

Evaluation of Using Salep and Chitosan Hydrocolloid as Stabilizers and Fat Replacer in Physicochemical and Rheological Features of Low-Fat Mayonnaise

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Received: 20 February 2017

Accepted: 3 May 2017

ABSTRACT: In the present study, salep and chitosan hydrocolloid were used as stabilizers and fat replacer in low-fat mayonnaise and rheological and physicochemical characteristics were evaluated. Fat has been substituted with 0.1, 0.2 and 0.3% chitosan and 6.4, 0.0, 0.7, 1.0 and 1.3% salep. High-fat mayonnaise sample or control with 67% fat and without salep and chitosan (FF), mayonnaise with 67% fat and 0.4% salep and 0.2% chitosan (F1), mayonnaise with 48% fat and 0.6% salep (F2), mayonnaise with 30% fat and 1.3% salep (F3), mayonnaise with 30% fat and 1% salep and 0.1% chitosan (F4), mayonnaise with 30% fat and 0.7% salep and 0.2% chitosan (F5) and mayonnaise with 30% fat and 0.6% salep and 0.3% chitosan (F6) were used as treatments of the present study. The results showed that salep and chitosan hydrocolloids could increase the viscosity of low-fat mayonnaise samples by absorbing water as well as substitution role for fat to increase the stability of low-fat mayonnaise. Among the produced samples, F1 and F2 had the most similarity to the control sample. All the samples (both control and low-fat samples) did not have significant microbial load and the pH and acidity were at the allowed level. Creamy and phase separation phenomenon was not observed among the samples. Based on the results, it can be concluded that salep and chitosan hydrocolloids are proper substitutes for common high-fat mayonnaises and can be employed in the formula of low-fat mayonnaise without changing the qualitative features of the product significantly.

Keywords: *Chitosan, Low-Fat Mayonnaise, Salep.*

Introduction

The presence of fat is necessary for human body and can act as an energy source and transfer the vital nutrients, therefore its consumption is inevitable in the diet. Oils play important roles in preparation, production and frying of foods. The consumption of high-fat diet can cause problems regarding the health namely blood pressure, obesity, cardiovascular diseases (Zaouadi *et al.*, 2015). Sauces as a

concentrated liquid have been used for flavoring. Sauces are classified into two classes of oil and non-oil sauces. Oil sauces based on their texture include:

1) concentrated decorative dressing such as mayonnaise, concentrated salad sauce, and similar low-calorie products.

2) Diluted and fluid decorative dressings such as French, Italian and thousand islands sauces. Some examples for non-oil sauces are fruit sauce or diluted sauces such as ketchup sauce. Mayonnaise is one of the

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oldest and popular sauces that have been consumed widely all over the world (Maghsoudi, 2005).

Hydrocolloids are high molecular weight hydrophilic biopolymers that are used in order to stabilize the emulsions and create optimal sensory characteristics in food products (Dickinson, 2003). Salep is one of the hydrocolloids effective in creating consistency and stability in food systems (Kaya & Tekin, 2001). Chitosan is another hydrocolloid that can be employed as a stabilizer and thickener in mayonnaise and other emulsion products (Karbasi *et al.*, 2006).

Materials and Methods

- Materials

The required ingredients for preparing mayonnaise sauce are chitosan from Acros Company U.S, salep from Local bazaar of Gilan province, modified starch from Roquette Company Spain, xanthan from Otilo Company China. The formulation consisted of soya bean oil, egg yolk, vinegar, mustard powder, salt, sugar and edible preservatives that were provided by the R&D center of Kadbanoo Food Industries, Tehran, Iran.

Materials required to carry out chemical and microbiological examinations consisted of NaOH, phenolphthalein indicator, acetic acid, diluter (ringer tablet), PCA culture medium, DGA culture medium, MRS both modified culture medium, lauryl sulfate broth culture medium, brila broth culture medium and rappaprot broth culture medium (all were obtained from Merck company – Germany).

- Formulation and preparation of samples

- Preparation of hydrocolloids

Before preparing different samples of mayonnaise, various concentrations of chitosan were hydrated separately. The ingredients used were based on the standard method of mayonnaise production.

