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Study of stability characteristics of sesame milk: effect of pasteurization temperature, additives, and homogenisation pressure

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RESEARCH ARTICLE

Abstract

Stability of oilseeds milk such as sesame milk is an important quality scale that is a necessity for consumer acceptance. This study addresses the effect of pasteurisation temperature (65, 75, 85 and 95 °C), xanthan gum (0, 0.03, 0.06 and 0.09 g/100 g), mono-diglyceride as an emulsifier (0, 0.3, 0.6 and 0.9 g/100 g), pH (6, 7, 8 and 9) and homogenisation pressure (0 and 6 MPa) on phases separation, viscosity, and colour of sesame milk. Quality attributes included suspension stability index, precipitation index, visual stability index, particle size, zeta potential, viscosity, and colour. Pasteurization temperature changed colour, phase separation and viscosity. Emulsifier (mono-diglycerides) concentration showed similar effects than pasteurization temperature, but to a lesser extent except for viscosity which was increased. Xanthan gum concentration had variable effects on phase separation, depending on the analytical technique. While, it had no effect on colour difference and viscosity. Homogenisation pressure and pH had minor effects on the characteristics of sesame milk. The analysis of the results showed that the optimal conditions for production of stable sesame milk are pasteurisation temperature of 74.70 °C, gum concentration of 0.09 g/100 g, emulsifier level of 0.44 g/100 g, pH of 6.68 and homogenisation pressure of 1.81 MPa.

Keywords: homogenisation, pasteurisation, phases separation, xanthan gum

1. Introduction

Recently, much consideration has been directed toward exploring the use of oilseeds for new food applications on the basis of their functional, nutritional and health properties (Afaneh *et al.*, 2011a,b; Quasem *et al.*, 2009). Sesame (*Sesamum indicume* L.) seed is one of the most important oil seed crops which contains approximately 20-25 g/100 g protein, 50 g/100 g fat, 14 g/100 g carbohydrate, 4-7 g/100 g water and 1-11 g/100 g fibre (Green, 1989). Linoleic and linolenic as essential fatty acids make up about 40.5 g/100 g of total fatty acids in sesame oil, also sesame seed has noteworthy amounts of characteristic lignans such as sesamin, sesamolin, sesaminol and others. Some lignans are known to have antitumor, antimitotic, and antivirus activities (Namiki, 2007).

Although sesame seeds are used in different ways around the world, but its per capita consumption is low. Thus, one of the methods for increasing sesame consumption is processing in different ways, such as production of sesame milk. Sesame milk is a dairy-free beverage that is relatively similar in colour and texture to cow's milk. This milk is created by soaking sesame seeds in water. The water and seeds are then ground together. This mixture is then drained and the resulting white turbid liquid is sesame milk. Development of sesame based milk beverages which contain trivial amount of beany flavour can overcome this problem which restricts consumption of soy-based dairy products and other vegetable milks (Afaneh *et al.*, 2011a). Generally, vegetable milks are rich source of highly valuable proteins, unsaturated fatty acids, soluble and insoluble dietary fibres and isoflavones (Akinola *et al.*, 2015). Furthermore, they are free of casein, lactose and cholesterol so they do not have problems of lactose intolerance and casein allergenicity (Ahmadian-Kouchaksaraei *et al.*, 2014).

Suspension stability is an important quality scale in oilseeds milk or beverage. Thus, optimisation of sesame milk stability for a desired period is a necessity for consumer acceptance. It has been reported that vegetable milk production is dependent on various process variables like pasteurisation temperature, gum and emulsifier levels, pH and homogenisation pressure. Shimoyamada *et al.* (2008) showed that heat treatment affected the dispersion stability of soy milk; also Tangsuphoom and Coupland (2005) stated homogenisation and heat treatment affected the colloidal stability of coconut milk. Hinds *et al.* (1997a) evaluated the effect of homogenisation pressure and also addition of stabiliser and emulsifier on suspension stability and viscosity of peanut beverage. These studies indicate that understanding the basic needs or optimisation of parameters is an important factor in achieving maximum stability.

