sample) and honey (310.3 mg GAE/kg sample). For the blend samples, the highest TPC level was in the sample (R9) prepared by the mixture of grape molasses and honey at the ratio of 75:25 (w/w) while the lowest value was in the sample (R8) by the mixture of sesame paste and honey at the ratio of 75:25 (w/w). Figure 1a shows the change in the TPC of the samples depending on the level of mixture component in the prepared blends. It is clear from the figure that TPC increased toward to the vertex of the grape molasses and decreased to the vertex of the honey. According to table, 100% grape molasses is inclusive blend which involves the highest results of TPC. Sesame paste and grape molasses mixtures at the level of 50:50 (w/w) showed higher TPC compared to blend of honey : grape molasses at the level of 50:50 (w/w) because ternary plot shows higher increment toward to the edge of sesame paste and grape molasses blends. Statistically significant differences were determined in terms of their TPC among the blends ($P < 0.05$). AA values of samples were also tabulated in Table 4 and it was seen that there was a significant positive correlation between TPC and AA ($r = 0.853$, $P < 0.05$). The highest AA value (18.91%) was determined in grape molasses sample while the lowest (0.306%) was in the sample having the lowest TPC among the blend samples. Ozturk *et al.* (2014) reported that there was a relationship between TPC and radical scavenging activity. Figure 1b shows the ternary plot of the change in the AA values of samples. As similar to the change in TPC depending on the mixture component, AA significantly increased toward to the vertex of the grape molasses and decreased toward to the edge of sesame paste and honey. Table 3 shows the predicted regression equations showing the effect of concentration of each mixture component and their interactions on bioactivity values of blend samples. High determination of coefficients showed that the constructed models for TPC and AA could be used to predict the bioactivity values of the blends depending on grape molasses, sesame paste and honey concentration (Table 3). As understood from the equations, the linear terms of all

