Sys Rev Pharm 2021;12(1):1142-1150
A multifaceted review journal in the field of pharmacy

Preparation of Healthy Mayonnaise by Using Plant and Animal Gums as Oil Replacer

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ABSTRACT
Mayonnaise is an appetizer common in Iraq, and the demand for it has increased in recent times with the trend of the societies towards fast food and prepared foods. Therefore, this study was conducted to reduce the number of calories in mayonnaise. The chitin was extracted from crab shells, where the acetyl groups were removed with an alkali treatment to prepare chitosan, tamarind gum was also extracted. The prepared hydrocolloids, represented by chitosan and tamarind gum at 3%, were used as a stabilizer, and fat replaces in the manufacture of reduced-fat mayonnaise (RFM) and light mayonnaise (LF) and was compared with full-fat mayonnaise (FFM). Thus, the quality of the prepared mayonnaise treatments was assessed by analyzing the chemical composition, and the caloric value. Furthermore, the physicochemical properties represented by (stability, heat stability, pH, and viscosity) were studied, and microscopy and sensory evaluation were performed. The results showed an increase in the moisture content of the mayonnaise treatments with a decrease in the percentage of fat, and that the caloric value of low-calorie mayonnaise decreased significantly in the treatments that had a lower fat percentage. Light mayonnaise LM1 (prepared with adding chitosan) and LM2 (prepared with the addition of tamarind gum seed) were observed to have a higher moisture content than FFM and generally, the moisture content increased with increasing the level of fat replacing. The caloric values of the treatments containing fat replacer were significantly decreased. Low-fat mayonnaise samples showed high stability. The highest stability values were obtained in the RFM1 (prepared by adding chitosan) and the lowest value of stability in the FFM. Slight significant differences were observed in the pH values between the treatments, and the pH value of FFM was less than the rest of the treatments. The optical microscope images in the samples of FFM showed that small droplets were efficiently packed between the larger ones, while in the RFM samples, larger size distributions and smaller droplets appeared. The treatment RFM1 obtained the highest mark for the sensory evaluation compared to the other treatments and the consistency values were affected by the viscosity of the mayonnaise treatments, as the higher viscosity value of the RFM1 treatment corresponded to the highest value of the consistency in the same treatment. It can be concluded from this research the possibility of using the chitosan and the tamarind gum as suitable replacers for the fat in preparing healthy, low-calorie mayonnaise, as they are healthy hydrocolloids, which are non-toxic, biodegradable, and easy to prepare steps, as they do not being caused an undesirable change in the specific properties of mayonnaise.

Keywords: Fat replacer, seed gum, mayonnaise, stability

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INTRODUCTION
Mayonnaise was found more than 100 years ago, and is one of the most preferred sauces in the world, as it has a relatively long shelf life of weeks or months under refrigerated storage conditions (Morley, 2016). Mayonnaise is an oil-in-water emulsion and is a semi-solid system that contains fat, between 70-80%, its usually prepared by mixing egg yolks, vinegar, oil, and spices (especially mustard), which may also contain salt, sugar, or sweeteners and other optional ingredients. Furthermore, it's an important source of protein, fats, and fat-soluble vitamins due to the presence of oil and eggs in it. Mayonnaise is relatively resistant to microbial damage due to its low pH and high-fat content (Abu-Salem and Abou-Arab, 2008; Thomareis and Chatziantoniou, 2011; Fenandesa and Salas-Mellado, 2018).

The high-speed mixer is usually used in mayonnaise production, as well as a homogenizer, and to stop the microbial activity, heat treatments are applied to the product before the packaging process and then storage. Freezing, heating, and heavy shaking must also be avoided during storage, and transportation periods to prevent the emulsion to collapse. Mayonnaise is a high-calorie food due to its high-fat content, while the vegetable oils usually used in its manufacture are corn and sunflower oil, as linoleic acid is abundant in them, which is one of the most important essential fatty acids that the body needs as well as the ease of its emulsification. Mayonnaise acidifiers, such as apple cider vinegar and lemon juice, can be used to help give the characteristic flavor to the final product, and these acids have a role in keeping mayonnaise from spoilage (Mouheb Eldean *et al.,* 2014).

The added proportions of oil and eggs play an important role in determining the texture of the resulting emulsion, and its viscosity, as well as the method of preparation used, and the equipment used with other additives. The balance of the proportions of salt, sugar, vinegar, and spices gives a smooth and rich flavor. However, the nature of the resulting emulsion affects the preservation of the prepared flavor, as the consistent emulsion, for example, results in a moderate flavor, while the weak emulsion shows sweetness and salinity with a weak flavor. The number of eggs used and the percentage of solids in it affect the viscosity and strength of the resulting emulsion, as the yolk contributes mainly to the emulsification of mayonnaise, and the substances responsible for emulsification in the yolk are lipoproteins (LDL and HDL), phospholipids and no bonded proteins such as phosvitin and livetin. The physical properties of mayonnaise include stability, rheology, appearance, and flavor.