Taguchi's style is entirely based on statistical design of experiments, and this can economically gratify the needs of problem solving and product/process design optimisation (Roy, 2001). The experimental design proposed by Taguchi involves using orthogonal arrays to organise the parameters affecting the process and the levels at which they should be varied. Instead of having to test all possible combinations like the factorial design, the Taguchi method tests pairs of combinations. This allows for the collection of the necessary data to determine which factors most affect product quality with a minimum amount of experimentation, thus saving time and resources (Pignatiello and Joseph, 1988). Some of the previous works applied the Taguchi method as a tool for design of experiment in various areas (Azin *et al.*, 2007; Molina-Rubio *et al.*, 2010; Oztop *et al.*, 2007).

In this article, the effect of different proportions of pasteurisation temperature, gum and emulsifier levels, pH and homogenisation pressure were optimised by Taguchi method. The main objective was to find a combination of best parameters to achieve high stability of sesame milk.

2. Materials and methods

Materials

The white dehulled sesame seeds were purchased from the local market in Mashhad, Iran and stored at 4 °C until used. Xanthan gum and mono-diglyceride were supplied by Sigma (St Louis, MO, USA) and Vista Tejarat Company (Tehran, Iran), respectively.

Preparation of sesame milk

Preparation of sesame milk was done according to the method of Ahmadian-Kouchaksaraei *et al.* (2014) with some modifications. Sesame seeds soaked in tap water containing 0.5 g/100 ml $NaHCO_3$ for at least 16 hours at room temperature. After draining, sesame seeds were washed with fresh tap water, integrated with water (water:sesame = 2:1), submerged in a steam jacketed kettle and blanched at 95 °C for 15 min, followed by draining the excess water and grinding in an adequate quantity of distilled water (water:sesame = 5:1) for about 20 minutes. Before filtering through a double-layered coarse cloth, the slurry remained at room temperature for about 1 hour. The pH of raw sesame milk was adjusted (6, 7, 8 and 9) with 2 N NaOH solution. Xanthan gum (0, 0.03, 0.06 and 0.09 g/100 g) and mono-diglyceride (0, 0.3, 0.6 and 0.9 g/100 g) were added to the mixture at 60 °C and blended for 5 minutes. The sesame milk was then homogenised (Stomacher homogeniser, type 400; Seward, Worthing, UK) twice at 55 °C and a pressure of 0 and 6 MPa. The homogenised milk was then pasteurised at 65, 75, 85 and 95 °C for 30 minutes and stored at 4 °C before further analysis.

Suspension stability index

Suspension stability of sixteen different formulations was determined after 5 days quiescent storage at 4 °C by withdrawing 30 ml portion of samples from the upper and lower 1/3 of a bottle. Then top to bottom ratio of total solids as the suspension stability index (SSI) was reported (Mepba *et al.*, 2006).

Precipitation index

Sesame milk samples were centrifuged in an Eppendorf centrifuge (model 5430; Brinkman Instruments, Westbury, NY, USA) at 2,500×*g* for 10 minutes then the supernatant was removed by placing the tubes upside down for half a minute. The resulting precipitate was weighed to calculate precipitation as a fresh weight percentage (Shimoyamada *et al.*, 2008). Since Precipitation index is a measure of particle sedimentation, the lower values of this parameter give better stability (Ahmadian-Kouchaksaraei *et al.*, 2014).

Visual stability index

Stability of samples visually was investigated after 15 days storage into glass test tube at 4 °C. Visual stability index (VSI) was determined by calculating height of sludge layer (HS) and the total height of milks (HT). If no separation layers were observed, the VSI was 1.00 (Hinds *et al.*, 1997a). This VSIwas defined by the following equation:

$$VSI = \frac{HS}{HT} \quad (1)$$

Measurement of particle size and zeta potential

The mean particle size in the sesame milk samples were measured by light scattering techniques with a nano particle size analyser (model Vasco3; Cordouan, Pessac, France) using 1.46 as refractive index of the scatterers in sesame milk and 1.333 as a refractive index of dispersant (water).

The milks were diluted 1:1000 with deionised water in order to acquire exact particle size measurements (Malaki Nik *et al.*, 2009). All measurements were duplicated for each sample and results are reported as averages.

Zeta potential (ZP) of particles was measured according to their eletrophoretic mobility using dynamic light scattering technique (model Zetacompact; CAD, Les Essarts-le-Roi, France) (Soleimanpour *et al.*, 2013). Each evaluation was achieved from the average of two ridings.

