Carbohydrate Polymers 87 (2012) 1611–1619

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Development of an optimal formulation for oxidative stability of walnut-beverage emulsions based on gum arabic and xanthan gum using response surface methodology

Seyed Mohammad Taghi Gharibzahedi, Seyed Mohammad Mousavi*, Manouchehr Hamedi, Faramarz Khodaiyan, Seyed Hadi Razavi

Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, University of Tehran, P.O. Box 4111, Karaj 31587-77871, Iran

ARTICLE INFO

Article history:
Received 29 July 2011
Received in revised form 4 September 2011
Accepted 23 September 2011
Available online 29 September 2011

Keywords:
Walnut oil-in-water emulsion
Xanthan gum
Lipid oxidation
Peroxide value
Droplet size
Response surface methodology (RSM)

ABSTRACT

The susceptibility of lipids to oxidation is one of the most fundamental problems in oil-in-water emulsions. A response surface methodology 5-level-3-factor central-composite rotatable design was applied to study the effects of key formula ingredients including walnut oil (WO, 3–6%, w/w), gum arabic (GA, 5–10%, w/w) and xanthan gum (XG, 0.05–0.15%, w/w) on lipid oxidation in walnut-beverage emulsions. During 30 days' storage, the oxidation process was monitored by evaluating the peroxide value, anisidine value and total oxidation (Totox) value in different emulsion formulations. Use of XG as a stabilizer at high concentrations considerably inhibited the oxidation of WO in the prepared emulsions. The experimental data were satisfactorily fitted to quadratic models using multiple regression analysis. The optimum conditions to obtain the minimum peroxide (0.923 mequiv. O_2/kg oil), anisidine (0.500) and Totox (2.347) values are met when a walnut-beverage emulsion is formulated with 3% WO, 10% GA and 0.12% XG.

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1. Introduction

Beverage emulsions are thermodynamically unstable systems due to the positive free energy needed to increase the surface area between the oil and water phases, and the density difference between these two phases. They tend to separate into two layers over time through a number of mechanisms, including creaming, coalescence, flocculation and Ostwald ripening (McClements & Decker, 2000; Mirhosseini, Tan, Hamid, Yusof, & Chern, 2009). It is possible to form emulsions that are kinetically stable (metastable) for a reasonable period of time by including substances known as emulsifiers and/or thickening agents prior to homogenization (Sun, Gunasekaran, & Richards, 2007). Gum arabic (GA) due to the excellent emulsifying properties is the most usually applied biopolymer emulsifier in beverage emulsions. It is surface active, adsorbs to interfaces between oil and water, and facilitates the production of small droplets by lowering the interfacial tension during homogenization (Buffo, Reineccius, & Oehlert, 2001). Xanthan gum (XG) is a non-linear anionic microbial heteropolysaccharide synthesized by aerobic fermentation of *Xanthomonas campestris*, and has a particularly complicated molecular structure. It is widely used as an emulsion stabilizer and thickener due to its excellent viscosity and dispersion characteristics (such as reversible shear thinning and its ability to disperse in either hot or cold water) (Krstonošić, Dokić, Dokić, & Dapčević, 2009). The use of GA biopolymer in combination with XG has revealed for increasing the density of oil droplets, controlling the rheological characteristics of the water phase, and attaining the long-term stability of beverage emulsions (Mirhosseini, Tan, Taherian, & Boo, 2009; Taherian, Fustier, & Ramaswamy, 2007). Vegetable oil is another critical structural component in the emulsion system. It is added to the formulation of beverage emulsions to achieve desirable optical and rheological properties in the finished emulsion-based product (Mirhosseini, Tan, Hamid, & Yusof, 2008). This study investigated the feasibility of using walnut oil (WO) as a functional ingredient in the production of concentrated beverage emulsions. The used oil in present study contained the following fatty acids (mol%): 0.406% C14:0, 8.82% C16:0, 0.153% C16:1, 3.11% C18:0, 25.17% C18:1, 50.18% C18:2, 12.07% C18:3 and 0.133% C20:1 as measured by gas chromatography of methyl esters (Gharibzahedi, Mousavi, Hamedi, & Ghasemlou, 2012). WO is a good source of essential fatty acids such as linoleic and α-linolenic acids. These compounds are known to reduce the risk of coronary heart disease, non-fatal myocardial infarction and certain type of cancer (Gharibzahedi, Mousavi, Hamedi, & Khodaiyan, 2011; Miraliakbari & Shahidi, 2008).

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* Corresponding author. Tel.: +98 261 2248804; fax: +98 261 2249453.
E-mail address: Mousavi@ut.ac.ir (S.M. Mousavi).

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doi:10.1016/j.carbpol.2011.09.067

Table 1
Experimental domain of central composite rotatable design (CCRD).

Independent variables	Symbol		Coded variables levels				
	Uncodified	Codified	−1.682 (−α)	−1	0	+1	+1.682 (+α)
Walnut oil concentration (WO, % w/w)	X_1	x_1	1.98	3	4.5	6	7.02
Gum arabic content (GA, % w/w)	X_2	x_2	0.03	3.3	7.5	10	11.7
Xanthan gum content (XG, % w/w)	X_3	x_3	0.02	0.05	0.10	0.15	0.18