Dietary fats provide, essential fatty acids, the precursors for prostaglandins as well as provide fat-soluble vitamins. Fats contribute to the main rheological and organoleptic properties as well as their nutritional value, and among the most important roles of fats in food are to improve flavor, give softness and viscosity, stabilize emulsification. Besides, give the quality of creaminess, texture, and good appearance of food (Aslanzadeh *et al.,* 2012; Sahurkar and Karadbhajne, 2018). However, increased consumption of dietary fats is associated with an increased risk of obesity, high cholesterol, coronary heart disease, some types of cancer, as well as gallbladder disease, and this has encouraged the trend towards low-fat food products (Worrasinchai *et al.,* 2006). Accordingly, recommendations refer to the need to reduce the overall amount of total fat consumed to less than 30% of calories and to reduce saturated fats to less than 10% of total energy consumption of people (Aslanzadeh *et al.,* 2012; Ognean *et al.,* 2006). Due to the risks of consuming lots of oils and fats, people tend to reduce the amount of fats in their diet, therefore replacing part of the fat with some other components that may play the role of the fat in foods is necessary (Aslanzadeh *et al.,* 2012).

Consumer demands have increased in recent years to obtain more natural, healthy, and nutritious foods. Attention has been focused on reducing the percentage of fat in traditional food products such as mayonnaise to obtain low-fat or no-fat products. Researchers have indicated the possibility of reducing the amount of fat in the emulsion by replacing the oil or fat droplets within the emulsion with non-fat materials, and these materials are usually polymers such as gum, starch, and protein. One of the most difficult challenges in producing low-fat mayonnaise is its flavor change, so flavor enhancers must be used when using fat replacer in mayonnaise production (Fernandesa and Salas-Mellade, 2018).

It has been shown that the effect of added oil on the rheological and sensory characteristics such as flavor, taste, texture, and hardness is difficult to compensate for in fat-free mixtures. Nevertheless, some materials can be used to increase the thickness of the water phase, and then compensate for oil excluded and these materials are called oil substitutes. Material such as modified starch, inulin, pectin, cellulose derivatives, carrageenan, whey protein isolate, konjac flour, oat dextrin, and some thickener is usually used to stabilize emulsifiers and to increase the viscosity of low-fat (low-calorie) mayonnaise (Choonhahirun 2008; Mouheb Eldean *et al.,* 2014). Various types of thickeners are used in the production of oil-in-water emulsions, either natural or chemically modified polysaccharides. Among the most common thickeners are xanthan (XG), starch, modified starch, carrageenan, alginate, Arabic gum, pectin, and guar gum (GG). These thickeners are used singly or mixed to reach the required texture, and the amount to be added is closely related to the desired texture of the final mayonnaise, the use of a mixture of glues types leads to better functional properties (Aslanzadeh *et al.,* 2012; Amin *et al.,* 2014). Fat replacers (FS) are generally categorized into four groups: fat substitutes (FS), fat mimetics (FMs), fat analogs, and fat extenders. Fat substitutes are synthetic compounds that have a chemical structure somewhat close to fats and have similar physiochemical properties. However, they are resistant to an analysis against digestive enzymes, and they are usually either indigestible or contribute to low calories on a per gram basis. Moreover, fat mimetics are ingredients that mimic the physical and sensory properties, FMs are also called texturizing agents, they need a high water content to give their functional properties. Fat analogs, which are compounds that have many properties of fats but have a variable digestibility and variable nutritional value. Fat extenders improve the fat function, which allows for a reduction in the normal amount of fat in the product. Carbohydrates or protein usually possess various functional properties that mimic some of the distinct physical-chemical features and desirable nutritional characteristics of fats such as viscosity, mouthfeel, and appearance (Chavan *et al.,* 2016; Ognean *et al.,* 2006). Several types of fat replacers can play a similar role as fats in foods, where carbohydrates are among the largest and oldest group that can be used as a substitute for fats (Nancy, 1997). Among the carbohydrates that are used in the manufacture of mayonnaise and other sauces is a dietary fiber taken from the shells and casings of cereal. In recent years, many studies have been conducted to produce low-fat mayonnaise and salad dressings.

Hydrocolloids are biopolymers of hydrophilic with high molecular weights that possess the properties of thickening, stabilization, the formation of coatings, water retention, and gelling, and are commonly used to stabilize the texture of emulsions and to give desirable sensory properties in food products (Bratu and Popescu, 2016).

Chitosan is a positively charged, homogeneous biopolymer, homogeneous in a composition consisting of repeating units of glucose amine and is derived from chitin, which is an essential component of the exoskeleton of crustaceans, insects, and fungi cell walls. It represents the most important chitin derivative obtained after removing the acetyl groups at the carbon atom site No. 2 of chitin under concentrated base conditions (Al-aubadi and Salman, 2009; El Knidri *et al.,* 2018). Chitosan contains active hydroxyl groups at the site of the carbon atom C6 and C3, and the amine group at the site of the carbon atom C2, and these groups add functional importance to chitosan, as it can be used as an antimicrobial and antioxidant (Hosseinnejad and Jafari, 2016). Also, chitosan is one of the types of colloids that can be used as a stabilizer and thickener in the manufacture of mayonnaise and emulsion products (Askari and Mostaghim, 2009).

