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Estimation of the Active and Functional Components of Basil Seed Gum Extract and their Use in Manufacturing of Yogurt

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A B S T R A C T

The current study aimed at extracting the gum of basil seeds and estimating its content of sugars and phenolic and active compounds, then studying the effect of its addition on some chemical and physical properties of yogurt made from whole milk (3 fat) during a storage period of 1,7,14 days . Gum on (D - Xylose, L-Arabinose, D-Glucose, D - Galactose, and D-Manose) was of percentages of 10.75, 10.71, 10.03, 7.56, and 2.82%, respectively. The results of the infra red spectrum analysis (FTIR) of the basil seed gum showed that it contained hydroxyl groups OH, the amine group - carbonyl groups C-HR and aldehyde groups CO. In contrast, the results of the diagnosis of phenolic compounds showed that the gum of basil seeds contained compounds of Chorogenic acid, Rutin, Gallic acid, Quercetin, and Ferulic acid Apigenin with percentages of 23.22, 18.24, 18.22, 17.98, 16.5, 13.04 and 11.42%, respectively. Besides, the addition of basil seed gum to the yogurt improved its properties as it decreased the pH values, moisture and whey separation while there was an increase in viscosity.

Introduction

The use of aromatic plants dates is back to ancient times as foodstuffs. They are used fresh or dry and have many forms of use, including various plant parts such as leaves, roots, flowers, seeds and peels, as well as crushed forms and extracts prepared from them(Alezandro,et al.,2011). Among these plants is Ocimum basilicum L. It is a common herbal plant that is consumed in large quantities due to the distinctive flavors it adds. Also, its leaves are especially consumed as food or as an additive to foods.

Basil seeds are a good source for obtaining gum, as when basil seeds are soaked in water, they swell and separate into a gelatinous mass because this layer contains polysaccharides, which produces a gum called basil seed gum(Osano,et al.,2014).BSG Basil seed gum BSG is a natural and new source It has great potential in various fields of the food industry.

Food products are based on foaming and emulsifying because it has high stabilizing and emulsifying properties, which makes it one of the important functional ingredients, and it can act as a foam stabilizer by increasing viscosity. Thus, BSG basil seed gum can be used in the food industry as a natural substance added to improve the structural properties of various foods(Razi, et al.,2019).

Yogurt occupies a large part in the dairy products market in terms of widespread consumption worldwide and for different ages and health groups. It is a fermented milk that can be added to the list of health products because it contains lactic acid bacteria that effectively contribute to the treatment of many intestinal disorders. In addition to its high nutritional value, and because it contains proteins, fats, and essential minerals, it can also be considered a low-calorie food that is eaten for health purposes (Fu,et al.,2018).

Dairy products are among the unique nutrients that have successfully been used to the manufacture of functional foods by adding herbs, spices, extracts, or other nutrients with health benefits to various dairy products (El-Sayed, & Youssef., 2019) .

Material and Methods

1-Extracting the Gum through Boiling Water:

The extraction was done by mixing basil seeds purified from impurities with boiled distilled water at a temperature of 100 °C for 30 minutes using a (Magnetic Stirrer Hotplate) in proportions (40-1)(and\ h). Then, the mixture was cooled to room temperature and then filtered by gauze. After that, ethanol was added at a concentration of 95% at a ratio of 2:1 (leachate/ethanol) (h / h), and the mixture was left for 24 hours at a temperature of (5°m) in the refrigerator. The separation was carried out in a centrifuge under refrigeration at a speed of 4500 rpm for 30 minutes. The precipitate was dissolved with a little distilled water and adjusting the pH to 7 using a sodium hydroxide solution (Noah) at a concentration of 0.5 M. Then, dried at 40°m for 24 hours, then grinding the gel with a grinder and keeping the powder in a glass containers airtight(Singer, et al.,2011).

1-1: Determination of Phenolic Compounds by High-Performance Liquid Chromatography (HPLC).

