EFFECT OF HYDROCOLLOID TYPE AND CONCENTRATION ON FLOW BEHAVIOUR AND SENSORY PROPERTIES OF URMU MULBERRY (*MORUS NIGRA* L.) SAUCES: A RESPONSE SURFACE APPROACH

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Abstract

Urmu mulberry sauces were prepared using three commercial hydrocolloids (Locust bean gum (LBG), Xanthan (XA), ι-Carrageenan (ι-CARR)). Response surface methodology was used to analyze the effect of hydrocolloid type and concentration on rheological responses of consistency (K), flow index (n), apparent viscosity (n_{app}) and sensorial responses of taste, sliminess, consistency and overall acceptability. Significant regression models were obtained with high determination coefficients (R^2>0.9554) for rheological responses of sauces prepared with XA and ι-CARR. No definite trend was observed for rheological responses sauces prepared with LBG. Greater stability of sauces prepared with negatively charged XA and ι-CARR may basically due to the electrostatic repulsion or higher viscosity as compared to sauces prepared with neutral LBG. All sauces took good consumer acceptance. The compromised optimum conditions obtained by numerical integration to prepare Urmu mulberry sauces were: sauces prepared with 0.30 % XA concentration which have good flow characteristics and satisfactorily consumer acceptance.

Key words: *sauce, hydrocolloid, rheology, sensory, optimization*

1. INTRODUCTION

Urmu mulberry (the local name in Southeast part of Turkey) is only grown in the province of Gaziantep, Urfa and Kahramanmaraş in Turkey. In Gaziantep region, it is very famous and it has relatively high consumption due to its traditional use as a natural medicine. Urmu mulberry is consumed as a fresh fruit, its sherbet is also produced and sold by hand sellers in Gaziantep, and it is used for mouth lesions, strengthen the teeth, diabetes, hypertension, etc. (Kara & Erçelebi 2013). In literature, there is no study about Urmu mulberry except studies of Kara and Erçelebi (2013). So, sufficient knowledge about Urmu mulberry has not been presented, and further studies are needed in literature.

Hydrocolloids may be used in food suspensions due to their functional properties: water-holding capacity, regulation of the rheological properties and ionization in aqueous solutions, leading to stabilization of insoluble particles. In terms of ionization, food gums may or may not have electrical charge (Genovese & Lozano 2001). In literature, there are several studies covers the using hydrocolloids to improve the cloud stability of juices (Yamasaki, Yasui & Arima 1964; Genovese & Lozano 2001; Koksoy & Kilic 2004; Liang et al. 2006; Erçelebi, Kara & İbanoğlu 2011). The use of two or more hydrocolloids in the formulation of single food is a common practice for the synergistic effect of combined use as they can impart better rheological properties to the product which may also bring about cost-reduction manufacturing (Kayacier & Dogan 2006). Studies mainly focuses on properties of polysaccharide blends, for instance, starch-xanthan in dessert strawberry sauces (Sikora et al. 2007), oat starch, xanthan, oat hydrolysate, potato starch combinations in sweet and sour sauces (Gíbińiski et al. 2006), modified starch or modified starch/xanthan mixture in commercial chili sauces (Gamonpilas et al. 2011), potato starch and xanthan gum in caramel sauces (Krystyjan et al. 2012), starch-xanthan in cocoa syrups (Sikora at al. 2003), guar gum dextrin in aqueous solutions (Pinder et al. 2003), alginate pectin gels (Walkenstroem et al. 2003), κ-carrageenan–xanthan gum gels (Dunsten et al. 2001), gellan-ι–carrageenan and gellan–xanthan gum gels (Rodriguez-Hernandes & Tecante 1999), and many others are available in literature.

In this paper, hydrocolloids of Locust bean gum (LBG), Xanthan (XA) and iota-carrageenan (*ι*-CARR) were used during sauce preparation to increase viscosity and physical stability of the sauces.

LBG, also known as carob or carubin, is a galactomannan (non-gelling neutral polysaccharides found in the endosperm of *Leguminosae*), and it is consisting of a β-(1→4)-mannan backbone to which single D-galactopyranosyl residues are attached via α-(1→6) linkages (Rocha et al. 2009). XA is a non-linear anionic microbial polysaccharide produced by aerobic fermentation of *Xanthomonas campestris* (Rodriguez-Hernandes & Tecante 1999). It is composed of a 1-4-linked β-D-glucose backbone substituted with a trisaccharide side chain that is composed of two mannoses and one glucuronic acid at every second residue (Higiro et al. 2007). Carrageenans are an anionic linear polysaccharides extracted from red seaweed (*Rhodophyceae*) (Černíková et al. 2008). Iota-carrageenan is composed of alterning α(1,3)-Dgalactose-4-sulphated and β(1,4)-3,6-anhydro-D-galactose-2-sulphate(Karbowiak et al. 2006) and it usually generates soft, elastic gels (Černíková et al. 2008). Generally, hydrocolloids and stabilizers have non-Newtonian rheology, and they impart non-Newtonian character to the emulsions even when the amount of the dispersed phase is low (Koocheki et al. 2009). Power law model has been frequently used to characterize the flow behavior of hydrocolloid solutions (Kayacier & Dogan 2006). Under this perspective, the objectives of this study are to examine the effects of hydrocolloid type and concentration on the rheological and sensory properties of Urmu mulberry sauces prepared with LBG, XA and *i*-CARR at varying concentrations. Evaluation of performance of these hydrocolloids was based on their effect on rheology and sensory properties.