The Tamarind (Tamarindus indica L.) is an evergreen tree that belonged to the genus Tamarindus and the family Caesalpinioideae. It is found in India, Southeast Asia, and tropical and subtropical regions. Tamarind seed polysaccharides (TSP), also known as tamarind gum, is a neutral xyloglucan (XG), the gum is composed of β- (1, 4)-D-glucan backbone with α- (1,6)-D-xylose branches that are partially substituted with β- (1,2) -D-galactose (Shao *et al.,* 2017).

The gum is found in the seeds of the tamarind and makes up 65% of the seed components. It is a hydrophilic polymer of high molecular weight and used for the gelling, thickening agent, and emulsifying agent in food, as well as used as a binder in the preparation of pharmaceutical tablets and as an emulsifier in many pharmaceutical formulations (Singh *et al.,* 2011) formulations. It has been found that tamarind gum is highly viscous, biocompatible, characterized by being non-toxic and non-irritant, possessing hemostatic activity, insoluble in organic solvents, and dispersed in warm water to form a very viscous gel. It possesses non-Newtonian materials, mucomimectc, mucoadhesive properties, and pseudoplastic properties (Gupta *et al.,* 2010). Due to the availability of chitin sources represented by marine organisms such as crab in Iraq, especially in Basra Governorate, as it is consumed in abundance, but its residual waste represented by the exoskeleton has a low economic value. Therefore, it is often discarded causing environmental pollution or may be used as animal feed or organic fertilizer (Parthiban *et al.,* 2017). Moreover, the tamarind seeds are a by-product of the manufacture of tamarind juices, as the fleshy part is used and the seeds are often thrown without making use of them. Thus, the research aims to use natural polymers extracted from a plant source (tamarind seeds) and an animal source (chitosan prepared from crab shells) in preparing low-calorie mayonnaise and studying it's chemical composition, physicochemical and sensory qualities.

MATERIALS AND METHODS

Materials

All the ingredients used in the mayonnaise industry were purchased from the local markets of Baghdad-Iraq, represented by corn oil (Al-Tunisa brand - Turkey), fresh table eggs (Diwaniyah - Iraq). Also, commercial white vinegar, 5-6% acetic acid (produced by Al-Badawi Company for Food Products. - Iraq), table salt, purity 99.9% (Mansour mark - Iraq), table sugar - sucrose (Al-Osra mark - Saudi Arabia) and mustard powder (Al-Tahona mark - Iraq).

The seeds of the tamarind were obtained from the local markets of Baghdad, where the seeds were separated from the fleshy part by hand, and then the seeds were washed with tap water and dried in an electric oven at a temperature of 100 °C/ 30 minutes. Then, they cooled at room temperature and milled for 1-0.5 minutes in a laboratory mill to separate the brown crusts from seed pulp, the pulp of seeds was milled and converted into a powder using a laboratory mill. The exoskeleton of crab (*Portunus pelagicus*) was obtained from the local markets of Basra-Iraq, which represent the by-products of cleaning this type of fresh crustaceans, which were placed in cooled containers and transferred to the laboratory. They were washed and cleaned with tap water to get rid of dust, impurities, and suspended materials, and then dried under the sun. The dried packages were packed in sealed polyethylene bags and kept at a temperature of 25 °C until laboratory experiments were carried out.

All chemicals used in the study were analytical grad, obtained from the British Drug Houses company BDH.

Methods

Extraction of gum from Tamarind Seed gum

The gum was extracted from the seeds of the tamarind according to the method mentioned by (Chawananorasest *et al.,* 2016) by removing the fat from 50 g of the seed powder using hexane. Then, the extraction was done using 20 g of defatted seed powder, and 200 ml of cold water was added to it to prepare the suspension, and 800 ml of boiling water was added to the mixture. The heating process was carried out by boiling heat for 20 minutes on a hot plate until a clear solution was obtained; it was left at room temperature overnight and centrifuged at 6000 xg for 20 minutes to separate foreign materials. The filtrate was separated and 95% ethanol was added to it with continuous mixing, then the sediment obtained was collected using a stainless steel sieve and dried in a heated oven at a temperature of 50 °C / 4 hours, and finally, the dried polysaccharide was kept in a glass container until it was used in the preparation of mayonnaise.

Chitin extraction

Chitin was extracted according to the method mentioned by (Ahyat *et al.,* 2017). The demineralization process was carried out by treatment with hydrochloric acid at a concentration of 0.1 N at a ratio of (15: 1) (solid: solvent, w/ v) with continuous mixing for 1 hour. Then the deproteinization process took place by heating the demineralized powder under reflux using a solution of NaOH at a concentration of 0.1 N at a ratio of (15: 1) (solid: solvent, w / v) at a temperature of 80 °C / 6 hours. Likewise, the pigments were removed using acetone (100: 1) (solid: solvent, w / v) with continuous mixing for 24 hours. The purified chitin was filtered, washed until neutral, and dried in an electric oven at a temperature of 60 °C. Finally, a white powder was obtained from the crab shells, after the steps of the demineralization process, deproteinization process, and removing stains.