According to Ngamsuk et al., (2019) three gm of crushed basil seed gum samples were well mixed with 60 ml of methanol/water (60/40) within 24 hours. Then, the mixture was filtered and the filtrate was concentrated under low pressure and temperature (40°) to a volume of 5 ml. The solution was analyzed with 2 ml of(NaOH) for 30 minutes, and the(pH) of the mixture was adjusted to 7 with 2NHCL. The phenolic acids were extracted by liquid-liquid extraction using ethyl acetate under low pressure, then the residue was dissolved in 7 ml of methanol and 10 microliters and then analyzed by HPLC.

Separation conditions included a Sykamn Hplc system (Germany) equipped with c18-ods(5m * 250 mm *(4.6)), then injected (100) µl of samples into the mobile phase system consisting of trifluoro acetic acid%0.01 + acetonitrile%95 trifluoroacetic acid (solvent (A)) and 5% acetonitrile + 0.01% trifluoroacetic acid (solvent (B) at1 ml/min.The internship program was as follows:

10%A from 0-5 minutes

25 A from 7-5 minutes

40%A from 7-13 minutes

Then back to the initial conditions, the detection of phenolic compounds was carried out using a visual detector for ultraviolet radiation at 278 nm, according to the following equation:

$$C_{sam} = \frac{C_{st}}{A_{st}} * \frac{A_{sam}}{Wt_{sam}}$$

C_{sam}: sample concentration

A_{st}: area of the standard form

A_{sam}: sample area.

D.F: Final size of the form

W_t: weight of the sample.

1-2: Determination of Monosaccharides by High-Performance Liquid Chromatography (HPLC):

According to Herchi,et al.(2012), one gm of basil seed gum was dissolved in 10 ml of methanol solution. The solution was mixed well, then mixed in a bath with ultrasonic waves for 10 minutes. The extract was then filtered through a filter with apertures of 0.2 microns and µL of filtrate into the

device column blue 20. The separation conditions that were used for the determination of sugars included column type 4.63×50 mm particle size 3 µm either. The mobile phase was a mixture of acetonitrile and deionized water in proportions 25:75 (h/h). The detection was done using a Refractive Index detector and the flow rate was 1.5 ml/min at a temperature of 25 °C. The values were obtained from quantitatively determining each sample's concentration by comparing the standard samples' apex area with the model's apex area, according to the equation.

$$\text{Sample concentration Dilution factor} * \frac{\text{Sample apex area}}{\text{apex area of the standard model}} = \text{Sample concentration}$$

(µg/mL) The.

1-3: Identification of Effective Functional Groups by FTIR:

The functional groups of the samples of basil seeds were identified using FTIR infrared spectrum analysis, by making tablets from the samples with potassium bromide (KBR) by mixing 40 mg of gum powder with 120 mg of KBR and mixing well with a ceramic mortar for 10 minutes, then taking 40 mg of the mixture and pressurized with a hydraulic press of the FTIR device at a pressure of 8 bar for 60 seconds. After which, the CDs were placed in a dryer inside an oven at 80 °C for 16 hours before being analyzed by the FTIR device at a frequency of 400-4000 cm - 1, and this is according to what reported by (Martinez,et al.,2017).

2: Manufacture of Yogurt:

1-2: Activate the starter:

The starter Yogurt Full, which is used in manufacturing milk is composed of bacteria having (*Sterp.thermophilus* + *Lact.bulgaricus*). This is done by taking (10) grams of skimmed milk powder (0% fat) in 100 ml of distilled water, heating it to a pasteurization temperature of 85°C, then letting it cool down to 40°C. The starter culture was then added to it at a ratio of 2-3%.

