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PRODUCTION OF LOW-ENERGY YOGURT FORTIFIED WITH CHIA GUM AND STUDY OF ITS STORAGE CAPABILITY

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Abstract
The current study aimed to produce a low-energy yogurt by using skim milk fortified with different proportions of vegetable gum (0.1, 0.2, 0.3 g gum / 1000 ml skim milk), in addition to the control treatment without any addition to milk. The effect of adding gum on the properties was studied. The effect of adding gum on the chemical, physical and microbiological properties of the resulting yogurt was studied. The results of the pH values and the total acidity percentage of the yogurt manufactured by adding vegetable gum indicated that there were no significant differences between the control treatment and the treatments to which the vegetable gum was added immediately after manufacturing, while a significant decrease was observed in the pH values with an increase in the total acidity percentage with an increase in the period of refrigerated storage for all treatments. The addition of vegetable gum extracted from chia seeds to skim milk improved the physical properties of yogurt, which included viscosity, automatic whey exudation, and water holding capacity during refrigerated storage periods of 21 days, compared with control treatment. The results of the microbial examinations also indicated a variation in the total count of bacteria for the yogurt fortified with vegetable gum as the total numbers of bacteria decreased with increasing the period of cold storage compared to the control treatment, which increased the total numbers of bacteria with increasing the period of cold storage. The results showed that the yogurt fortified with vegetable gum and the control treatment were free of coliform bacteria, yeasts and molds and psychrophilic bacteria, starting from the day of manufacture until the end of the 21-day storage period.

Keywords: skim milk, nutritional value, gum, shelf life

Introduction
Chia seeds have been widely used in various food applications such as yogurt, cereals, juices, bread, pastries, and field peanut butter in different countries around the world including New Zealand, Canada, Chile, Mexico, Australia, and the United States of America (Ali *et al*., 2012).

As of 2011, chia seeds are so popular as a food that more than 72 products based on this grain have reached the global market in the form of snacks, seasonings, yogurts, bakery products, etc. (Fernandes., 2021).

Chia seeds are used in food industries that need to stabilize their weak texture, as the gum of chia seeds is located in the first three layers of the seed layer. When the seeds are moistened, fibers begin to appear, and it is a unique phenomenon for the formation of a gelatinous mass rich in sugars, where the percentage of sugars reaches (71.22 %) (Muñoz *et al*., 2012).

Nowadays, low-fat foods are widely produced as this may help reduce the risk of several diseases such as cardiovascular disease (CVD) and obesity (Felisberto *et al*., 2015). Fat substitutes are substances that look, feel, and taste similar to fat, some contain calories, others are low-calorie or calorie-free. The three main categories of fat substitutes are carbohydrates, protein, and fats. Common carbohydrate-based fat substitutes include cellulose and gum, and protein-based fat substitutes, such as egg whites, milk, and milk serum (Hamdia, *et al*., 2020). Fat is replaced in low-fat food products, as food hydrocolloids are mainly added to diets to modify rheological properties, increase stability, or reduce calories, as many previous experiments were conducted on enriching yogurt using vegetable gum such as okra fruits, quince seeds, and psyllium seeds (Nikoofar *et al*., 2013).

The Food and Drug Administration FDA., (1996) indicates that consumption of full-fat yogurt, which contains at least 3.25% fat, can lead to the development of obesity and other health problems worldwide, including cardiovascular disease. and metabolic disorders (Monsters and Saris., 2014), Consequently, consumers tended towards foods with low fat or fat free (FDA, 1996). Since milk fat is the main factor that determines the quality of yogurt, so reducing a small amount of fat has severe effects on the structure and texture of low-fat yogurt, which includes a weak texture and structure, in addition to the whey separating; (Lee and Lucey, 2010 ; Mistry and Hassan, 1992).

Among the different methods that can be used to reduce the fat content and stabilize the texture of yogurt without compromising its quality, the use of hydrocolloids has attracted the attention of producers, which has led to the use of several types of hydrocolloids as additives globally (Yousefi and Jafari .., 2019 ; Seth *et al* ...,2018 ; Nguyen *et al* 2017). Therefore, the current study aimed to produce a low-energy yogurt by using skim milk and using gum extracted from chia seeds as a substitute for fat to obtain a yogurt with good qualities.

