A mixture design approach to optimizing low cholesterol mayonnaise formulation prepared with wheat germ protein isolate

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Revised: 15 April 2014 / Accepted: 21 April 2014 / Published online: 10 May 2014
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Abstract The aim of this study was to optimize the mixture proportion of low cholesterol mayonnaise containing wheat germ protein isolate (WGPI) and xanthan gum (XG), as emulsifying agents in mayonnaise preparation. The mayonnaise prepared with different combinations of WGPI, egg yolk (0–9 % of each component) and XG (0–0.5 %). The optimized mixture proportions of low cholesterol mayonnaise were determined by applying the optimal mixture design method to acquire the mayonnaise with proper stability, texture, rheological properties and sensory scores. Optimum values of WGPI, XG and egg yolk in the mixture were found to be 7.87 %, 0.2 % and 0.93 %, respectively (of 9 % egg yolk). The WGPI, due to unique functional properties, had the greatest effect on properties of mayonnaise samples. Moreover, combination of XG and WGPI, improved the stability, heat stability, viscosity, consistency coefficient and textural properties of product. However, the overall acceptance was maximum in a mixture contained high amount of WGPI and XG and low amount of egg yolk. The results of this research showed the feasibility of preparation a low cholesterol mayonnaise by application a

desirable combination of WGPI, XG, and egg yolk, with comparable properties those of the conventional mayonnaise.

Keywords Low cholesterol mayonnaise · Mixture design · Wheat germ protein isolate · Xanthan

Introduction

Mayonnaise is a typical oil in water emulsion prepared from vegetable oil, egg yolk, vinegar, sugar, salt, mustard and a variety of food additives (Juszczak et al. 2003). Among its ingredients, egg yolk is most critical in term of stability of the mayonnaise. Egg is considered a high profile ingredient because of its high nutritional value and multifunctional properties, including emulsification, coagulation, foaming, and flavor product (Narsimhan and Wang 2008) (Table 1).

The desire to replace eggs in food systems was brought about by a multitude of concerns from consumers, and processors desired to have low cholesterol foods (Liu et al. 2007). Therefore, several protein products such as Whey protein (Takeda et al. 2001) and soy protein (Rir et al. 1994), have been evaluated as emulsifying agents in O/W emulsions. Wheat germ, a by-product of the flour milling industry, is reported to be one of the most potential and excellent source of vitamins, minerals, dietary fiber, proteins, and some functional micro-compositions at a relative low cost (Zhu et al. 2006).

Defatted wheat germ, is a high nutritive value protein material, which contains more than 30 % protein, mainly in the form of albumin and globulin, indicates remarkable functional properties (Gomez et al. 2012).

Previous studies have indicated high solubility index, favorable emulsifying properties, foaming capacity, and hydrophobicity of wheat germ protein isolate (WGPI), that make it a

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Table 1 Mixtures composition in the mayonnaise formulated with egg yolk, wheat germ protein isolate, and xanthan gum in a three-component constrained optimal mixture design

Formulation	Ingredient proportion X ₁ (EY)	(g/100 g mayonnaise)	
		X ₂ (WGPI)	X ₃ (XG)
1	4.38	4.38	0.25
2	9.00	0.00	0.00
3	8.50	0.00	0.50
4	0.00	9.00	0.00
5	6.44	2.19	0.38
6	0.00	8.50	0.50
7	8.75	0.00	0.25
8	4.50	4.50	0.00
9	0.00	8.75	0.25
10	2.19	6.44	0.38

EY egg yolk, WGPI wheat germ protein isolate, XG xanthan gum

useful ingredient for various food products (Ge et al. 2000; Hassan et al. 2010).

In order to prepare low cholesterol mayonnaise, application of protein, such as whey protein isolate along with some thickeners has been reported. However, there is no published data on application of WGPI with xanthan gum (XG) to replace egg yolk (EY) in mayonnaise formulation. Therefore, the present study was conducted to prepare low cholesterol mayonnaise by application a desirable combination of WGPI, XG, and egg, with comparable properties those of the conventional mayonnaise.

In the proposed approach, the WGPI can be used as EY substitute, because of unique functional properties and natural nutrients that can compare to EY. Furthermore, the best combination of WGPI, XG, and EY was obtained by Mixture Design approach to produce low cholesterol mayonnaise.

Material and methods

Chemical analysis

The proximate composition of raw wheat germ (RWG), defatted wheat germ flour (DWGF), WGPI and mayonnaise samples were determined according to official methods (AOAC 2005).