Chitosan preparation

Acetyl groups were removed from chitin powder prepared by deacetylation process according to the method reported by (Ahyat *et al.,* 2017) by using the conventional heating method. The samples were mixed with a NaOH solution at a concentration of 50% (w / v) at a ratio of (20: 1) (solid substance/solvent) with mixing for 6 hours at a temperature of 110 ° C. After the end of deacetylation process, the filtration was done to remove the NaOH solution, then pure chitosan was washed using deionized water until reaching neutralization and dried in an electric oven at a temperature of 60 ° C.

Preparation of mayonnaise

This study was conducted in the laboratories of the Department of Food Sciences at the College of Agricultural Engineering Sciences at the University of Baghdad. (FFM), (RFM), and (LF) samples were prepared according to the method of (Mozafari *et al.,* 2019) as mentioned in Table 1. The dry ingredients represented by salt, sugar, and mustard (except gum) were mixed, then 1/3 of the vinegar amount was added to it and mixed using the Arab Mixer MX900 with a power of 250 watts at a low speed for 10 minutes. Then, the egg yolks were added and the mixture was mixed for an additional 10 minutes. Similarly, the gum and water were added and mixed again for 10 minutes, with an addition of 1/3 corn oil with continuous mixing and the remaining amount of vinegar, finally, the oil was added slowly with continuous mixing for 10 minutes. A different sample of mayonnaise was transferred into sterile 300 ml glass containers with lids and stored in the refrigerator until laboratory analysis. Mayonnaise treatments containing 75% fat are classified as commercial mayonnaise, and mayonnaise containing 56.25% fat mayonnaise is classified as reduced-fat mayonnaise, while mayonnaise containing 37.5% fat is classified as light mayonnaise.

Evaluate the quality of mayonnaise

Composition analysis and Caloric value

The percentages of moisture, fat, and ash were estimated by methods according to standard methods 920.116, 938.06, and 920.117 mentioned in (AOAC, 2000), respectively. The percentage of protein was estimated by the Kjeldahl method, and the percentage of carbohydrates was calculated by subtracting the sum of the percentages of moisture, ash, fat, and protein from 100%. The tests were conducted with three replications. The calorific value (kcal / 100g) was calculated for each treatment of mayonnaise-based on the following equation (Liu *et al.,* 2007):

$$\text{Caloric value} = (4 \times \text{protein}) + (9 \times \text{fat}) + (4 \times \text{carbohydrate})$$

Table 1. formulation of mayonnaise samples (w/w)

Ingredients (g)	FFM (control)	Mayonnaise containing Chitosan		Mayonnaise containing Tamarind gum	
		RFM1	LM1	RFM2	LM2
Corn oil	75.00	56.25	37.5	56.25	37.5
Egg yolk	7.27	7.27	7.27	7.27	7.27
Chitosan	-	3	3	-	-
Tamarind gum	-	-	-	3	3
White vinegar	12	12	12	12	12
Mustard powder	1	1	1	1	1
Sugar	2.5	2.5	2.5	2.5	2.5
Salt	1	1	1	1	1
water	1.23	16.98	35.73	16.98	35.73

FFM (control): full-fat mayonnaise without chitosan and Tamarind gum in its formulation.
RFM1: reduced-fat mayonnaise containing 56.25% fat and 3% chitosan
LM1: light mayonnaise containing 37.5% fat and 3% chitosan
RFM2: reduced-fat mayonnaise containing 56.25% fat and 3% Tamarind gum
LM2: reduced-fat mayonnaise containing 37.5% fat and 3% Tamarind gum

Determination of pH

A 10 g sample was placed in baker and the pH values of the mayonnaise treatments were estimated at 25 ° C using a pH meter (model CyberScan 500). (Ghazaei *et al.,* 2015).

Microscopic examination

A glass slide was covered with a sample of mayonnaise for each treatment and examined with a light microscope (Microscope Olympus DP12, B41TF, Japan). A digital camera connected to a microscope obtained the microstructure image of the mayonnaise with a 40 X magnification. (Ghazaei *et al.,* 2015).

Stability test

A 15 g of mayonnaise (F0) samples were placed in test tubes, closed with plastic plugs, and centrifuged at 2000 xg for 10 minutes. The weight of sediment (F1) was measured according to the following equation:

$$\text{stability rate } (\%) = \left({}^{F1}/_{F0}\right) \times 100$$

To measure the stability of mayonnaise towards temperature, samples were placed in test tubes and exposed to a temperature of 50 °C for 48 hours, then the stability towards temperature was calculated based on the above-mentioned equation (Mozafari *et al.,* 2017).

Determination of viscosity

The viscosity of the mayonnaise samples was measured with a Brookfield viscometer using a spindle 64 at 4 ° C.

Sensory evaluation

The sensory evaluation of the mayonnaise samples was carried out after one day of storage at room temperature for the characteristics of aroma, color, and consistency. Besides, the overall acceptance by specialized professors and graduate students in the Department of Food Sciences/College of Agricultural Engineering Sciences-University of Baghdad, where the total number of assessors was 20. The evaluation was done based on the 5-points hedonic method, and the value (5) was considered the best and (1) the worst. Samples (10 g) were presented at room temperature in plastic cups after coding them randomly with three numbers. Water was provided to rinse the mouth after each evaluation, and the jury was asked to evaluate the samples according to the indicators mentioned in the sensory evaluation form.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of different factors in study parameters. Duncan's multiple range test (1955) to compassion between means in this study.