- Formulation of mayonnaise samples

First, water and powder materials (salt, sugar, spices, etc.) and eggs were put into a blender and if the formula had chitosan, hydrated chitosan was added, then starch and other saleps were added as well as small quantity of oil that was gradually added as drop and then as a thin layer. After production of an emulsion with proper texture, vinegar was added to the mixture. The final mixture was homogenized by the homogenizer (3000r/min for 5 min). The formulation is presented briefly in Table 1.

- Analysis of chemical compounds and evaluation of calorie

Moisture content was determined by weighting the samples prior and after drying according to the Iranian National Standard number 2454.

Protein and fat determination was carried out according to Laboratory Techniques in Food Analysis, (D. person, 1973).

Ash was determined according to Chen *et al.*, 1998.

Total carbohydrates were obtained by difference.

pH measurements were carried out using pH meter and acidity was measured according to the Iranian National Standard No. 2454.

Hunter Lab Colorimeter was employed to determine the colors of various samples according to Iranian National Standard.

The water activities of the produced samples were determined according to the Iranian National Standard.

Texture evaluation and the creamy index was calculated according to Amiri *et al.*, 2010.

Emulsion sustainability was determined according to Nikzadeh *et al.* (2012).

Brookfield viscometer Model DV0 III, England: was employed to determine the viscosity according to Tolouee *et al.* (2010).

Bostwick consistometer was used to determine and measure the consistency.

Texture features were measured by surface analysis device (Brookfield Co. with loading cell 4500 gr) according to Amiri *et al.* (2010).

Sensory evaluations, were carried out by 15 trained evaluators based on Hedonic scale method 5 point according to (Very good-good-acceptable-weak- very weak), based on the following description; eventually, to be able to do statistical considerations, evaluative results were calculated and reported digitally (Jeia *et al.*, 2001).

Microbiological tests concerned with the total count of microorganisms were carried out according to the Iranian National Standard No. 5272.

Mold and yeast were determined according to the Iranian National Standard No. 997.

Heterofementative Lactobailli bacteria were obtained according to the Iranian National Standard No. 2965.

E. coli according to the Iranian National No. 2946.

Salmonella detection and determination was carried out according to the Iranian National Standard No. 1810.

The microbiological features of mayonnaise sauce should correspond to the standard presented in Table 2. The data analysis was carried out in a completely accidental plot using SPSS 18.

Table 1. Formulation of mayonnaise samples in this study (gr/100gr mayonnaise)

components	Control (F _F)	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆
Soya bean oil	67	48	48	30	30	30	30
Egg yolk	7	7	7	7	7	7	7
Mustard powder	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Salt	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sugar	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Edible preservatives	8.16	8.16	8.16	8.16	8.16	8.16	8.16
Xanthan	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Modified starch	2	2	2	2	2	2	2
Salep	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Chitosan	9.28	9.28	9.28	9.28	9.28	9.28	9.28
Edible preservatives	0	0.4	0.6	1.3	1	0.7	0.6
Xanthan	0	0.2	0	0	0.1	0.2	0.3

F_F = high-fat sample without salep and chitosan in its formulation

F₁ = mayonnaise sample in which its 28.4% fat was substituted with 0.4% salep and 0.2% chitosan.

F₂ = mayonnaise sample in which its 28.4% fat was substituted with 0.6% salep.

F₃ = mayonnaise sample in which its 55.2% fat was substituted with 1.3% salep.

F₄ = mayonnaise sample in which its 55.2% fat was substituted with 1% salep and 0.1% chitosan.

F₅ = mayonnaise sample in which its 55.2% fat was substituted with 0.7% salep and 0.2% chitosan.

F₆ = mayonnaise sample in which its 55.2% fat was substituted with 0.6% salep and 0.3% chitosan.