Measurement of cholesterol content of mayonnaise samples

Extraction of total lipid and saponification

Accurately 1 g mayonnaise was weighed into a 50 ml volumetric flask and 1 g sea sand was added to complete saponification. Then, 20 ml freshly prepared 1.0 M methanolic

potassium hydroxide solution and 10 ml isopropanol were added. In the next step, this solution was heated in a water bath at 60 °C under a reflux condenser for 30 min while stirring with a magnetic stirrer. After cooling the turbid solution, 50 ml isopropanol was added and the fluid was filtered through Watmann no. 5 A filter paper. The clear sample solution was retained for the assay Wrolstad et al. (2005). Enzymatic measurement was used to determine the cholesterol content of different solutions by using Pars Azmoon diagnostic kits.

Raw materials

RWG was supplied by roller flour mill, Gorgan, Iran. XG was purchased from Sigma Aldrich Company. Other ingredients of mayonnaise formula such as commercial mixture containing vegetable oil, vinegar, salt, sugar, and mustard powder were acquired from local market. All other chemical used were of analytical grade.

Preparation of defatted wheat germ flours (DWGF)

RWG was defatted with n-hexane (1:10 w/v) for 8 h, and air dried at room temperature (25 °C). The defatted wheat germ meal was milled using a laboratory-scale hammer mill (Perten, 3100, Germany) and the resulting flour (DWGF) was sieved through a 100 mesh screen.

Preparation of wheat germ protein isolate (WGPI)

WGPI was prepared according to the procedure described by Ge et al. (2000), with some modifications. DWGF was dispersed in 0.5 M NaCl solution (1:10, w/v) and stirred for 30 min at ambient temperature. The suspension was adjusted to pH 10 with 1 M NaOH. After stirring for 30 min, the suspension was centrifuged at 8,000 rpm for 20 min at 4 °C using a refrigerator centrifuge (Hanil, combi 514R, Korea). The supernatant was adjusted to pH 4 with 1 M HCl to precipitate the proteins and centrifuged again at 8,000 rpm for 20 min at 4 °C. The precipitates were washed with deionized water, dispersed in a small amount of deionized water, and adjust to pH 7. The dispersed product was freeze-dried. Protein powder sealed in air-tight polyethylene and kept in a freezer (−20 °C) until experiments.

Preparation of mayonnaise

The amount of various ingredients in percentage (w/w) in mayonnaise formula were: vegetable oil 65, vinegar 7 (11 % w/v acetic acid), salt 1, sugar 5, mustard 0.3, additives (sodium benzoate, potassium sorbate and citric acid 0.03) and water 12.61. The amount of WGPI, XG and EY varied totally 0–9 (0–9 for WGPI and EY and 0–0.5 for XG) according to

the Mixture Design approach. WGPI and XG were replaced according to egg yolk dry matter.

The mayonnaise samples were prepared using a standard mixer (Kenwood, AW34655, Germany). In order to mayonnaise preparation, the WGPI and/or egg yolk, and half of the water were mixed together, followed by the addition of the dry matters. Then a small portion of the total oil was added. The ingredients were admixed for about 5 min. Then, one third of vinegar was gradually blended in the mixer. Finally, the remaining oil, water and vinegar were slowly added and admixed in a blender.

Mayonnaise samples were transferred to glass bottles and stored in refrigerator (4 °C) until analysis.

Emulsion stability measurement

Fifteen grams (F_1) of each sample placed in test tubes (15 mm internal diameter, and 125 mm height) which were tightly sealed with plastic caps and centrifuged for 30 min at 5,000 rpm to remove the top oil layer. The weight of precipitated fraction (F_2) was measured, and the emulsion stability of mayonnaise was expressed as:

$$\text{Emulsion stability}(\%) = (F_2/F_1) \times 100$$

For measurement of heat stability, each sample transferred to test tubes and stored at 50 °C for 48 h. They were, then, centrifuged for 10 min at 3,000 rpm. Finally, heat stability was characterized according the above equation.

Rheological analysis

The rheological measurements were performed in a rheometer (Physica MCR 301, Anton paar, Austria). Flow properties of mayonnaise samples were determined at 20 °C using a parallel stainless steel plate having a diameter of 20 mm, in the shear rate range of 0.05–450 s^{-1} .

