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Preliminary Phytochemical Evaluation and chemical Composition of Gum Isolated from Fenugreek Seeds

Bushra B. G. Al-Shamari, Rawdhah M. A. Al-Ali, and Ali A. S. Al-Sahi

Department of Food Sciences, College of Agriculture, University of Basrah, Iraq

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Abstract:

The present study aimed to extract the gum from fenugreek seed and studying the chemical composition, the preliminary phytochemical, and the flow properties. The extraction was conducted under different conditions (temperature, soaking period, and mixing ratios of seeds: water). The highest yield was of the fenugreek gum was 30.44% around 1:25w/v at 40 C° for 4 h. Preliminary qualitative chemical test for gum shows the presence of carbohydrate, protein, amino acids, fixed oil, fats, and Ruthenium red while Arabic gum and Acacia showed that carbohydrates were only found, the fenugreek gum had 9.4 % moisture, 2.3 % ash, 2.49% protein and 0.9% fat. The total polysaccharide content of isolated gum was determined spectrophotometrically using the phenol–sulphuric acid method. The total polysaccharide content was 84.75%. Arabic gum and Acacia had 5.44, 5.56 % moisture, 3.74, 2.975% Ash, 0.0% protein, 0.0% fat and 90.80, 91.25% carbohydrates. The gum showed good flow properties it has good viscosity of 72.40 CP compared to the Arabic gum and Acacia 9.1, 9.61 CP higher concentration of NaCl and CaCl caused a larger decrease in viscosity, increasing or decreasing the pH and temperature led to the decrease of viscosity, and micrometric properties such bulk density, tapped density, Hausner's ratio, Carr's index, particle size and Angle of repose Bulk density was found to be 0.37 g/ml, tapped density was 0.58 g/ml, Carr's index was 36.20%, Hausner's ratio 1.56 and Angle of repose was found to be 34.02.

Keywords: Fenugreek seeds gum, yield, Phytochemicals, Extraction, chemical analysis

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Introduction

Fenugreek is an erect annual plant, botanically known as *Trigonella foenum graecum* (Family: Leguminosae). The seed of fenugreek contains 3-6% moisture, 25-30% protein, 20-25% insoluble fibers, and 3-4% Ash. The potential fenugreek gum could be significant in the food industry such as a spica vegetable and medicinal plant (Roberts et al, 2012). Fenugreek gum is extracted with water or dilute alkali from the endosperm or ground whole seed. From

various reports the yield can be from 13.6 to 38%, depending on cultivar and extraction procedure (Repin, 2016). The galactomannan is polysaccharides composed of linear (1-4) – D- mannan backbone with varying amounts of single D-galactose units attached to the main backbone by (1-6) glycosides bond (Kay, 2016). Fenugreek seed produces high viscosity gum at low concentration levels which do not dissolve in water and form a viscous tacky mass when exposed to fluids (Kumar *et al.*, 2014). Fenugreek gum also has a

strong surface activity in solution, making it a strong emulsifier and stabilizer for oil in water emulsions. In the food industry, fenugreek gum is less utilized for its thickening, stabilizing, and emulsifying properties, compared to other gum, however, it is an ideal candidate to be used in baking, bread making, gravies and soups, chocolate, and ice cream (Seetharaman *et al.*, 2016; kay, 2016). Has been extensively used in the pharmaceutical industry as a binder, gelling agent, emulsifier, and suspending (Mundhe et al, 2012).

The aim of the study is to study the effect the extraction was conducted under different conditions (temperature, soaking period, and mixing ratios of seeds: water). on the yield gum, a chemical composition such moisture, protein, fat, ash and carbohydrate, micrometric properties such bulk density, tapped density, Hausner's ratio, carr's index, particle size and angle of repose

Material and methods

Fenugreek seeds was obtained from the local market in Basrah city. The seeds were powdered using a coffee grinder. Arabic gum was obtained from BDH (England). All the other solvent reagents and chemicals used were analytical grades.

Extraction of gum

The fenugreek seeds gum was extracted by the method described by Nazni and vigneswar (2014). The fenugreek powder was soaked in distilled water in a ratio (1:10 w/v) and kept under shaking for 4h at 40 C°, the viscous solution obtained was filtered through a muslin cloth. Ethanol (99%) was added in the ratio 1:1 to precipitate out gum present the gum was dried in the oven at temperature 40-45 C°. The dried gum was stored in an airtight container.