2. MATERIALS AND METHODS

2.1. Materials

Urmu mulberry (*Morus nigra* L.) fruits were collected in Gaziantep, Southeast Anatolia, Turkey. Pectolytic enzyme, Panzym XXL, was kindly gifted by Sinerji A.Ş., Mersin, TURKEY. Locust bean gum (LBG) (G-0753), xanthan hydrocolloid (XA) (G-1253) and *ί*-carrageenan (*ί*-CARR) (C-1138) were purchased from Sigma Chemical Company. Buffer salts, potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate were purchased from Analar analytical reagent; BDH chemical Ltd. All the other reagents were of analytical grade.

2.2. Preparation of juices and concentrates

All the foreign materials such as pieces of branches and leaves and also unripe and damaged fruits were removed from Urmu mulberry samples by hand. The cleaned fruits were washed under cold tap water, stalks and seeds were removed if it is available. Fruits were ground by using a laboratory blender. Juice was immediately filtered through muslin to remove pulp from the juice. Then the juice was depectinized with 1.0 % (w/v) Panzym XXL at 50 °C for 2 h. The depectinized juice was allowed to rest at 4°C for 24 h. The juice was again filtered through five layers muslin and finally double layer filter paper to obtain a clear juice. Clear juices were concentrated using BÜCHİ Rotary Evaporator (Rotavapor R-3 model, BÜCHI Loborthechnik AG, Flawil, Switzerland) at 40 °C up to 45 °Brix.

2.3. Preparation of sauces

Phosphate buffer to dissolve hydrocolloids and, stock hydrocolloid solutions used in sauce preparation were prepared as follows:

Phosphate buffer, 0.05 M and pH 7.0: Phosphate buffer was prepared by dissolving potassium dihydrogen orthophosphate (3.76 g) and disodium hydrogen orthophosphate (3.44 g) in 2 liters twice distilled water and, pH was adjusted to 7.0 by using 0.1 M NaOH and 0.1 M HCI.

Preparation of stock hydrocolloid solutions: LBG, XA and *ί*-CARR stock solutions were prepared by dispersing the powders in phosphate buffer and vigorously stirring for 30 min at room temperature by magnetic stirrer, followed heating less than 50 °C for XA and *ί*-CARR and, up to 80 °C for LBG until the solution became clear.

Sauces were prepared in the laboratory with previously prepared fruit juice concentrates. For each fruit juice concentrate, sauces prepared as without hydrocolloid and with hydrocolloids of LBG, XA and *i*-CARR at three different hydrocolloid concentrations (0.15, 0.30 and 0.60 % (w/v) by using stock hydrocolloid solutions. In each case, to make 20 ml of sauce, appropriate quantity of stock hydrocolloid solution was dissolved in related fruit juice concentrate via vortex in the test tube.