MATERIALS AND METHODS

The raw materials used in the research

Powdered skim milk (0% fat) of the type (REGAILIA) from the local markets of Al-Najaf Governorate was used. Strains of the bacteria *Lactobacillus bulgaricus Subsp delbrueckii and Streptococcus Salivarius Subsp thermophilus* produced by the Italian company SACO were used in the manufacture of yogurt treatments.

Manufacture of low-energy yogurt with adding different percentages of chia seed gum

Yogurt was made according to the method used by Tamime and Robinson., (1999). Skimmed milk powder was dissolved at a recovery rate of 12%, or 120 g / 1000 ml of water. The milk was divided into four equal parts, as the first part was left without any addition, as it was considered as control treatment (TC), as for the remaining three sections, different proportions of chia seed gum were added to them separately, at the rate of (0.1, 0.2, 0.3) g / 1000 ml of milk, represented by the treatments T1, T2, and T3 respectively. The samples of the treatments were mixed with an electric mixer to ensure their mixing well, and then the milk was exposed to a temperature of 90 C^o for 10 minutes, and then cooled to a temperature of 42 C^o. After that, all treatments were inoculated with the starter *Lactobacillus bulgaricus Subsp delbrueckii* and *Streptococcus Salivarius Subsp thermophilus* after activation with direct addition and with the quantity indicated by the producing company at a rate of 3%, and were filled in plastic containers of 150 ml and incubated at a temperature of 42 ±2 C^o until complete coagulation, then it was removed from the incubator and transferred to the refrigerator for cooling and preservation at a temperature of (5±1) C⁰ until the necessary tests are performed after 0,7, 14, and 21 days of manufacture.

Physical Tests of Yogurt

pH Determination

Estimate the pH of the yogurt samples by placing a pH meter sensor directly in the yogurt sample (A.O.A.C., 2012).

Determination of Total Acidity

The total acidity of the manufactured yogurt samples was estimated according to the method described in (A.O.A.C., 2008).

Rheological examinations of yogurt

Viscosity Determination of

The apparent viscosity of the yogurt samples was estimated at a temperature of 10 C⁰ after 0, 7, 14 and 21 days of refrigerated storage using a Brookfield DVII viscometer with a number of cycles of 10 revolutions / minute and a volume of 150 ml for the sample, leave the spindle to rotate inside the sample for 60 seconds after the gel has been broken by moving it 10 times clockwise and 10 times counterclockwise., and the reading was taken three times according to the rate in centipedes units (Donkor *et al*., 2007).

Determination of Whey Separation

The whey separation was estimated by putting 50 ml of yogurt in a cup at an angle of 45 degrees for two hours at a temperature of 5 C⁰. The exuded whey was withdrawn from the surface using a syringe, then the cup was weighed again, and the operation was performed within a period of 10 seconds to avoid excessive exudation, according to what he mentioned by (Amatayakul., 2006).

Determination of Water Holding Capacity

The water holding capacity was estimated by exposing 10 g of the yogurt sample to centrifugation at a speed of 3000 cycles/min for 60 minutes at a temperature of 10 C⁰, after which the filtrate was removed and the weight of the remaining wet sediment was calculated. The water holding capacity was calculated as a ratio between the weight of the remaining sediment and the weight of the original sample (Parnell-Clunies *et al*., 1986).

Microbiological Tests

Microbiological tests were conducted for the yogurt samples after (0, 7, 14, 21) days of refrigerated storage at a temperature of 5 °C, which included the total count of bacteria, coliform bacteria, Psychrophiles bacteria, yeasts and molds, by taking 10 grams of yogurt and adding it to 90 ml of peptone water. The sample was mixed well, then 1 ml was taken from it and transferred to the first test tube containing 9 ml of peptone water, thus obtaining the first dilution 1:10. Then 1 ml of the first dilution was transferred to a test tube containing 9 ml and mixed well to obtain the dilution, the second 1:100 and so on until reaching the seventh dilution (APHA, 1984).

Total Bacterial Count

The total number of bacteria was estimated according to the method of pouring using Nutrient agar culture medium, and the dishes were incubated at a temperature of 37 °C for a period of (24-48) hours, according to the method mentioned by Moghimi and Mogadam (2017).

Psychrophiles Bacteria The Total Count of

The total number of Psychrophiles bacteria was estimated according to the pouring method using Nutrient agar culture medium, and the plates were incubated at 7 °C for 10 days according to the method mentioned by Moghimi and Mogadam (2017).