Effect of Extraction condition on the Recovery.

The effect of extraction was analyzed. The extraction conducted under different conditions and the gum yield was calculated. The best dilution with maximum gum yield was selected for further analysis using different soaking periods such 4, 6, and 8 h, and gum yield in percentage was analyzed.

Extraction conditions

Powder : water	Temperature
1:10	40 C°
1:15	60 C°
1:20	80 C°
1:25	

Chemical analysis

Determination of moisture content. The extracted gum was dried in an oven for 3 h at 105 C° according to the following AOAC (1984).

Determination of protein content. Nitrogen was determined by a semi-micro khjeldan (Pearson, 1970). Protein content was determined by multiplying nitrogen percent by the factor 6.25.

Determination of Ash content. The ash content was determined according to AOAC (1984) method oven-dried gum was weighted and was ignited in a furnace at 550 C° until light gray Ash produced.

Determination of Fat content. Fat was determined using soxhlet according to AOAC (1984).

Determination of polysaccharide content. The total polysaccharide contents of fenugreek gum were determined by using the phenol – sulphuric acid method according to the following Pawar & Jadhav (2015).

Preparation of standard curve

Preparation of blank solution: To a test tube containing 1 ml of distilled water, 1 ml of 5% phenol was added followed by the addition of 5 ml of concentrated sulphuric acid.

Preparation of standard solution

A stock solution of 100 mg/ml of glucose was prepared by dissolving 0.01 g of the standard glucose in distilled water (100 ml) Aliquots were withdrawn from the stock solution to get sugar concentration 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml and 100 mg/ml to every 1 ml of sugar solution, 1ml of 5% phenol solution was added followed by addition of 5ml of concentrated sulphuric acid. The absorbances were determined after 10 minutes at 488nm against blank.

Preparation of gum solution

0.1g of gum powder was dissolved in 100 ml of distilled water. From this 1 ml was used for sugar analysis, to determinate the polysaccharide content in

fenugreek seeds gum and Arabic gum, and Acacia. 1ml of 5% phenol added to the 1ml of gum solution followed by the addition of 5 ml concentrated sulphuric acid. The absorbances were determined after 10 minutes at 488 nm against a blank. The experiment was carried out in triplicate (i.e Test-1, Test-2, and Test-3)

Determination of purity of fenugreek gum.

Preliminary tests of gum were performed to confirm the purity of gum obtained according to Poonkotha and Selvis (2015). Assie tal, (2017)

Test for carbohydrate

a- Molisch test

This test is used to confirm the presence of carbohydrate in the mucilage 1ml of 1% gum solution has been placed in a clean test tube, then, two drops of the freshly prepared molisch reagent were introduced. Finally, concentrated sulfuric acid has been added gradually to the side of the tube to form a layer above the aqueous solution. A violet ring was observed at the junction of two layers

b- Barfoed s test: To the 1% gum solution 1ml of barfoed reagent was added and boiled in a water bath for 5. Brick-red precipitate formation shows the presence of carbohydrate.

c- benedict s test : To the 1% gum solution added 1ml Benedict s reagent and boiled in a water bath for 5. green reddish-brown precipitate indicates the presence .

d- Bial test: to the 1% gum solution added 3 drops of HCl and 1.5 ml bial reagent and boiled in a water bath for 3. The appearance of green color indicates the presence of fifty sugars.

e- Iodine test: To the 1% gum solution added 1ml 0.2 N iodine solution in a test tube and the mixture has been observed no color was developed in solution confirm the presence of starch.

Red ruthenium test

0.1 g of dried gum was placed on a slide with 3 drops of red ruthenium solution was added and the whole has been observed under a microscope appearance of pink color indicates the presence plant gums

Test for Amino acid/protein

Ninhydrin test: Heated the 1 ml of 1% gum solution and 1 ml of Ninhydrin solution in a boiling water bath for 10 minutes. The appearance of purple color shows the presence of amino acids

Test for phenolic compounds: To 1 ml of 1 % gum solution added 1 ml of 1% neutral ferric chloride solution formation of blackish-blue color indicates the presence of phenolic compounds.