RESULTS AND DISCUSSION

Chemical composition of chitosan and tamarind gum

Table 2 showed the chemical composition of chitosan prepared from crab and tamarind gum. It is clear from the Table that the percentage of moisture was 8.15 and 10.12%, while the protein percentage was 1.06 and 0.90%, whereas the ash percentage was 0.78 and 1.87%, respectively. Further, the percentage of fat and total carbohydrates in the tamarind gum were 0.21 and 87.01%, respectively.

Table 2. The chemical composition of chitosan prepared from crab and tamarind gum seed

Component (%)	Chitosan	Tamarind gum
Moisture	8.15±0.19	10.12±0.28
Protein	1.06±0.07	0.90±0.03
Fat	N.D	0.10±0.05
Ash	0.78±0.02	1.87±0.90
Total carbohydrate	N.D	87.01±0.9

N.D, Not determined
Values are means of three replicates

The decrease in the ash content in chitosan is due to the increase in the time of removing the acetyl groups because of alkaline treatment with NaOH solution. The ash content of the prepared chitosan indicates the efficiency of the demineralization process, which is an essential step in the preparation of chitin. However, this process led to the removal of the largest amount of calcium carbonate and calcium phosphate, whose concentration in the shells reaches 30-50%. The ash content of high-quality chitosan should not exceed 1% (No and Lee, 1995). The low ash and protein content is an indicator of chitosan and Trisodium phosphate (TSP) purity.
The high moisture content of chitosan is attributed to its high ability to absorb moisture, as chitosan is a hygroscopic material, and commercial chitosan products contain less than 10% moisture (Salman and Khaleel, 2009).

Chemical analysis and caloric values

Table 3 showed the chemical analysis and caloric values of FFM (control treatment), RFM, and LM prepared by adding chitosan and tamarind gum. It is evident from the below Table that the moisture content of samples increased with decreasing fat percentage, and that the caloric value of low-calorie mayonnaise decreased significantly in the treatments that have a less fat percentage. Moreover, it was observed that LM has a higher moisture content (P≤0.05) than FFM, and generally, the moisture content increased with the increase in the level of fat substitution.
In this regard, (Mun *et al.,* 2009) noticed a significant decrease in caloric values by adding modified starch instead of fat in mayonnaise, and stated that this decrease in calories is associated with an increase in the level of moisture and the indigestible nature of the modified starch.

No significant differences (P> 0.05) were observed between FFM and LM treatments in protein content in samples, and slight significant differences were observed for ash values, this might be attributed to the types and levels of the fixed components in all treatments except for fat, water, and hydrocolloid.
The caloric values of the samples containing the fat substitute decreased significantly, as the values of mayonnaise LM1 and LM2 decreased significantly (P≤ 0.05) with the increase in the level of fat substitution.
Akoh and Min (2002) found that the moisture level in LF and prepared with the addition of a carbohydrate-based fat substitute increased significantly with the decrease in oil content, which was compensated for by the increased humidity level.
The low caloric values of mayonnaise may be attributed to replacing the oil in FFM with water and the hydrocolloids represented by chitosan and tamarind gum seed (which are calorie-free materials because they are neither digested nor absorbed in the human digestive system) to prepare RFM. Water is an essential component when replacing fats by using fat substitutes of carbohydrate origin, as the water is a gel-like substance in the presence of hydrocolloids, which leads to the improved texture of RFM (Amin *et al.,* 2014).
The percentage of carbohydrates in the mayonnaise treatments LM1 and LM2 is higher than the control due to the side chains of gums, which increase the proportion of carbohydrates. Those hydrocolloids, which are used as a type of fat substitute, can give functional properties due to their binding to the water molecules inside the emulsion, and because the gel formed when the gum is mixed with water has a high percentage of moisture, these hydrocolloids are suitable for use in the food industry (Amiri Aghdaee *et al.,* 2011).

Table 3. Proximate chemical analysis (%, w/w) and caloric values of mayonnaise samples

Samples	Moisture	Ash	Protein	Fat	Carbohydrate	caloric values (Kcal/100g)
FFM	20.02 ±0.81 c	1.20 ±0.07 b	1.21 ±0.05 a	75.50 ±2.78 a	2.07±0.07 b	692.62 ±15.42 a
RFM 1	33.05 ±1.42 b	1.25 ±0.08 ab	1.21 ±0.05 a	57.00 ±2.63 b	7.49 ±0.66 a	547.80 ±13.79 b
LM 1	50.90 ±3.56 a	1.28 ±0.08 ab	1.22 ±0.06 a	38.80 ±1.86 c	7.80 ±0.82 a	385.28 ±13.06 c
RFM 2	32.10 ±1.77 b	1.24 ±0.05 ab	1.20 ±0.03 a	57.81 ±2.92 b	7.64 ±0.57 a	555.69 ±22.37 b
LM 2	51.80 ±2.08 a	1.30 ±011 a	1.22 ±0.05 a	38.90 ±1.56 c	6.78 ±0.71 a	382.10 ±14.09 c
Means having with the different letters in the same column differed significantly. * (P≤0.05).						