Viscosity

Viscosity of 15 ml sesame milk was determined at 25 °C using a Bohlin rotational viscometer (Bohlin model Visco 88; Bohlin instruments, Cirencester, UK). Measurements were carried out with C30 measuring spindle at the constant shear rate of 50 (s^{-1}) (Ahmadian-Kouchaksaraei *et al.*, 2014).

Colour changes

Sesame milk changes in coloration were demonstrated by using a chroma-meter (chroma meter model CR-410; Minolta Co. Ltd., Osaka, Japan). Calibration readings for reference were done with the white plate: L*=98.14; a*=-0.23; b*=1.89. Samples (15 ml) were transferred into black-jacketed polystyrene petri dishes. The colour was recorded using CIE-L*a*b* uniform colour space (CIE-Lab), where L* indicates lightness, a* indicates hue on a green (-) to red (+) axis, and b* indicates hue on a blue (-) to yellow (+) axis. Total colour difference (ΔE) was calculated from the following formula (Altunakar *et al.*, 2004):

$$\Delta E = [(L^* - L^*_{ref})^2 + (a^* - a^*_{ref})^2 + (b^* - b^*_{ref})^2]^{1/2} \qquad (2)$$

Reference values refer to the white plate (L*=98.14; a*=-0.23; b*=1.89).

Experimental design, optimisation and verification procedures

Taguchi method was used for optimisation of variables (Benito-Román *et al.*, 2011; Sun *et al.*, 2012). The pasteurisation temperature, gum and emulsifier levels, pH and homogenisation pressure as shown by Table 1, were studied. To perform the Taguchi method, 16 different experiments, by using L16 orthogonal array, were run.

For each of the response variables, a quadratic polynomial model was used to data fitting. In this study, predictor variables were allowed to be at any level within the range of the design. All experiments were performed in triplicate. Statistical significance of the terms in the regression equations was investigated. The significant terms in the model were determined by analysis of variance (ANOVA) for each response. In addition, the coefficient of determination (R^2), adjusted R^2 (adj. R^2), coefficient of variation (CV), significant probabilities and percentage contribution were computed to check the model adequacy. The percentage contribution by each of the process parameter in the total sum of the squared deviations can be used to evaluate the importance of the process parameter change on the performance characteristic. Equation 3 expresses the percentage contribution (P):

$$P = \frac{SS_d}{SS_T} \qquad (3)$$

Table 1. Variables and levels used in Taguchi design.

Run	A: pasteurisation temperature (°C)	B: gum (g/100 g)	C: emulsifier (g/100 g)	D: pH	E: homogenisation pressure (MPa)
1	65	0	0	6	0
2	65	0.03	0.3	7	0
3	65	0.06	0.6	8	6
4	65	0.09	0.9	9	6
5	75	0	0.3	8	6
6	75	0.03	0	9	6
7	75	0.06	0.9	6	0
8	75	0.09	0.6	7	0
9	85	0	0.6	9	0
10	85	0.03	0.9	8	0
11	85	0.06	0	7	6
12	85	0.09	0.3	6	6
13	95	0	0.9	7	6
14	95	0.03	0.6	6	6
15	95	0.06	0.3	9	0
16	95	0.09	0	8	0

Where SS_d and SS_T are the sum of the squared deviations and the total sum of squared deviations, respectively.

The quadratic equation was applied to build surfaces for the variables. The software Design Expert, version 7.0.0 (State-Ease Inc., Minneapolis, MN, USA) was utilised to analyse the results. The models fitted in this study could also be utilised for optimisation purposes using the desirability function. The idea is that this desirability function acts as a penalty function that leads the algorithm to regions where we can find the desired response variable values. The factor levels that take to a maximum or a minimum of the response variable are called 'optimum points'. Optimisation was based on generation of the best results for evaluated properties of sesame milk.

The characteristics of sesame milk were determined after production under optimal condition. In order to determine the validity of the model, the experimental and predicted values were compared by paired t-test using Minitab 15 software (Minitab Inc., State College, PA, USA).