2-2: Manufacture of full-fat Yogurt:

Yogurtwas made according to Tamime & Robbinson.,1985 where we filtered raw cow's milk (full fat 3.3%) by means of gauze to get rid of impurities, treated it at 85 m for 15 minutes, then divided the milk into three parts for each basil seed gum and a sample Comparison; viz. Cooling the milk to a temperature of 60-65 °C, adding the proportions of basil seed gum (0.2% and 0.5%), while continuing to stir to dissolve it, cooling the milk to the incubation temperature 42-40 °C. 3% starters were added and mixed well, then placing the samples in the incubator for 3-4 hours at a temperature of 40-42°C. It is removed from the incubator and kept refrigerated until the necessary tests were done.

3: Chemical Estimates of Yogurt:

3-1: Moisture Determination:

According to the method mentioned in AOAC (2008), the moisture content of the Yogurt samples to which the gum of basil seeds was added and the comparison samples were estimated by using the drying oven at a temperature of 100 °C until the weight is stable, using the following equation:

$$\mathbf{100*} \frac{\text{Weigh the sample with the dish before drying} - \text{Weigh the sample with the dish after drying.}}{\text{the weight of the sample before drying}} = \%\mathbf{Moisture}$$

3-2: Fat Determination

Mindfulness was estimated according to what mentioned by the Kerber method(Ling.,1963), where we put 10 ml of sulfuric acid (H2SO4) in a Kerber tube in a manner that quietly touched the walls of the tube, we added 11 ml of the milk sample, we added 10-12 ml of distilled water, we added 1 ml of amyl alcohol with cleaning the nozzle of the tube and tightly closing the tube, then we slowly moved the tube to mix the ingredients with.

3-3: Determination of pH:

According to what was mentioned by Emeje et al.,(2011), the pH value was estimated using the pH meter by inserting the entire electrode into a sample of milk and then reading the device.

3-4 Protein Determination

Based on the method mentioned in AOAC., 2008 using the Micro Kiel Dahl method which was conducted in the central laboratory belonging to the College of Agriculture/ University of Mosul, by using a conversion factor of 6.38 to extract the percentage of protein in the raw material and according to the equation :

protein% =nitrogen *6.38

nitrogen

$$\% = \frac{(\text{Plank work} - \text{sample in ml}) \text{ x } 0.1 \text{ x } 0.014}{\text{weight of the sample (un)}} * 100$$

4: Physical determinations of yogurt:

1-4: Whey Separation (synersis):

According to what was mentioned by Amatayakul et al. (2006), urinary perfusion was estimated by placing the milk cartons at an angle of 45 for 60 minutes at a temperature of 5C and

withdrawing the separated in using a medical syringe weighing the box again.

$$100* \frac{\text{weight of the sample (un)}}{\text{of the model before drawing}} = \text{the } \textit{weight}\%.$$

4-2: Determination of Viscosity:

According to Donkor et al. (2007) with some modifications, the viscosity of yogurt samples was estimated at 10 °C during the storage periods on the days 1, 7, and 14, by using a device (Brook Field Engineering Lab Inc. Stoughton Mass) using spindle No. 3 with a number of basil seeds at the beginning by soaking the basil seeds in water (1g of seeds / 35ml water at a temperature of 69°C and 6 PH, but we noticed that the percentage of a quarter was low. Thus, we changed the temperature of the water for soaking the seeds to 100°C and PH. This, in turn, gave a percentage a higher quarter, and the centigrade unit took the reading.

Results and Discussion

Basil Seed Gum Extract:

