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A practical optimization on salt/high-methoxyl pectin interaction to design a stable formulation for Doogh

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ABSTRACT

Doogh is a traditional Iranian fermented drink. Response surface methodology-central composite design (RSM-CCD) was used to determine optimum formulation for the production of Doogh stabilized by high methoxyl pectin (HMP). The effects of HMP (0.28–0.42% wt) and salt (0.4–1.1% wt) concentrations on the stability, viscosity, volume-weighted (D_{43}) and surface-weighted (D_{32}) mean diameters and Span were investigated. Graphical response surface plots were applied to locate the optimum point. The optimum composition for stable Doogh production was found to contain (% wt) HMP 0.4 and salt 0.62. These optimum conditions yielded the stability of 96.34%, viscosity of 2.05 mPa s, D_{43} of 11.922 µm, D_{32} of 0.966 µm and Span value of 2.140. There was no significant difference between the physical, rheological and sensory attributes of Dooghs produced under optimum conditions and commercial samples.

1. Introduction

Doogh is a typical Iranian drink produced from yoghurt by adding potable water, starter culture, salt and some aqueous extracts of local herbs (Foroughinia, Abbasi, & Hamidi, 2007). This dairy product is exported and consumed in Afghanistan, Armenia, Azerbaijan, Balkans, Turkey and to a lesser extent in other parts of Middle East and central Asia (Codex Alimentarius Commission, 2009). The most important quality problem in international trade of Doogh and the other acidified milk drinks (AMDs) is their physical instability as phase separation (Azarikia & Abbasi, 2010). However, this phenomenon due to the dilution extent is greater than in most other yogurt-based beverages like ayran.

Overall, the caseins (CSNs) are solubilized as CSN micelle forms at neutral pH conditions. The acidification process is lead to aggregation and precipitation CSNs by the changing their charge from negative to positive. At this condition, negatively charged stabilizers such as anionic polymers adsorb to positively charged CSNs, thereby generating electrostatic repulsive forces and steric repulsive forces between particles, which contribute to the stabilization of AMDs (Tuinier, Rolin, & de Kruif, 2002). Many researchers investigated the effect of various polysaccharide-hydrocolloids to prevent the aggregation or precipitation of milk proteins in AMDs during processing and storage (Azarikia & Abbasi, 2010; Foroughinia et al., 2007; Kiani, Mousavi, & Mousavi, 2010; ; Kiani, Mousavi, Razavi, & Morris, 2010). Mohammadifar, Musavi, and Williams (2007) also pointed out that polysaccharides can modify the functional behavior of milk proteins and prevent serum separation in acidified milk beverages.

Pectin as a multi-functional polysaccharide is one the most commonly used hydrocolloids in the food industry. It is an anionic polysaccharide mostly derived from citrus fruits. Pectin consists of a backbone of galacturonic acid partly methylesterified and branches of arabinose, galactose and xylose (Lam, Shen, Paulsen, & Corredig, 2007). This gelling polysaccharide is usually classified by the degree of esterification (DE) in high-methoxyl pectin (HMP), if half or more of the carboxyl groups are esterified, and low-methoxyl pectin (LMP), if less than half of the carboxyl groups are esterified. It is negatively charged at acidic pH (>3.5). The overall charge of pectin and its charge distribution on the chain determine the polysaccharide functionality as a film constituent. The gelling agent of HMP is considerably used in jams and marmalade (Willats, Knox, & Mikkelsen, 2006), however, it stabilizes acidified dairy beverages, drinkable yogurts, fruit-juice-containing milks, and fruit-flavored, protein-fortified drinks (Pereyra, Schmidt, & Wicker, 1997). Kiani, Mousavi, Razavi, et al. (2010) studied effect of gellan, alone and in

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Table 1
The CCRD matrix and, experimental and predicted values for the response variables.