3. Results and discussion

As a quality control of samples obtained, some physicochemical properties were determined. The results of ANOVA for properties of sesame milk with the corresponding coefficients of multiple determinations (R^2) are shown in Table 2. As it can be seen, probabilities (*P*-value) of significant coefficients are shown. In Table 3, the percentage contribution of each individual factor and interaction that demonstrated significant influence ($P<0.05$) was shown, as well as the residual error, obtained from Taguchi's analysis. A polynomial regression was used to test the effect of several factors (two at a time) and to obtain the response surface. The predicted values for properties of sesame milk are calculated according to the mathematical models given in Table 4. These high coefficients are an evidence of the applicability of the regression model between the ranges of variables included. In general, the regression models were highly significant with satisfactory R^2 that varied from 0.898 to 0.999, indicating that the models are sufficiently accurate for predicting the aspects of sesame milk samples. Moreover, CV describes the extent to which the data were dispersed. The CV for each parameter was within the acceptable range. Since CV is a measure of expressing standard deviation as a percentage of the mean, the small values of CV give better reproducibility. In general, a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model (Daniel, 1991).

Suspension stability index

It was seen that SSI of samples was affected by pasteurisation temperature, mono-diglyceride concentration and pH. The pasteurisation temperature was the factor that showed the maximum influence (9.17%) among main factors as can be

Table 2. Probability values of regression coefficients of predicted quadratic polynomial models for sesame milk properties.

Source	Suspension stability index	Precipitation index	Visual stability index	Particle size (μm)	Zeta potential (mV)	Viscosity (Pa.s)	ΔE[2]
Model	0.0004	<0.0001	0.0004	0.0011	0.0001	0.0030	<0.0001
A-pasteurisation	0.0012	0.0009	0.0013	ns	<0.0001	0.0031	<0.0001
B-gum	ns[1]	0.0008	0.0012	0.0385	0.0244	ns	ns
C-emulsifier	0.0056	0.0004	0.0295	ns	ns	0.0014	<0.0001
D-pH	0.0278	ns	0.0032	0.0168	ns	0.0372	ns
E-homogenisation	ns	0.0034	ns	ns	ns	ns	ns
AB	0.0062	ns	0.0020	ns	ns	0.0023	<0.0001
AC	0.0022	0.0006	0.0050	0.0198	ns	0.0009	0.0022
AD	0.0006	0.0207	0.0020	ns	0.0466	0.0029	<0.0001
AE	0.0060	ns	0.0025	ns	0.0280	0.0020	0.0259
BC	ns	0.0224	0.0039	0.0084	ns	0.0037	0.0002
BD	0.0033	ns	0.0007	0.0002	ns	0.0021	ns
BE	0.0001	0.0258	0.0002	0.0015	ns	0.0032	ns
CD	0.0031	0.0092	0.0040	0.0039	ns	0.0041	0.0012
CE	0.0176	0.0007	0.0003	0.0046	ns	0.0008	ns
DE	0.0018	ns	ns	0.0001	0.0262	0.0045	0.0008
Coefficient of variation	4.36	5.35	0.58	8.98	7.18	7.19	11.31

[1] No significant effect at $P<0.05$.
[2] ΔE = total colour difference.

Table 3. Percentage contribution of the different factors in the production of sesame milk.

Factor	Percentage contribution						
	Suspension stability index	Precipitation index	Visual stability index	Particle size (μm)	Zeta potential (mV)	Viscosity (Pa.s)	ΔE[1]
A-pasteurisation	9.17	13.38	5.04	0	58.34	5.11	20.45
B-gum	0	13.92	5.38	2.73	8.22	0	0
C-emulsifier	3.17	19.52	0.21	0	0	11.15	29.00
D-pH	0.99	0	1.98	4.22	0	0.41	0
E-homogenisation	0	7.39	0	0	0	0	0
AB	2.93	0	3.27	0	0	6.99	13.82
AC	5.92	16.30	1.26	3.89	0	18.52	3.57
AD	14.33	3.01	3.17	0	6.03	5.43	11.65
AE	3.02	0	2.53	0	7.72	7.95	1.19
BC	0	2.88	1.65	5.84	0	4.28	9.44
BD	4.60	0	9.13	24.69	0	7.57	0
BE	42.54	2.66	40.93	11.74	0	4.94	0
CD	4.77	4.59	1.60	8.11	0	3.92	4.62
CE	1.39	15.00	23.84	7.56	0	20.19	0
DE	6.98	0	0	28.88	7.97	3.51	5.43
Residual error	0.18	1.35	0.01	2.35	11.72	0.03	0.83

[1] ΔE = total colour difference.

Table 4. Polynomial equations and correlation coefficients to test the influence of production factors on sesame milk properties.