Strong interest in the functional food market is growing as result of consumer demand for foods that contain some health-promoting components beyond traditional nutrients. The polyunsaturated fatty acids (PUFAs) most commonly found in functional foods are generally in the form of dispersed lipids. Use of oils high in n-3 PUFAs in foods is limited due to their high susceptibility to oxidation (Donnelly, Decker, & McClements, 1998; McClements & Decker, 2000). Lipid oxidation is an unfavorable process in most foods such as beverage emulsions because it leads to the expansion and development of potentially toxic reaction products and undesirable changes in the quality and characteristics of foods, such as taste, flavor, texture, appearance, shelf life and nutritional value (Frankel, Satue-Gracia, Meyer, & German, 2002; McClements & Decker, 2000; Osborn & Akoh, 2004). Duh, Yen, and Yen (1999) concluded that the oxidative stability of soybean oil-in-water emulsions can be improved by adding emulsifiers. Donnelly et al. (1998) found that controlling the type and concentration of emulsifier and the size of oil droplets at the oil–water interface can increase the oxidative stability of emulsified menhaden oil. Shimada, Fujikawa, Yahara, and Nakamura (1992) and Sun et al. (2007) also demonstrated that the presence of stabilizers such as XG can retard lipid oxidation in o/w emulsions.

Response surface methodology (RSM) is a collection of useful mathematical and statistical procedures that possess the advantage of reducing number of experimental trials needed to scrutinize multiple factors and their interactions in comparison to other approaches (Myers & Montgomery, 2002). Tiliu and Yang (2011) optimized the emulsification and microencapsulation efficiency and oxidative stability of evening primrose oil (EPO) with different emulsifiers during storage using RSM. However, to the best of our knowledge, there is no specific study on the effect of various concentrations of the main emulsion components on the oxidative stability of WO-in-water beverage emulsions by RSM. Therefore, this study aimed to determine the optimum formulation of walnut-beverage emulsions with RSM for minimizing oxidative damage.

2. Materials and methods

2.1. Materials

Potassium sorbate, sodium benzoate and food grade citric acid (anhydrous) were obtained from Chisso Co. (Tokyo, Japan), Fars Chemical Industry Co. (Shiraz, Iran) and Kimia Gharb-Gostar Industry Co. (Kermanshah, Iran), respectively. GA was purchased from Merck KGaA (Darmstadt, Germany). XG was provided from Sigma–Aldrich (Oakville, ON, Canada). WO was obtained by pressing Toyserkan cultivar walnuts. The oil expression was carried out with a screw press (Model NA 21 T, Zeith, Kerman, Iran), with a 5 mm restriction die and a screw speed of 20 rpm.

2.2. Emulsion preparation

For the preparation of walnut-beverage emulsions, the method of Gharibzahedi et al. (2012) was adopted with minor modifications. 20 WO-in-water emulsions were composed of GA (5–10%, w/w), XG (0.05–0.15%, w/w), WO (3–6%, w/w), citric acid (0.4%, w/w), potassium sorbate (0.1%, w/w), sodium benzoate (0.1%, w/w) and deionized water. These components were prepared for the optimization procedure based on a central rotatable composite design (CCRD) (Table 1). To prepare the water phase, potassium sorbate, sodium benzoate and citric acid were dispersed in deionized water (≈60 °C) using a blender (IKA-WERK, RW 20 DZM, Staufen, Germany). During mixing, GA was gradually added to the deionized water (≈60 °C), and the solution was mixed for an extra 5 min to further facilitate hydration. To achieve full hydration, the mixture was kept at room temperature overnight. XG solution was prepared separately by dissolving XG in deionized water with shaking and then mixed with the GA solution by using a high-speed blender. While the water phase was mixing, the cold-pressed WO without addition of weighting agents was slowly added to the water phase to prepare an initial coarse emulsion (Mirhosseini et al., 2008). To obtain fine emulsification with small average droplet size (<1 μm) and narrow particle-size distribution, pre-emulsions were subjected to pre-homogenization using a high shear blender (IKA-WERK, Ultra Turrax, T25 basic, Staufen, Germany) for 3 min and passed through a high-pressure homogenizer (APV 2000, Denmark) three times (30, 25 and 20 MPa). At least two separate emulsions were prepared for each treatment.

2.3. Droplet size measurement

Mean droplet size of WO-in-water emulsions was determined by laser light scattering (Mastersizer 2000S, Malvern Instruments Ltd., Malvern, UK). Sample analysis was carried out during 30 days based on Mie theory (Gharibzahedi et al., 2012; Huang, Kakuda, & Cui, 2001). The concentrated emulsions were diluted with deionized water (1:100) to avoid multiple scattering effects, and placed directly into a metal jar that circulated the sample through the measuring-glass cuvette. A laser beam directed through the diluted emulsions was scattered by the droplets in a characteristic pattern according to their size and detected by an array of photodiodes located behind the cuvette (Buffo et al., 2001). Size distribution was characterized by volumetric percentage and mean particle size of the emulsion droplets obtained using the surface-weighted mean diameter:

$$D_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}$$

where n_i is the number of droplets of diameter d_i (Tangsuphoom & Coupland, 2009).

2.4. Optical microscopy

Emulsions were gently agitated in a glass test tube to ensure homogeneity prior to analysis. A drop of emulsion sample was diluted with 1 g/l SDS solution and placed on a microscope slide, covered with a cover slip (Koocheki, Kadkhodaee, Mortazavi, Shahidi, & Taherian, 2009). Microstructure was visualized by phase-contrast light microscopy (Leica Galen III, Germany) at 40× magnifications and recorded by means of a digital camera (Mavica FD88, Sony company, Japan) mounted on the microscope. Images were made immediately after preparation of emulsion and 30 days after being stored at temperature room.

Table 2
Three-factor central composite rotatable design (CCRD) used for RSM with experimental and predicted values for the independent variables.