The process of extracting the gum from the basil seeds was carried out with boiling water at 100°C, and this in turn gave good results. So every 100 gm of basil seeds gave a quarter rate of 1.288%. This means that the amount of 1.580 kilos of basil seeds gave 20.3566 gm of basil seed gum. Our results agreed with Razavi, et al. (2009) through their study of the optimal conditions for extraction of basil seed gum which found that the amount of soaking water did not have a significant effect on the percentage of the quarter, while the temperature and PH had a significant effect on the percentage of the quarter and the quality of the extracted gum, as the higher the temperature from 85 and the PH from 7, the percentage increased by a quarter and the properties of the extracted gum were good. And this was confirmed by Hossein-Parvar,et al. (2010) that the extraction temperature has a significant effect on the quantity and quality of the gum produced. It was also noted that the extracted gum was brown , and this may be due to the presence of tannins, according to what was mentioned by Barbary et al. (2009),or it may be due to the difference in the chemical composition of the seeds and their nature, in addition to the difference in the extraction solutions and the difference in the temperature used in the extraction. Also, these results came in agreement with Somboonpanyakul,et al's., (2006) that the pH effected the yielding of the extracted gum. As the reason may be due to the increase in the pH, the carboxyl groups and their association with water molecules increased. It is important to note that the extracted gel with boiled water which was precipitated with ethanol. It was dark in color, and this may be due to the different cultivars extraction and sedimentation condition.

Identification of Phenolic Aggregates by HPLC:

Table (1) shows the results of the HPLC which showed many phenolic compounds in the gum of basilseeds
as shown in Figure (1). Ferulic acid showed the lowest percentage compared with other compounds 11.42%, Gallic acid 18.24%, Rutin 18.22%, Apigenin 13.04%, Chlorogenic acid 17.98%, and Catechine 16.5% performance on some phenolic compounds and determination of their proportions. Our results agreed with Shaygannia,et al's.(2021) as they confirmed that basil seed gum contained gallic acid at an estimated rate of 16.87 mg / g. Calderón Bravo, et al.(2021) confirmed that basil seed gum contained gallic acid and several phenolic compounds in different proportions and this was in agreement with our results.

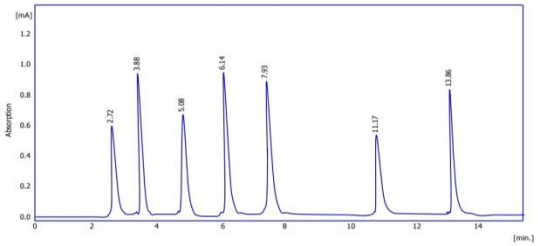

Figure 1. Identification of phenolic compounds by high-performance liquid chromatography (HPLC) of basil seed gum.

Table 1. Phenolic compounds and their ratios in the gum of basil seeds.

%Percentage	Phenolic compounds
23.22	Qurcetine
18.24	Gallic acid
18.22	Rutin
17.98	Chorogenic acid
16.5	Catechine
13.04	Apigenin
11.42	Ferulic acid

Identification of Monosaccharides by High Performance Chromatography (HPLC).

Table (2) shows the components of the gum of basil seeds from monosaccharides using high-performance chromatography (HPLC), which are shown in the diagram Figure (2) for the gum of basil seeds. Basil seed gum contains xylose and arabinose sugars, which were in high proportions as they reached (10.75 and 10.71%, respectively),

while the content of mannose was the lowest among sugars compared with the rest of the sugars, reaching 2.82%. lactose.%7.56.

These results agree with what was indicated by Hosseini-parvar et al. (2010), that the high glucose content compared with mannose sugar in basil seed gum explained the high solubility of this gum.

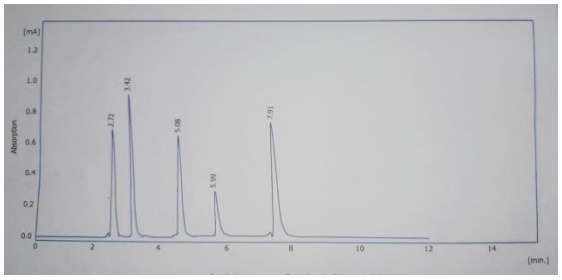

Figure 2. Results of monosaccharide analysis of basil seed gum using high-performance liquid chromatography (HPLC).

Table 2. Monosaccharide analysis of basil seed gum %.

