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CHEMICAL AND BIOLOGICAL ANALYSIS OF MAYONNAISE PREPARED FROM PROTEIN CONCENTRATES OF LOCAL VARIETIES OF BROAD BEAN AND COWPEA

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ABSTRACT

This study was conducted to make free cholesterol mayonnaise and meet market needs without harmful side effects for those suffering from atherosclerosis diseases and for people who have cholesterol phobia. Local varieties of broad bean and cowpea were used to prepare mayonnaise with high nutritional content. Five mixtures of mayonnaise were prepared (B from raw broad bean powder, C from raw cowpea powder, D from broad bean protein concentrate, E from cowpea protein concentrate and F from 50% of broad bean protein concentrate and 50% cowpea protein concentrate. Sensory tests were done on the five mixtures and just D and E mixtures with control treatment A were chosen as it achieved the highest sensory evaluation and tests were done on the chosen mixtures. Results of chemical analysis showed that E, D and A mixtures was recorded 5.62, 6.55 and 5.18% protein content, 8.64, 8.54% carbohydrates content, 48.23, 50, 47.27% moisture, 0.37, 0.27, 0.26% ash, 32.3, 30.03, 38.5% fat, 4.80, 4.30, 4.03% fibers and 0, 0, 17% cholesterol respectively.

Keywords **:** Free cholesterol mayonnaise, broad bean, cowpea, protein concentrate.

Introduction

Mayonnaise is one of most popular food appetizers spreading on dining tables around the world, as the demand for it increased in recent years. Mayonnaise provides human with protein, fat, fat-soluble vitamins and carbohydrates, and it considered an oil-water emulsifier and its oil content reaches 70-80% (Depreea and Savage, 2001). There are two type of mayonnaise, the first one obtained from egg by emulsifying one or more types of vegetable oils in a water medium using lemon juice or vinegar with the use of egg yolks (Shaker, 2018). The second type is also made by emulsifying one or more types of vegetable oils in a water medium using lemon juice or vinegar without egg (Mohammed, 2018). Emulsification is one of the basic elements in the manufacture of mayonnaise, as it important to reduce tension on the surface between water and oil, in addition to the emulsifier stability component. In the past, only eggs were used, but with the increasing interest in producing free cholesterol foods, it was necessary to use alternatives to egg yolks by using an emulsifying agent with keeping all properties of mayonnaise in order to ensure the quality of manufacturing process (Nikzade *et al*., 2012). Mahshid *et al*. (2015) mentioned that it is possible to manufacture low cholesterol mayonnaise by using wheat germ protein and xanthan gum which gives mayonnaise with suitable stabilizing properties. Mayonnaise consists of water, salt, sugar, mustard powder, egg yolk, oil, vinegar and whole egg or dried egg (Widerström and Öhman, 2017). There are also some other methods to make mayonnaise free of eggs (Shaker, 2018) by using water, salt, sugar, mustard powder, white vinegar, Arabic gum, starch, oil and lupine isolated protein as an emulsifying agent instead of egg. Thus, current study aims to manufacture free cholesterol mayonnaise from local legumes (broad bean and cowpea) and its protein concentrates in comparison with mayonnaise in market.

Materials and Methods

This study was conducted in laboratories of Department of Food Sciences, Faculty of Agriculture, University of Kufa, Najaf, Iraq, the chemical analysis were done in Najaf Directorate Agriculture laboratories and the biological analysis were done in central health laboratory at Al-Hakim hospital.

Broad bean and cowpea seeds

Seeds of Iraqi broad bean and cowpea were obtained from local markets in Najaf province then cleaned to get rid of dust and impurities after that seeds were washed three times and soaked in water for 10 minutes. Seeds were sun dried for two days and then dried in an oven at 50ºC for 20 h to get rid of moisture then grinned and put in polyethylene plastic bags and kept at 4-5^{0}C (Arbo, 1988).

Preparation of defatted powder

Defatted powder of broad bean and cowpea was prepared according to Gustavo *et al*. (2012) procedure by treated the powder of broad bean and cowpea with hexane by magnetic mixing device for 3 h then filtrate was separated to take the solvent and filter it again. The product was collected and the defatted left to dry then put in polyethylene plastic bags and kept at -18ºC until used.

Preparation of protein concentrate

Protein concentrate of defatted broad bean and cowpea seeds powder was prepared following Ogunwolu *et al*. (2009)

method by mixing the defatted powder with 70% ethanol in 1:10 ratio (weight/volume) with shaking for 2 h by magnetic mixing device then the filtration was done under vacuum using Buechner funnel. Precipitate was washed with distil water many times and left to dry then grinned and put in polyethylene plastic bags and kept at -18ºC until used.