Table 2. Microbiological features of mayonnaise sauce

No.	Feature	Allowed maximum (digit/gr)
1	Total count of microorganisms	1000
2	Heterofementative Lactobailli bacteria	Negative in 0.1
3	Salmonella	Negative in 25 gr
4	Staphylococcus aureus	negative
5	E. coli	negative
6	Mold	100
7	Yeast	100

Results and Discussion

Table 3 shows the results of chemical analysis of various mayonnaise sauces. Low-fat mayonnaise sauce has lower calorie than the control sample (FF), therefore its calorie is 25-52% lower than the high-fat sauce (control treatment). The result of the mean comparison concerned with calorie indicated that most calories is attributed to the control treatment and the least is concerned with F5 sample; although F3, F4, F5, and F6 did not have statistically significant differences.

The results concerned with the pH is presented in Figure 1 that indicates the treatment, has the highest value and had significant difference with other treatments. Control treatment had the least value.

Figure 2 presents the acidity where control (FF) and treatment F6 have the highest and lowest concentrations respectively.

The results of mean comparison for various treatments on index characteristics L^* , a^* and b^* are present in Figure 3, 4 and 5.

Table 3. The results of chemical analysis (%) and calorie production (kcal/100g)

Treatments	Fat	Protein	Moisture	carbohydrate	ash	calorie
Control (F _F)	0.35 ^a ±67.56	0.01 ^c ±2.00	0.33 ^c ±27.73	0.03 ^f ±1.75	0.02 ^c ±0.96	2.99 ^a ±623.0
(F ₁)	0.55 ^b ±49.62	0.01 ^{bc} ±2.01	0.58 ^b ±45.01	0.03 ^e ±2.38	0.01 ^c ±0.98	5.07 ^b ±464.12
(F ₂)	0.22 ^b ±49.83	0.01 ^c ±2.00	0.33 ^b ±44.72	0.03 ^d ±2.47	0.02 ^c ±0.98	1.98 ^b ±466.35
(F ₃)	0.14 ^c ±30.81	0.01 ^c ±2.00	0.19 ^a ±63.00	0.08 ^a ±3.08	0.01 ^a ±1.10	1.52 ^c ±297.62
(F ₄)	0.26 ^c ±30.92	0.01 ^{bc} ±2.01	0.28 ^a ±63.25	0.03 ^b ±2.79	0.02 ^b ±1.03	2.51 ^c ±297.47
(F ₅)	0.13 ^c ±30.88	0.01 ^b ±2.03	0.12 ^a ±63.46	0.01 ^c ±2.61	0.01 ^b ±1.02	1.19 ^c ±296.46
(F ₆)	0.33 ^c ±30.94	0.01 ^a ±2.05	0.31 ^a ±63.42	0.03 ^c ±2.58	0.01 ^b ±1.01	2.88 ^c ±296.96

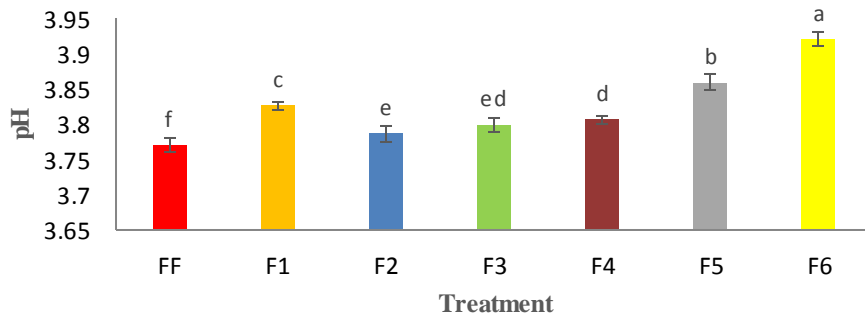


Fig. 1. Mean comparison of treatments for pH values using Duncan test at 95% level.

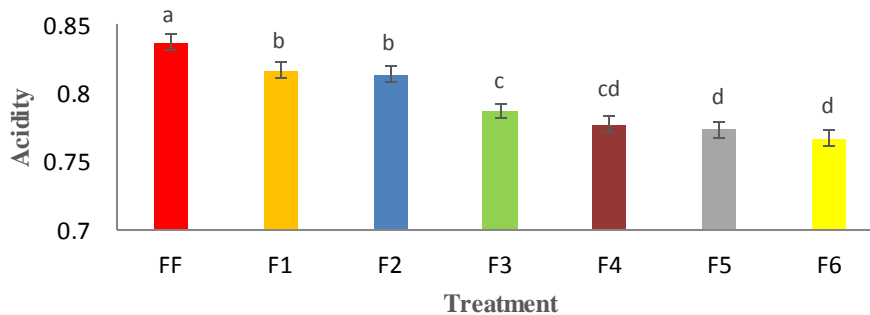


Fig. 2. Mean comparison of treatments for acidity characteristic using Duncan test at 95% level.