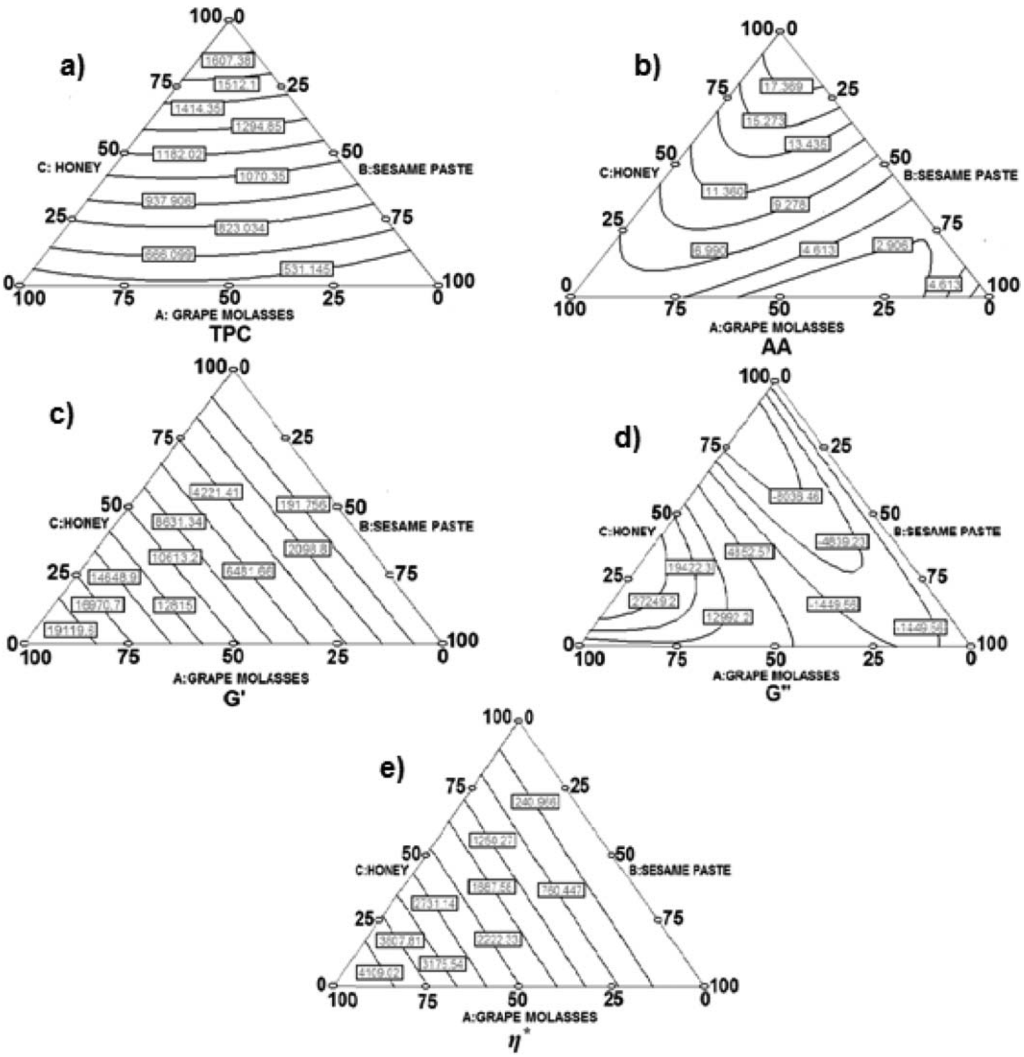

FIG. 1. TERNARY CONTOUR PLOTS SHOWING THE EFFECTS OF PROCESSING COMPONENTS ON BIOACTIVE PARAMETERS G', G'' AND η^* GRAPE MOLASSES/SESAME PASTE/HONEY BLENDS

mixture components in the predicted models for bioactivity properties were found to be significant ($P < 0.05$). As a conclusion, the mixture component type used in the prepared blend has significant effect on the bioactive activity of the final product. It was obvious from these results that grape molasses had a major effect on phenolic content and AA. The reason for this effect is undoubtedly their contents; according to literature; grape molasses contains high amounts of sugar, mineral and organic acid in addition to sesame paste is rich source in dietary fiber, niacin, calcium, iron, phosphorous, thiamin and sesamol (Ustün and Tosun 1997; Demirözü *et al.* 2002; Sengül *et al.* 2005) and honey contains organic acids (gluconic acid, acetic acid, etc.), vitamins (ascorbic acid) and phenolic substances such as flavonoids and carotenoids (Habibi-Najafi and Alaei 2006; Gharehyakheh *et al.* 2014).

Viscoelastic Properties of Blends

Loss modulus, storage modulus, complex viscosity, complex modulus and tangent delta values were determined for the each sample and the viscoelastic properties of samples were shown in Table 3. Storage modulus and loss modulus values of all samples were determined as a function of frequency and generally, an increase in the frequency increased the storage and loss modulus values of the samples (Fig. 2). Grape molasses and sesame paste exhibited elastic behavior, showing that G' value was higher than G'' values. However, honey samples exhibited viscous behavior. It was found that G'' was significantly ($P < 0.05$) higher compared to G' value in honey (R3) and the blends rich in honey. Kayacier *et al.* (2014) and Yilmaz *et al.* (2014) reported that the loss modulus values of honey were tremendously higher than storage modulus, showing that honey is weak gel like liquid product