The Total Count of Coliform Bacteria

The total number of coliform bacteria was estimated according to the pouring method using MacConkey Agar culture medium, and the plates were incubated at 37 °C for a period of (24-48) hours (Moghimi and Mogadam (2017).

Mold and Yeast Count

The total number of yeasts and molds was estimated according to the pouring method using Potato Dextrose Agar culture medium, and the dishes were incubated at a temperature of 22 ^{0} C for 5 days (APHA, 1978).

RESULTS AND DISCUSSION

Chemical properties of low-energy yogurt with adding different proportions of chia seed gum

pH

The results shown in Table (1) show the pH values of the control treatment (TC) yogurt, and the yogurt treatments that have different percentages of chia seed gum added T1, T2, and T3, as the value after processing directly for the treatment (TC) (4.74). The result is higher compared to what was found by El-Galeel *et al*. (2017) who indicated that the pH of the non-fat yogurt after processing was 4.43, and Sahan *et al*., (2008) indicated that the pH of the yogurt after processing was 4.40, while the values of the pH of the yogurt treatments that added different concentrations of chia seeds gum (T1, T2, T3) were 4.71, 4.69, and 4.67, respectively, but when storing, a decrease was observed in the pH values of all the treatments with an increase in the period of refrigerated storage, so the values were after 21 days for the TC treatment (4.48), and the yogurt with adding different percentages of chia gum (T1, T2, and T3), reaching(4.45, 4.44, and 4.31) respectively, and this is consistent with what Al-Zarfi (2021) indicated about a decrease in pH values during storage. The results of the statistical analysis indicate that there are significant differences ($P<0.05$) in the pH values between the control treatment and the rest of the gum-fortified treatments during the storage period of 21 days. The reason for the decrease in the pH during the storage period in yogurt is attributed to the continued activity of the starter bacteria during storage, but in a slow manner (Al-Sheikh., 2018).

Total Acidity Percentage

The results shown in Table (1) show the total acidity values (calculated on the basis of lactic acid) for the control treatment yogurt (TC) and the yogurt treatments with adding different concentrations of chia seed gum T1, T2, T3, as the total acidity after processing directly of TC treatment reached (0.85) %, and this result was similar to what was indicated by El-Galeel *et al*., (2017) that the total acidity of non-fat yogurt amounted to (0.80%). As for the total acidity of the yogurt treatments with added Chia seeds gum T1, T2, T3, it amounted to (0.82, 0.83, and 0.85)

% respectively. The results of the study indicated that there was no effect of adding Chia seeds gum on the total acidity percentage compared with the control treatment immediately after processing, and the results of the statistical analysis of the total acidity indicated that there were significant differences for all treatments with the advancement of the storage period. The total acidity of the (TC) treatment after 21 days reached 0.88%, while the yogurt treatments fortified with Chia gum T1, T2, and T3 reached after 21 days of storage (0.86, 0.88, and 0.90)% respectively. This is consistent with what was found by Shaghaghi *et al*., (2013) who indicated an increase in acidity from 0.78% on the first day to 0.92% at the end of the 28-day storage period.

Table (1) Chemical properties of yogurt treatments added to different percentages of chia seed gum

Treatment	Yogurt age (day)	% Acidity	pH
Control (TC)	0	0.85	4.74
	7	0.86	4.68
	14	0.86	4.54
	21	0.88	4.48
T1 (0.1 g of gum/1000 ml of milk)	0	0.82	4.71
	7	0.84	4.66
	14	0.84	4.48
	21	0.86	4.45
T2 (0.2 g of gum/1000 ml of milk)	0	0.83	4.69
	7	0.86	4.66
	14	0.87	4.50
	21	0.88	4.44
T3 (0.3 g of gum/1000 ml of milk)	0	0.85	4.67
	7	0.86	4.61
	14	0.88	4.45
	21	0.90	4.31
($P < 0.05$) L.S.D value		0.165	0.166

*Each number in the table represents an average of three replications
*($P < 0.05$) significant difference.