Formulation	Point type	Independent variables			Peroxide value (mequiv. O_2/kg oil) (Y_1)		Anisidine value (Y_2)		Totox value (Y_3)	
		X_1	X_2	X_3	Experimental[a]	Predicted	Experimental[a]	Predicted	Experimental[a]	Predicted
1	Fact	−1	−1	−1	1.230	1.223	1.121	1.094	3.581	3.541
2	Fact	1	−1	−1	1.440	1.431	1.621	1.588	4.501	4.451
3	Fact	−1	1	−1	1.020	1.060	0.607	0.587	2.647	2.707
4	Fact	1	1	−1	1.060	1.048	0.782	0.817	2.902	2.913
5	Fact	−1	−1	1	0.910	0.938	0.594	0.555	2.414	2.433
6	Fact	1	−1	1	1.260	1.236	0.615	0.631	3.135	3.105
7	Fact	−1	1	1	0.790	0.815	0.359	0.388	1.939	2.019
8	Fact	1	1	1	1.070	1.093	0.691	0.714	2.831	2.901
9	Center	0	0	0	1.140	1.103	0.491	0.473	2.771	2.679
10	Center	0	0	0	1.050	1.103	0.512	0.473	2.612	2.679
11	Center	0	0	0	1.220	1.153	0.585	0.602	3.025	2.908
12	Center		0	0	1.170	1.153	0.553	0.602	2.893	2.908
13	Axial	−1.68	0	0	1.090	1.045	0.954	0.985	3.134	3.077
14	Axial	1.68	0	0	1.350	1.370	1.487	1.459	4.187	4.199
15	Axial	0	−1.68	0	1.410	1.423	1.335	1.381	4.155	4.229
16	Axial	0	1.68	0	1.120	1.082	0.851	0.808	3.091	2.972
17	Axial	0	0	−1.68	1.610	1.610	1.854	1.877	5.074	5.099
18	Axial	0	0	1.68	1.350	1.325	1.142	1.121	3.842	3.772
19	Center	0	0	0	1.320	1.330	0.954	1.027	3.594	3.688
20	Center	0	0	0	1.270	1.330	1.112	1.027	3.652	3.688

[a] Mean of triplicate determinations.

2.5. Oxidizing conditions and evaluation of oil oxidation

The procedure described by Osborn and Akoh (2004) was employed for the analysis of the oil oxidation progression in emulsion samples with minor modification. Oxidation was monitored over a 28-day period. In brief, 15 ml aliquots of the WO-in-water beverage emulsions were stored in covered test tubes and then allowed to oxidize at a thermostated water bath (50 °C). In order to determine the levels of hydroperoxide and aldehyde present in the oil, triplicate 15 ml WO-in-water emulsion samples were taken periodically. By adding isooctane/isopropanol (3:2, v/v), vortexing 3 times for 10 s each, and followed by centrifugation at 1000 rpm for 5 min at room temperature, WO was extracted from these emulsions. The upper layer was removed and the solvent was evaporated under nitrogen gas. Then, the oil samples were weighed in order to accurately determine oxidation progression in each tested sample.

Peroxide values were determined using the International Dairy Foundation method as described previously by Moayedi, Rezaei, Moini, and Keshavarz (2011). Briefly, about 0.25 g of obtained oil was weighed into a glass test tube and dissolved in 9.7 ml of a mixture of chloroform/methanol (4:1, v/v). Then, a drop of ammonium thiocyanate solution (30%, w/v) and a drop of ferrous chloride solution (0.35%, w/v) were added. The absorbance of the mixture was measured at 500 nm using a UV–visible spectrophotometer (U-5100 model, Hitachi, Japan), after standing for 5 min. In addition, a calibration curve was constructed using ferric chloride solutions containing 3–15 μg of Fe^{3+}. The anisidine value was determined at 350 nm in a 1.0 cm cell of a solution containing 1.0 g of oil in 100 ml

Table 3
Analysis of variance for the effect of the independent variables on the dependent variables and the regression coefficients of the fitted quadratic equations obtained from experimental results.

Source	DF	Peroxide value (mequiv. O_2/kg oil)			Anisidine value			Totox value		
		Coefficient	Sum of squares	*p*-Value	Coefficient	Sum of squares	*p*-Value	Coefficient	Sum of squares	*p*-Value
Model	9	1.195	0.480	0.0002	0.701	1.989	<0.0001	3.092	7.063	<0.0001
Linear										
β_1	1	0.096	0.127	0.0001	0.140	0.271	<0.0001	0.333	1.521	<0.0001
β_2	1	−0.101	0.141	<0.0001	−0.170	0.396	<0.0001	−0.373	1.905	<0.0001
β_3	1	−0.084	0.098	0.0003	−0.224	0.689	<0.0001	−0.394	2.122	<0.0001
Quadratic										
β_{11}	1	−0.043	0.027	0.0135	0.068	0.068	0.0028	–	0.004	ns
β_{22}	1	–	0.010	ns	–	0.008	ns	–	0.013	ns
β_{33}	1	0.048	0.033	0.0077	0.167	0.401	<0.0001	0.264	1.004	<0.0001
Interaction										
β_{12}	1	–	0.007	ns	–	0.001	ns	–	0.030	ns
β_{13}	1	0.047	0.018	0.0329	–	0.012	ns	–	0.023	ns
β_{23}	1	–	0.009	ns	0.149	0.178	0.0001	0.219	0.384	0.0003
Residual	8		0.021			0.030			0.086	
Lack-of-fit	5		0.015	0.4171		0.017	0.6263		0.063	0.3626
Pure error	3		0.006			0.013			0.023	
Total	19		0.704			3.270			11.225	
R^2		0.956			0.985			0.988		
Adj-R^2		0.907			0.968			0.974		
CV		4.37			6.75			3.09		