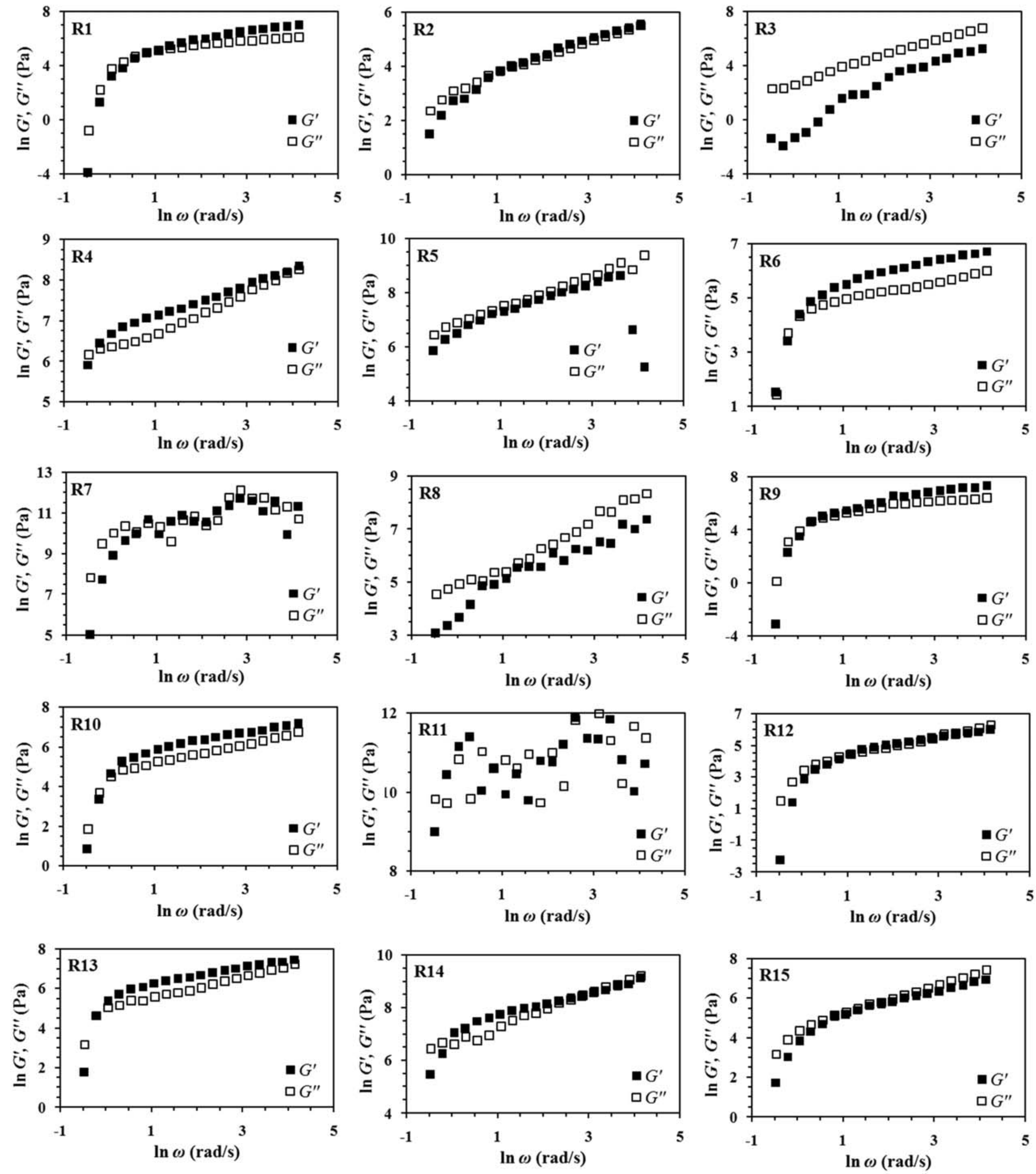

FIG. 2. *G′* (STORAGE MODULUS) AND *G″* (LOSS MODULUS) VALUES OF GRAPE MOLASSES/SESAME PASTE/HONEY BLENDS AS A FUNCTION OF ANGULAR FREQUENCY (ω). R1–R15, THE EXPERIMENTAL BLENDS (RUNS) FROM 1 TO 15

because of its Newtonian behavior. Storage modulus values of samples at 1 Hz ranged between 12.49 and 48,460 Pa. The lowest storage modulus value was in the honey sample (R3) while the highest was in blend sample prepared by sesame paste : honey at the level of 75:25 (w/w). The differences between the all storage modulus values of samples were determined to be significant statistically ($P < 0.05$) As can be seen clearly from the Fig. 1, elastic modulus increased tremendously toward to the vertex of honey and there was a decrease toward to the edge of grape molasses-honey. Loss