Rheological characteristics of fat free yogurt with adding different proportions of chia seed gum

Viscosity

Table (2) indicated the viscosity values of the control treatment (TC) and the yogurt treatments fortified with chia seed gum, as the viscosity value of the control treatment (TC) was (1310) centipoise, while the viscosity values of the yogurt treatments fortified with chia seed gum T1, T2, T3 increased significantly with an increase in the percentage of vegetable gum addition reached (1637.7, 1965.3, and 2260.3) centipoise respectively, as it is noted that the yogurt treatments fortified with chia seeds gum are superior compared to the control treatment, and this result is expected due to the addition of gum. The results of the table also indicated an increase in the viscosity values for all yogurt treatments with the progression of the storage period, as the viscosity value after 21 days of storage for the control treatment (TC) reached (1582.3) centipoise, while the viscosity values for the yogurt fortified with chia gum reached for T1, T2, T3 (1840, 2209.3 , 2541.7) centipoise, respectively, after 21 days, and this result is consistent with what was mentioned by Shaghaghi *et al*, (2013)who indicated an increase in the viscosity of the yogurt treatment from 2123 centipoise after one day of manufacture to 2307 centipoise after 21 days of refrigerated storage, and this may be due to the low pH of yogurt, which leads to an increase in its hardness and then an increase in viscosity (Walstra *et al*, 2006). Nowrouzi & Rashidi ., (2021) indicated that the viscosity value of yogurt that contains gum from belingo seeds at a rate of (0.1%) amounted to (1169.49) Pascal. sec. Achanta *et al*., (2017) indicated a significant increase ($P<0.05$) in the values of the viscosity of yogurt fortified with oat concentrate by increasing the percentage of adding protein concentrate.

Automatic Whey Exuding

Table (2) shows the automatic whey exudation of the control yogurt and the yogurt treatments fortified with chia seed gum, as the amount of whey exuded for the control treatment (TC) immediately after manufacturing was 9 .69 ml/100 ml, and for the yogurt treatments fortified with chia gum (T1, T2, T3) (6.63, 5.90, 5.30) ml / 100 ml, respectively, and it is noted from the results of the table that the quantities of whey exuded decreased upon storage, so the values after 21 days for the TC treatment were 9.14 ml / 100 ml, and for the treatments for yogurt fortified with chia gum were (6.23, 5.39, 5.06) respectively. Achanta *et al* ., (2007) indicated a decrease in the rate of whey exuded in selenium-fortified yogurt of 105 ml / 300 g compared to the control treatment of 67.121 ml / 300 g. The results of the table also indicated that there was a significant decrease in the amount of whey exuded with the progression period of refrigerated storage. This result agreed with the findings of Çelik, (2007) who indicated a decrease in the percentage of whey exuded of yogurt from 8.55% on the first day to 3.51% on the 21st day of storage, and attributed this to the metabolic activity of the starter bacteria and to the decrease in net pressure inside the protein mold, which reduces maturity (Guler and Akın ., 2007).

The results showed that the quantities of whey exuded for the yogurt fortified with chia gum were significantly lower compared to the control treatment, and this is expected because the addition of chia gum which will absorb the quantities of water and then reduce the exudation. The results of the statistical analysis indicated that there were significant differences ($P<0.05$) in the exudation percentage between the control treatment and the yogurt treatment fortified with chia seeds gum immediately after processing, as well as during the storage period of 21 days.

Water holding capacity

Table (2) shows the percentage of the water holding capacity of the control yogurt treatments (TC) and the yogurt treatments fortified with different percentages of chia seed gum T1, T2, T3, as it is clear from the table that the water holding capacity of the control treatment (TC) immediately after processing reached 48.30%, while the ability to hold water for the yogurt treatments with added Chia gum (T1, T2, T3) reached (50.10, 53.20, and 56.30%) respectively, as the yogurt fortified with gum excelled in its ability to hold water compared to the control treatment, as the ability to hold water increased with an increase in the concentration of chia gum. This indicates that fortifying the yogurt with chia gum increased the ability of the protein network to hold water compared to the (TC) treatment. The same table also indicated that there was an increase in the ability to hold water with an increase in the storage period, as the ability to treat the control (TC) after 21 days reached (53.30%), while the treatments for yogurt fortified with chia gum T1, T2, T3 were (55.20, 56. 20, 58.80%) respectively, and the reason for this may be due to the effect of the decrease in moisture content of the yogurt treatments (Al-Sheikh., 2018). The results of the statistical analysis indicate that there are significant differences ($P<0.05$) between the control treatment and all the treatments fortified with chia gum immediately after processing and also during cold storage periods.