%Percentage	Sugar
10.75	D-Xylose
10.71	L-Arabinose
10.03	D-Glucose
7.56	D-Galactose
2.82	D-Manose

Diagnosis of Functional Aggregates by FTIR:

The (FTIR) test was carried out for the purpose to diagnosing and determining the effective functional aggregates of the gum. The results of the diagnosis of the gum of basil seeds using the FTIR (spectroscopy) technique, which is illustrated by the spectrogram in Figure (3), showed the appearance of curves with different frequencies. This technique is a method that can be used to study the functional properties to reveal the structural composition of the gum as the absorbance triangle at a frequency of 3419.79 cm 'hydroxyl groups (OH) of the gum of basil seeds. This is considered an important functional group in monosaccharides for water solubility and stability the structural structure of the internal structure of the molecule and at the absorbance, 2926.01 cm' the appearance of amine groups N-H. And this indicated the presence of protein in the gum and when the absorbance is 2862.36 cm, it showed the apperance of asymmetric aliphatic H- groups and the appearance of the symmetrical aliphatic CH group at an absorbance of 1423.47 cm, which increased the affinity for water molecules. The gum also contains carbonyl CO groups at an absorbance of 1627.92 cm. And our results agreed with(Gahruie, et al.,2017 ;Ghumman. et al.,2022) when estimating the effective functional groups of basil seed gum and confirming that the gum contains the protein by the appearance of an amine group.

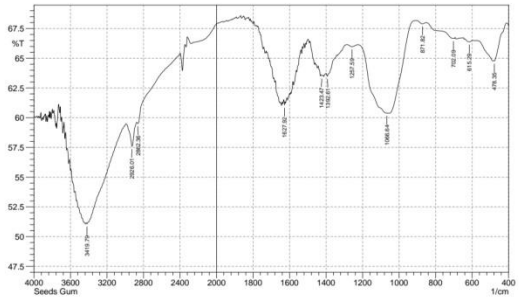

Figure 3. The spectrogram of basil seed gum extracted using (FTIR-Spectro scopy) method. Moisture determination.

Moisture Value:

Table (3) shows that the highest moisture value was recorded on the 14th day of the storage period, which amounted to 89.32%. It is clear that the samples to which basil seed gum were added at a rate of 0.2 and 0.5% recording lower results for humidity. And this confirmed what (Hussain,et al.,2019) mentioned that the gum works to sequester water interacts with moisture, which reduced the percentage of moisture in samples containing gum.

Table 3. Percentage of moisture in yogurt to which basil seed gum was added.

Basil seed gum 0.5	Basil seed gum 0.2	Comparison sample	Transaction Tode
86.32 ab	87.30 ab	ab88.86	1 day
87.62 ab	86.32ab	87 .32ab	7 days
88.11ab	87.40 ab	89.32a	14 days

Similar letters indicate that there are no significant differences at the probability level of 0.05

Fat Determination

Table (4) percentage of fat in the yogurt samples was made from full-fat milk. There are no significant differences between the comparison sample and the samples to which basil seed gum was added at the rates of addition of 0.2 and 0.5%. However, some slight increases were observed in the percentage of fat in the samples to which the gum of basil seeds was added, and the highest value of fat was recorded in the sample of milk to which the gum of basil seeds was added by 0.5%, and this agrees with what was mentioned by (Connolly et al. 2013;Wen et al., 2019), showing

that the fat in BSG replaces the milk fat and increases the fat content.

Table 4. percentage of fat in full-fat yogurt samples to which basil seed gum was added

Basil seed gum 0.5	Basil seed gum 0.2	Comparison sample	Transaction Tode
3.500 a	3.400a	3.300a	1 day
3.500 a	3.400 a	3.300a	7 days
3.500a	3.400 a	3.300a	14 days

Similar letters indicate that there are no significant differences at the probability level of 0.05

PH Determination:

Table (5) shows the results of analyzing the pH values. It is noticed that there are no significant differences among the samples. It is also noted that the addition of basil seed gum BSG led to a slight decrease in the pH. This confirms what was mentioned by (Tamime & Deeth,1980) because the pH in the curd is affected by total solids content, pH, and fermentation temperature.