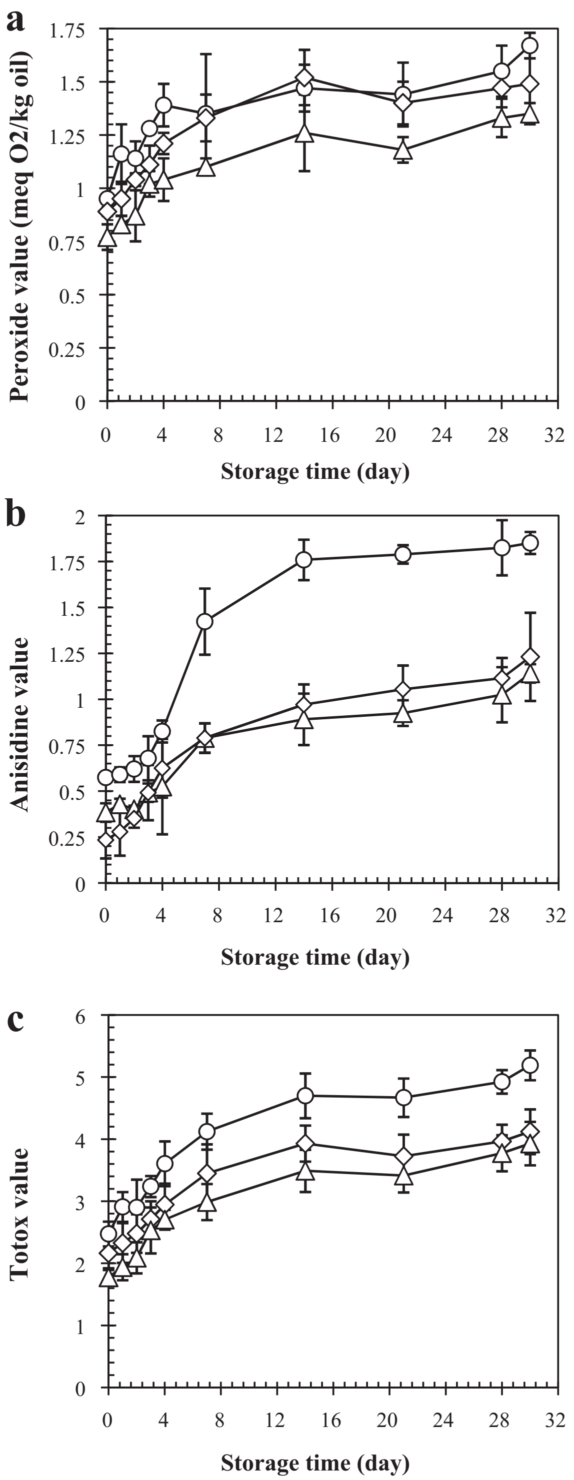

Fig. 1. Changes in peroxide value (a), anisidine value (b) and Totox value (c) over storage time at 25 ± 2 °C in WO-in-water beverage emulsions prepared with the different concentrations of XG (0.05%, w/w, ○; 0.1%, w/w, ◇; 0.15%, w/w, △) and the same contents of WO (=3%, w/w) and GA (=10%, w/w) as a function of storage time. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

of isooctane according to the method CD 18-90 of the AOCS (1998). The total oxidation (Totox) value was determined from following equation:

$$\text{Totox value} = 2\,(\text{peroxide value}) + \text{anisidine value}$$

2.6. Experimental design and statistical analysis

The software Design-Expert (trial version 7.1.6, Stat-Ease Inc., Minneapolis, USA) was used for experimental design, regression analysis of the experimental data and quadratic model building. A central-composite rotatable design (CCRD) was employed to identify the reasonable relationship existing between the response functions and the formulation variables, as well as to determine those conditions that optimized the production of concentrated beverage emulsions based on WO. The influence of three main emulsion components namely WO (3–6%, w/w, X_1), GA (5–10%, w/w, X_2), and XG (0.05–0.15%, w/w, X_3) on the lipid oxidation (peroxide value (Y_1), anisidine value (Y_2) and Totox value (Y_3)) in model beverage emulsions formulated with WO was studied by using the CCRD. Our preliminary studies showed that the addition of the concentration levels studied to the emulsion formulation resulted in desirable changes of physical and chemical stability of walnut-beverage emulsions. For statistical calculations, the relation between the coded values and actual values are described by Eq. (1):

$$x_i = \frac{X_i - X_0}{\Delta X} \tag{1}$$

where x_i is the coded value of the variable, X_i is the actual value of the variable, X_0 is the actual value of X_i at the center point, and ΔX is the step change value of the variables. A second-order polynomial regression model was used to express the dependent variables as a function of the independent variables, which is given by Eq. (2):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon \tag{2}$$

where X_1, X_2 and X_3 represent the levels of the factors according to Table 1 and $\beta_0, \beta_1, \ldots, \beta_{23}$ represent coefficient estimates with β_0 having the role of a scaling constant. The adequacy of model was examined accounting for coefficient of determination (R^2), adjusted-R^2 (adj-R^2) and coefficient of variation (CV). Statistical significance of the terms in the regression equations was checked. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The significances of each of the coefficients in the empirical model were selected or rejected based on the p-value. For the graphical analysis of the independent variable interactions, the use of 3D surface plots of the regression models was highly recommended (Myers & Montgomery, 2002). Thus, these plots were depicted from the fitted polynomial equations to explain the interactive effects of the independent variables with the response variable. Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