TABLE 5. MEAN VALUES FOR SENSORY PROPERTIES OF GRAPE MOLASSES/SESAME PASTE/HONEY BLENDS

	Sensory properties Viscoelastic properties							
Blends	Color	Oiliness	Spreadability	Firmness	Adhesiveness	Mouth coating	Taste	Overall acceptance
1	8.2a	8.2a	5.3cde	6.5bcdef	5.6bcde	5.8cd	8.5a	7.9ab
2	8.0ab	8.0ab	6.9abc	6.2cdef	4.8de	6.7abc	7.1bcdefg	7.0abcd
3	7.9abc	6.4cd	7.4a	7.7ab	7.5a	7.2abc	8.2abc	8.1a
4	6.1ef	6.3cd	6.4abcd	8.0a	6.4abcd	7.2abc	7.5abcde	7.6ab
5	5.6fg	6.0de	5.0de	6.6bcdef	5.1cde	5.9bcd	5.9gh	5.7ef
6	6.7bcdef	4.9e	5.3cde	4.0g	5.6bcde	4.9d	6.7defg	5.8def
7	4.5g	5.9de	3.1f	6.6bcdef	4.1e	5.1d	5.2h	5.0f
8	6.6cdef	6.2cd	6.3abcd	7.5ab	6.6abc	7.0abc	7.3abcdef	7.0abcd
9	6.7bcdef	4.8e	4.3ef	5.7ef	5.1cde	5.1d	6.2fgh	6.1cdef
10	6.3ef	6.5cd	5.9abcde	6.9abcde	5.9abcd	6.1abcd	7.0cdefg	7.3abc
11	6.2ef	6.8bcd	4.8def	7.0abcd	4.8de	6.4abcd	6.3efgh	6.0def
12	6.5def	5.8de	5.6bcde	5.4f	6.2abcd	6.3abcd	7.0cdefg	6.7bcde
13	7.8abcd	7.3abc	7.3ab	6.0def	7.2ab	7.1abc	7.8abcd	7.5ab
14	7.2abcde	6.6cd	7.0abc	7.3abc	7.1ab	7.4ab	8.3ab	6.7bcde
15	7.8abcd	6.8bcd	7.4a	6.7bcde	7.0ab	7.5a	8.2abc	7.0abcd

a–g Different superscript lowercase letters show differences between the rows (mixtures) ($P < 0.05$).

modulus values of the samples also changed depending on the mixture component significantly ($P < 0.05$) and the lowest loss modulus value (108 Pa) was in the sample of sesame paste. The highest value (16,899 Pa) was recorded for the blend prepared with sesame paste and honey (R11). The viscous behavior of honey samples systematically increased as function of angular frequency. Domination viscous behavior of samples indicated weak particle–particle interactions and there was no network formation in honey samples. Complex viscosity values of samples were also affected by the mixture component significantly ($P < 0.05$). Addition of honey, grape molasses and sesame paste to the blends increased viscosity of the final products (Table 3). Specially, addition of the sesame paste in blends increased viscosity tremendously (Fig. 2e). In the blends coded as R7–R11 containing %75 sesame paste was present, complex viscosity was the highest. However, the lowest complex viscosity was observed in the blends coded as R3–R12 containing 75–100% honey (Table 3). These results indicated that sesame paste was the component having the highest increasing effect on the consistency of the blends samples. Furthermore, binary interactions of sesame paste had more effect on increasing viscosity in the blends. This effect can be attributed to interactions between protein-carbohydrates in sesame paste blends. Effect of concentration of each mixture component and their interactions on viscoelastic parameters was indicated by the predicted model equations presented in Table 3. As is seen R^2 values calculated for G', G'', G^*, η^* and tan δ values were 0.83, 0.91, 0.90, 0.90 and 0.96, respectively, indicating that the constructed models could be used to predict the dynamic mechanical properties of the blends depending on grape molasses, sesame paste and honey concentration (Table 3). It is clear from the equations that the linear terms in all regression models were found to be significant ($P < 0.05$). It was reported that the viscosity of sesame paste known as tahin increased with the increase of honey addition significantly ($P < 0.05$). Honey is rich in sugar which is responsible for the viscosity of honey, whereas sesame paste is basically structured oil and protein[13]. The increase in the viscosity is of course is also related to solid content because the higher solid content generally cause increment in the viscosity because of molecular movements and interfacial film formation (Bhattacharya *et al.* 1992; Maskan and Göğüş 2000; Alpaslan and Hayta 2002). The other viscoelastic parameters namely complex modulus and tangent delta were also significantly affected by the mixture components ($P < 0.05$).