The apparent viscosity was determined as a function of shear rate. The flow curve data were adjusted to Power law model (R^2 higher than 0.97), using slidewrite software (Version 2.0.1) to following equation:

$$\tau = K\dot{\gamma}^n$$

where τ is the shear stress (Pa), K is the consistency coefficient (Pa s^n), $\dot{\gamma}$ is the shear rate (s^{-1}), n is flow behavior index (dimensionless).

Microscopic structure

Microstructure of mayonnaise samples was observed using a microscope equipped with digital camera (Olympus DP12, B41TF, Japan). A drop of each sample was placed on a

microscope slide. Pictures of the mayonnaise microstructure were obtained at the magnification of 40× by a digital camera connected to the microscope (Mun et al. 2009). The mean particle diameter of each sample was calculated by using Image J software (1.43 u).

Texture analysis

Mayonnaise samples were stored in refrigerator for 24 h until texture analysis. The measurement were carried out using a Texture Analyzer (CT3 Brookfield, USA) equipped with a 24.5 mm diameter cylindrical probe at 25 °C. The container (20 mm internal width and 64 mm depth) was carefully filled by the samples. One cycle was applied, at the constant cross-head velocity of 1 mm/s, to a sample depth of 25 mm, and then returned. From the resulting force–time curve, the values of texture attributes, ie. firmness, adhesiveness, and cohesiveness were obtained, using texture software.

Color characteristics

The mayonnaise samples were measured for color in the L , a , b system using a Lovibond calorimeter (CAM 100, Great Britain). In this color system, L represents the lightness, and a and b are the color coordinates. In this study the L values of mayonnaise samples were measured.

Sensory evaluation

After 1-day storage at room temperature, sensory characteristics of mayonnaise samples including appearance, texture, taste, odor and overall acceptance were evaluated by 12 panelists of students of food science and technology department at Gorgan University, on 5-point hedonic scale, (1 = the least; 5 = the most).

Experimental design and data analysis

A three component, optimal mixture design was used for the experiments, because the component had different range; WGPI and EY 0–9 g, and XG 0–0.5 g of 100 g mayonnaise (0–100 % substitution of EY). Components of the mixture were EY (X_1 or A), WGPI (X_2 or B) and XG (X_3 or C). Components were expressed as fractions of the mixture, and sum ($X_1 + X_2 + X_3$) of the proportions was one. The ten points consisted of two-single ingredient treatments, five two-ingredient mixtures and three three-ingredient mixtures (Table 1). The Design-Expert (8.0.1) software was used to determine the optimum proportions of the mayonnaise formulation.

Mixture experiment models were developed and other statistical analyses of data were performed using the Design Expert (8.01) software. Scheffe's canonical linear, quadratic, and cubic equations [Eqs. (1) to (3)] were used to represent the

fitted response values, using manual multiple regression analyses as described by Cornell (2002).

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \quad (1)$$

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (2)$$

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (3)$$

Where Y is the predictive dependent variable (stability, heat stability, *K*, *n*, viscosity, firmness, cohesiveness, adhesiveness, color and overall acceptance). β_1 , β_2 , and β_3 , β_{12} , β_{13} , β_{23} , and β_{123} are corresponding parameter estimates for each product term produced for the prediction models for EY, WGPI, and XG, respectively (determined according to Cornell (2002)); and X is the proportions of pseudo-components. The statistical significance of each equation was determined by Analysis of Variance (ANOVA) at 5 % level. Other data were analysed with SPSS (version 18) software.

Results and discussion

Proximate chemical composition of RWG, DWGF and WGPI

Proximate chemical composition results show DWGF contains a relatively high amount of protein compared with RWG (31.42 %, and 27.71 % wb, respectively). Defatting resulted in a significant ($P < 0.05$) reduction of fat of wheat germ (10.49 to 0.75 %). RWG and DWGF contain a large amount of carbohydrates (56.37 and 53.27 %, respectively) (Table 2). These results are similar to previous findings reported by Zhu et al. (2006).

Proximate chemical composition and caloric values of mayonnaise samples

The proximate composition and caloric values of mayonnaise samples and the optimized formulation are listed in Table 3. The protein content significantly ($P < 0.05$) increased with addition of WGPI. The content of ash in mayonnaise sample containing higher levels of WGPI was significantly different among samples, which could be due to the higher ash content of WGPI. However, there was no significant difference in the case of moisture, fat and carbohydrate contents of mayonnaise samples. The caloric value of egg free mayonnaise (F4) was significantly lower than that of control (F2). However, caloric values of other samples were in the nearly same ranges. According to the results, cholesterol content of mayonnaise samples containing WGPI as egg yolk replacer was rationally decreased.