3. Results and discussion

3.1. Fitting the response surface models

The experiments were performed according to the CCRD matrix under the defined conditions and the responses obtained from the experimental runs are given in Table 2. Using the CCRD, 20 sets of tests with appropriate combinations of WO concentration (X_1), GA content (X_2) and XG content (X_3) were conducted. The results in Table 2 were fitted to second-order polynomial models by applying multiple regression analysis for three response variables including peroxide value (Y_1), anisidine value (Y_2) and Totox value (Y_3). As shown in Table 3, the models were highly significant with very low p-values ($p < 0.0001$). Moreover, the "fitness" of the models was investigated through the lack-of-fit test ($p > 0.05$), which indicated the suitability of models to accurately predict the variation (Myers

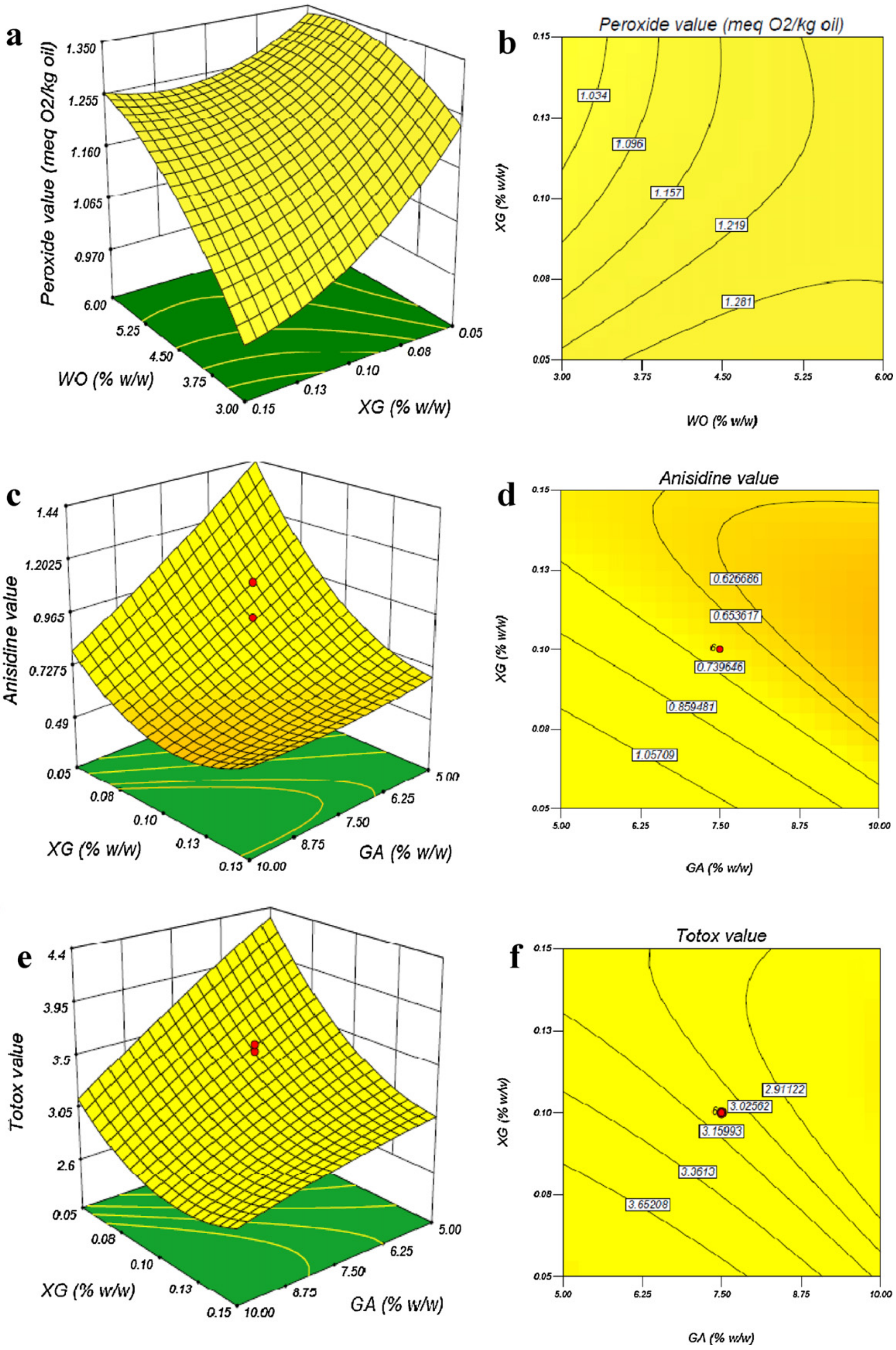

Fig. 2. Response surface plots and contour plots showing the significant ($p < 0.05$) interaction effects on the variation of peroxide value (a and b), anisidine value (c and d) and Totox value (e and f) of WO-in-water beverage emulsion.

& Montgomery, 2002). A measure of the model's overall performance referred to as the coefficient of determination and denoted by R^2 must be considered (Gharibzahedi et al., 2011). The R^2 were found to be 0.956, 0.985 and 0.988 for peroxide value, anisidine value and Totox value, respectively (Table 3). The adjusted R^2 values (0.907–0.974) were also indicated the significance of the models. At the same time, the values of the CV were 3.09–6.75%, indicating high degree of precision and reliability of the experimental values (Table 3).

3.2. Effects of formulation variables on peroxide value

As illustrated in Table 3, the linear effect of all independent variables on the emulsions' peroxide value (Y_1) was highly significant. The quadratic effect of WO concentration and XG content was significant at the 5% and 1% level, respectively (Table 3). The results also indicated that among the interactions, only the interaction effect of WO with XG was significant ($p < 0.0001$). The individual optimization procedure showed that the optimum content of WO, GA and XG for the minimum peroxide value ($Y_1 = 0.908$ mequiv. O_2/kg oil) was around 3% (w/w), 10% (w/w) and 0.15% (w/w), respectively.