Assays were performed in triplicate

Stability test

Emulsion stability usually involves preventing coalescence of the oil droplets, flocculation, and creaming. Creaming does not occur in mayonnaise that is high in fat by 80% because the droplets are close together and thus cannot move. However, in products with low-fat content, creaming does not occur due to the addition of thickening substances such as gum or starch to the aqueous phase to slow down the movement of the droplets.

Low-fat mayonnaise samples in the current study showed high stability due to the viscosity of the aqueous phase, mainly due to the addition of chitosan and tamarind gum, which led to slow the movement of oil droplets.

Table 4 showed the results related to stability that the highest values of stability were 95.84% in the RFM1 mayonnaise mixture, and the lowest value of stability in the FFM mayonnaise mixture was 88.00%. Egg yolk proteins and phospholipids act as excellent emulsifiers; accordingly, it gives high stability to the control model prepared by adding egg yolk without adding gum or chitosan.

Mayonnaise, as is the case with all high-fat foods, is sensitive to spoilage due to the auto-oxidation that occurs to unsaturated and polyunsaturated fatty acids in the oil. The stability of mayonnaise depends on the type of oil used. Salt, vinegar, and mustard are very important components in the development of flavor and stability and appear to influence the rate of oil oxidation in the emulsion. The stability of mayonnaise as a successful and generally balanced emulsifier depends on several factors, including the amount of added egg yolk and viscosity. Likewise, the relative size of the fat phase to the aqueous phase, the mixing method, and the speed used, the quality of the aqueous phase. Further, the arrangement of adding the ingredients and the temperature used (Thomareis and Chatziantoniou 2011, Rahmati *et al.* ., 2012; Farahmand, 2014; Mirzanajafi-Zanjani *et al.,* 2019).

Several factors affect the instability of the emulsifier, including frozen or dried egg yolk, amount of sucrose and added salt, amount and purity of the oil, pH of emulsion, the addition of spices (Mun *et al.,* 2009; Fernandesa and Salas-Mellado, 2018). Table 4 also showed the results related to stability towards temperature, and the highest stability of the RFM1 mayonnaise mixture was 86.57%, while the lowest heat stability was 80.00% in FFM. The stability of RFM is due to the increase in the viscosity of the continuous phase and the formation of a weak gel network due to the addition of gum and chitosan.

In addition, the stability of mayonnaise can be improved by increasing the energy during manufacture, which leads to the formation of small droplets. Therefore, the good stability of RFM is attributed to the initial small droplets that were stabilized by adding chitosan and gum. It has been observed that the use of xanthan, Guar gum, and some other proteins increases stability while creaming does not occur in RFM due to the increased viscosity of the continuous phase and reduced movement of the fat droplets (Nikzade *et al.,* 2012). There is a direct relationship between the increase in the percentage of gum and stability. Thus it is assumed that the droplets of fat are flocculated and the increase in the viscosity of the continuous phase contributes to the stability of RFM prepared by adding gum. This phenomenon may occur in most biopolymers that help to stabilize the droplets as a result of physical and chemical interactions such as electrostatic interactions and hydrogen bonding (Mozafari *et al.,* 2019).

Viscosity

Table 4 showed that the viscosity values of the RFM and LM mayonnaise treatments prepared by adding chitosan and tamarind gum ranged between 126-108 cP, and it was 79 cP in the FFM mayonnaise factor. It was observed that the highest viscosity value is for RFM1.

Erçelebi and Ibanoğlu (2010) noted an increase in viscosity with the presence of guar gum, indicating the importance of a hydrocolloid to give a thickness of the continuous phase, which consistent with the present study. (Aslanzadeh *et al.,* 2012) found that treatments of RFM prepared with the addition of different proportions of modified wheat bran showed the characteristics of pseudoplastic behavior.

pH

Microorganism growth does not occur in mayonnaise due to the high-fat content and low water content, as well as the presence of acetic acid, which leads to a decrease in the pH value (Lin *et al.,* 2014). The pH of mayonnaise also clearly affects the composition of the emulsion, at the isoelectric pH of the yolk proteins the viscoelasticity and stability of mayonnaise are at the highest value. If the proteins on the surface of the droplets are overloaded, this will lead to an inability to absorb any other protein, and the droplets will repel each other, preventing flocculation. The critical pH that affects the shelf life of mayonnaise is 4.1, which means that when the pH is higher than 4.1, this will significantly increase the rate of microorganism growth in the system (Fernandesa and Salas-Mellado, 2018). Amin *et al.,* (2014) also indicated that mayonnaise should have a pH of 4.1 or less to ensure that it is free from the growth of Salmonella. According to (Lin *et al.,* 2014), the natural pH of mayonnaise is about 3.70 and the FFM treatment is closest to this value. The components of mayonnaise affect its pH, as sugar and salt cause a decrease in the pH value, while oil and mustard increase its value, and the reason may be the acidic behavior of sugar and the alkaline behavior of mustard components under the acidic conditions of mayonnaise (Mouheb Eldean *et al.,* 2014).