	Independent variables		Response variable				
	HMP (%wt)[b]	Salt (%wt)[c]	Stability (%)	Viscosity (mPa s)[d]	D_{43} (μm)	D_{32} (μm)	Span
Run (experimental)[a]							
1	0.30 (−1)	0.50 (−1)	81.7 ± 0.8	1.56 ± 0.06	14.91 ± 0.59	1.263 ± 0.009	2.515 ± 0.261
2	0.40 (+1)	0.50 (−1)	99.7 ± 1.3	2.29 ± 0.11	12.67 ± 2.36	0.912 ± 0.021	2.312 ± 0.152
3	0.30 (−1)	1.00 (+1)	74.2 ± 2.4	1.22 ± 0.05	24.87 ± 1.65	2.035 ± 0.056	6.542 ± 0.051
4	0.40 (+1)	1.00 (+1)	82.1 ± 0.9	1.81 ± 0.41	15.83 ± 0.25	1.077 ± 0.009	2.169 ± 0.009
5	0.35 (0)	0.75 (0)	98.3 ± 0.3	1.65 ± 0.02	12.79 ± 0.69	0.991 ± 0.004	2.164 ± 0.321
6	0.35 (0)	0.75 (0)	99.5 ± 0.7	1.82 ± 0.09	13.12 ± 0.78	1.092 ± 0.121	2.456 ± 0.212
7	0.35 (0)	0.75 (0)	97.8 ± 1.6	1.71 ± 0.16	12.56 ± 3.12	1.011 ± 0.054	2.312 ± 0.023
8	0.28 (−1.41)	0.75 (0)	61.7 ± 1.6	1.12 ± 0.21	20.68 ± 3.64	1.856 ± 0.011	3.451 ± 0.012
9	0.42 (+1.41)	0.75 (0)	81.9 ± 1.2	2.17 ± 0.05	12.79 ± 4.14	0.984 ± 0.093	2.140 ± 0.096
10	0.35 (0)	0.40 (−1.41)	84.2 ± 0.2	1.59 ± 0.03	13.59 ± 0.44	1.412 ± 0.124	3.123 ± 0.002
11	0.35 (0)	1.10 (+1.41)	69.4 ± 0.5	1.32 ± 0.03	20.56 ± 0.26	1.543 ± 0.005	5.489 ± 0.412
12	0.35 (0)	0.75 (0)	91.8 ± 0.6	1.47 ± 0.32	13.25 ± 1.29	1.241 ± 0.006	3.412 ± 0.063
13	0.35 (0)	0.75 (0)	94.4 ± 0.1	1.54 ± 0.06	12.84 ± 3.31	1.110 ± 0.130	3.261 ± 0.012
14	0.35 (0)	0.75 (0)	93.8 ± 0.9	1.47 ± 0.14	12.66 ± 1.54	1.315 ± 0.014	2.187 ± 0.126
Run (predicted)							
1	0.30 (−1)	0.50 (−1)	79.79	1.52	15.24	1.33	2.14
2	0.40 (+1)	0.50 (−1)	98.46	2.22	13.04	1.00	2.62
3	0.30 (−1)	1.00 (+1)	73.33	1.22	24.39	1.92	6.03
4	0.40 (+1)	1.00 (+1)	81.90	1.92	15.38	0.98	2.34
5	0.35 (0)	0.75 (0)	99.94	1.72	12.89	1.05	2.45
6	0.35 (0)	0.75 (0)	99.94	1.72	12.89	1.05	2.45
7	0.35 (0)	0.75 (0)	99.94	1.72	12.89	1.05	2.45
8	0.28 (−1.41)	0.75 (0)	63.23	1.03	20.76	1.88	4.03
9	0.42 (+1.41)	0.75 (0)	82.48	2.02	12.82	0.98	1.76
10	0.35 (0)	0.40 (−1.41)	85.99	1.74	13.07	1.29	3.13
11	0.35 (0)	1.10 (+1.41)	69.72	1.31	21.20	1.69	5.69
12	0.35 (0)	0.75 (0)	91.93	1.53	12.84	1.20	2.82
13	0.35 (0)	0.75 (0)	91.93	1.53	12.84	1.20	2.82
14	0.35 (0)	0.75 (0)	91.93	1.53	12.84	1.20	2.82

[a] Mean ± standard deviation (n = 3).
[b] The five levels (−1.41, −1, 0, +1 and +1.41) of HMP concentration represented 0.28%, 0.30%, 0.35%, 0.40% and 0.42% wt, respectively.
[c] The five levels (−1.41, −1, 0, +1 and +1.41) of salt concentration represented 0.40%, 0.50%, 0.75%, 1.00% and 1.10% wt, respectively.
[d] Reported at a shear rate of 46.9 1/s.

combination with HMP, on the structure and stability of Doogh. They by using many treatments suggested that 0.05 wt% gellan in combination with 0.25 wt% HMP could be suitable in increasing the shelf-life period of Doogh. Azarikia and Abbasi (2010) and Ghorbani Gorji, Mohammadifar, and Ezzatpanah (2011) also found that gum tragacanth due to the functional characteristics of its soluble and insoluble fractions can considerably increase viscosity and stability of Doogh formulated with the different concentration of milk fat. However, there is still no optimization study concerning HMP and its interaction with other components in the literature which is important for scale-up and commercialization purposes.

Response surface methodology (RSM) is a mathematical/statistical based technique which is useful for analyzing the effects of several independent variables on the response. This method solves multivariate data, which is found from appropriately designed experiments to solve multivariate equation simultaneously (Gharibzahedi, Mousavi, Hamedi, & Ghasemlou, 2012a). Moreover, the main advantage of RSM is to reduce number of experimental trials needed to evaluate multiple variables and their interactions. Therefore, RSM is less laborious and time-consuming than the classical methods required optimizing a process (Gharibzahedi, Mousavi, Hamedi, Khodaiyan, & Razavi, 2012b).

The popularity of this nutritious and refreshing drink has significantly increased during last decade in Iran and some other countries, which led to its large industrial scale production. However, little data have been published on the practical optimization of Doogh formulation and its critical physical characteristics. Therefore, the aim of this work was to optimize Doogh formulation with the highest stability and the lowest particle size using RSM.

2. Materials and methods

2.1. Materials

Fresh skim milk was provided from the Pegah Dairy Co. (Iran, Tehran). It was contained 2.0 ± 0.1% fat, 2.8 ± 0.2% protein and 4.7 ± 0.2% lactose. A commercial reference culture (Yo-Mix™ 502) for traditional acidic yogurt was obtained from Gemak-Danisco Co. (Ankara, Turkey). Citrus pectin was provided from Sigma Chemical Co. (St. Louis, MO, USA). The composition determined according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2007) was as follows: pectin content (ash-free, dried) 91.4% w/w, galacturonide content 88.0% w/w (percent of pectin) and DE 72.2%. Sodium chloride (99.5% purity) and lactic acid were from Merck Chemical Co. (Darmstadt, Germany).