Emulsion stability measurement

Emulsions containing high fat content are usually stable, because the droplets are so closely packed together and they cannot freely move. Thus, creaming or flocculation that is destabilizing agent does not occur (Mun et al. 2009). Because of the minimum amount of fat content (65 %) required for a commercial formulation of mayonnaise, mayonnaise samples showed a suitable stability. The low cholesterol mayonnaise samples showed a higher stability than control. It might be due to the increased viscosity of the aqueous phase from the addition of xanthan gum to the WGPI to slow down the droplet movement (Sathivel et al. 2005). Table 3 shows the experimental results for the mayonnaise mixtures. The highest stability values were obtained for samples containing higher amount of XG and WGPI (F3, F4, F5, F6 and F7). On the other hand, the lowest stability value was observed in the F8, contained 50 % egg yolk replacement and free from xanthan gum. However, all mayonnaise samples represented high stability values (higher than 95 %). Similar results were reported by Herald et al. (2009), that optimized the composition of low cholesterol mayonnaise using whey protein concentrate-fenugreek gum blend at 50 % and 100 % replacement. A possible reason for this event might be related to

Table 2 Proximate chemical composition of raw wheat germ (RWG), defatted wheat germ flour (DWGF) and wheat germ protein isolate (WGPI) (g/100 g, wb)

	Moisture content	Ash	Fat	Protein ($n \times 5.7$)	Carbohydrate (by difference)
RWG	11.02±0.09a	4.50±0.005a	10.49±0.2a	27.71±0.13c	56.28
DWGF	9.88±0.45b	4.68±0.22a	0.75±0.4b	31.42±0.26b	53.27
WGPI	3.20±0.19c	4.68±0.22a	0.75±0.4b	85.33±0.47a	8.29

Values are means of three replicates ± SE. Values followed by the same letter in each column are not significant different at $P \leq 0.05$ by ANOVA and Duncan's test

Table 3 Chemical composition analysis (% w/w), caloric values and cholesterol content (mg/100 g) of mayonnaise samples and the optimized formulation

Samples	Moisture content	Fat	Carbohydrate (by difference)	Protein	Ash	Caloric values*	Cholesterol content
1	24.62±0.83 ^a	67.31±0.31 ^a	5.33±0.07 ^a	1.90±0.08 ^b	0.835±0.005 ^f	634.71±3.43 ^{ab}	30.90±0.21 ^f
2	24.51±0.79 ^a	68.17±0.20 ^a	5.31±0.17 ^a	1.24±0.01 ^d	0.814±0.006 ⁱ	639.73±2.54 ^a	63.37±0.22 ^a
3	24.18±0.50 ^a	67.89±0.47 ^a	5.90±0.01 ^a	1.88±0.85 ^b	0.818±0.005 ^h	639.49±4.60 ^a	30.87±0.43 ^c
4	25.39±0.24 ^a	66.38±0.30 ^a	4.77±0.15 ^a	2.61±0.030 ^a	0.860±0.004 ^a	626.94±4.60 ^b	0 ⁱ
5	24.51±0.88 ^a	67.71±0.10 ^a	5.20±0.45 ^a	1.78±0.08 ^{bc}	0.823±0.003 ^g	637.15±3.10 ^{ab}	45.30±0.04 ^d
6	24.60±0.77 ^a	66.81±0.05 ^a	5.90±0.22 ^a	1.99±0.08 ^b	0.856±0.002 ^b	632.85±1.67 ^{ab}	0 ⁱ
7	24.68±0.43 ^a	67.96±0.73 ^a	4.82±0.55 ^a	1.44±0.09 ^{cd}	0.818±0.005 ^h	636.68±10.03 ^{ab}	61.74±0.23 ^b
8	24.16±0.48 ^a	67.26±0.68 ^a	5.59±0.68 ^a	1.83±0.10 ^b	0.836±0.003 ^f	635.02±9.32 ^{ab}	31.88±0.34 ^c
9	24.65±0.72 ^a	66.79±0.10 ^a	5.93±0.41 ^a	2.06±0.20 ^b	0.858±0.003 ^b	633.07±2.63 ^{ab}	0 ⁱ
10	24.63±0.28 ^a	67.12±0.02 ^a	5.42±0.03 ^a	1.88±0.85 ^b	0.847±0.007 ^c	633.28±0.98 ^{ab}	15.37±0.09 ^g
Optimized formulation	24.64±0.71 ^a	67.75±0.2 ^a	4.83±0.23 ^a	1.93±0.03 ^b	0.848±0.005 ^d	636.79±2.74 ^a	6.55±0.61 ^h