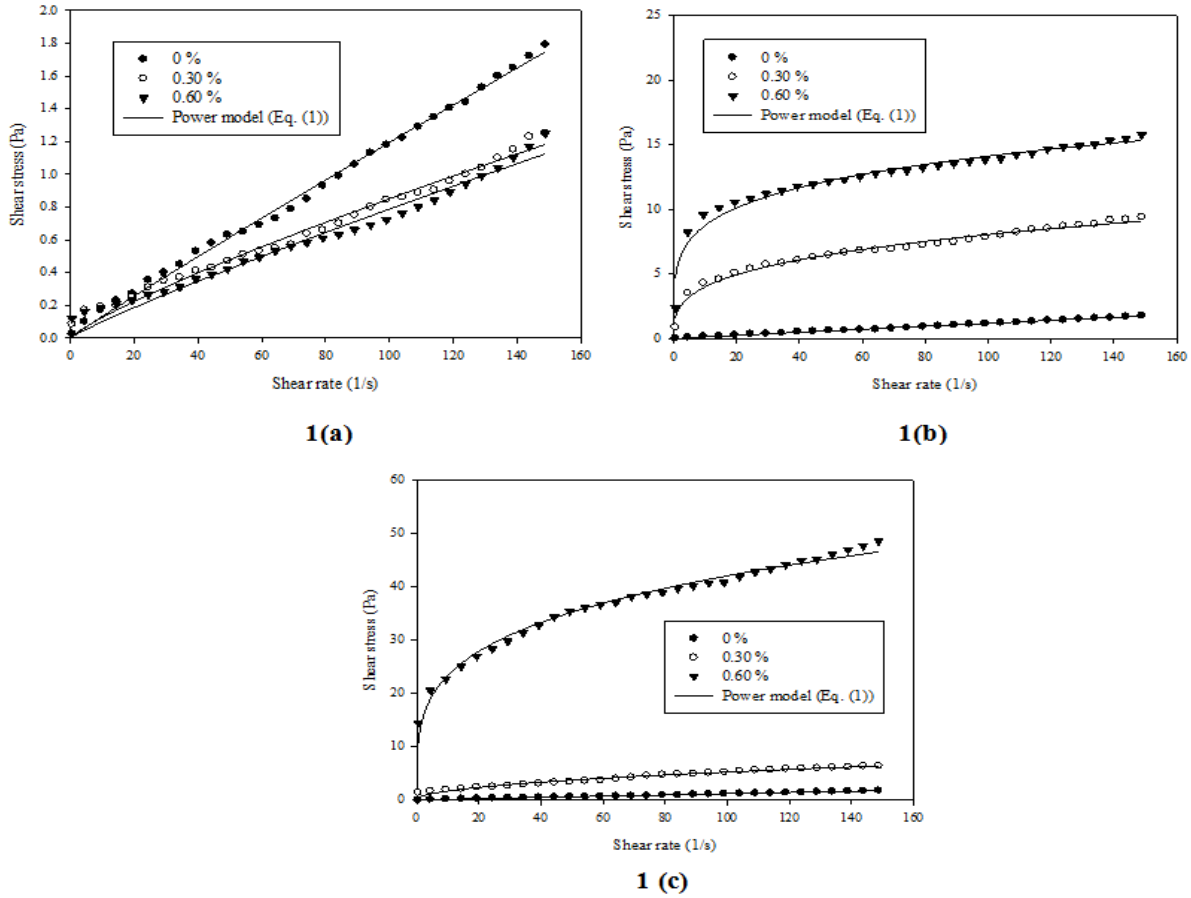


Fig. 1. Flow curves of Urmu mulberry sauces at different gum concentrations: **(a)** prepared with LBG; **(b)** prepared with XA; **(c)** prepared with *i*-CARR

2.4. Rheological measurements

The rheological measurements were performed at 25±0.01 °C by using a CVOR Rheometer (Bohlin, Malvern, Worcestershire, United Kingdom) equipped with Peltier Temperature Controller Unit. The measuring system consisted of a cone and plate sensor with a diameter of 2 cm and cone angle of 2°. Shear rate range was 0–300 s^{-1} within 600 s. For each measurement, 1 ml of sample was carefully poured over the plateau of rheometer. Each measurement was done in duplicate. Rheological parameters (shear stress, shear rate, apparent viscosity) were obtained from the Bohlin CVOR 150 data analysis software. Experimental flow curves were fitted to power law model (Krokida 2001):

$$\sigma = K\gamma^n \quad (1)$$

where σ is the shear stress (Pa), γ is the shear rate (s^{-1}), *K* is the consistency index (Pas^n), and the exponent *n*, called the flow behavior index (dimensionless). The parameters (*K*, *n*) of the power law model (Eq. (1)) were estimated by linear regression iterative procedure of the SigmaPlot (SigmaPlot 10.0 Windows version, SPSS Inc.). If *n is* less than 1, the material is shear-thinning (pseudoplastic), if *n* is more than 1, then material is shear thickening (dilatant) (Krokida 2001). Since for non-Newtonian

fluids, apparent viscosity, η_{app}, is reported as a function of shear rate (Rha 1978), in this study, it was computed at shear rate of 50 s^{-1} using power law parameters (K, n) due to the following equation:

$$\eta_{app} = K\gamma^{n-1} \quad (2)$$

The shear rate of 50 s^{-1} was selected because of its being known as effective oral shear rate (Marcotte, Hoshahili & Ramaswamy 2001).

2.5. Sensory analyses

The panel of 10 trained persons (seven females and three males) was selected among the staff of Food Engineering Department of the Gaziantep University in Turkey. Approximately 5 ml of samples was served in plastic cups, coded with three digits chosen at random. The evaluation was called in different sensations for each hydrocolloid type with two replications in every sensation. Six samples were served in each sensation according to experimental design.

An evaluation form with a 9-point Hedonic scale (Elibol 1988) from 1=disliked extremely to 9=liked extremely (1-disliked extremely, 2-disliked very much, 3-disliked moderately, 4-disliked slightly, 5-neither liked nor disliked, 6-liked slightly, 7 liked moderately, 8-liked very much and 9-liked extremely) was used by participants to assess the acceptability of the sensory qualities (taste, sliminess (liquid and slippery like slime), consistency), and the overall acceptability of sauces. Participants were supervised during the test session and individual assessment was followed throughout the test, i.e., communication between panelists was not allowed. Panelists washed their mouths with drinking water during intervals of evaluations. Scores below 5 was taken as the unacceptable limit for rejection of samples for the responses of taste, consistency and overall acceptability, while, scores above 5 was taken as the unacceptable limit for rejection of samples in terms of sliminess.

2.6. Experimental design

Response Surface Methodology (RSM) was chosen to build some mathematical models, using one factor response surface design with two replicates at center points, making it possible to quantitatively interpret and describe the relationship between the hydrocolloid type and concentration resulting in 6 samples for each hydrocolloid. Response variables were consistency (K), flow index (n), apparent viscosity (n_{app}), taste, sliminess, consistency and overall acceptability. The data was analyzed by using a commercial statistical package, Design-Expert (version 6.0, Statease Inc., Minneapolis, MN, USA). The effects of unexplained variability in the observed response due to extraneous factors were minimized by randomizing the order of experiments. The behavior of the system was explained by the following second degree polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_{11} X_1^2 \quad (3)$$

where Y is response, β_0 is the constant coefficient, β_1 is the linear coefficient, β_{11} is the quadratic coefficient and, X_1 is the coded values of the independent variable. The adequacy of the regression model was checked by R^2, adjusted R^2, Adequate Precision and Ficher's F-test (Montgomery 2001).