Mayonnaise manufacturing method

Mayonnaise was prepared by well mixing of emulsifier, salt, sugar, mustard, pepper, gum, starch, water and vinegar in a blender with adding oil continuously to avoid the separation of materials at a moderate temperature and then put in sealed sterilized containers (Shaker, 2018) and kept in refrigerator to do the sensory evaluation (Table 1).

Table 1 : The various blends prepared for manufacture of mayonnaise.

50% broad bean concentrate 50% cowpea concentrate	Cowpea protein concentrate mayonnaise	Broad bean protein concentrate mayonnaise	Raw cowpea mayonnaise	Raw broad bean mayonnaise	Control sample	Ingredients
50	50	50	50	50	-	Oil (ml)
-	-	-	-	-	-	Egg (g)
5	5	5	5	5	-	Water (ml)
2.5	2.5	2.5	2.5	2.5	-	Sugar (g)
5	5	5	5	5	-	Starch (g)
2	2	2	2	2	-	White vinegar (ml)
3	3	3	3	3	-	Gum (g)
2	2	2	2	2	-	Mustard (g)
1.5	1.5	1.5	1.5	1.5	-	Salt (g)
0.5	0.5	0.5	0.5	0.5	-	White pepper (g)
-	-	-	-	10	-	Raw broad bean (g)
5	-	10	-	-	-	Broad bean protein concentrate (g)
-	-	-	10	-	-	Raw cowpea (g)
5	10	-	-	-	-	Cowpea protein concentrate (g)

Sensory evaluation

Sensory evaluation for the mixture of mayonnaise was done by ten volunteers according to Reddy *et al*., (2005) method by given ten degrees for each property (color, smell, taste, texture and general acceptance).

Moisture determination

Moisture percentage was estimated following A.O.A.C. (2008) method.

Protein determination

Keldahl method was used to estimate (A.O.A.C, 2008).

Fat determination

Soxholet was used to estimate fat ratio (A.O.A.C, 2008).

Carbohydrates determination

Carbohydrates were estimated as follows:

Carbohydrates % = 100 – (protein % + fat % + ash % + fibers %).

Ash determination

Ash ratio was estimated following A.O.A.C., (2008) method.

Fibers determination

Fibers percentage was estimated following A.O.A.C., (2008) method.

Cholesterol estimation

Cholesterol percentage was estimated following Al-Salhi, (2012) procedure.

The total number of bacteria

Kassem *et al*., (2011) method was used to calculate the total number of bacteria as follows: Nutrient agar medium was put in pour plate and incubated at 37^0C for 24 to 48 h.

Total molds and yeasts

Malt agar medium was put in petri dishes then dishes were incubated at 22^0C for 2 to 4 days (Anderwa, 1992).

Total coliform

0.1 ml of dilution was put in petri dishes then Macconkey agar was added, after that plates were inculpated for 24 h in 37^0C and red colonies only were counted (Kassem *et al*., 2011).

***Salmonella* spp.**

0.1 ml of dilution was put in petri dishes then *Salmonella Shlgella* agar, after that plates were inculpated for 24 h in 37^0C and colonies grow in the plate were counted (Kassem *et al*., 2011).

Statistical analysis

The experiment was arranged using complete block randomized design (RCBD) for sensory evaluation and the significant differences between treatments were tested using Duncan test, at $P \leq 0.05$ then data were analyzed using GenStat V12.

Results and Discussion

Sensory evaluation

Table 2 showed the sensory evaluation results of mayonnaise mixtures and control treatment for color, smell, taste, texture and general acceptance properties. Results

indicated that there were significant differences between studied mixtures of mayonnaise. Statistical analysis results showed that prepared mixture E was significantly exceled other mixtures followed by D mixture, as it received the highest degree of acceptance by volunteers, so these two mixtures were chosen and the rest were excluded. These results are in agreement with Mahshid at al., (2015) findings who reported that mayonnaise sample contains wheat germ protein and xanthan gum received the highest degree in sensory evaluation of myaonnaise.

Table 1 : The sensory evaluation of studied mayonnaise samples.

General acceptance	Texture	Taste	Smell	Colour	
9.12 a*	9.01 a*	9.01 a*	8.20 a*	8.24 a*	Control sample A
5.87 F	5.40 e	5.40 F	5.48 d	5.84 c	Raw broad bean mayonnaise B
6.24 e	5.77 f	5.77 e	5.77 d	6.04 c	Raw cowpea mayonnaise C
7.67 c	7.49 c	7.49 D	7.24 c	7.24 d	Broad bean protein concentrate mayonnaise D
8.19 b*	7.91 b*	7.91 b*	7.61 b*	7.53 b*	Cowpea protein concentrate mayonnaise E
7.19 D	7.06 d	7.06 C	7.02 c	6.90 d	50% broad bean protein concentrate 50% cowpea protein concentrate F

*Different letters in the same column mean there were significant differences between treatments.