2.2. Doogh preparation

For the Doogh preparation, the method of Kiani, Mousavi, and Mousavi (2010) was adopted with slight modifications. The used milk was pasteurized in a water bath at 80 ± 1 °C for 30 min. After the cooling to 40 °C, it was inoculated by the commercial reference culture and transferred to plastic cups, incubated at 43 °C until the pH reached 4.3 ± 0.1. In order to measure the total solids content (TSC) of the produced yogurt, about 1.0 g sample on a boiling bath was evaporated and then oven-dried at 100 °C to a constant weight. In the next step, the resulting yoghurt was diluted and blended using a classic blender (PBI International, Milan, Italy). HMP (0.28–0.42% wt, Table 1) as used stabilizing agent in this study was gradually added in deionized water. The HMP–salt mixture by adding salt (0.40–1.10% wt, Table 1) was produced, heated (80 °C for

15 min), and cooled down to ambient temperature. To achieve full hydration, the mixture was kept at room temperature overnight. In this step, the obtained solutions were added to the partial diluted yoghurt kept at room temperature. The TSC standardized at 4% wt for all formulations, including solids originated from yoghurt, HMP and salt. Afterward, the mixture passed through a high-pressure homogenizer (APV 2000, Denmark) at 185 ± 5 bar. At least two separate samples were prepared for each treatment.

2.3. Stability evaluation

In order to determine serum separation, freshly prepared samples (15 ml) were transferred to cylindrical tubes (internal diameter 15 mm, height 120 mm), and stored for 30 days at 4 °C. Each phase has a clear appearance that could be easily detected visually. The amount of supernatant volume was measured and expressed as a percentage of the total sample volume (15 ml).

2.4. Weight fraction determination

The weight fraction was evaluated as described by Laurent and Boulenguer (2003). Briefly, 15 g of Doogh was centrifuged at 13,000 × g for 2 h in 30 ml screw cap tubes. The upper layer or supernatant was decanted and then centrifuge tubes were left upside down for 30 min prior to determining the sediment weight (SW). The weight fraction (WF) was expressed in percent as (Eq. (1)):

$$\mathrm{WF} = \frac{\mathrm{SW} \times 100}{15} \qquad (1)$$

2.5. Viscosity measurement

Viscosity of the prepared samples was measured using a steady stress rheometer (Brookfield DV-II, LV Viscometer, USA) equipped with the ULA spindle, as previously recommended by Gharibzahedi, Mousavi, Hamedi, and Khodaiyan (2013a). The experiments were carried out at 25 ± 2 °C and different rotational speeds depending on their torque values. After primary experiments, the appreciate torque value found between 10% and 100% of the measuring range to obtain reliable results. Tests were performed in triplicate immediately after production of the samples. The power law model was applied to determine the consistency coefficient and the flow behavior index of the samples using the shear stress data obtained from increasing shear rate measurements as follows (Eq. (2)):

$$\sigma = K\dot{\gamma}^n \qquad (2)$$

where σ is shear stress, K is the consistency coefficient, $\dot{\gamma}$ is shear rate, and n is the flow behavior index.

2.6. Particle size characteristics

Particle size of the samples 1 day after preparation was measured by light scattering, using a Mastersizer 2000S (Malvern Instruments Ltd., UK). Sample analysis was carried out after sample preparation and during storage time, based on the principle of Fraunhofer (Huang, Kakuda, & Cui, 2001). Size distribution was characterized by volumetric percentage and mean particle diameter obtained by volume-weighted mean diameter (D_{43}) and surface-weighted mean diameter or Sauter diameter (D_{32}) of the Doogh particles, based on the following equations (Gharibzahedi, Mousavi, Hamedi, & Ghasemlou, 2012; Gharibzahedi, Mousavi, Hamedi, Khodaiyan et al., 2012):

$$D_{43} = \left(\frac{\sum n_i d_i^4}{\sum n_i d_i^3}\right) \qquad (3)$$

$$D_{32} = \left(\frac{\sum n_i d_i^3}{\sum n_i d_i^2}\right) \qquad (4)$$

where n_i is the number of droplets of radius d_i.

The Span is the distribution width of the particles in the dispersion, which was calculated using the following equation (Eq. (5)) (Koocheki & Kadkhodaee, 2011):

$$\mathrm{Span} = \left(\frac{d(\upsilon, 90) - d(\upsilon, 10)}{d(\upsilon, 50)}\right) \qquad (5)$$

where $d(\upsilon, 10)$, $d(\upsilon, 50)$ and $d(\upsilon, 90)$ are diameters at 10%, 50%, and 90% cumulative volume, respectively. In other words, $[d(\upsilon, 90) - d(\upsilon, 10)]$ is the range of the data, and $d(\upsilon, 0.5)$ is the median diameter.

Moreover, deviation from the median which is an indicative of polydispersity was reported as uniformity as previously described by Gharibzahedi, Razavi, and Mousavi (2013b).