2.7. Statistical Analysis

Results were given as mean ± standard deviations of 3 independent determinations. One-way analysis of variance (ANOVA) was used to compare means. Differences were considered significant at $p< 0.05$. The parameters (K, n) of the power law model (Eq. (1)) were estimated by linear regression iterative procedure of the SigmaPlot (SigmaPlot 10.0 Windows version, SPSS Inc.). All other statistical analyses were performed with a commercial statistical package, Design-Expert (version 6.0, Statease Inc., Minneapolis, MN, USA).

Table 1. Parameters from power law model ((Eq. 1)) fitting; consistency coefficient, *K*, and flow behaviour index, *n*, and apparent viscosity, η_{app} calculated (Eq. (2)) at 50 s^{-1} and sensory data of Urmu mulberry sauces

Gum added	Gum conc. (%) (w/v)	Consistency index (Pa.s^n) *K*± *SE*	Flow index (Dimensionless) *n*± *SE*	R^2	(50/s) (Pa.s) η_{app} ±*SE*	Taste ± *SE*	Sliminess± *SE*	Consistency± *SE*	Overall Acceptability± *SE*
LBG	0	0.0146±0.0011	0.9572±0.0157	0.9966	0.0123±0.008	7.00±0.00	4.15±0.21	6.00±0.00	7.00±0.00
	0.30	0.0186±0.0024	0.8302±0.0283	0.9840	0.0096±0.0007	7.15±0.21	5.20±0.14	7.30±0.00	7.15±0.21
	0.60	0.0126±0.0024	0.8975±0.0410	0.9710	0.0084±0.0006	6.00±0.00	5.90±0.14	5.95±0.07	6.15±0.07
XA	0	0.0146±0.0011	0.9572±0.0157	0.9966	0.0123±0.0008	7.00±0.00	4.00±0.00	6.00±0.00	7.00±0.00
	0.30	1.9963±0.0838	0.3027±0.0095	0.9842	0.1305±0.0092	7.45±0.07	5.50±0.71	7.85±0.07	8.15±0.07
	0.60	5.4440±0.2396	0.2069±0.0101	0.9593	0.2446±0.0173	6.25±0.07	6.50±0.00	6.40±0.14	6.55±0.07
***ί*-CARR**	0	0.0146±0.0011	0.9572±0.0157	0.9966	0.0123±0.0008	7.00±0.00	4.05±0.07	6.00±0.00	7.05±0.07
	0.30	0.5403±0.0515	0.4938±0.0210	0.9711	0.0746±0.0052	6.70±0.14	5.10±0.00	7.25±0.21	7.20±0.28
	0.60	12.9588±0.4195	0.2552±0.0074	0.9848	0.7033±0.0498	5.10+0.14	6.35+0.07	5.35+0.21	5.25±0.35

LBG: Locust bean gum
XA: Xanthan gum
ί-CARR: ί-carrageenan
SE: Standard error
R^2: Determination coefficients

Table 2. Analysis of variance results for fitted second-order polynomial models for the response variables

			K	*n*	η_{app}	Taste	Sliminess	Consistency	Overall acceptability
Locust bean gum (LBG)									
	Model	F -value	27.59	2.01	26.57	52.11	97.35	1248.44	34.90
		p-value	0.0063*	0.2797^{ns}	0.0067*	0.0047*	0.0006*	< 0.0001*	0.0084*
	Lack of fit		0.1542^{ns}	-	*0.3133*^{ns}	-	*0.3161*^{ns}	*0.3081*^{ns}	-
	Adequate precision		7.879	2.832	8.928	13.279	17.090	53.000	10.954
	Adj-R^2		0.8417	0.2873	0.8364	0.9534	0.9507	0.9960	0.9313
	R^2		0.8734	0.5724	0.8691	0.9720	0.9605	0.9968	0.9588
Xanthan gum (XA)									
	Model	F -value	269.42	190.32	555.60	220.50	42.86	227.40	408.50
		p-value	0.0004*	0.0007*	< 0.0001*	0.0006*	0.0028*	0.0005*	0.0002*
	Lack of fit		-	-	*0.8475*^{ns}	-	*0.5305*^{ns}	-	-
	Adequate precision		32.437	25.348	40.827	29.394	11.339	28.660	39.192
	Adj-R^2		0.9908	0.9870	0.9911	0.9887	0.8933	0.9891	0.9939
	R^2		0.9945	0.9922	0.9929	0.9932	0.9146	0.9934	0.9963
***ί*-Carrageenan (*ί*-CARR)**									
	Model	F -value	382.87	85.77	349.79	156.50	1154.18	62.17	33.64
		p-value	0.0002*	0.0008*	0.0003*	0.0009*	< 0.0001*	0.0036*	0.0088*
	Lack of fit			*0.0641*^{ns}			*0.4740*^{ns}		
	Adequate precision		34.572	16.041	33.810	23.270	58.843	15.513	10.423
	Adj-R^2		0.9935	0.9443	0.9929	0.9842	0.9957	0.9607	0.9289
	R^2		0.9961	0.9554	0.9957	0.9905	0.9965	0.9764	0.9573