Polynomial equation	R^2	Adj. R^2
suspension stability index = 6.4140 – 0.1001 × pasteurisation – 3.9762 × emulsifier – 0.7936 × pH – 0.3202 × pasteurisation × gum + 0.0267 × pasteurisation × emulsifier + 0.0128 × pasteurisation × pH – 0.0004 × pasteurisation × homogenisation – 2.7821 × gum × pH – 0.2741 × gum × homogenisation + 0.2695 × emulsifier × pH + 0.0049 × emulsifier × homogenisation – 0.0054 × pH × homogenisation	0.999	0.994
precipitation index = 14.6733 – 0.1491 × pasteurisation – 24.1135 × gum + 36.5680 × emulsifier – 0.0792 × homogenisation – 0.2863 × pasteurisation × emulsifier + 0.0489 × pasteurisation × pH – 40.0463 × gum × emulsifier + 0.4034 × gum × homogenisation – 1.5103 × emulsifier × pH + 0.0706 × emulsifier × homogenisation	0.994	0.981
visual stability index = +1.0136 – 0.0145 × pasteurisation + 37.7455 × gum – 1.5568 × emulsifier – 0.1328 × pH – 0.2103 × pasteurisation × gum + 0.0103 × pasteurisation × emulsifier + 0.0028 × pasteurisation × pH – 0.0002 × pasteurisation × homogenisation – 2.8976 × gum × emulsifier – 1.8168 × gum × pH – 0.1376 × gum × homogenisation + 0.0764 × emulsifier × pH + 0.0105 × emulsifier × homogenisation	0.999	0.998
particle size = -2177.8492 + 39420.3082 × gum + 237.9369 × pH – 20.5576 × pasteurisation × emulsifier – 6413.0130 × gum × emulsifier – 4736.4459 × gum × pH – 103.6699 × gum × homogenisation + 271.2183 × emulsifier × pH + 9.2680 × emulsifier × homogenisation + 5.7540 × pH × homogenisation	0.964	0.91
zeta = -68.2017 × 1.0535 × pasteurisation – 264.9556 × gum – 0.2302 × pasteurisation × pH – 0.0047 × pasteurisation × homogenisation – 0.1171 × pH × homogenisation	0.898	0.847
viscosity = 0.2772 – 0.0026 × pasteurisation + 0.1920 × emulsifier – 0.0491 × pH – 0.0391 × pasteurisation × gum – 0.0037 × pasteurisation × emulsifier + 0.0005 × pasteurisation × pH + 0.0000 × pasteurisation × homogenisation – 0.6177 × gum × emulsifier + 0.2663 × gum × pH – 0.0066 × gum × homogenisation + 0.0171 × emulsifier × pH – 0.0014 × emulsifier × homogenisation – 0.0003 × pH × homogenisation	0.999	0.997
total colour difference = -26.9749 + 0.5703 × pasteurisation – 23.5158 × emulsifier + 5.6425 × pasteurisation × gum + 0.1390 × pasteurisation × emulsifier – 0.0903 × pasteurisation × pH + 0.0011 × pasteurisation × homogenisation + 126.3772 × gum × emulsifier + 1.9197 × emulsifier × pH + 0.0369 × pH × homogenisation	0.989	0.973

observed in Table 3. Figure 1A shows that SSI was highest at 75 °C of pasteurisation temperature. As the pasteurisation temperature in the range of 75-85 °C, the SSI was decreased. However, temperatures above 85 °C resulted in the increase of SSI. Other researchers reported a similar trend in peanut beverage, due to the maximum formation of protein gels and hydrophilic protein-lipid complexes at 75 °C (Hinds *et al.*, 1997a). α-globulin is the major constituent (65-70%) of the proteins in the sesame seed with the isoelectric pH of 4.9. Near the isoelectric point (IEP) of sesame proteins, droplet aggregation and decline of suspension stability in sesame milk samples occurs. Therefore, when the pH is far from the protein's IEP, the charges on the droplets increase (repulsive forces between droplets increase) that lead to raising stability against droplet aggregation (White *et al.*, 2008). Whereas dissociation of α-globulins at the pH of above 8 leads to increase of protein particles and subsequently decrease of contribution charge on them. So decrease of repulsive force between particles leads to decline of SSI (Prakash and Nandi, 1977). Although the other main factors, i.e. gum concentration and homogenisation pressure, did not have a significant effect (P<0.05) on SSI but the interaction of them showed the most significant percentage contribution (42.54%). The second factor having influence in the SSI was the interaction of pasteurisation temperature and pH (14.33%). According to ANOVA results for SSI, except the gum-emulsifier interaction, all other interactions were found to be significant (P<0.05).