2.7. Sensory analysis

Sensory characteristics of the produced samples in the optimum formulation including taste, odor, homogeneity, mouthfeel and overall acceptability were evaluated by 30 testers including graduate students and staff members of *Tehran University's Food Engineering Department* who were familiar with the characteristic qualities of the commercial Dooghs. Panelists were seated in sensory booths with standard lighting. A hedonic 5-point structured scale, in which 5 corresponded to "most liked" and 1 to "most disliked". Each sample was presented twice, and the samples were presented in random order.

2.8. Experimental methodology

In order to find out the best Doogh formulation for the industrial applications, a series of experiments were performed base on RSM using the software Design-Expert (trial version 7.1.6, Stat-Ease Inc., Minneapolis, USA). Fourteen Dooghs were established by central-composite rotatable design (CCRD) with two independent variables including HMP (X_1) and salt (X_1) concentrations at five levels of each variable (Table 1). The range of independent variables and their levels is presented in Table 1, which was based on the results of preliminary experiments. The stability (Y_1), viscosity (Y_2), D_{43} (Y_3), D_{32} (Y_4) and Span (Y_5) were the dependent variables. The value of responses in each trial was average of triplicates. Data from CCRD were analyzed by multiple regressions to fit the following quadratic polynomial models (Eq. (6)):

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_{12} x_1 x_2 + \alpha_{11} x_1^2 + \alpha_{22} x_2^2 \qquad (6)$$

The coefficients of the polynomial were represented by α_0 (constant term), α_1 and α_2 (linear effects), α_{11} and α_{22} (quadratic effects) and α_{12} (interaction effects).

The significance of the equation parameters for each response variable was also assessed by F-ratio at a probability (P) of 0.05. The terms found to be statistically non-significant ($P > 0.05$) were removed and the experimental data was refitted to only the significant ($P < 0.05$) factors to obtain the final reduced model. The adequacy and fitness of mathematical models was tested by analysis of variance (ANOVA). Adequacy of regression models were evaluated by determination of R^2, adjusted R^2, lack of fit and adequate precision (ADP) as previously recommended by Gharibzahedi, Mousavi, Hamedi, and Ghasemlou (2012), Gharibzahedi, Mousavi, Hamedi, Khodaiyan et al. (2012).

Table 2
Regression coefficients, R^2, adjusted R^2, probability values for dependent variables.

Regression coefficient	Constant	Linear		Quadratic		Interaction	R^2	R^2 (adj)	Lack of fit[a]	Regression (*P*-value)	ADP
	α_0	α_1	α_2	α_{11}	α_{22}	α_{12}					
Stability (%, Y_1)	95.93	6.81	−5.75	−9.54	−7.04	−2.52	0.982	0.969	0.0569^{ns}	<0.0001	25.47
Viscosity (mPa s, Y_2)	1.62	0.35	−0.15	–	–	–	0.926	0.912	0.1760^{ns}	<0.0001	23.27
D_{43} (μm, Y_3)	12.87	−2.80	2.87	1.97	2.14	−1.70	0.991	0.985	0.0669^{ns}	<0.0001	32.90
D_{32} (μm, Y_4)	1.13	−0.32	0.14	0.11	0.14	−0.15	0.926	0.874	0.1212^{ns}	0.0008	10.99
Span (Y_5)	2.63	−0.80	0.90	–	0.80	−1.04	0.908	0.842	0.3130^{ns}	0.001	11.05

[a] ns: Not significant.

2.9. Model verification

Experimental data for the stability, viscosity, D_{43}, D_{32} and Span of Doogh were obtained by conducting the experiments under the recommended optimum formulation. Verification of the response surface model was achieved by comparing the experimental value obtained from an independent set of samples with the predicted value obtained from the optimized model.

3. Results and discussion

3.1. Statistical analysis and the model fitting

By employing multiple regression analysis on the experimental data, the predicted responses for the stability (Y_1), viscosity (Y_2), D_{43} (Y_3), D_{32} (Y_4) and Span (Y_5) can be obtained by the following relationships (Eqs. (7)–(11)):

$$Y_1 = 95.93 + 6.81x_1 - 5.75x_2 - 9.54x_1^2 - 7.04x_2^2 - 2.52x_1x_2 \quad (7)$$

$$Y_2 = 1.62 + 0.35x_1 - 0.15x_2 \quad (8)$$

$$Y_3 = 12.87 - 2.80x_1 + 2.87x_2 + 1.97x_1^2 + 2.14x_2^2 - 1.70x_1x_2 \quad (9)$$

$$Y_4 = 1.13 - 0.32x_1 + 0.14x_2 + 0.11x_1^2 + 0.14x_2^2 - 0.15x_1x_2 \quad (10)$$