Hydroperoxides are primary oxidation components that have a lower half-life than secondary oxidation components (Fomuso, Corredig, & Akoh, 2002). Many researchers believe that the most common reason for oxidative instability is the interaction between lipid hydroperoxides located at the droplet surface and transition metals from the aqueous phase (McClements & Decker, 2000; Mei, McClements, & Decker, 1998; Paraskevopoulou, Boskou, & Paraskevopoulou, 2007). Mancuso, McClements, and Decker (1999) mentioned that iron is the major prooxidant of lipid oxidation in o/w emulsions. XG, due to its ability to chelate metal ions at negatively charged pyruvate sites in the structure, can quench oil peroxidation by inactivation of Fe^{2+} ions present in the emulsion system (Karadjova, Zachariadis, Boskou, & Stratis, 1998; Shimada et al., 1992; Sun et al., 2007). Moreover, increasing viscosity in the aqueous phase by adding XG inhibits diffusion of prooxidants such as oxygen, and slows down the movement of oil droplets, hence decreasing the rate of lipid oxidation (Shimada et al., 1992). As Fig. 1a shows, the peroxide value of emulsions was significantly decreased by increasing XG content.

The emulsions with high WO contents had higher peroxide values compared to the low-WO emulsions (Fig. 2a and b). It may also be due to the high concentrations of unsaturated fatty acids especially mono-unsaturated fatty acids (oleic acid) at higher WO levels (Shahidi & Wanasundara, 1998; Waraho, McClements, Decker, 2011). Waraho et al. (2011) found that the monounsaturated fatty acids due to more linear geometry compared to di- and tri-unsaturated fatty acids had more ability to promote lipid oxidation in soybean oil-in-water emulsions. Therefore, the high presence of these fatty acids with more linear molecular structure could permit them easier availability to the interface area of emulsion droplets and followed by increases the lipid oxidation rate in the emulsions. However, Osborn and Akoh (2004) obtained that the emulsions containing 10% oil had significantly higher peroxide values compared to the emulsions with oil content of 30%. This discrepancy might be attributed to differences in the molecular arrangements of the fatty acids within surfactant micelles (Miyashita, Tateda, & Ota, 1994). Therefore, an increase of XG content in range and initial concentration of WO were associated with minimum peroxide value (Fig. 2a and b).

Fig. 3a–c shows the results of variations during storage in the droplet-size distribution of the emulsions prepared with 3% WO and 15% XG in various concentrations of GA. A monomodal size distribution with a mean droplet diameter of 0.855–0.952 μm was observed when concentration of GA emulsifier was increased to 10% (w/w). Some of researchers have determined that lipid oxidation spreads faster in emulsions with small droplets than in emulsions with large droplets (McClements & Decker, 2000; Osborn & Akoh, 2004). Since the majority of emulsions had no significant difference for the droplet size values ($p > 0.05$), therefore, there is no dependence of the oxidation rate on droplet size. Fomuso et al. (2002) reported similar results in the case of fish-oil-based structured lipid emulsions. It is possible that emulsifier content, rather than droplet size distribution, caused the changes in oxidation characteristics found in that study.

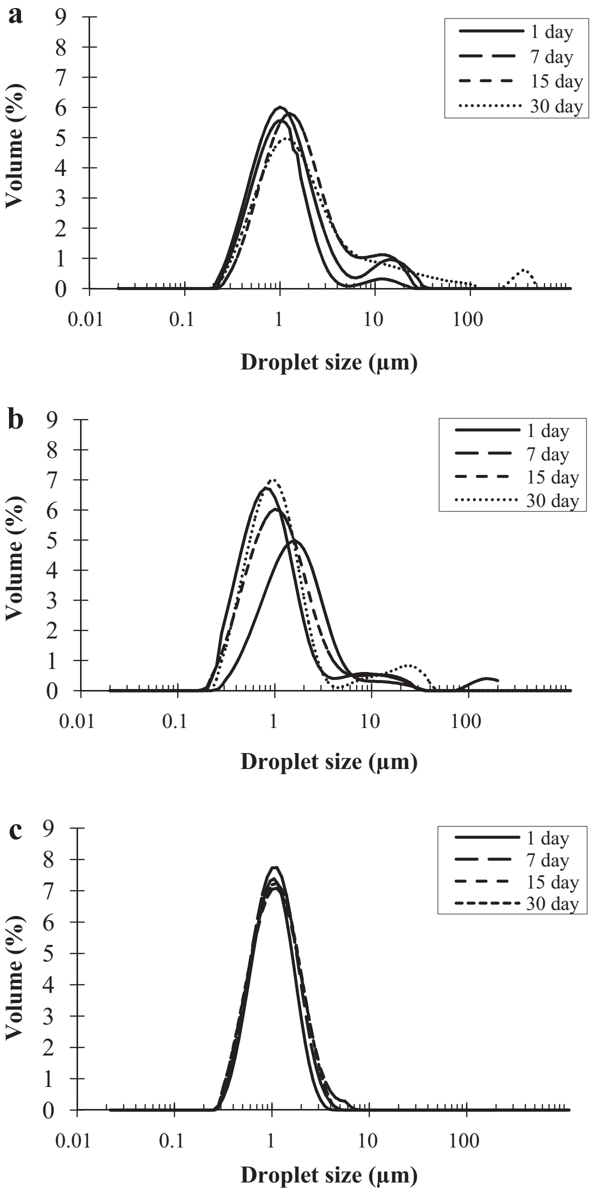

Fig. 3. Change in the droplet-size distribution of the emulsions prepared with 3% (w/w) WO and 15% (w/w) XG in different concentrations of GA (a, 5%, w/w; b, 7.5%, w/w; c, 10%, w/w) during 30 days' storage at 25 ± 2 °C.