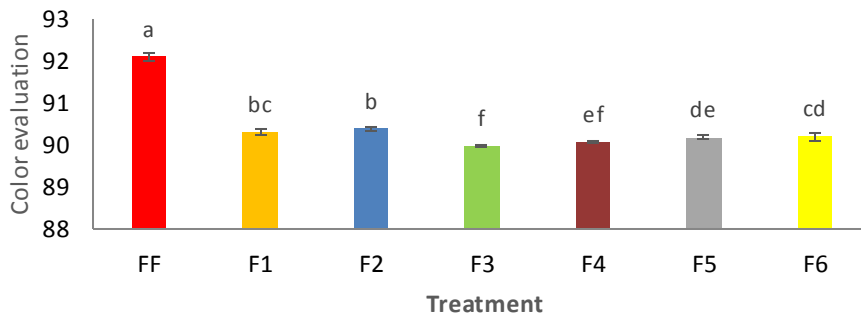


Fig. 3. Mean comparison of treatments for L* index characteristic using Duncan test at 95% level.

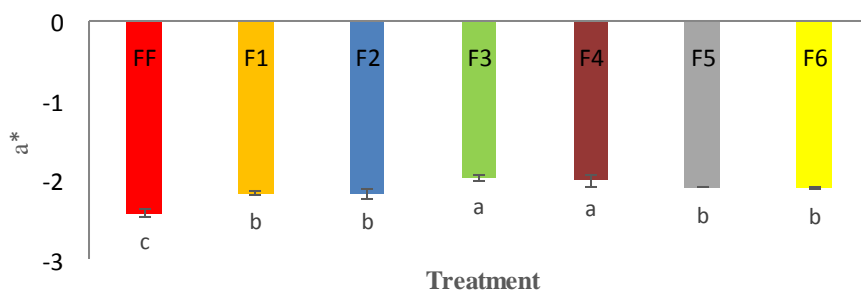


Fig. 4. Mean comparison of treatments for a* index characteristic using Duncan test at 95% level.

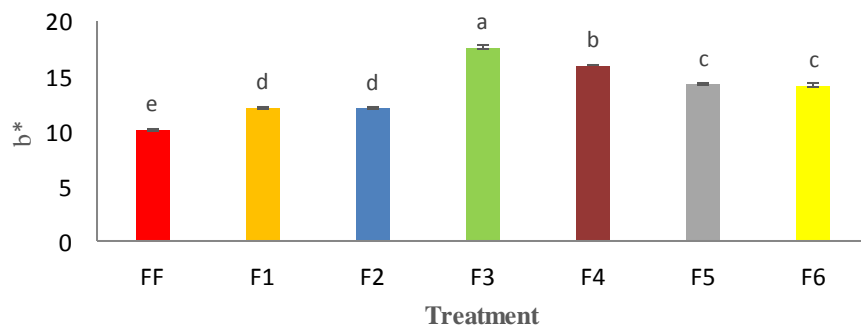


Fig. 5. Mean comparison of treatments for b* index characteristic using Duncan test at 95% level.

The water activity results are present in Figure 6 that indicated treatment F4, F5 and F6 had the highest activity and there were not significant differences among the treatments.

Consideration on samples' appearance and texture showed that there was no sign of emulsion break down and two-phase formation and also there was no sign of cream among the samples.

The results of mean comparison on emulsion sustainability are shown in Figure 7 where treatments F3, followed by 4 had

the highest while control treatment had the lowest.

By application of Bostwick method based on cm/30s (Figure 8) the highest consistency was related to F3 treatment.

The results concerned with viscosity (Pascale/ s) are presented in Figure 9 that indicates treatment 3 and 4 had the highest values.