* Significant at 95 % confidence level

Table 3. Estimated regression coefficients of fitted second-order polynomial models for the response variables

		K	*n*	η_{app}	Taste	Sliminess	Consistency	Overall acceptability
Locust bean gum (LBG)								
Coefficient								
Constant	β_0	+0.019*	+0.83^{ns}	+0.010*	+7.15*	+5.08*	+7.30*	7.15*
Linear (Gum Concentration)	β_1	-	-0.030^{ns}	-1.950E-003*	-0.50*	+0.87*	-	-0.43*
Quadratic (Gum concentration)^2	β_{11}	-4.775E-003*	+0.097^{ns}	-	-0.65*	-	-1.32*	-0.57*
Xanthan gum (XA)								
Coefficient								
Constant	β_0	+2.00*	+0.30*	+0.13*	+7.45*	+5.33*	+7.85*	+8.15*
Linear (Gum Concentration)	β_1	+2.71*	-0.38*	+0.12*	-0.38*	+1.25*	+0.20*	-0.23*
Quadratic (Gum concentration)^2	β_{11}	+0.73*	+0.28*	-	-0.82*	-	-1.65*	-1.37*
***i*-Carrageenan (*i*-CARR)**								
Coefficient								
Constant	β_0	+0.54*	+0.57*	+0.075*	+6.70*	+5.18*	+7.25*	+7.20*
Linear (Gum Concentration)	β_1	+6.47*	-0.35*	+0.35*	-0.95*	+1.15*	-0.33*	-0.90*
Quadratic (Gum concentration)^2	β_{11}	+5.94*	-	+0.28*	-0.65*	-	-1.57*	-1.05*

* Significant at 95 % confidence level

3. RESULTS AND DISCUSSION

3.1. Rheological analyses

3.1.1. Flow curves of Urmu Sauces

The parameters (*n*, *K*) obtained by fitting the power law model to the shear stress versus shear rate (Eq. (1)), and determination coefficients, R^2, and calculated η_{app} of sauces (Eq. (2)) at 50 s^{-1} by fitting power law model were presented in Table 1. The model appeared to be suitable for describing the flow behavior of sauces with high correlation coefficients (0.9593- 0.9966). Typical flow curves of sauces prepared without and with hydrocolloid at selected hydrocolloid concentrations of 0.30 and 0.60 % (w/v) were shown in Fig. 1. The curves in the figures represent the Power law model fitted to the measured data.

There was no definite trend in *K*, *n* and η_{app} values for the sauces prepared by LBG and, it can be concluded that LBG did not show definite change in flow properties of the related sauces (Table 1). All sauces prepared with XA and *i*-CARR showed non-Newtonian, shear thinning behavior (n<1). Similar shear-thinning behavior also reported by Sikora et al. (2007) in dessert sauces thickened by starch-xanthan hydrocolloid combinations. For all sauces prepared with XA and *i*-CARR, an increase in hydrocolloid concentration was accompanied with an increase in pseudoplasticity, shown by a decrease in *n* values, a measure of departure from Newtonian flow (Table 1). The smaller the *n* values the greater the departure from Newtonian behavior (Farhoosh & Riazi 2007).

3.1.2. Regression results and analysis of the adequacy of the fitted model

The independent variable and dependent variables were analyzed to get regression equation, which was an empirical relationship between the response and test variable in coded units, and used to examine the goodness of the fitted model. ANOVA (Table 2) was performed to determine the lack of fit and the significance of the linear and quadratic effects of the independent variable on the dependent variables. Insignificant model terms were omitted and, the models were reanalyzed.

ANOVA of quadratic model (Eq. (3)) were carried out to fit mathematical models to the experimental data of consistency index (*K*) of sauces prepared by LBG, XA and *i*-CARR. The coefficients of the models and other statistics were given in Table 3 and Table 2, respectively. The model *F* and *p* values

of 27.59 and 0.0063, 269.42 and 0.0004 and 382.87 and 0.0002 for sauces prepared by LBG, XA and i-CARR, respectively, are implied that the models are significant (p<0.05). A determination coefficient, R^2, is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit and, for a good fit of model, R^2 should be at least 0.80 (Sin et al. 2006). It is also the proportion of the variability in the response variables, which is accounted for by the regression analysis. That is, when R^2 approaches unity, the better the empirical model fits the actual data and, the smaller the value of R^2, the less relevant the dependent variables in the model have in explaining the behavior variation (Sin et al. 2006). R^2 and adjusted R^2 values of the model were 0.8734 and 0.8417, 0.9945 and 0.9908, and 0.9961 and 0.9935 for K of sauces prepared by LBG, XA and i-CARR (Table 2), respectively, which were in close agreement. Adjusted R^2 is a variation of the ordinary R^2 statistic that reflects the number of factors in the model (Montgomery 2001). The Adequate Precision values were 7.879, 32.437 and 34.572 for K of sauces prepared by LBG, XA and i-CARR, respectively (Table 2). Adequate Precision is the signal to noise ratio and it gives you a factor by which you can judge your model whether it is an "adequate" or not to navigate through the design space and whether it is able to predict the responses, in addition, values greater than 4.0 is desirable (Montgomery 2001), which supports our findings. However, only the model for K of sauce prepared by LBG showed insignificant lack of fit (Table 2). The lack of fit test is a measure of the failure of a model to represent data in the experimental domain at which points were not included in the regression (Gan et al. 2007). Due to the goodness of other statistics related to models, significant lack of fit values were ignored to reject the models to define design space, since the final conclusion related to the model should be made due to the overall evaluation of all statistics. The same assumption was used for all other design models.