Values are means of three replicates ± SE. Values followed by the same letter in each column are not significant different at $P \leq 0.05$ by ANOVA and Duncan's test

*Caloric values = (4× protein) + (9× fat) + (4× carbohydrate)

protein–polysaccharide interaction that significantly reduces the interfacial tension of oil and water and forms a thick interfacial film on oil droplets, which creates a stable emulsion. It has already reported by Abu Ghoush et al. (2008). They showed similar results by application of iota-carrageenan and wheat protein as an emulsifier alternative to egg yolk in a model mayonnaise system.

The heat stability was significantly higher for samples containing higher levels of WGPI or XG, while control formulation and formulation with 50 % egg yolk replacement and free from xanthan gum, represented lower heat stability (F2 and F8, respectively).

The stability and heat stability responses were well fitted to the special cubic and quadratic models with 0.96 and 0.9 determination coefficient, respectively. These models had

high predicted R -square (R^2), low standard deviation (SD) and low predicted value for sum of squares ($PRESS$), thus, independent and dependent variables were well fitted to them (Cornell 2002). Two components interactions (AB) and three component interactions (ABC) had significant influence ($P > 0.01$, $P > 0.05$, respectively) on the stability response of samples. In addition, interaction of two components (AB) had significant effect on heat stability responses (Table 4). According to Fig. 1a, the highest values were obtained in the B vertex, AB and AC edge, where mayonnaise formulations contained high XG and WGPI. In the contour plot of heat stability response, B vertex and AC edge represented the highest values (Fig. 1b).

Emulsifier agents such as proteins and polysaccharides which increase the viscosity and slow down the migration rate

Table 4 Regression coefficients and correlation for the adjusted model to experimental data in mixtures design

Variable	Coefficients							R^2
	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	β_{123}	
Stability	98.57	99.71	−384.02	−11.64**	537.93	511.56	264.25*	0.96
Heat stability	90.14	94.79	−1529.11	−24.01**	1833.23	1699.04	−	0.90
Viscosity	3.09	4.11	194.12	−3.05*	−199.14	−212.55	62.26	0.95
K	20.00	126.08	2135.40	−160.44*	−1986.42	−3331.66	−	0.947
n	0.35	0.13	−5.43	0.33*	5.31	7.03	−	0.968
Firmness	104.44	274.25	21050.55	−301.86*	−20785.1	22503.91-	−	0.940
Cohesiveness	0.486	0.486	0.0277	−	−	−	−	0.70
Adhesiveness	6.42	24.76	2205.01	−35.55*	−2235.11	−2385.60	−	0.91
Color	87.15	81.52	55.73	−	−	−	−	0.90
	3.52	3.60	−19.41	−2.33**	27.25	24.83	76.57*	0.947
Overall acceptance	3.52	3.60	−29.45	−2.28**	38.06	35.20	70.03*	0.98

β_1 : EY, β_2 : WGPI, β_3 : XG

* Significant at 0.05 level

** Significant at 0.01 level

Fig. 1 Ternary contour plots for stability (a) and heat stability (b) of mayonnaise samples formulated with egg yolk, WGPI (wheat germ protein isolate) and XG (xanthan gum)

of oil droplets by generating sufficient strong repulsive between droplets, can prevent oil droplet coalescence, that cause emulsion instability (Mun et al. 2009). The lowest viscosity and stability was observed in formulation 8 in which WGPI was present at 4.5 % (50 % replacement) and XG was absent. On the other hand, in other formulation with 100 % egg yolk replacement and without XG, stability was increased. These results might be a result of antagonistic effect between egg yolk protein and wheat germ protein. This finding is in agreement with Herald et al. (2009) results which showed 50 % egg yolk replacement with protein (WPI¹ and WPC²) based mayonnaise treatments, had significantly lower viscosities than samples with 100 % egg yolk replacement. However this is contradictory to finding reported by Daugaard (1993). He found out no synergistic or antagonistic effects between egg yolk and whey protein, when cold processing was used. There was an antagonistic effect, however, when the process involved the heating of the proteins. The results of physical and heat stability experiments of samples containing high levels of WGPI and XG, clearly indicated the synergistic effect of xanthan gum and wheat germ protein isolate.