It was not observed through the results in Table 4 that there were significant differences in the pH values between the treatments, as they ranged between 3.80 - 3.88. The pH value of the control treatment, which represents FFM, was less than the rest of the treatments, as it was 3.71. The increase in pH values for the rest of the treatments was attributed to a significant increase in the level of moisture with an increase in the proportion of fat replacement, which led to an increase in the pH values due to dilution of acetic acid in the continuous phase. These results are consistent with (Askari and Mostaghim, 2019; Amin *et al.,* 2014; Karas *et al.,* 2002). In terms of health, vinegar is used as an acidulant to reduce the pH and to reduce the risk of micro-organisms, and preserve the product from microbial damage. Also, is a source of flavor, especially if used in the appropriate quantity and concentration, as the use of a large amount affects the flavor of mayonnaise, while the water in the vinegar is part of the emulsion composition (Aqueous phase).

Table 4. Stability, heat stability, pH and viscosity for each formulation.

Samples	Stability	Heat stability	pH	Viscosity
FFM	88.00 ± 2.64 b	80.00 ±2.83 c	3.71 ±0.08 a	79.00 ±2.57 c
RFM1	95.84 ± 2.08 a	86.57 ± 2.77 a	3.80 ±0.11 a	126.00 ± 12.07 a

LM 1	92.07 ±3.21 ab	84.64 ±1.97 ab	3.88 ±0.15 a	110.33 ±8.55 b
RFM 2	94.22 ±2.98 ab	83.25 ±2.04 ab	3.82 ±0.09 a	123.00 ±8.21 a
LM 2	90.00 ±2.52 ab	81.25 ±3.47 bc	3.88 ±0.15 a	108.00 ±7.04 b
Means having with the different letters in the same column differed significantly. * (P≤0.05).				

Each value in the table is the mean of the Mean of three replicates

Optical microscope observation

Microscopic examination was used to provide information about the exact composition of mayonnaise samples prepared using different concentrations of hydrocolloids. Figure 1 shows the microphotographs of mayonnaise treatments prepared FFM, RFM and LF by adding chitosan and tamarind gum.

Mayonnaise is composed of oil droplets spread throughout the aqueous medium. However, the properties of mayonnaise vary widely with different prepared mixtures due to the different components and microstructure. Factors such as the amount and distribution of emulsifying and stabilizing agents, droplet size, fat concentration, and aqueous phase viscosity are important indicators in estimating the exact composition of mayonnaise (Mun *et al.,* 2009). The microstructures of the FFM, RFM, and LM mayonnaise samples were assigned after storing the prepared samples for 24 hours. The optical microscope images showed that in the samples of FFM, small droplets were efficiently packed between the larger ones, and this microscopic composition creates a concentration of droplets above the theoretical maximum for packing monodisperse spherical particles, while in the RFM samples, larger size distributions and smaller droplets appeared. This result indicated that RFF might possess a variety of micro-structures depending on production conditions and chemical composition.

An ideal emulsion consists of spherical droplets packed together in a continuous phase. In mayonnaise, a high-fat content and close packing may affect the oil droplet (Depree and Savage, 2001).