Table (2): The rheological properties of the control treatment yogurt and the yogurt fortified with different percentages of chia seed gum during the storage period at a temperature of (5 ±1) ˚C for a period of 21 days

Treatment	Yogurt age (Day)	water Holding Capacity (%)	Automatic whey Exuding (ml/100 ml)	Viscosity (centipoise)
Control (TC)	0	48.30	9.69	1310
	7	50.11	9.36	1506.3
	14	52.00	9.24	1551.3
	21	53.30	9.14	1582.3
T1 (0.1 gram of gum/1000 ml of milk)	0	50.10	6.63	1637.7
	7	52.66	6.37	1752
	14	53.00	6.29	1804
	21	55.20	6.23	1840
T2 (0.2 gram of gum/1000 ml of milk)	0	53.20	5.90	1965.3
	7	54.30	5.85	2102.7
	14	55.30	5.79	2166
	21	56.20	5.39	2209.3
T3	0	56.30	5.30	2260.3
	7	57.30	5.17	2419

(0.3 gram of gum/1000 ml of milk)	**14**	**58.10**	**5.22**	**2491.7**
	21	**58.80**	**5.06**	**2541.7**
(P < 0.05) L.S.D value		**0.166**	**0.096**	**110**

*Each number in the table represents an average of three replications
*(P < 0.05) significant difference.

Microbiological tests of the yogurt fortified with different percentages of chia seed gum

Table 3 shows the results of estimating the total number of bacteria, the number of coliform bacteria, the number of Psychrophiles bacteria, the total number of yeasts and molds for the control (TC) yogurt and the treatments for yogurt fortified with chia seed gum (T1, T2 and T3) immediately after manufacturing and during storage at a temperature of (5 ± 1) °C for a period of 21 days. It is clear from the results that the total number of bacteria immediately after processing (TC) treatment was 8.20 logarithmic cycles. Al-Badrani (2016) indicated that the total number of full-fat yogurt was 60 x 10^7 cfu / g. As for the total number of bacteria for the yogurt treatments fortified with chia gum (T1, T2, and T3) immediately after manufacturing, it was (8.16, 8.13, 8.09) logarithmic cycle respectively.

The results of the table also indicated an increase in the value of the total number of bacteria for the control yogurt treatment with the progression of the storage period, as the value of the total number of bacteria after 21 days of storage for the control treatment (TC) was (8.79), a logarithmic cycle, while the values of the total number of bacteria for the yogurt fortified with chia gum T1, T2, T3 were (7.91, 7.89, 6.93) logarithmic cycle respectively. After 21 days, it is clear from the results that there is a decrease in the values of the total number of bacteria for the yogurt fortified with chia gum, the reason may be due to the presence of materials that work to withdraw water, so it becomes unavailable in a free form for microorganisms, which restricts its activity and inhibits its effectiveness in yogurt during the storage period (Fernondez, 1997), the reason may also be due to the inhibitory ability of the gum against some types of bacteria, which may have affected the growth of bacteria during the storage period. The results of the same table also indicated that the control yogurt treatment and the gum-fortified yogurt treatments were free of coliform, Psychrophiles bacteria and yeasts and molds during all periods of refrigerated storage, and the reason may be due to following the health conditions during manufacturing. The results of the statistical analysis indicate that there are significant differences (P>0.05) between the control treatment and all the treatments of yogurt fortified with chia gum immediately after processing, as well as during refrigerated storage at a temperature of (5 ± 1) °C.

Table (3)The microbiological tests of the control yogurt treatment and the yogurt treatments fortified with different concentrations of chia seed gum stored at a temperature of (5 ±1) ˚C for a period of 21 days.

Treatment	Yogurt age (Day)	Total count of bacteria	Total count of Coliform bacteria	Total count of Psychrophiles bacteria	Total count of yeasts and molds
Control (TC)	0	8.20	0	0	0
	7	8.35	0	0	0
	14	8.65	0	0	0
	21	8.79	0	0	0
T1 (0.1 gram of gum/1000 ml of milk)	0	8.16	0	0	0
	7	7.83	0	0	0
	14	7.89	0	0	0
	21	7.91	0	0	0
T2 (0.2 gram of gum/1000 ml of milk)	0	8.13	0	0	0
	7	7.78	0	0	0
	14	7.81	0	0	0
	21	7.89	0	0	0
T3 (0.3 gram of gum/1000 ml of milk)	0	8.09	0	0	0
	7	6.77	0	0	0
	14	6.83	0	0	0
	21	6.93	0	0	0
(P < 0.05) L.S.D value		0.1583			

*Each number in the table represents an average of three replications
*(P < 0.05) significant difference.

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