¹ Wheat Protein Isolate

² Whey Protein Concentration

Rheological analysis

Flow rheogram of mayonnaise samples formulated by various amounts of WGPI, XG, and EY is presented in Fig. 2. For all mayonnaise samples, relationship of shear stress and shear rate was nonlinear and they showed a shear thinning behavior. In concentrated emulsions, the droplets are close enough together to interact with each other which may lead to the formation of a three-dimensional network of aggregated droplets and consequently to a higher viscosity. As the shear rate is increased, the hydrodynamic forces cause aggregates to become deformed and eventually disrupted which results in a reduction in the viscosity (McClements 1999).

Apparent viscosity investigation at the middle shear rates is useful for sensory and mouth feel evaluation (1–100 s⁻¹). Apparent viscosity of samples were found to be significantly ($P < 0.05$) different at shear rate of 50 s⁻¹.

The Power law model was able to properly explain flow characteristics of mayonnaise samples. Rheological data of samples were adequately fitted to the model with high determination coefficients (96.8–94.7). The model fitting flow equation parameters of different mayonnaise samples are presented in Table 4. Flow behavior index values of samples were determined in the range of 0.123–0.355, which indicates the samples are pseudoplastic fluids. Rheological characteristics of all samples were shear thinning and the apparent viscosity of them decreased with increasing shear rate (Table 3). The consistency coefficient (K) of the samples varied between 21.59 and 135.2 Pa s^{*n*} and K values of samples were found to be significantly ($P < 0.05$) different with respect to the mentioned Power law parameters. The highest K value observed in F4 (9 % WGPI), that indicates a more viscous fluid (Laca et al. 2010).

These results are consistent with finding of Sathivel et al. (2005) that observed shear thinning behavior of mayonnaise made from Arrowtooth flounder soluble protein powder as

Fig. 2 Flow rheograms of different mayonnaise formulations

egg substitute. In the other study, all investigated mayonnaise samples containing lupin protein and xanthan gum, showed a shear thinning behavior (Raymundoa et al. 2002).

Figure 3c indicates the apparent viscosity contour values increased toward the WGPI vertex and the lowest viscosity values observed in middle of AB edge (F8). Also, A vertex indicated low apparent viscosity.

The K values were the highest and n values were the lowest for F4 which egg yolk was replaced by WGPI totally. However, the lowest K and the highest n values were observed in F2 in which only the egg yolk exists, and F8 with 50 % egg yolk replacement and free from XG. The results showed that WGPI had the great increasing effect on the consistency of the mixture samples. Combination of XG and WGPI, also increased consistency of samples. In contour plots, the highest K , but the lowest n values were observed at BC edge and B vertex. The lower values of K and higher values of n occurred at A vertex and the middle of AB edge, where xanthan gum

was absent. From this point of view, it can be concluded that WGPI alone or with XG, not only maintained the structure of mayonnaise, but had a greater effect on the viscosity and flow parameters of mayonnaise samples compared to egg yolk.

Texture analysis

According to texture analysis results mayonnaise sample with 9 % WGPI, had the hardest texture (F4). Also, the mayonnaise sample containing 8.5 % WGPI and 0.5 % XG represented high firmness (F6), whereas lower firmness value was observed in formulation containing 9 % egg yolk (F2) and F8 with 4.5 % WGPI. Firmness response was well fitted to quadratic model. The two-component interaction (AB) had a significant influence on firmness (Table 4). According to Fig. 4a, the B vertex represented the highest value of firmness response, and from B vertex to A vertex, firmness was reduced. However, the AC edge, represented the high firmness.

Fig. 3 Ternary contour plots Power law parameters [flow behavior index (a), consistency coefficient (b)], and viscosity at shear rate of 50 s^{-1} (c) of mayonnaise samples formulated with egg yolk, WGPI (wheat germ protein isolate) and XG (xanthan gum)

Fig. 4 Ternary contour plots for firmness (a), adhesiveness (b), and cohesiveness (c) of mayonnaise samples formulated with egg yolk, WGPI (wheat germ protein isolate) and XG (xanthan gum)

Adhesiveness was significantly higher for F4 and lower for F8. Adhesiveness response was well fitted to quadratic model with 0.91 determination coefficient. Higher adhesiveness values were obtained in the B vertex and decreased toward A vertex. Formulation with XG, also represented higher adhesiveness (Fig. 4c).