Regarding the hardness, the experimental treatments did not have any significant differences and based on the results of the mean comparison, which is observable in

Figure 10, all the experimental treatments were classified into one group.

The results of the sensory evaluation of mayonnaise samples, in respect of color, taste, texture, consistency, spreadability, palatal sense and total acceptance using Duncan test at 95% are shown in Table 4. Based on the results, from the appearance FF

and F1, and color FF, F2 and F1, and taste FF, F3, F2 and F1, and texture FF and F1, and consistency FF, F3, F4, F5 and F6, and rub pussies control treatment, and palatal sense FF, F1, F2 and F6 and total acceptance aspect control treatment had the highest score.

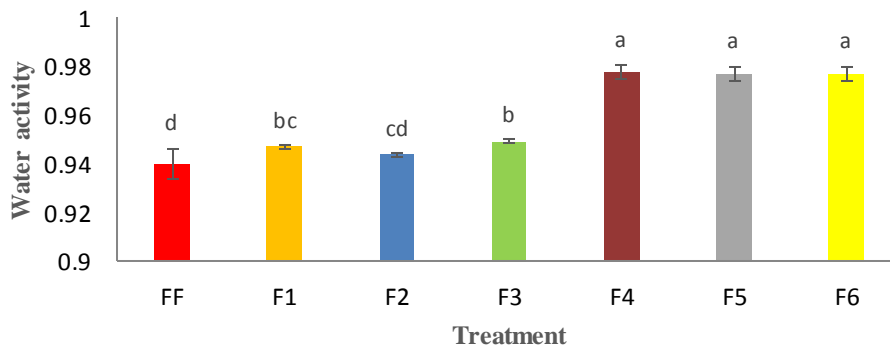


Fig. 6. Mean comparison of treatments concerned with water activity using Duncan test at 95% level.

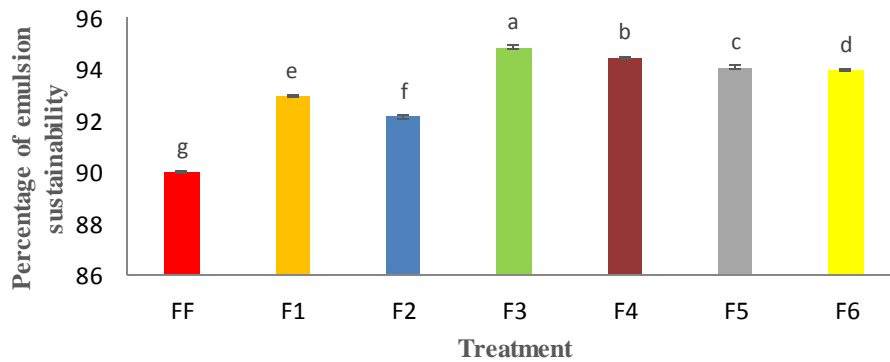


Fig. 7. Mean comparison of treatments for emulsion sustainability using Duncan test at 95% level.

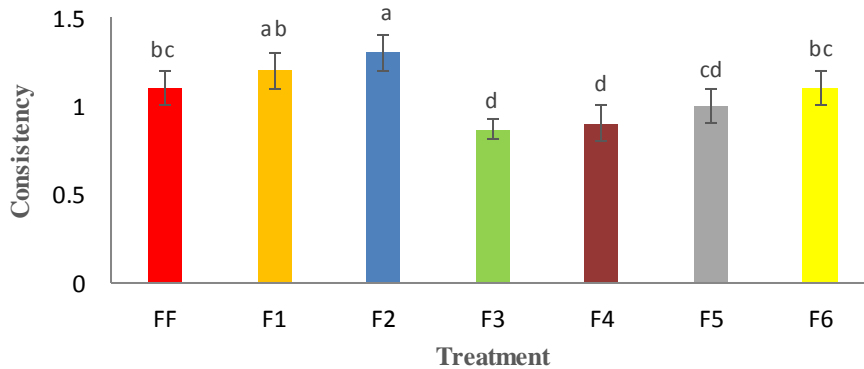


Fig. 8. Mean comparison of treatments concerned with consistency by Bostwick method using Duncan test at 95% level.