Precipitation index

It can be seen that the precipitation index of samples was affected by all of the main factors except for pH. The emulsifier was the factor that showed the maximum influence (19.52%) among main factors (Table 3). As can be observed in Figure 1B, the precipitation index increased gradually as the emulsifier level was increased. Emulsifier would reduce the surface tension at the interface between water, oil and protein in the beverage that resulted in decrease of precipitation (Tan, 2004). The next important coefficients were interactions of emulsifier with pasteurisation temperature and homogenisation pressure with percentage contributions of 16.3 and 15%, respectively. Also, the xanthan gum individually had key effect on the precipitation index of sesame milk samples. The reduction of precipitation index (increase of stability) by xanthan gum could be related to water binding capacity of gums (Sánchez *et al.*, 1995).

Visual stability index

This parameter affected by all of the main factors except for homogenisation pressure. Among main factors, pasteurisation temperature and gum were the factors that showed the maximum influences (about 5%) as can be observed in Table 3. It may be attributed to formation of protein-polysaccharide and protein-lipid complexes as consequences of stabiliser addition and heating at >85 °C, respectively. Similar results were observed in other studies about the VSI of peanut beverage that significantly increased as stabiliser level was increased and also heating at pasteurisation temperatures higher than 85 °C (Hinds *et al.*, 1997b). In addition, interaction of gum and homogenisation pressure had the highest contribution percentage among all of the coefficients (40.93%). The ANOVA results are also shown that almost all of interactions were found to be significant (P<0.05).except the interaction of pH-homogenisation pressure.

Particle size

Knowledge of the particle size of a suspension is useful for predicting its long-term stability. According to Table 3, it is evident that among significant main factors, pH had the most influence on the particle size of samples. Increase of pH up to 8 resulted in the increase of particle size, due to increase of negative charges on protein particles and adsorption of environmental particles with opposite charges on the surface of them (Colic and Fuerstenau, 1997). While at pH>8, dissociation of α-globulins occurs. Figure 1D shows that particle size gradually increased until pH=8 but it decreased after that point. As mentioned above, it might be due to the fact that average of IEP of sesame seed proteins is about pH=8. In addition, interaction of pH and homogenisation pressure had the most contribution (28.88%) on the particle size. The next important coefficients were interactions of pH with homogenisation pressure and gum concentration with contribution percentages of 28.88 and 24.69%, respectively. Also, xanthan gum had a significant effect on the particle size. It appears that the additional gum up to level of 0.06 g/100 g was able to coat a greater surface area and resulted in the formation of greater number of smaller particles (Huang *et al.*, 2001) but higher concentration resulted in the increase of particle size (Figure 1D).

Zeta potential

The determination of the ZP is important in the study of suspension stability. It is a significant variable to obtaining beverage stability or destroying it. ZP is explained as the electrical potential which exists at the hydrodynamic plane of shear encircling a charged particle and is basically the potential at the point in space where low molecular weight ions stop to move with the particle and stay within the encompassing solvent (McClements, 2004). Basically, when all the particles have a large positive or negative ZP (where the positivity and negativity is greater or lower than +30 and -30 mV), they will repel each other and the dispersion is stable (Carneiro-da-Cunha *et al.*, 2011). As can be seen in Figure 1E, most of ZP values are lower than -30 mV that lead to stability of sesame milk. Also, Table 2 showed that ZP is significantly influenced by pasteurisation temperature