Chemical analysis of mayonnaise

Statistical analysis results showed that E mayonnaise mixture was significantly exceled other treatments in protein percentage which reached 6.55% compare to 5.18 and 5.62% in A and D mixtures respectively. The reason for increasing protein percentage is cowpea contains high percentage of protein, while there was significant reduction of fat percentage in mixture E amounted 30.03% in comparison with 38.5 and 32.3% respectively in A and D mixtures, this increasing of fat ratio in mixture A is related to high fat percentage in egg yolk in addition to the oil that used to manufacture mayonnaise. D mixture was significantly exceled other mixtures in dietary fibers as it recorded 4.80% compare to 4.03 and 4.30% respectively in A and E mixtures, this increasing of fibers ratio in mixture D is related to high percentage of fibers in broad bean. Mixture E was recorded the highest percentage of moisture which reached 50%, while there were no significant differences between A and D mixture that recorded 47.27 and 48.23% respectively, the reason for increase moisture percentage in mixture E is related to increase the ability of cowpea seeds to absorb water. There were no significant differences in ash percentage between A and E mixtures as it recorded 0.26 and 0.27% respectively compare to D mixture which recorded 0.37%. There were also no significant differences in carbohydrates percentage between E and D mixtures as it recorded 8.54 and 8.64% respectively compare to 4.75% in A mixture, this increasing of carbohydrates percentage in E and D mixture is related to increase carbohydrates in broad bean and cowpea seeds and decrease in in egg. Cholesterol percentage was recorded 17% in A mixture, while it recorded 0% in E and D mixtures, this is due to the lack of cholesterol in vegetable proteins (Almusawy, 2014; Johary *et al*., 2015).

Table 3 : Chemical content results of mayonnaise samples.

Raw materials	Contents percentage %						
	Protein	Fat	Dietary fibers	Ash	Moisture	Carbohydrates	Cholesterol
Control sample	5.18 B	38.5 C	4.03 a	0.26 a	47.27 b	4.75 a	17 B
Cowpea mayonnaise	6.55 a*	30.3 a*	4.30 b	0.27 a	50 a	8.54 b	0 a*
Broad bean mayonnaise	5.62 B	32.3 B	4.80 c*	0.37 b	48.23 b	8.64 b	0 a*

Microbiological analyses of mayonnaise samples

Table 4 results of the total number of bacteria showed no significant differences between treatments as it recorded 1.3×10^5 in A, 1.67×10^5 in E and 1.67×10^5 in D, and this is in conformity with Iraqi standard specifications No. 1517 in 2012. Statistical analysis results also showed no significant differences between treatments in the total coliform as it recorded 1.33×10^2 in A, 1.67×10^2 in E and 1.67×10^2 in D. There were no significant differences between treatments in the number of yeasts and molds as it recorded 1.33×10^2 in control treatment, cowpea protein concentrate and broad bean protein concentrate, and this is in conformity with Iraqi standard specifications No. 1517 in 2012.

There was no growth of *salmonella* in studied samples of mayonnaise which is considering a good indicator in food quality. Many studies have been shown that acid type is affected and killed microorganisms; for instance, acetic acid has a bactericidal more than citric acid on *Clostridium perfringens*, *Staphylococcus autres* and *Salmonella* spp. in hand-made mayonnaise as the role of acetic acid depends

mainly on lowering pH. Salt (NaCl) also has an effective role as one of mayonnaise components, in addition, it gives the taste to mayonnaise and provides an unsuitable environment for microbial growth by reducing water stress, as pH, water stress and the percentage of sodium chloride in mayonnaise all together provide inappropriate environment for the growth of pathological bacteria (Lee, 2004).

Table 4 : Microbial analyses of mayonnaise samples.

	Control sample	Cowpea protein concentrate mayonnaise	Broad bean protein concentrate mayonnaise
Total number of bacteria CFU	1.3×10^5	1.67×10^5	1.67×10^5
Total coliform CFU	1.33×10^2	1.67×10^2	1.67×10^2
Total yeasts and molds CFU	1.33×10^2	1.33×10^2	1.33×10^2
Salmonella CFU	Neg	Neg	Neg

Conclusion

Current study indicates the possibility of using local broad bean and cowpea and its protein concentrates as an emulsifier instead of egg yolks in the manufacture of foods including mayonnaise, and there are no significant differences in the ability of storage at 4 to 5^0C. The microbial tests showed acceptable results in terms of the total number of coliform bacteria, yeasts, molds and salmonella as well as being free of pathogenic bacteria. The preparation of mayonnaise from legumes by using it as an emulsifier had the preference in chemical and health tests (free of cholesterol) but the control sample was exceled in sensory evaluation.

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