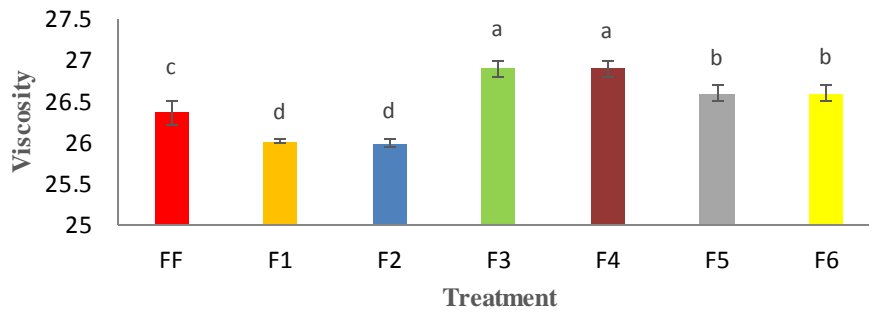


Fig. 9. Mean comparison of treatments for viscosity using Duncan test at 95% level.

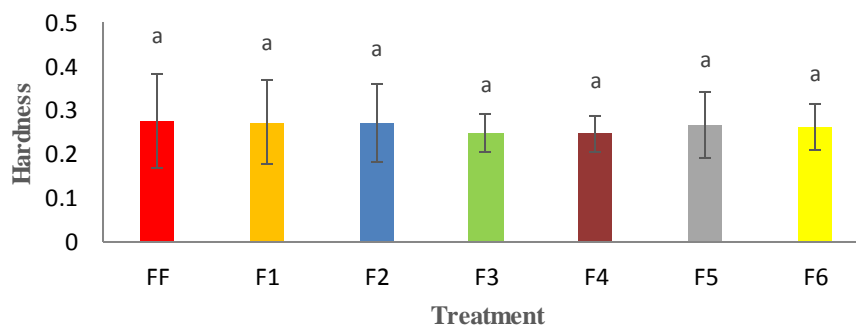


Fig. 10. Mean comparison of treatments for hardness using Duncan test at 95% level.

Table 4. The results of mean comparison relating to sensory evaluation of various mayonnaise samples

Treatments	Appearance	Color	Taste	Texture	Consistency	spreadability	Palatal sense	Total acceptance
Control	3.80±0.15 ^a	4.06±0.21 ^a	3.93±0.14 ^a	4.33±0.16 ^a	3.46±0.03 ^{ab}	4.46±0.14 ^a	3.93±0.02 ^a	4.00±0.18 ^a
F1	3.40±0.16 ^{ab}	3.80±0.20 ^{ab}	3.80±0.14 ^{ab}	4.00±0.02 ^a	3.13±0.24 ^{bc}	3.87±0.21 ^b	3.67±0.08 ^{ab}	3.46±0.09 ^b
F2	3.00±0.17 ^{bcd}	3.86±0.08 ^a	3.53±0.11 ^{abc}	3.40±0.08 ^b	2.93±0.26 ^c	3.27±0.16 ^c	3.53±0.15 ^{ab}	3.13±0.11 ^{bc}
F3	3.06±0.16 ^{bcd}	3.20±0.19 ^c	3.40±0.17 ^{abc}	2.80±0.14 ^c	3.80±0.03 ^a	2.40±0.02 ^d	2.53±0.13 ^c	2.80±0.12 ^{cd}
F4	2.53±0.09 ^d	3.33±0.21 ^{bc}	3.06±0.09 ^c	2.13±0.17 ^d	3.67±0.02 ^a	2.53±0.01 ^d	2.60±0.16 ^c	2.46±0.02 ^d
F5	3.20±0.01 ^{bc}	3.60±0.07 ^{abc}	3.20±0.11 ^c	3.27±0.19 ^{bc}	3.46±0.07 ^{ab}	3.13±0.21 ^c	3.26±0.02 ^b	3.13±0.09 ^{bc}
F6	2.60±0.19 ^{cd}	3.66±0.23 ^{abc}	3.33±0.09 ^{bc}	3.13±0.02 ^{bc}	3.40±0.18 ^{ab}	3.20±0.02 ^c	3.53±0.06 ^{ab}	3.06±0.02 ^{bc}

The E.coli, Heterofermentative Lactobacilli bacteria, and Salmonella tests were negative for all the samples. The results of mold and yeast were lower than 10 for all the treatments. The total count was lower than 100 and there was not any significant difference among the treatments.