$$(11) Y_5 = 2.63 - 0.80x_1 + 0.90x_2 + 0.80x_2^2 - 1.04x_1x_2$$

As considered in the above equations, ANOVA indicates that the second-order polynomial models were highly significant and adequate to represent the actual relationship between the responses (stability, D_{43}, D_{32} and Span) and the significant variables (Table 2). However, the linear regression equation was significantly fitted to predict the effect of independent variables on the variation of viscosity ($P < 0.0001$). Also, no indication of significant ($P > 0.05$) lack of fit was observed for the final reduced models, thus ensuring satisfactory fitness of the response surface model to the significant ($P < 0.05$) independent variable effects (Table 2) (Gharibzahedi, Razavi, & Mousavi (2013c)). Regression coefficients, R^2, adjusted R^2, lack of fit, ADP and *P*-values for five dependent variables are tabulated in Table 2. A high R^2 was obtained for all regression models (0.908–0.982). It was suggested that R^2 should be at least 0.80 for a good fitness of a model (Joglekar & May, 1987). However, a large value of R^2 does not always imply that the regression model is a good one. Adjusted R^2 is a modification of R^2 that adjusts for the number of explanatory terms in a model. Unlike R^2, the adjusted R^2 increases only if the new term improves the model more than would be expected by chance (Koocheki & Kadkhodaee, 2011). The values of adjusted R^2 were 0.969, 0.912, 0.985, 0.874 and 0.842 for the stability, viscosity, D_{43}, D_{32} and Span, respectively (Table 2). ADP measures the signal-to-noise ratio. A ratio greater than 4 is desirable (Gharibzahedi, Razavi, & Mousavi, 2013b). ADP value for the presented models was between 10.99 and 32.90 (Table 2). On the other hand, the most important diagnostic test to determine the adequacy of a model will be the normal probability plot of the studentised residuals (Samavati & Manoochehri Zadeh, 2013). The residual plots indicated a normal distribution, as no response transformation was needed and there was no apparent problem with normality in all cases (Fig. 1a–e). Table 1 also showed that no significant difference was observed between the experimental and the predicted values.

3.2. Effects of HMP and salt on the independent variables

Table 3 illustrates that except for the quadratic effect of HMP on the D_{32}, all the linear, quadratic and interaction effects of HMP and salt were significant for the five response variables ($P < 0.05$; $P < 0.001$; $P < 0.01$; $P < 0.0001$). It can be seen that the variable with the largest effect on the stability was the quadratic effect of HMP concentration, followed by the quadratic term of salt content and linear effect of HMP concentration (Table 2). As shown in Fig. 2a, the variation of the stability could be explained as a nonlinear function of the HMP and salt concentrations. Increases in salt content resulted in reduced the stability, although this index increased gradually with increased HMP concentration (Fig. 2a). The individual optimum region to obtain a desirable stability (98.76%) for Doogh was estimated to be achieved by a set level of 0.37% wt and 0.63% wt for HMP and salt, respectively.

The intensity of interactions between CSN micelles and pectin is pH-dependent (Liu, Akihiro, & Corredig, 2006). The most produced Dooghs in this study had a pH in the range of 3.9–4.2 that is favorable for ideal stabilization of CSN dispersions because the CSN and pectin have optimum opposite net charges. However, the instability at low HMP concentrations is probably because of the bridging flocculation phenomenon which occurs as the surface of the CSN particles are only partially covered and the particles can then be linked by polymer bridges (de Kruif & Tuinier, 2001; Dickinson, 1998; Maroziene & de Kruif, 2000; Syrbe, Bauer, & Klostermeyer, 1998). Therefore, in order to achieve long-term stability by preventing sedimentation of CSN aggregates, the presence of a weak gel network at high concentrations of HMP is necessary (Boulenguer & Laurent, 2003; Tromp, de Kruif, van Eijk, & Rolin, 2004). This multilayer biopolymer matrix formed in the serum phase is supposed to restrict the suspended CSN particles from colliding, and thus the weak gel is considered to be strong enough to overcome gravity (Miquelim, Lannes, & Mezzenga, 2010). Besides the electrosorption of pectin onto CSN particles, the Doogh stabilization by HMP occurs also by steric repulsion between the CSN aggregates. This fact could be attributed to the entropy of the protruding chains of HMP as loops and dangling tails, which were adsorbed to the CSN aggregate surface (Jensen, Rolin, & Ipsen, 2010). Some researchers also reported that pectin molecules in acidic milk beverages can interact with CSN through calcium ions and prevent their aggregation, sedimentation and hence serum separation by ionic and steric stabilization (Atamer et al., 1999; Lucey, Tamehana, Singh, & Munro, 1999). The negative effect of high salt concentrations on the stability may be explained as a salting out effect (Ambjerg Pedersen & Jorgensen, 1991). At this condition, some of the water molecules