Sensory Properties of Blends

As is known, during product formulation, optimization of sensory analysis of the product is escapable since it determines acceptance and rejection of the product according to consumers' response. All sensory properties of the GSHB were generally affected by concentration of each mixture component concentration (Table 5); therefore, those results should be considered depending on the consumer requirement during formulation step. Established model equations and R^2 values are also tabulated in Table 3 where findings indicated that dependent parameters (color, oiliness, spreadability, firmness, adhesiveness, mouth coating taste and overall acceptance) can be victoriously predicted using generated models. Due to a dilution effect of sesame paste on the sweetness of grape molasses and honey, sesame paste may increase the sensory properties of blends[13]. The adhesiveness

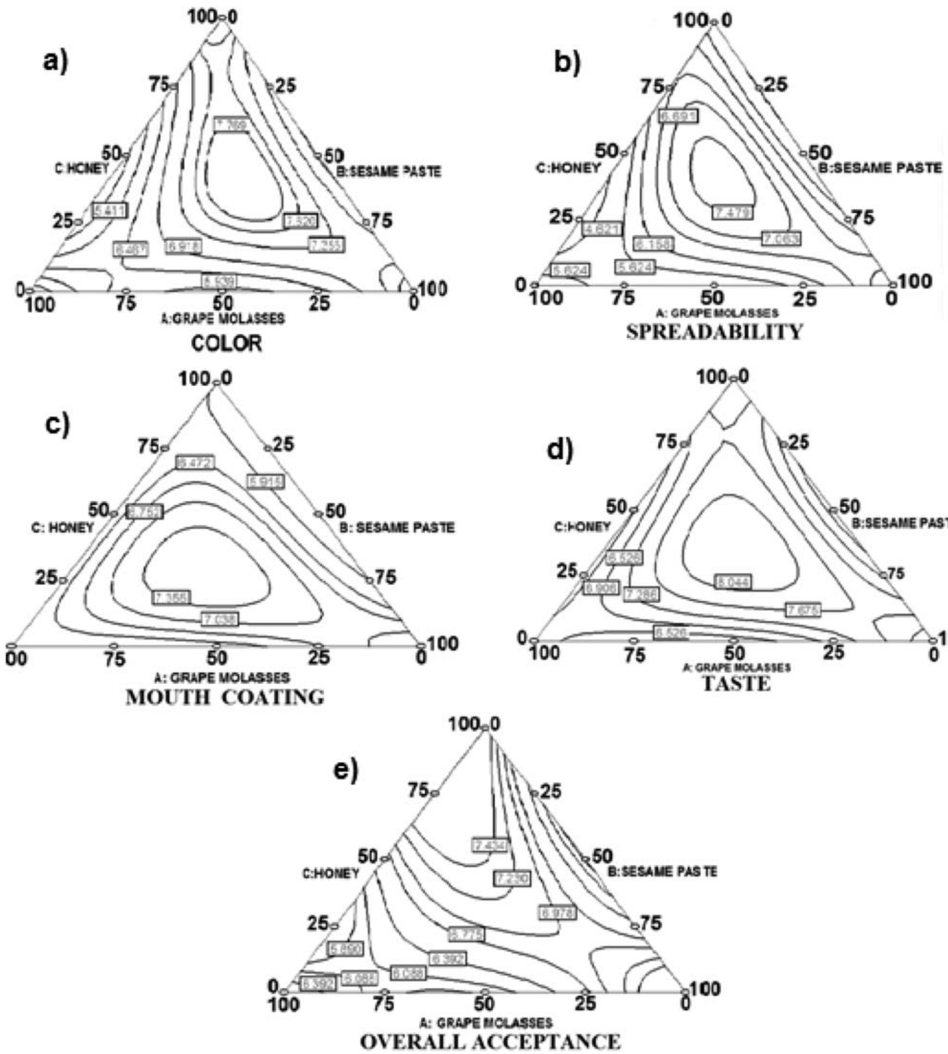

FIG. 3. TERNARY CONTOUR PLOTS SHOWING THE EFFECTS OF PROCESSING COMPONENTS ON SENSORY PROPERTIES OF GRAPE MOLASSES/SESAME PASTE/HONEY BLENDS