Variables in their coded form (Table 3) are permitted a direct interpretability of the effects (linear, quadratic) of the independent variables derived from the statistical analysis. From Table 3, for the K of sauces prepared with LBG, only quadratic effect of hydrocolloid concentration (X_1^2) was significant (p<0.05) with negative sign (-4.77E-0.003), indicating that, there is a maximum in K of Urmu mulberry sauce prepared with LBG, but it starts to decrease above this point. Since the value of coefficient was small, this effect would be small, also. For K of sauces prepared with XA and i-CARR, both linear and quadratic effect of hydrocolloid concentration were verified to be statistically significant (p<0.05) (Table 3). Hydrocolloid concentration had positive linear effect on K for these two sauces, that is, increasing hydrocolloid concentration will increase K of sauces. Quadratic effect of hydrocolloid concentration was positive, that is, there was a minimum K for the sauces in the range of hydrocolloid concentration considered in the study.

ANOVA results for flow index (n) of quadratic model (Eq. (3)) indicated that models for sauces prepared by XA and i-CARR were significant (p<0.05) whereas, the regression model for sauces prepared with LBG was insignificant (p>0.05). However, only the model for sauce prepared by i-CARR showed insignificant lack of fit (p>0.05) (Table 2). R^2 and adjusted R^2 values of the model were, 0.9922 and 0.9870, and 0.9554 and 0.9443 for sauces prepared by XA and i-CARR (Table 2), respectively, which were satisfactory. The Adequate Precision values were 25.348 and 16.041 for sauces prepared by XA and i-CARR, respectively, which were desirable (Table 2). For sauces prepared with XA, hydrocolloid concentration had significant (p<0.05) negative linear (X_1) effect on n which means increasing hydrocolloid concentration will decrease the flow index, and had significant (p<0.05) positive quadratic (X_1^2) effect on n, that is, minimum n will be seen in the range of hydrocolloid concentration studied (Table 3). Hydrocolloid concentration had only significant (p<0.05) negative linear (X_1) effect on n of the sauces prepared with i-CARR.

Regression analyses for apparent viscosity (n_{app}) indicated that, models for sauces prepared by LBG and XA and i-CARR gave significant (p<0.05) fits. Except sauces prepared by i-CARR, models had insignificant lack of fit (Table 2). Good fitted models for the apparent viscosity of the sauces prepared by LBG, XA and i-CARR were achieved with high R^2 (> 0.8691), good accordance between R^2 and adjusted R^2 values, good adequate precisions (>4.0) (Table 2). Table 3 shows that hydrocolloid concentration had only significant (p<0.05) negative linear (X_1) effect on n_{app} of sauces prepared by LBG, while, having significant (p<0.05) positive linear (X_1) effect on n_{app} of sauces prepared by XA.

For the case of Urmu mulberry sauces prepared with *i*-CARR, hydrocolloid concentration had both significant (p<0.05) positive linear (X_1) effect and quadratic (X_1^2) effect on n_{app} (Table 3).

3.1.3. Interaction of fruit juice and hydrocolloids

Generally, the electrostatic repulsion is a good way of stabilization of particles in dispersed systems since juice particles are negatively charged after depectinization, addition of food gums with negative charge is expected to increase electrostatic repulsive forces between particles (Genovese & Lozano 2001). In this study, XA and *i*-CARR are negatively charged ionic hydrocolloids, while LBG is a nonionic hydrocolloid with no charge, which may probably accounts for better stability and flow regimes of sauces prepared with XA and *i*-CARR compared to LBG. This result conformed to previous studies. Yamasaki, Yasui and Arima (1964) found that negatively charged colloids (like sodium alginate, carboxymethylcellulose, and arabic gum) in concentrations as low as 0.05%, completely inhibit apple juice clarification. Genovese and Lozano (2001) found that the greater stabilizing effect of carboxymethylcellulose in cloudy apple juice was basically due to its electronegativity. Zhang, Li and Cao (2004) also found that alginate-sudium with negative charge had an effective action in preventing sedimentation in combined vegetable juice. Liang et al. (2006) found decrease of pulp sediment amount after addition of negatively charged ionic polysaccharides (xanthan, sodium carboxymethylcellulose, gellan) and the increase of pulp sediment amount after addition of a nonionic polysaccharide with no charge (guar) to reconstituted carrot juice.

When we compared to sauces prepared with those negatively charged ionic hydrocolloids (XA and *i*-CARR), sauces prepared with XA showed better flow properties and stable sauces. This structural differences, that is, XA has a three dimensional network consisting of the unique rigid, rod-like conformation (Gamonpilas et al. 2011) while *i*-CARR has linear conformation (Karbowiak et al. 2006).Also, *i*-carrageenan has gelling ability (Černíková et al. 2008), while XA is a non-gelling (Rodríguez-Hernández & Tecante 1999). Due to gelling ability of *i*-CARR, sauces prepared with higher *i*-CARR concentrations showed elastic but highly viscous jells, which was unacceptable. This structural advantage of non-gelling XA may result in better interaction with juice components, easy hydration and stable sauces with good flow properties.