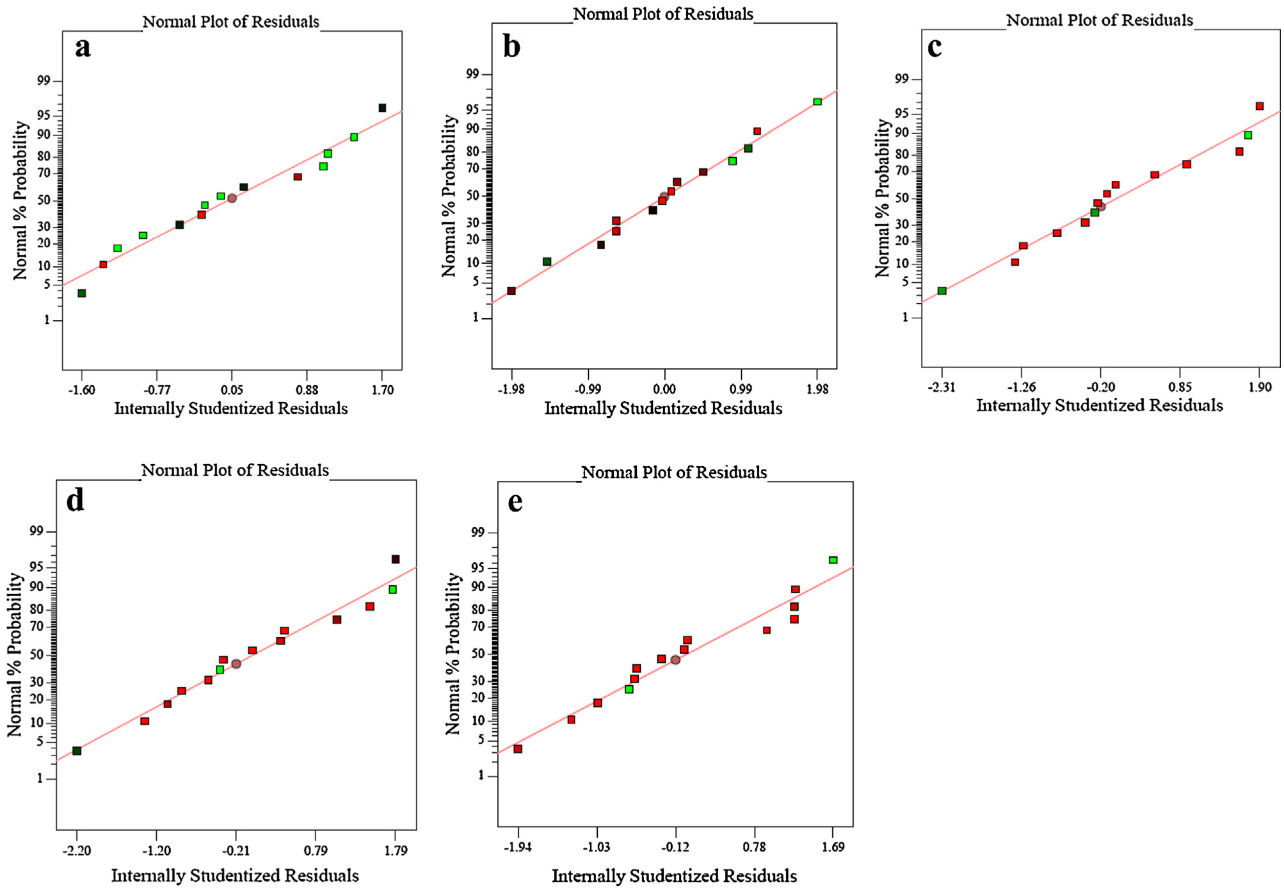

Fig. 1. Normal probability of internally studentized residuals of the response variables (a, stability; b, viscosity; c, D_{43}; d, D_{32}; e, Span).

are attracted by the NaCl ions, which reduce the number of available water molecules to interact with the charged biomacromolecules present in the system. Wang, Lee, Wang, and Huang (2007) studied effects of salt concentration and initial β-lactoglobulin/pectin ratio in the protein–polysaccharide coacervates. They found that higher salt concentrations along with reduction of the total biopolymer concentrations in the protein/polysaccharide coacervates lead to a more watery coacervate structure which is ascribed to the screening of the electrostatic interaction between the protein and the polysaccharide (Ru, Wang, Lee, Ding, & Huang, 2012; Weinbreck, Tromp, & de Kruif, 2004).

The linear regression equation was fitted to predict the effects of HMP and salt on the variation of viscosity leading to achieve the optimum viscosity value (Fig. 2b). The quadratic and interaction effects were shown to be non-significant on viscosity (Table 2). However, the linear effect of HMP concentration on Doogh viscosity was stronger than the linear term of salt content. Other researchers also found that the apparent viscosity of acidic milk drinks enhanced when pectin concentration was increased (Amice-Quemeneur, Haluk, Hardy, & Kravtchenko, 1995; Atamer et al., 1999; Koksoy & Kilic, 2004; Lucey et al., 1999). This fact is probably because of the HMP ability to induce formation of a weak network structure in Doogh (Tromp et al., 2004). At high level of HMP, more HMP to cover the CSN particles and also to interact with water could be available for increasing the resistance of the fluid against flow (Dickinson, 1998; Syrbe et al., 1998). The low diffusivity in this condition reduces the physical instability due to decrease the collisions between Doogh particles. The authors applied the power law model to study the flow behavior of Doogh. This model was previously used to model the shear stress data of a similar Turkish product (Koksoy & Kilic, 2004) and fermented milk beverages (Janhoj, Bom Frost, & Ipsen, 2008; Penna, Sivieri, & Oliviera, 2001) at increasing

Table 3
The significance of each response variable effect showed by using *F*-ratio and *P*-value in the fitted models.

Regression coefficient	Statistical parameters	Linear		Quadratic[a]		Interaction
		x_1	x_2	x_1^2	x_2^2	x_1x_2
Stability (%, Y_1)	*P*-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0424
	F-ratio	89.25	63.74	161.59	87.97	6.14
Viscosity (mPa s, Y_2)	*P*-value	<0.0001	0.001	–	–	–
	F-ratio	106.77	19.60	–	–	–
D_{43} (μm, Y_3)	*P*-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0002
	F-ratio	254.72	267.21	116.36	137.40	46.85
D_{32} (μm, Y_4)	*P*-value	0.0001	0.013	0.036	0.014	0.040
	F-ratio	55.38	10.79	6.69	10.45	6.31
Span (Y_5)	*P*-value	0.004	0.002	0.84[ns]	0.005	0.006
	F-ratio	17.29	21.87	0.04	15.64	14.55

[a] ns: Not significant.