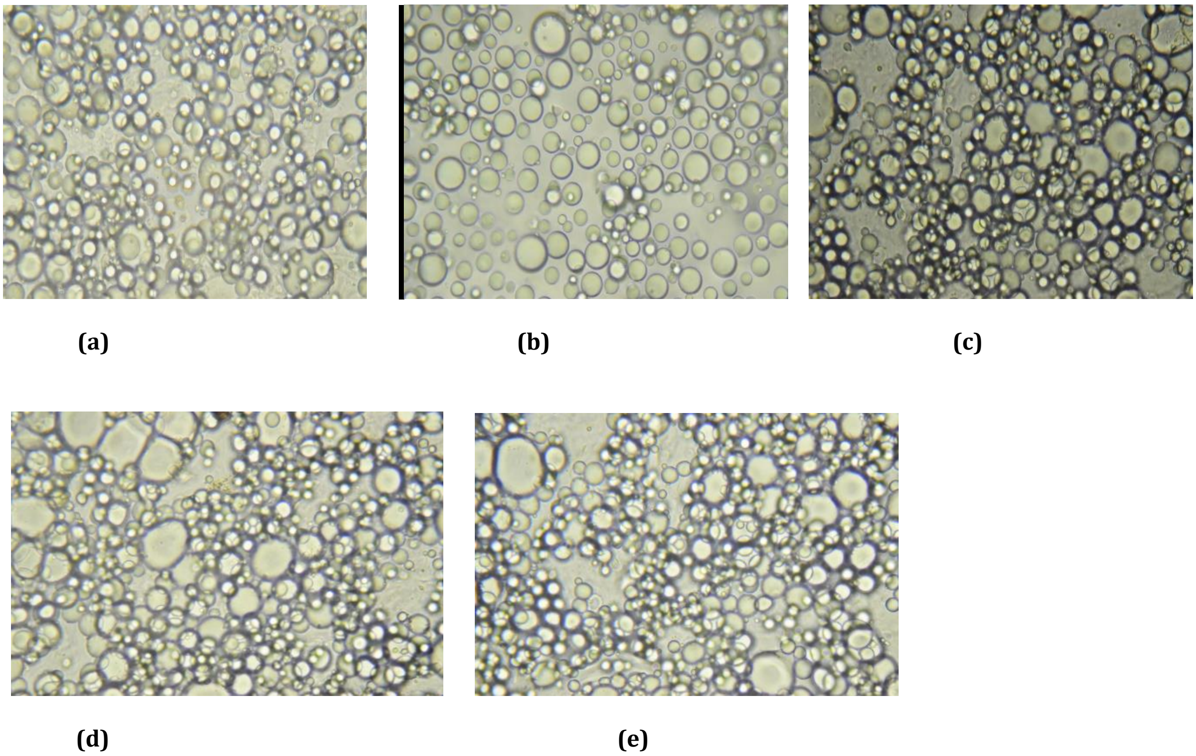

Figure 1. Microphotograph of different mayonnaise formulation, taken by using a 40x magnification. (a) FFM (b) RFM1 (c) RFM2 (d) RFM3 (e) RFM4

Sensory evaluation

Table 5 showed the results of sensory evaluation of prepared FFM, RFM, and LF mayonnaise treatments by adding chitosan and tamarind gum. The results showed that there were significant differences between the different treatments at (P≤0.05). The highest consistency, aroma, and overall acceptance were 4.7 for RFM1 treatment, while the lowest consistency, aroma, and overall acceptance were 4.1, 4.1, and 4.2 for the control treatment, LM2, and RFM2, respectively. The treatment RFM1 got the highest color evaluation score of 4.4, while the treatment RFM2 got the lowest color evaluation score. In general, the mayonnaise treatments prepared by adding chitosan RFM1 and LM1 were better compared to the mayonnaise treatments prepared by adding tamarind gum, which is RFM2 and LM2.

Color is one of the important quality characteristics of mayonnaise and the first criterion by which the product is rejected or accepted by the consumer (Fernandesa and Salas-Mellado, 2018). The yellow color of mayonnaise is mainly attributed to egg yolk carotenoids. Oil and mustard do not contribute to the color provided by the egg yolk (Abu-Salem and Abou-Arab, 2008). Chitosan did not affect the color of all mayonnaise treatments because it was off white, as it did not cause the taste and color to change, so the RFM1 treatment gave a high value. The high water content of LF mayonnaise dilutes the color of the model, resulting in an off white color that is more receptive to assessors compared to the yellow color of FFM mayonnaise. This contrasts with what (Liu *et al.,* 2007) found, that FFM with a dark yellow color was more receptive compared to off-white. Mayonnaise contains balanced proportions of salt, vinegar, and spices (mustard) that contribute to its distinct flavor. Mayonnaise has a sour taste due to the presence of vinegar in its composition (Abu-Salem and Abou-Arab, 2008). Nikade *et al.,* (2012) stated that the addition of xanthan as a stabilizer led to an increase in the general acceptability of LF mayonnaise, which is consistent with the results obtained in the current study.

Table 5. Sensory evaluation of mayonnaise formulation

Samples	Consistency	Aroma	Color	Overall acceptance
FFM	4.1 ±0.05 c	4.6 ±0.08 a	4.3 ±0.07 a	4.4 ±0.08 ab
RFM1	4.7 ±0.09 a	4.7 ±0.08 a	4.4 ±0.09 a	4.7 ±0.06 a
LM 1	4.3 ±0.07 bc	4.6 ±0.06 a	4.2 ±0.06 ab	4.5 ±0.08 ab
RFM 2	4.5 ±0.08 ab	4.2 ±0.04 b	3.8 ±0.04 b	4.2 ±0.05 b
LM 2	4.2 ±0.04 bc	4.1 ±0.04 b	4.0 ±0.06 b	4.3 ±0.07 ab

Means having with the different letters in the same column differed significantly. * (P≤0.05).

CONCLUSION

At present, consumer demands are increasing for more healthy, low-fat, low-cholesterol, and allergen-free food products. It can be concluded from the current study that chitosan prepared from sea crab and tamarind gum seed plays an important role as a substitute for fats in the manufacture of mayonnaise and as a stabilizer for the emulsion at the same time. Replacing the fats leads to the production of mayonnaise with desirable sensory qualities with the possibility of obtaining good consistency, high stability, low calories, as well as good viscosity due to the ability of these materials to absorb water. Among the advantages of using chitosan and tamarind gum are that they are hydrocolloids that contain dietary fiber, which is beneficial to human health and are characterized by being safe, non-carcinogenic, biodegradable, and inexpensive. Therefore, it can be recommended to use them in preparing low-fat mayonnaise without changing the properties of the final product.

ACKNOWLEDGEMENT

The researcher thanks the Ministry of Science and Technology- Department of Environment and Water for assisting in carrying out some laboratory experiments related to the research.

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