and spreadability value of blends increased by the addition of grape molasses to sesame paste. Akbulut *et al.* (2012) obtained similar findings about spreadability. Also the addition of grape molasses to sesame paste increased color and oiliness values of blends. On the contrary, according to Alpaslan and Hayta (2002) oiliness had been decreased with addition of grape molasses to sesame paste. The highest oiliness value must be 100% sesame paste in blends. But in this study, it can be seen that the grape molasses has highest oilness score (Table 5). Honey indicated more viscous characteristic which influenced more mouth coating than the others and addition of honey in blends indicated more mouth-coating properties (Table 5). Overall acceptance of the GSHB was significantly affected by all of the linear factors in the following order from greatest effect to lowest effect: honey, sesame paste and grape molasses. Figure 3 shows the ternary contour plots for color, spreadability, mouth coating, taste and overall acceptance parameters.

Multiple Response Optimization

Foods are very complex products; therefore, during the optimization of formulation, many factors should be taken into consideration meanwhile since there are many factors determining quality of the product. Accordingly, in the present study, MROs were performed to optimize formulation of GSHB. Three different optimization criteria were determined. One of them was bioactive properties: the blend including 30.28% of sesame paste and 69.72% of honey had the highest TPC and AA (Table 6). Regarding viscoelastic properties, the sample containing 34.01% of grape molasses, 4.89% of sesame paste and 61.10% of honey had the highest viscoelastic parameters' (G', G'', η^*, G^* and tan δ) value. The

TABLE 6. MULTIPLE RESPONSE OPTIMIZATION PROCEDURE APPLIED TO FIND OPTIMUM VALUES FOR BIOACTIVE, VISCOELASTIC AND SENSORY PARAMETERS OF GRAPE MOLASSES/SESAME PASTE/HONEY BLENDS

	Multiple response optimization									
	Minimization					Maximization				
Parameters	Minimum levels	Grape molasses (%)	Sesame paste (%)	Honey (%)	Desirability	Maximum levels	Grape molasses (%)	Sesame paste (%)	Honey (%)	Desirability
Bioactive properties										
Total phenolic content*	445.8	0.00	30.28	69.72	0.941	1,739	100	0.00	0.00	0.996
Antiradical activity†	0.72					19.1				
Viscoelastic properties										
G' (Pa)	597.1	34.01	4.89	61.10	1.000	19,739	14.27	85.73	0.00	0.342
G'' (Pa)	2,442					32,491				
η^* (Pa s)	183.8					4273				
G^* (Pa)	1,155					26,847				
tan δ	0.321					1.289				
Sensory properties										
Color	6.4	57.31	0.00	42.69	0.718	7.8	34.66	34.11	31.23	0.83
Oiliness	4.7					7.1				
Spreadability	4.7					7.6				
Firmness	4.7					6.3				
Adhesiveness	5.3					7.4				
Mouth coating	5.1					7.6				
Taste	6.3					8.4				
Overall acceptance	5.8					7.2				

*Total phenolic content values were expressed as mg GAE/kg sample.
†Antiradical activity values were expressed as % inhibition.

last criterion was selected as sensory properties of the samples and the blend comprised of 57.31% of grape molasses and 42.69% of honey had the highest sensory scores.

CONCLUSIONS

Honey, sesame paste and grape molasses are widely consumed in breakfast. Blending of them provides advantage in many aspects. By this way, it is possible to fabricate products with functional characteristics, sweet and unique aroma, which can motive people of all ages to consume this novel natural product. Concentration of honey, sesame paste and grape molasses plays an important role in determining quality characteristics of the blend; namely, chemical, bioactive, rheological and sensory properties. Therefore, optimization of product formulation is vital. In this study, SLMD was accomplishedly performed to observe change in those quality parameters as a function of honey, sesame paste and grape molasses concentration. Almost all parameters were significantly affected by concentration of them. MRO technique was conducted to simultaneously optimize quality parameters of the blend, which is very important for the food industry in many aspects. By means of mixture design, it is possible to produce blend depending on intended use (consumer group) and storage condition considering quality parameters of the final product. In addition, this natural and functional blend can be used in formulation of different food products such as biscuits, cake, chocolates, etc.

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