Chemical compounds and calorie of low-fat sauces that are produced by chitosan and salep and also high-fat mayonnaise (control treatment) are shown in Table 3. By substituting salep with oil in low-fat mayonnaise, total moisture percentage increased significantly, while its fat content is reduced. Control sample had the least amount of moisture and highest amount of

fat; F3, F4, F5 and F6 had the highest amount of moisture and the least amount of fat. Enhancement of moisture in low-fat mayonnaise is because of using chitosan and salep in this study, which are hydrocolloid with high moisture absorption. The highest concentrations of protein were in F5 and F6 and the least were related to control, F2 and F3 samples.

The highest amount of ash was observed in F3 and the least was in control, F1 and F2 samples. The reason can be attributed to the presence of the tangible mineral impurity in consumed salep and chitosan. The amount of carbohydrate slightly is increased by increasing the substitution amount of gums

with oil. F3 sample had the most carbohydrate while control sample had the least level. In fact, carbohydrates of low-fat mayonnaise samples was slightly higher than control sample; because the subsidiary chain of gums included some carbohydrate. In low-fat samples, enhancement of gum can reduce the calorie production significantly. These hydrocolloids, which are used as a type of fat substitution, are able to show some of the function features of fat by bonding water molecules inside emulsions. As the gel, which is the mixture of gum and water, has the high amount of moisture, these hydrocolloids don't absorb in human digest system. Therefore, they are suitable to be used in food formulation (Amiri Aghdaee *et al.*, 2011).

pH and acidity are important factors in salad sauces; based on Iranian National Standard, their level should be adjusted. because enhancement of pH or reduction of acidity may provide the possibility of the growth of bacteria such as *Staphylococcus aureus*.

Among all the color factors, brightness level (L^*) of mayonnaise samples affects customers' acceptance level significantly. As it is observed in Figure 3, the brightness of all low-fat samples had significant difference with the control sample (FF) ($p < 0.05$) and all were less bright than the control sample. It can be concluded that the reduction of consumed oil in mayonnaise sauce and enhancement of hydrocolloids in its formulation can reduce the brightness index; that is in agreement with the results of Amiri Aghdaee *et al.* (2011). As it is observable on Figures 4 and 5, a^* (tendency to red color) and b^* (tendency to yellow) in F3, F4, F5, and F6 were higher than low-fat mayonnaise and control sample. This can be attributed to the presence of higher amount of saiep and chitosan in the formulation. As gum concentration is higher in the samples and they have color impurity, a^* and b^* indexes were higher.

As it is observable in Figure 6, the most amounts of water activity were related to samples F4, F5, and F6; these three treatments did not have any significant differences between each other. F1 and F3 samples were classified in the second group. In addition, the least amount of water activity was due to the control (FF). The reason for high amount of water activity in F4, F5, and F6 samples can be the lack of proper chitosan hydration in these samples and high level of their moisture level.

Based on the results of appearance and texture, no sample shows signs of break, two phases or cream. The reason of high sustainability is the presence of different hydrocolloids in the formulation of mayonnaise sauce. Basically, the viscosity of continuous phase has been increased and the size of emulsion particles has been reduced under the effect of enhancement of hydrocolloid's concentration (Niknia, 2010), as a result emulsion phase sustainability has been improved. As it can be observed in Figure 7, the highest concentration of emulsion sustainability percentage was related to F3 followed by F4. Moreover, the least amount of this feature was due to the control sample (FF). The reason of high percentage of emulsion sustainability in F3 can be the high percentage of its hydrocolloids. The reason for the higher amount of emulsion sustainability in F1 than F2 and also F5 than F6 can be attributed to the synergistic effect of saiep and chitosan on each other that has increased the sustainability of F1 and F5. As it is shown in Figure 8, the results of Bostwick consistency detector device indicated that the addition of the higher amount of hydrocolloids in the formulation of low-fat mayonnaise reduces Bostwick digit; in another word, it can lead to significant enhancement of samples' consistency. F3 and F4 had higher amount of hydrocolloids; therefore, the least amount of Bostwick digit or the most amount of consistency was due to these samples. Amir