Higher viscosity of the hydrocolloids is another constituent for the suspending stabilization of particles in dispersed system (Genovese & Lozano 2001). When compared to the sauces without hydrocolloid and sauces prepared with XA and *i*-CARR, hydrocolloid addition led to rise in K, thereby resulting in an increase in the η_{app} of sauces. In this study, LBG even caused the reduction in apparent viscosity (η_{app}) of sauces when compared to the sauces prepared without hydrocolloid (Table 1). Actually, LBG has many uses in the food industry due to its ability to form very viscous solutions at relatively low concentrations and, its high stability upon pH or heat processing, which was granted by its neutral character (Camacho, Martinez-Navarrete & Chiralt 2005). It can be concluded that LBG may not able to show its potential thickening ability in this study. In the studies of Wei, Wang and Wu (2001) related to flow properties of fruit fillings, it have been reported that under certain circumstances addition of some type of hydrocolloids may even decrease the η_{app} of prior system. In addition, they stated that when hydrocolloids are not properly hydrated, their potential for increasing viscosity does not fully appear.

3.2. Sensory analyses

The sensory scores of the response variables, i.e., taste, sliminess, consistency and overall acceptability were shown in Table 1. To evaluate whether optimum hydrocolloid concentration was found or not, perceived sliminess in mouth which is directly related to hydrocolloid concentration was added as a critical parameter to sensory analyses. In general, all sauces prepared in this study take good consumer acceptance since they had scores higher than 5 for taste, consistency and overall acceptability (Table 1), even prepared with or without hydrocolloid. This is basically caused by special aroma (sour taste and refreshing flavour) of Urmu mulberry. This also supports that the sauce

preparation procedure which was concentration with rotary vacuum evaporator and then preparation of sauces at room temperature (25 °C) may have helped to save this special aroma and smell. As supposed, hydrocolloid addition resulted in increasing slimy feel in the mouth independently from hydrocolloid type. From Table 1, it can be concluded that hydrocolloid addition up to 30 % was found to be accepted but, for higher concentrations it caused reduction in sensory quality by loss in mouth feel with increased sliminess.

3.2.1. Regression results and analysis of the adequacy of the fitted model

Regression analyses for taste indicated that models for sauces prepared by LBG, XA and *i*-CARR were significant *(*$p<0.05$), however, for none of them lack of fit value was computed by the model (Table 2). R^2 and adjusted R^2 values of the model were, 0.9720 and 0.9534, 0.9932 and 0.9887, and 0.9905 and 0.9842 for sauces prepared by LBG, XA and *i*-CARR (Table 2), respectively, which were satisfactory. The Adequate Precision values were 13.279, 29.394 and 23.270 for sauces prepared by LBG, XA and *i*-CARR (Table 2), respectively, all which were desirable since all of them were greater than 4.0. Sauces prepared by LBG, XA and *i*-CARR, showed similar trends for change in taste with varying hydrocolloid concentration. That is, hydrocolloid concentration had significant ($p<0.05$) negative linear (X_1) effect on taste which means increasing hydrocolloid concentration will decrease the taste, while having significant ($p<0.05$) negative quadratic (X_1^2) effect on taste, i.e., maximum taste will be perceived in the range of hydrocolloid concentration studied (Table 3).

ANOVA for sliminess of sauces prepared by LBG, XA and *i*-CARR gave statistically significant *(*$p<0.05$) fits with insignificant lack of fits ($p>0.05$) (Table 2). These good fitted models were supported by with high R^2 (> 0.9146), good accordance between R^2 and adjusted R^2 values, desirable Adequate Precisions (>4.0) (Table 2). Hydrocolloid concentration had only significant ($p<0.05$) positive linear (X_1) effect on the sliminess of sauces prepared by LBG, XA and *i*-CARR (Table 3). As supposed by theoretically increasing hydrocolloid concentration will increase slimy feel in the mouth which will not be accepted by the consumer.

The regression analyses of consistency indicated that models for sauces prepared by LBG, XA and *i*-CARR were significant *(*$p<0.05$) with satisfactorily R^2 and adjusted R^2 values and good Adequate Precisions (>4.0) (Table 2). Table 3 shows that for the sauces prepared with LBG, only quadratic effect of hydrocolloid concentration (X_1^2) was significant ($p<0.05$) with negative sign (-1.32), indicating that, there is a maximum in the consistency of Urmu mulberry sauce prepared with LBG, but it start to decrease above this point. For sauces prepared with XA and *i*-CARR, both linear and quadratic effect of hydrocolloid concentration were verified to be statistically significant ($p<0.05$) (Table 3). Hydrocolloid concentration had significant ($p<0.05$) positive linear (X_1) effect and significant ($p<0.05$) negative quadratic (X_1^2) effect in the sauces prepared with XA. For Urmu mulberry sauces prepared with *i*-CARR, hydrocolloid concentration had significant ($p<0.05$) negative linear (X_1) and quadratic (X_1^2) effects.