The results of the present study showed that high contents of GA not only protect the emulsion droplets against coalescence (Fig. 4), but also act as an efficient barrier to the penetration and diffusion of prooxidants at the o/w interface, inhibiting lipid oxidation into the droplets (Table 3). These findings were generally in agreement with those reported by Coupland and McClements (1996) and Silvestre, Chaiyasit, Brannan, McClements, and Decker (2000). Matsumura, Satake, Egami, and Mori (2000) and Matsumura et al. (2003) also

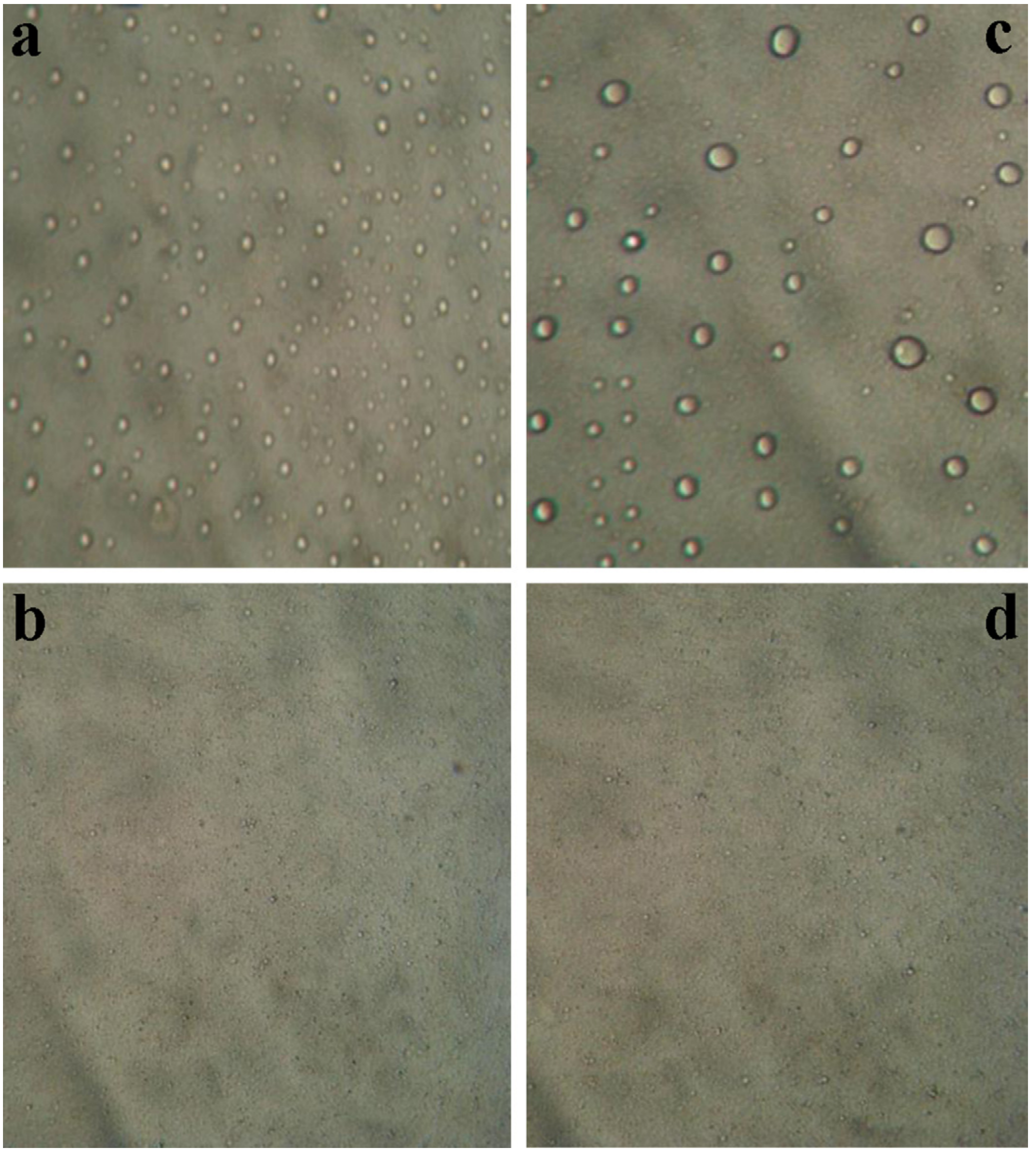

Fig. 4. Microstructure of WO-in-water beverage emulsions containing low (5%, w/w; (a) fresh emulsion and (c) after 30 days' storage) and high (10%, w/w; (b) fresh emulsion and (d) after 30 days' storage) concentrations of GA with 3% (w/w) WO and 15% (w/w) XG.

demonstrated the antioxidative effect of GA on lipid oxidation in methyl linoleate or methyl oleate emulsions containing β-casein or other surfactants such as Tween 20. The inhibitory effect of GA on lipid oxidation in oil-in-water emulsions can probably be attributed to the presence of peptide moieties in its structure (Matsumura et al., 2003). In other words, the radical-scavenging activity of GA found in this study was due to the physical protection of lipids from the lipoxygenase enzyme and radical by the adsorption of GA molecules to the oil-droplet surface (Matsumura et al., 2000).