Kavi, (2004) and Tolouee *et al.*, (2010) had similar reports about using Maltodextrin, pectin, and gums to produce low-fat mayonnaise with more consistency and viscosity; as it is observable in Figure 9, the results of mean comparison for viscosity (Pascale/s) indicated that the most amount of this feature was observed in F3 and F4 sample. F5 and F6 sample were classified in the second group. Control treatment ranked in the third group and the least amount of this feature was observed in F1 and F2 samples without any significant differences. The results of this test indicated the enhancement of continuous phase viscosity under the effect of hydrocolloids' enhancement. Experimental treatments did not have any effects on hardness; based on the results of the mean comparison (Figure 10), higher levels of hardness among F3 and F4 were not significant, and all the experimental treatments were classified in the same group.

The results of sensory evaluation of mayonnaise samples are shown in Table 4. Based on the results, from appearance aspect (FF) and F1, from color aspect FF, F2 and F1, from taste aspect FF, F3, F2 and F1, from texture aspect FF and F1, from consistency aspect FF, F3, F4, F5 and F6, from rub pussies control treatment, from palatal sense FF, F1, F2 and F6 and from total acceptance aspect control, F1 and F5 had the most score. The major reason of high score of color and appearance in control, F1 and F2 is their more brightness than other samples and this is in line with the data of color evaluation (L^* index). The other reason can be attributed to more moisture existing in low-fat mayonnaise samples that reduce the score of appearance and color (Amir Aghdaee *et al.*, 2011). From the taste point of view, control, F1, F2 and F3 had significant differences with other samples and the reason can be the higher level of water activity of other samples in comparison with aforementioned samples.

As chitosan prevent changing taste and color, it is expected that F4, F5, and F6 have higher score; this might be in contrary to lack of proper chitosan hydration in aforementioned samples. Moreover, the reason of higher score of taste in F3 in comparison with F4, F5 and F6 can be attributed to the higher level of consumed hydrocolloid. From texture aspect, control, F1 and F2 gained the highest scores, while it was expected that samples containing higher hydrocolloids have better texture. The reason might be due to the lack of proper hydration of hydrocolloids in water that changes the texture of final produce. From the consistency point of view, F3, F4, F5 and F6 gained the most score; this result was in line with the findings of Bostwick consistency detector device; its reason can be the higher amount of hydrocolloids and enhancement of continuous phase viscosity. From palatal sense, control treatment, F1, and F2 had the most score; its reason can be the higher amount of oil in these samples that might attribute to the synergistic activity of salep and chitosan on each other. Eventually, total acceptance showed that control, F1 and F5 gained the highest score; for control treatment, it was the result of all sensory features except consistency, for F1 it was affected by the appearance, taste and palatal sense and for F5 it was affected by appearance, texture, and consistency.

For microbial considerations of mayonnaise samples, first E.coli test should be carried out on its components and on the final product. The results of these tests showed that as the presence of E.coli in all powder materials were negative, the final product is not contaminated with E.coli. The results of Salmonella and Heterofementative Lactobailli bacteria were negative for all the samples. The results of mold and yeast tests were standard for all treatments. According to the lack of significant difference in the microbial test, it is not possible to consider the preventive role of chitosan that was used

in 4 out of 7 samples. About its anti-microbe feature, chitosan reaction with positive load and bacterial cell membrane composition including phospholipid and polysaccharide with negative load can damage cell membrane and change its permeability.

Conclusion

The results of present study show that chitosan and salep have fat substitution role and can reduce calorie level; in addition they can enhance viscosity of samples by absorbing water. Although reduction of fat content and addition of hydrocolloids as a substitution for fat reduced color and some sensory features of mayonnaise, these reductions were not significant and the results were similar. Based on the results of this study, it can be concluded that salep and chitosan are proper substitutions for common high-fat sauces and they can be employed in formulation of low-fat mayonnaise without changing qualitative features of the final product significantly.

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