ANOVA for the overall acceptability indicated that models for sauces prepared by LBG, XA and *i*-CARR were significant *(*$p<0.05$). For none of them lack of fit value was defined by the model. Quadratic models for the overall acceptability of the sauces prepared by LBG, XA and *i*-CARR had high R^2 (> 0.9573), good accordance between R^2 and adjusted R^2 values, good Adequate Precisions (>4.0) (Table 2). For all sauces prepared by LBG, XA and *i*-CARR, similar trends were observed for the change in the overall acceptability with respect to hydrocolloid concentration. That is, hydrocolloid concentration had significant ($p<0.05$) negative linear (X_1) effect on overall acceptability, which means increasing hydrocolloid concentration will decrease the overall acceptability, while having significant ($p<0.05$) negative quadratic (X_1^2) effect on overall acceptability, i.e., maximum overall acceptability will be observed in the range of hydrocolloid concentration studied (Table 3). Similar trends have been observed for change in taste with varying hydrocolloid concentration, from which it can be concluded that perceived taste and overall acceptability may have good correlation.

3.3. Compromised optimum conditions and verification

The polynomial regression equations for preparation of Urmu mulberry sauces using hydrocolloids of LBG, XA and *i*-CARR at varying concentrations revealed that LBG did not show definite change in flow properties of the related sauces So, the compromised optimum conditions were then determined using Design-Expert (version 6.0, Statease Inc., Minneapolis, MN, USA) for sauces prepared by XA and *i*-CARR. For high viscosity and good mouth-feel characteristics of sauces, the hydrocolloid choice should be a hydrocolloid system having low flow index (*n)* value, since high *n* value tends to feel slimy in the mouth (Farhoosh & Riazi 2007). In this respect, the final sauce would be considered optimum, if the sensory scores of taste, consistency, overall acceptability, and rheological scores of consistency (*K*), apparent viscosity (n_{app}) are as high as possible, whereas hydrocolloid concentration, flow index (*n*) and sliminess should be as low as possible. Therefore, compromised optimum condition criteria applied using numerical optimization technique for each hydrocolloid were as follows: (1) maximum sensory scores of (taste, consistency and overall acceptability) for highest consumer satisfaction; (2) maximum consistency (*K*) maximum apparent viscosity (n_{app}) for good flow regimes; and (3) minimum hydrocolloid concentration, flow index (*n*) and sliminess for good mouth feel characteristics. Overall optimum condition for sauces prepared by *i*-CARR was sauces prepared with 0.41% *i*-CARR concentration with 3.71 consistency (*K*), 0.44 flow index (*n*), 0.24 apparent viscosity (n_{app}), 6.27 score for taste, 5.61 score for sliminess, 6.92 score for consistency, 6.73 score for overall acceptability resulted in 0.47 desirability. Overall optimum condition for sauces prepared by XA was sauces prepared with 0.31% XA concentration with 2.13 consistency (*K*), 0.28 flow index (*n*), 0.13 apparent viscosity (n_{app}), 7.43 score for taste, 5.39 score for sliminess, 7.86 score for consistency, 8.14 score for overall acceptability resulted in 0.65 desirability. Verification of this optimum condition should be performed by recovery experiments at optimum conditions. In this study, the regression analyses clearly revealed that hydrocolloid concentrations higher than 0.30 % was not found acceptable, so recovery experiments for sauces prepared with 0.41 % *i*-CARR concentration were not needed to be performed since they were regarded as unfeasible. As 0.31 % XA concentration is very close to central point of 0.30 % XA concentration of experimental design matrix, sauces prepared with 0.30 % XA concentration was found to be the best option to prepare Urmu mulberry sauce with no need of verification experiments. Gamonpilas et al. (2011) reported that XA is an effective stabilizer which has a three dimensional network consisting of the unique rigid, rod-like conformation. Pseudoplastic characteristics of XA gives the advantage in terms of organoleptic quality , e.g. flavour release and mouth-feel, in food products and promotes mixability, pumpability and flowability characteristics which are important factors for the design of flow systems, which support our findings.

4. CONCLUSIONS

Rheological observation gave good and easy interpretation of effect of hydrocolloid type and concentration. As compared to sauces prepared with neutral LBG, addition of food gums with negative charge (XA and *i*-CARR) showed better stability and good flow regimes, which may partially accounted by the electrostatic repulsion or higher viscosity . Sauces prepared with XA and *i*-CARR showed good flow characteristics and adequate viscosity up to 0.30 % hydrocolloid concentrations, but at higher concentrations they caused reduction in product quality via loss in mouth feel with increased sliminess. Overall conclusion was depending on sensory attributes since the consumer is the ultimate judge and sauce characteristics need to be assessed in relation to the acceptability of the product by the consumer. In general, all sauces prepared in this study take good consumer acceptance even prepared with or without hydrocolloid. This is basically caused by special aroma (sour taste and refreshing flavour) of Urmu mulberry. However, common limitation with all sensory data should be keept in mind when taking the results of this study to represent the preference of consumers at large scale. Finally, sauces prepared with 0.30 % XA concentration was found to be the best option to prepare Urmu mulberry sauce having good flow characteristics and satisfactorily consumer acceptance.

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