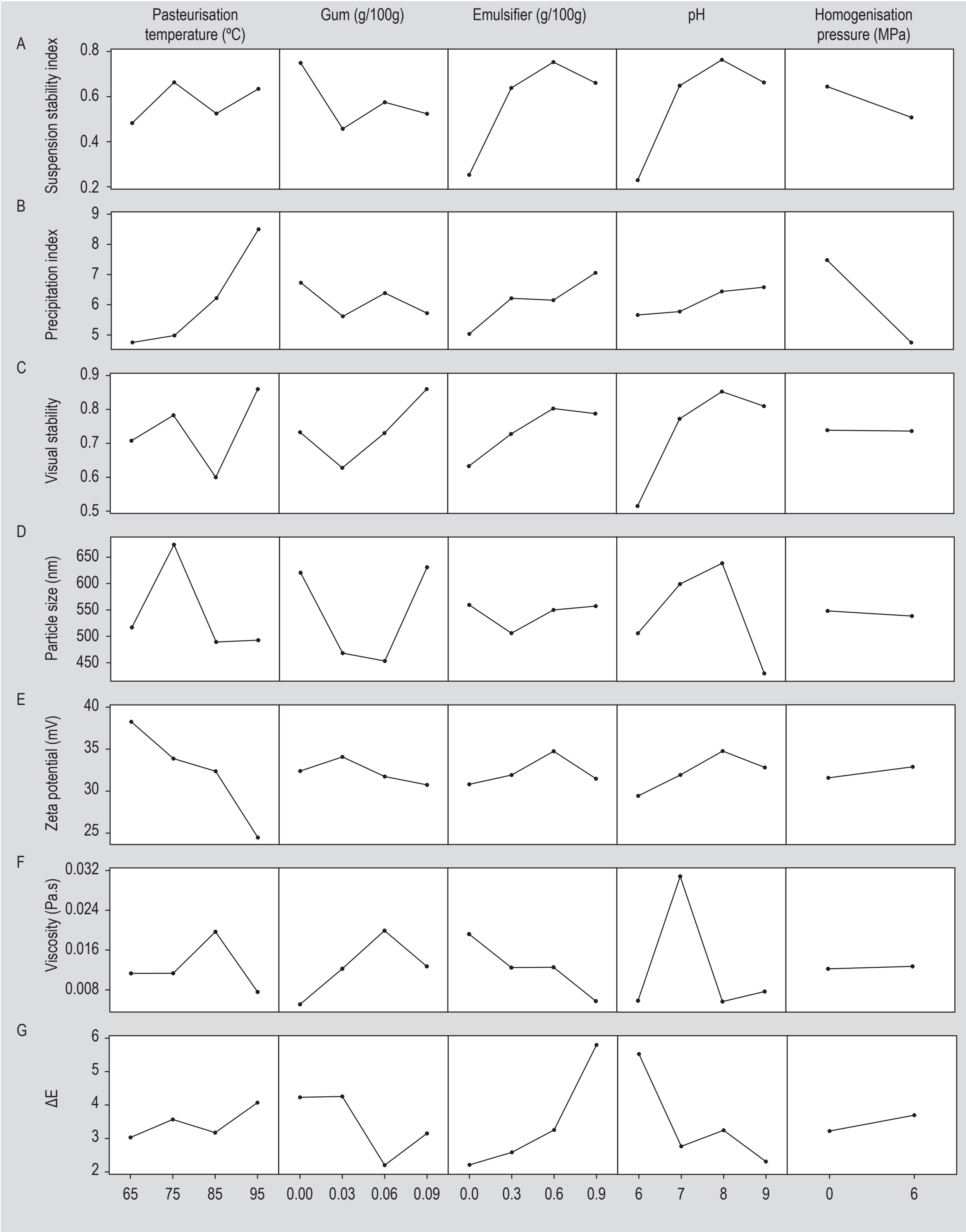

Figure 1. Effect of production parameters on properties of sesame milk.

and gum concentration as linear terms. Pasteurisation temperature had the most contribution percentage among terms (58.34%). It may have promoted an unfolding of the protein moiety to expose hydrophobic amino acids. It lead to the increase of positive surface charge of droplets followed by increasing the positive charged ZP (Buffo *et* *al.*, 2001). The increase of xanthan gum content leads to increase the negative surface charge of droplets. As shown in the results, 0.03% concentration of xanthan gum provided decrease ZP but it was increased above that level. It may be attributed to the reason that the increase of positive charges as a consequence of pasteurisation avoids the negative effect of xanthan gum on ZP.