Table 5. pH values for yogurt samples to which basil seed gum was added

Basil seed gum 0.5	Basil seed gum 0.2	Comparison sample	Transaction Tode
4.60 a	4.70a	4.80 a	1 day
4.80 a	4.70 a	4.90 a	7 days
4.80a	4.70 a	4.50 a	14 days

Similar letters indicate that there are no significant differences at the 0.05 probability level

Protein Determination

Table (6) shows that there are significant differences among all the values of the samples of the manufactured yogurt to which the gum of basil seeds was added at the rates of addition of 0.2 and 0.5%. During the storage period of 1, 7 and 14 days, we noticed that the highest value was recorded in the samples of full-fat yogurt to which the gum of basil seeds was added. By 0.5%, the values during storage period 1, 7, and 14 were 21.88, 21.89, and 21.90, respectively. This agrees with what was mentioned by(Naibaho & Korzeniowska, 2021) that adding BSG at several levels improves the protein level.

Table 6. shows the percentage of protein in full-fat yogurt samples to which basil seed gum was added

Basil seed gum 0.5	Basil seed gum 0.2	Comparison sample	Transaction Tode
21.88a	21.06defg	20.82i	1 day
21.89a	21.08cdefg	20.84hi	7 days
21.90a	21.09bcdef	20.86ghi	14 days

Similar letters indicate that there are no significant differences at the 0.05 probability level

Whey Separation (synersis) Value:

Table (7) shows the results of whey separation, where it was noted that there are significant differences between the samples and during the days of storage. The highest value of whey separation was observed in the comparison sample on the 14th day of storage when it was recorded at 23.92. It was noticed that the addition of basil seed gum (BSG) to the curd samples significantly reduced the whey separation rate, and this agrees with what was mentioned by (Rafe et al.,2013) where he explained that the addition of basil seed gum BSG made the gel network finer and the pores smaller. this allowed water to hang, i.e. its ability to retain water increases, and the gel becomes more solid. The higher the percentage of BSG, the greater the ability to retain water, as well as the fat content effect, as the higher the fat content, the greater the ability to retain water, as the fat content causes the decrease in the increase in urethral separation due to the weak structure, and this is consistent with what he mentioned (Akgun ,et al.,2016) .

Table 7. Percentage of whey separation for yogurt samples to which basil seed silage was added.

Basil seed gum 0.5	Basil seed gum 0.2	Comparison sample	Transaction Tode
6.65j	9.18 ij	15.93 c-f	1 day
10.61hi	13.11 fgh	19-54bc	7 days
15.28def	18.09 bcd	23.92 a	14 days

Similar letters indicate that there are no significant differences at the 0.05 probability level.

Viscosity Value:

Table (8) shows the results of viscosity estimation. It is noticed that there are significant differences among all values. The highest value was recorded in the curd sample to which basil

seed gum was added by 0.5% and on the 14th day of storage, as the BSG contains fibrous fibers that have a spherical structure resembling cotton, the mucilage is a gelatinous network, and this agrees with what was indicated by (Rafe, et al.,2013) .

Table 8. shows the viscosity values of yogurt samples to which basil seed gum was added.

Basil seed gum 0.5	Basil seed gum 0.2	Comparison sample	Transaction Tode
1640.00d	1150. 00 k	870 .00 n	1 day
1780.00b	1600.00e	1060 .00m	7 days
1840.00a	1740. 00c	1140 .00 l	14 days

Conclusion

It was concluded that basil seed gum BSG gave good results from the rheological point of view of the manufactured product, as it worked to reduce whey separation and moisture content of the samples and worked to increase viscosity and added a high nutritional value to what it contains of components

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Competing Interests
There are no competing interests.

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