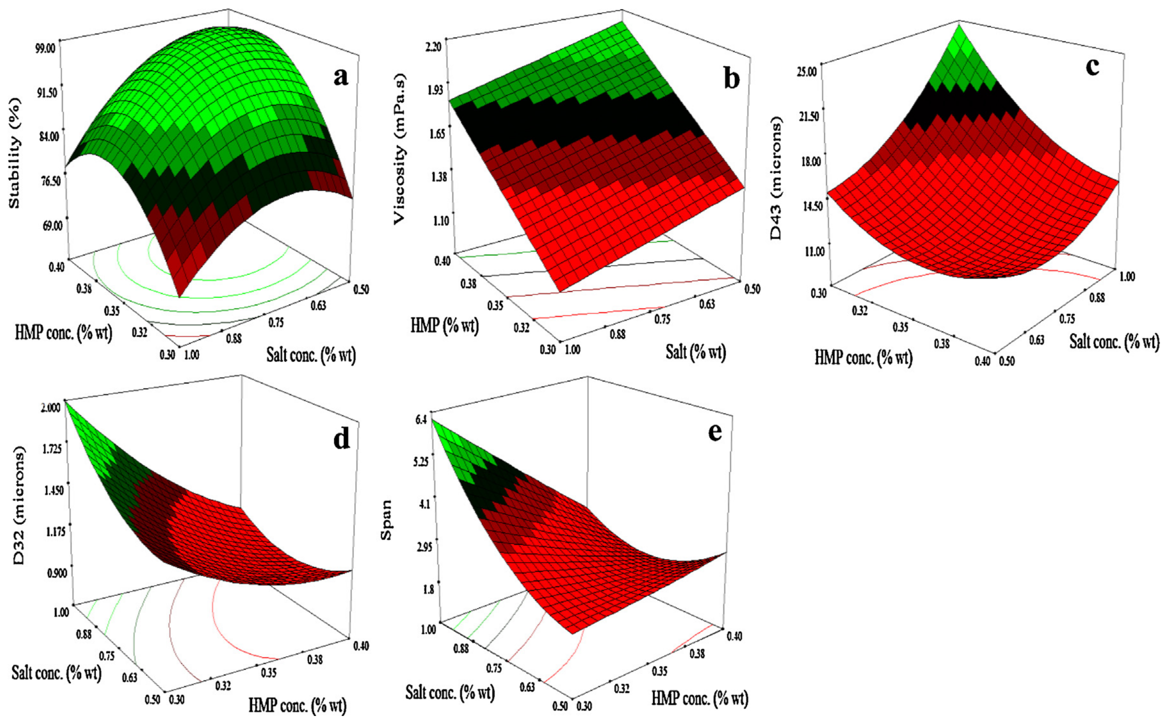

Fig. 2. 3D plots for the interaction effects of independent variables on the stability (a), viscosity (b), D_{43} (c), D_{32} (d) and Span (e).

shear rate. The Power law model had a satisfactory R^2 (>0.99) in most Dooghs in elucidating the shear stress–shear rate relationship (data not shown). A Newtonian behavior was also observed at all Dooghs formulated with 0.3–0.4% wt HMP. As observed in Fig. 2b, the Dooghs with high salt contents had lower viscosity values compared to the low-salt Dooghs. This fact could be attributed to the salt role in formation of more watery coacervate structure by inducing an imbalance in electrostatic interactions. Therefore, as the particle interactions were weakened, the CSN network might have become more shear sensitive exhibiting a higher thixotropy degree compared to Dooghs without added salt (Koksoy & Kilic, 2004). Schkoda, Hechler, and Kessler (1999) also demonstrated the addition of NaCl up to a concentration of 1.5% w/w led to a decrease in the apparent viscosity of acidified CSN gels. A Doogh containing 0.40% wt HMP and 0.50% wt salt was predicted to provide maximum viscosity (2.13 mPa s) using response surface plots and response optimizer.

Fig. 2(c–e) reveals the interaction between HMP and salt on the particle size characteristics of Doogh. From these plots it can be seen that when HMP concentration was increased from 0.30% to 0.40% wt, these properties decreased in a parabolic manner. In addition, salt concentration also showed a significant increase for the particle properties of Doogh. From the results illustrated in Table 2, it can be also concluded that variables with the largest effects on the D_{43}, D_{32} and Span were the linear terms of salt, the linear term of HMP and the interaction effect of HMP–salt, respectively. In this investigation, the lowest response for particle size characteristics (D_{43}, 12.08 μm; D_{32}, 0.923 μm and Span, 1.864) was observed when the Doogh was formulated with 0.40% wt HMP and 0.76% wt salt. A bimodal distribution of size with a large average diameter was observed at low concentrations of HMP (Fig. 3). Moreover, uniformity of the particles was significantly decreased by increasing the HMP from 0.734 to 0.514 ($P < 0.05$).