The highest cohesiveness value observed in F4. By increasing amount of WGPI, cohesiveness overallly increased. Cohesiveness response was fitted to linear model. According to Fig. 4b, the highest cohesiveness values were obtained in B vertex.

Low cholesterol mayonnaise samples containing higher levels of WGPI and XG, had more firmness, adhesiveness, and cohesiveness than samples with EY. This might be due to increase in viscosity of the emulsions containing high levels of WGPI and XG. The viscosity of the samples can

partially but not totally, reflects the texture analysis parameters (Liu et al. 2007). Addition of protein–gum combination as egg yolk substitutes, significantly increases viscosity and forms stable O/W emulsion and consequently, the oil droplets are kept apart by the particulate protein–gum and coalescence become less than formulation containing egg yolk (Abu Ghoush et al. 2008). Takeda et al. (2001) reported that gluten is an excellent emulsifying agent at acidic pH, because of glutenin and gliadin fractions forming a viscoelastic protein film around the oil droplets preventing coalescing. Similar results were reported in the literature in which the composition of low cholesterol mayonnaise, stabilized by wheat protein isolate and mixture gums as egg substitutes. They indicated that firmness increased with protein and gums concentration (Herald et al. 2009).

Fig. 5 Optical microscope of different mayonnaise formulations ($\times 40$)

Microscopic structure

Microstructure of mayonnaise is affected by different factors such as type and concentration of emulsifying and stabilizing agents, size of droplets, viscosity of the water phase, and oil content (Laca et al. 2010). Microstructure of mayonnaise samples was determined after 24 h of preparation (Fig. 5). A monodisperse (uniform droplets) emulsion was observed in samples prepared with high proportion of WGPI and XG (F4, F6, F9, F10). The mean particle diameter of these samples was below 20 μm . Samples containing higher proportions of EY exhibited polydisperse (different droplet size) emulsions (F1, F2, F3, F5, F7, F8).

Generally, microstructure of mayonnaise affects rheological properties. Hence, a decrease of droplet diameter leads to a great contact surface area between droplets, and therefore to an increased viscosity ($P < 0.05$) (Liu et al. 2007).

Color characteristics

Lightness of mayonnaise has a major impact on the perceived appearance of the product. It has been shown that emulsion color changes from grey to an increasingly bright white as the droplet size decreases, likely due to an increase in light scattering (Chantrapornchai et al. 1999). Lightness value of mayonnaise samples are shown in Table 3. Though samples

containing WGPI had smaller droplet size, their lightness was lower than other samples, which might be due to light brown color of WGPI. This result is in agreement with Worrasinchai et al. (2006) findings about low fat mayonnaise samples prepared with β -glucan. However, this is contradictory to the results reported by Chantrapornchai et al. (1999) and McClements and Demetriades (1998).

Fig. 6 Ternary contour plots for color (L) of mayonnaise samples formulated with egg yolk, WGPI (wheat germ protein isolate) and XG (xanthan gum)

Table 5 Sensory scores of mayonnaise samples

Mixture	Appearance	Taste	Odor	Texture	Overall acceptance
1	3.50±0.23 ^{ab}	3.25±0.27 ^a	3.25±0.17 ^{bc}	3.66±0.14 ^{bc}	3.50±0.10 ^b
2	3.50±0.23 ^{ab}	3.41±0.28 ^a	3.00±0.20 ^c	3.50±0.19 ^{cd}	3.50±0.10 ^b
3	4.16±0.16 ^a	3.58±0.228 ^a	3.16±0.19 ^{bc}	3.91±0.14 ^{ab}	3.67±0.075 ^{ab}
4	3.08±0.31 ^c	3.25±0.21 ^a	4.08±0.21 ^a	4.33±0.18 ^a	3.60±0.10 ^b
5	3.66±0.18 ^{ab}	3.50±0.194 ^a	3.25±0.14 ^{bc}	3.83±0.16 ^{ab}	3.75±0.10 ^{ab}
6	3.25±0.30 ^{bc}	3.33±0.22 ^a	3.83±0.11 ^{ab}	4.17±0.22 ^{ab}	3.60±0.10 ^b
7	3.83±0.16 ^{ab}	3.66±0.22 ^a	3.00±0.24 ^c	3.50±0.15 ^{cd}	3.67±0.08 ^{ab}
8	3.41±0.22 ^{bc}	3.08±0.193 ^a	3.25±0.18 ^{bc}	3.25±0.27 ^d	3.00±0.005 ^c
9	3.16±0.11 ^{bc}	3.25±0.17 ^a	4.16±0.71 ^{ab}	4.16±0.16 ^{ab}	3.60±0.10 ^b
10	3.50±0.14 ^{ab}	3.50±0.228 ^a	4.00±0.15 ^{ab}	3.75±0.27 ^{ab}	3.90±0.005 ^a