3.3. *Effects of formulation variables on anisidine value*

Anisidine value is an indicator used to determine the secondary reaction products during lipid oxidation by evaluating the level of 2-alkenals in the oil (White, 1995). Secondary oxidation products are responsible for the quality and sensory characteristics of oxidized oils (Osborn & Akoh, 2004).

The optimization results showed that WO concentration, XG content and GA content had significant ($p < 0.0001$) linear effects on the anisidine value of walnut-beverage emulsions (Table 3). The quadratic terms of WO and XG concentrations were significant ($p < 0.01$; $p < 0.0001$). The interaction between GA concentration and XG content was also found to be significant ($p = 0.0001$). The most significant ($p < 0.05$) effect on anisidine value was revealed to be the linear effect of XG followed by the linear term of GA and quadratic effect of XG (Table 3). As shown in Fig. 2c and d, the variation in the anisidine value of the walnut-beverage emulsion could be explained as a nonlinear function of the XG and GA. The highest concentrations of GA and XG were predicted to achieve the minimum anisidine values (Fig. 2c and d).

Analysis of anisidine values showed that the emulsions formulated with 0.10 and 0.15% XG presented better protection against lipid oxidation than those formulated with 0.05% XG (Fig. 1b), which was in agreement with the results for peroxide values. This may be due to the fact that adding XG to the emulsion may, by increasing the viscosity of the aqueous phase, enhance the adsorption of the constituent protein of GA on the oil droplets and slow the diffusion of prooxidants to oil droplet surface, thus decreasing the decomposition of lipid hydroperoxides into secondary products (Shimada et al., 1992; Sun & Gunasekaran, 2009; Sun et al., 2007). Sun et al. (2007) also reported that the anisidine value of whey protein isolate oil-in-water emulsions stabilized by adding

Table 4
Optimum conditions of formulation variables and, predicted and experimental values of the responses at these conditions.

Independent variables	Optimum condition	Response variables	Optimum condition	
			Experimental[a]	Predicted
WO concentration	3% (w/w)	Peroxide value	0.978 ± 0.154	0.923 (mequiv. O_2/kg oil)
GA content	10% (w/w)	Anisidine value	0.522 ± 0.036	0.500
XG content	0.12% (w/w)	Totox value	2.321 ± 0.121	2.347

[a] Mean ± standard deviation ($n = 3$).

about 0.15% XG changed negligibly during storage time. The individual optimum condition also indicated that minimum anisidine value would be obtained for a walnut-beverage emulsion containing 3.03% (w/w) WO, 10% (w/w) GA and 0.12% (w/w) XG.

3.4. Effects of formulation variables on Totox value

The Totox value is an evaluation of both primary and secondary oxidation products. This value is often considered practical for estimating the extent of oxidative deterioration of food lipids, as it combines evidence about an oil's past history with its present state (Sun, Wang, Chen, & Li, 2011). Fig. 1c confirms that the Totox values of extracted WO from prepared emulsions had the same trend as the peroxide and anisidine values. In general, lower Totox values in the emulsions prepared using high concentrations of XG may be due to its higher hydrogen-donating ability and its efficiency in lessening the oxygen consumption at the interfacial surface between oil and water (Kishk & Al-Sayed, 2007).

As can be seen in Table 3, the linear effect of WO exhibited a significant ($p < 0.0001$) positive effect on the Totox value (Y_3); in contrast, the variation of Totox value was negatively influenced by the concentrations of GA and XG ($p < 0.0001$). The quadratic term of XG content was significant ($p < 0.0001$). The mutual interaction of GA content with XG concentration was also significant ($p < 0.001$). Fig. 2e and f shows the interaction between GA and XG contents. When GA concentration was increased from 5 to 10% (w/w), the Totox value decreased in a parabolic manner. The XG concentration revealed a nonlinear decrease for the emulsion's Totox value. The results suggested that a walnut-beverage emulsion containing 3% (w/w) WO, 10% (w/w) GA and 0.12% (w/w) XG would result in the minimum Totox value ($Y_3 = 2.347$).

3.5. Optimization and validation of RSM results

The optimal values of the selected variables were obtained by solving the regression equations using Design-Expert software. According to the RSM analysis, the minimum oxidative stability can be achieved at a WO concentration of 3% (w/w), GA content of 10% (w/w), and XG content of 0.12% (w/w). This optimum condition provides the lowest value of peroxide value = 0.923 mequiv. O_2/kg oil, anisidine value = 0.500 and Totox value = 2.347 (Table 4). In order to validate the optimization results, three additional experiments were carried out at the optimum conditions within the experimental range obtained above (Table 4). No significant difference ($p > 0.05$) was found between the experimental and predicted values, which indicates the high accuracy of the presented models.

4. Conclusion

This study used RSM to determine the optimal formulation of WO-in-water beverage emulsions to maximize oxidative stability. The effects of various levels of WO, GA and XG on the lipid oxidation of walnut-beverage emulsions were studied by central rotatable composite design (CCRD). The results showed that the second-order polynomial models with high R^2 (>0.95) can be used to predict the optimal formulation for any given conditions within the experimental range. An increase of GA and XG contents in range and initial concentration of WO were associated with high oxidative stability in the emulsions.

Acknowledgments

This study has been partially supported by a grant provided by the Council for Research at the Campus of Agriculture and Natural Resources of the University of Tehran and the Research Council of the University of Tehran. Gratitude is expressed to the ZamZam Iran Co. (Tehran, Iran) for providing the laboratory facilities and financial assistance. Also, the authors gratefully thank Mr Seyed Jalil Gholami and Mrs Mehrnoosh Farid, Department of Fisheries and Environmental Sciences of University of Tehran, for assistance in carrying out the optical microscopic analysis.

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