Viscosity

Table 2 clearly exhibited that viscosity was significantly (P<0.05) influenced by pasteurisation temperature, emulsifier and pH. According to Figure 1F, addition of xanthan gum up to level of 0.06% leads to increase of viscosity but it decreases at higher concentrations. Therefore, insignificant effect of xanthan gum on viscosity contributed to this manner. Among main factors, the emulsifier was the most determinant factor (11.15% of influence) on viscosity. Emulsifier acts as a lubricant and thereby reduce internal friction between the components that led to decreasing of viscosity (Bueschelberger, 2004). The second factor having influence on viscosity of samples was the pasteurisation temperature. The highest viscosity value was observed at 85 °C probably followed by formation of lipid-protein complexes (Sivanandan *et al.*, 2010). According to Table 2, all of the two-way interactions were found to be significant (P<0.05).

Colour analysis

Colour is an important factor influencing consumer's acceptability of a product. According to the ANOVA results, ΔE of sesame milk increased significantly (P<0.0001) as pasteurisation temperature and emulsifier concentration increased (Figure 1G). Table 3 shows emulsifier concentration had the most influence (29%) on colour. It can be also seen that the pasteurisation temperature had the second influence (20.45%) on colour. The increase in ΔE with increase of pasteurisation temperature was due to a slight decrease in L* value and a further increase in a* value. As it has been reported by Hinds *et al.* (1997b), beverages treated with heat processing were slightly darker and less bright than control samples. It could be due to Maillard browning reactions between the free amino acids and reducing sugars. Also increase in ΔE with increase of emulsifier concentration was due to remarkable increase in L* value that induces lightness of sesame milk. This probably was due to the whitening properties of mono-diglyceride that usually adds to coffee whiteners as an ingredient (Malundo and Resurreccion, 1994).

Optimisation procedure and verification of results

Multiple response optimisations were carried out to measure the optimum levels of independent variables to reach the desired response goals. It can be concluded from the results that, among three parameters analysing stability of sesame milk (SSI, precipitation index, and VSI), VSI was the most powerful and useful technique to determining opacity and syneresis of sesame milk and to show the most important changes brought by processing conditions. Because its model had the lowest CV and residual error and the highest R^2 and adj. R^2. Therefore, VSI was chosen between them and was desired maximal. Other parameters were fixed to intermediate level. Then, the optimal conditions were extracted by Design Expert software. The final result for this optimisation suggested that a process containing pasteurisation temperature of 74.70 °C, gum concentration of 0.09 g/100 g, emulsifier level of 0.44 g/100 g, pH of 6.68 and homogenisation pressure of 1.81 MPa could be a good mixture of these five components in order to achieve the best properties of sesame milk.

Afaneh *et al.* (2011b) reported that the best heat treatment for obtaining acceptable dispersion stability of sesame milk was 85 °C for 5 min. This difference with our results might be due to the fact that the analysed parameters of this study was relatively different with them which resulted in difference between optimisation point i.e. time and temperature of pasteurisation.

This new process was submitted to the same experimental procedures applied as those from the beginning of this study (Table 5). There was no significant difference between the estimated and observed values (P<0.05), suggesting a good fit between the models to the experimental data.

4. Conclusions

In this research it was studied the effect of pasteurisation temperature, gum and emulsifier concentration, pH and homogenisation pressure on the stability indexes, particle size, ZP, viscosity and colour of sesame milk samples. From the analysis, it is evident that the pasteurisation temperature, gum and emulsifier concentration had the high contributions among main factors. Pasteurisation

Table 5. Predicted and experimental values of the response variables at optimum process.

Experimental values	Predicted values	Response variables
0.95±0.04	0.93	suspension stability index
4.71±0.05	4.61	precipitation index
0.99±0.002	1.009	visual stability index
494±0.16	496.33	particle size (µm)
31.16±0.22	31.68	zeta potential (mV)
0.024±0.018	0.021	viscosity (Pa.s)
0.62±0.07	0.65	total colour difference

temperature was the most significant factor affecting the SSI and ZP. xanthan gum concentration was the most determinant factor for visual stability and emulsifier concentration had the most influence on precipitation index and viscosity. Pasteurisation temperature had an effect on all parameters, except particle size. Emulsifier (mono-diglycerides) concentration showed similar effects than pasteurisation temperature, but to a lesser extent except for viscosity which was increased. Xanthan gum concentration had variable effects on phase separation, depending on the analytical technique. While, it had no effect on colour difference and viscosity. Finally, those results achieved under the optimal conditions revealed a maximum stability for sesame milk. It was concluded that Taguchi design method could be a suitable way for optimisation of sesame milk production.

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