This fact showed that the HMP content was not sufficient to completely cover the positively charged CSN particles and caused bridging flocculation as well as CSN aggregation (Liu et al., 2006). This result was generally in agreement with the finding obtained by Maroziene and de Kruif (2000) and Tuinier et al. (2002). It is hypothesized that the high concentrations of salt especially Cl^- counterions by locally decreasing the CSN surface charge and the presence of electrostatic forces led to increase protein–protein interactions and large aggregates of the CSNs (Tran & Rousseau, 2013). As discussed in earlier parts, the salt addition at high concentrations can prevent HMP–CSNs complexation by the screening electrostatic interactions. This fact led to increase self-aggregation of CSNs and the sedimentation. Thus, the higher level of WF in Dooghs containing 1% wt salt was reasonable than those had 0.5% wt salt.

3.3. Optimization and verification

Optimum conditions for the production of stable Doogh was determined to obtain maximum stability and viscosity with minimum values for the particle size characteristics. In the present work, HMP and salt concentrations were selected in the range of 0.28–0.42% and 0.40–1.10% wt, respectively. Using the "response optimizer" option of Design-Expert software; the optimum values

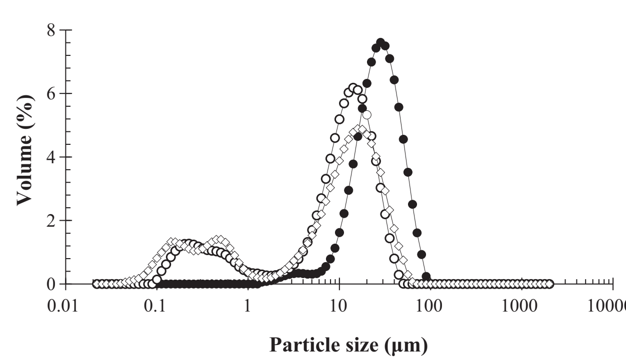

Fig. 3. Particle size distributions of Dooghs containing 0.50 wt% salt at different concentrations of HMP after 24 h of storage (0.30 wt% (□), 0.35 wt% (○), 0.40 wt% (●)).

Table 4
Evaluation of some sensory properties of Doogh produced under optimum conditions in comparison with commercial Doogh.

Doogh type[b]	Sensory characteristics[a]				
	Taste	Odor	Homogeneity	Mouthfeel	Overall acceptability
Optimum sample	4.73 ± 0.04[a]	4.20 ± 0.14[b]	4.59 ± 0.07[a]	4.62 ± 0.05[a]	4.85 ± 0.09[a]
Commercial sample	4.71 ± 0.15[a]	4.48 ± 0.11[a]	4.61 ± 0.04[a]	4.79 ± 0.08[a]	4.81 ± 0.08[a]

[a] Values in the same columns followed by different letters (a and b) are significantly different ($P < 0.05$).
[b] Without aqueous extracts of local herbs.

of the two factors studied were 0.40% wt HMP and 0.61% wt salt. The stability, viscosity, D_{43}, D_{32} and Span in this combination predicted 96.34%, 2.05 mPa s, 11.992 μm, 0.966 μm and 2.140 μm, respectively. This prediction was associated with a high overall desirability (=0.915).

In order to validate the adequacy of the model equations, three verification experiments were carried out at the optimum conditions. The corresponding response values predicted for the stability, viscosity, D_{43}, D_{32} and Span of optimum Doogh (0.40% HMP and 0.62% salt) were examined to be 97.12 ± 0.25%, 1.92 ± 0.32 mPa s, 9.871 ± 0.120 μm, 0.925 ± 0.048 μm and 2.101 ± 0.044, respectively. Moreover, a very low sedimentation rate (0.83%) was observed in the Dooghs prepared with optimum formulation. The results indicate that the model used can identify formulation conditions for the production of stable Doogh.

3.4. Sensory evaluation

Table 4 shows a comparative evaluation on the sensory attributes of two Doogh samples of commercial and prepared with optimum formulation. As considered in this table, there was no significant difference between the taste, homogeneity, mouthfeel and overall acceptability of commercial samples and the samples stabilized with 0.4% HMP and 0.62% salt ($P > 0.05$). However, in the preliminary sensory assessments, high concentrations of the HMP (0.4% wt) were found to adversely affect the odor of the Doogh. This fact can be improved by adding aqueous extracts of local herbs. The panelists evaluated that consistency of the prepared samples under optimum formulation is ideal for the consumers. Penna et al. (2001) also reported that a high consistency coefficient can positively increased the sensory acceptability of lactic beverages.

4. Conclusion

The objective of this study was to develop a stable Doogh that contains HMP and salt using RSM-CCRD as the optimization technique. Physical stability, apparent viscosity and particle size characteristics including D_{43}, D_{32} and Span were analyzed. The results revealed that on increasing the HMP concentration further the CSN micelles become fully coated and attraction between the particles is lowered. Enhancing the concentration of added salt decreased the consistency and accelerated the serum separation in Doogh during storage period. The results also proved the importance of ionic strength of NaCl in controlling the particle size and rheological characteristics of Doogh. The optimum formulation in order to enhance the stability and viscosity and to minimize the particle size and its polydispersity was found to contain (% wt) HMP 0.40 and salt 0.62. Under these conditions, the stability, viscosity, D_{43}, D_{32} and Span of Dooghs were 96.34%, 2.05 mPa s, 11.922 μm, 0.966 μm and 2.140 μm, respectively. Confirmation of experimental results was found to be close to the prediction derived from the RSM models. It can be concluded that RSM is a powerful tool for design and optimization of suitable formulation for the production of stable Dooghs.

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