1 = the least/ lowest; 5 = the most/ highest

Values are means of three replicates ± SE. Values followed by the same letter in each column are not significant different at $P \leq 0.05$ by ANOVA and Duncan's test

Figure 6 indicates A vertex and AC edge represented the highest value of *L* response and the lowest *L* value observed in B vertex, indicating mixtures containing EY and XG, exhibited a shiny bright yellow color, whereas low cholesterol mayonnaise samples were evaluated as too pale and dense, which is related to samples containing WGPI.

Sensory properties

Sensory evaluation scores of the mayonnaise samples are shown in Table 5. The appearance score of F4 sample was significantly ($P < 0.05$) lower than other samples, which might be due to the effect of WGPI on the color of mayonnaise. However, WGPI and XG did not significantly affect the taste attribute. Samples with minimum amount of EY or free from EY (F4, F6, F9, and F10) got higher scores for odor attribute, which probably related to the effect of raw egg smell. F3, F4, F5, F6, F9, and F10 samples showed higher significant ($P < 0.05$) texture scores. This might be because of higher amount of WGPI and XG. The texture attribute scores were

well correlated with the data derived from instrumental analysis (Fig. 4). The highest overall acceptance score belonged to F10 formulation, followed by F3, F7 and F5. However, the lowest overall acceptance was given to sample containing equal proportion of WGPI and EY and free from XG. On the other hand, overall acceptance was significantly ($P < 0.01$) affected by two component interactions (AB). Also, three component interactions (ABC) significantly ($P < 0.05$) affected the sensory parameter (Table 4). Figure 7 indicates that the mayonnaise samples containing high amount of WGPI and XG and low amount of EY, were more acceptable by the panelists. Generally, significantly negative effect of WGPI on the color attribute score of mayonnaise samples resulted in decrease in overall acceptance of those samples. However, WGPI performed the egg yolk's role well in mayonnaise. Thus, it is reasonable to produce a low cholesterol mayonnaise ever with relatively lower color and overall acceptance scores. On the other hand, it should be noted that all mayonnaise

Fig. 7 Ternary contour plots for overall acceptance of mayonnaise samples formulated with egg yolk, WGPI (wheat germ protein isolate) and XG (xanthan gum)

Fig. 8 Overlayed contour plot of stability, heat stability, rheological parameter (n , K and viscosity), textural properties (firmness, adhesiveness, cohesiveness), color and overall acceptance for mayonnaise samples

samples in this study gained sensory attribute score of more than 2.5, which are generally considered acceptable.

Mixture proportion optimization

Figure 8 shows the overlaid contour plot giving value of each independent variable measured for mayonnaise samples as a response, that are concluded solving the previous equation (Table 4). 7.87, 0.2 and 0.93 (g/ 9 g egg yolk) of WGPI, XG, and EY, respectively are the best solution to make low cholesterol mayonnaise. According to these results, higher proportion of WGPI and XG, in addition to low contents of EY is needed to obtain low cholesterol mayonnaise.

Conclusion

One of the most significant findings to emerge from experimental results and contour plots is that increasing proportions of WGPI and XG improved the stability, heat stability, firmness, adhesiveness, cohesiveness, viscosity, consistency coefficient and overall acceptance. However, high amounts of WGPI adversely affected the mayonnaise appearance and color leading to the significantly lower sensory quality as compared with mayonnaise containing egg yolk. Thus, the use of combination of WGPI, XG, and egg yolk, improve the appearance and color of mayonnaise samples, which are important factors to consumer acceptance.

The findings of this study indicates that, the use of desirable combination of WGPI, XG, and egg yolk, allows the preparation of a low cholesterol mayonnaise which shows similar characteristics to those of a typical mayonnaise, and mixture design approach is a suitable method to optimize the low cholesterol mayonnaise formulation.

Acknowledgments The authors gratefully acknowledge Tabarestan Technology Incubator, Saari, I.R. Iran for cooperation in rheological measurements and technical assistance.

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