Test for alkaloids

Wagner s test: To 1 ml of 1 % gum solution was added 2 drops of Wagner reagent. formation of reddish-brown precipitate indicates the presence of alkaloids

Test Flavonoids

Alkaline reagent test/NaOH test: To 1% gum solution added 2 drops of sodium hydroxide solution. Appearance yellow color which disappeared after adding 0.5N HCl indicates the presence of flavonoids.

Test for glycosides

Borntrager s test: Added 3 drops of 1N sulphuric acid to 1 ml gum solution. Boiled and added 1 ml of chloroform, the chloroform layer is treated with 1 ml of ammonia, the formation of red color of the ammonical layer shows the presence of glycosides.

Test for fix oils and fats

Saponification test: To 1 ml of gum solution added 2drops of 0.5N alcoholic potassium hydroxide along with a one drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours, the formation of soap indicates the presence of fixed oils and fats

Test for sterols

Salkowski test: 1 ml of concentrated sulphuric acid was added to 1 ml of 1% gum solution and mixed well. 1 ml of chloroform was added, the appearance of red color in the lower layer indicates the presence of sterols

Evaluation of micromeretic studies of gums

Viscosity measurement

The flow time for a 1% aqueous solution of fenugreek gum was determined using capillary viscometer size C at 25 C° Yusuf (2011). The relative viscosity was calculated using the equation

Relative viscosity (25C°) = $(T-T^{\circ}) / T^{\circ}$

Where: T is the flow time of gum solution (sec.)

T° is the flow time of distilled water (sec.)

Bulk and tapped densities

A 2 g quantity of each of the powder was placed in a 10 ml measuring cylinder and the volume, V0 occupied by each of the samples without tapping was noted. After 100 taps on the table, the occupied volume V100 was read according to Emeje *et al.* (2011).

Hausner index

This was calculated as the ratio of tapped density to bulk density of the sample according to Farooq *et al.* (2014).

Compressibility index

This was calculated using the following equation according to Farooq *et al.* (2015).

Compressibility = $\frac{\text{Tapped density} - \text{Bulk density}}{\text{tapped density}} \times 100$.

Angle of repose

The angle of repose was calculated according to Farooq *et al.* (2015), finely powdered 5g was passed through a funnel fixed on a stand at a specific height upon graph paper according to Farooq *et al.* (2015), and calculations were done as per equation

$$\tan \theta = \frac{\text{Height}}{\text{radius}}$$

$$\theta = \tan^{-1} \frac{h}{r}$$

θ = angle of repose

h= height of pile

r=radius of pile

Particle size determination

Dried Powdered gum was determined using an optical microscope according to Gupta *et al.* (2015), and calculated using the equation.

Size of the particle = No. of Particles in eyepiece \times Calibration factor

$$\text{Calibration factor} = \frac{\text{Stage reading}}{\text{ocular reading}} \times 0.01$$

Effect of temperature, pH, and electrolytes on viscosity

Temperature

To determine the effect of temperature on viscosity of 1% w/v of fenugreek gum at 25 - 80 C° according to Norbrillinda *et al.* (2014)

PH

To measure the viscosity at different pH (3-10). PH of 1% solution of fenugreek solution was measured using pH meter at 25 C° with pH 3 and 10 buffers for calibration, according to Norbrillinda *et al.* (2014).

Salt

The effect of chloride calcium and chloride sodium (0.2-1) molar on the viscosity of 0.5% solution gum according to Elijah *et al.* (2010).

Statistical analysis

All the chemical analysis were made triplicate. These data were analyzed using SPSS 2012, analysis of variance (Anova). Comparative analysis between parameters was carried out by L.S.D. With probability (P < 0.05).

Results and Discussion

The results are illustrated in Figure 1 depicted the effect of ratios of powder: water on the yield of Fenugreek gum. The highest yield was obtained from 1:25 w/v 20.44% whereas the minimum yield was observed for 1:10 v/w. The result showed significant differences in yield content in the fenugreek gum. These values are lower than those previously reported by Kumar *et al.*, (2009). That the yield of fenugreek gum was 31% for 1:10 effect temperature.

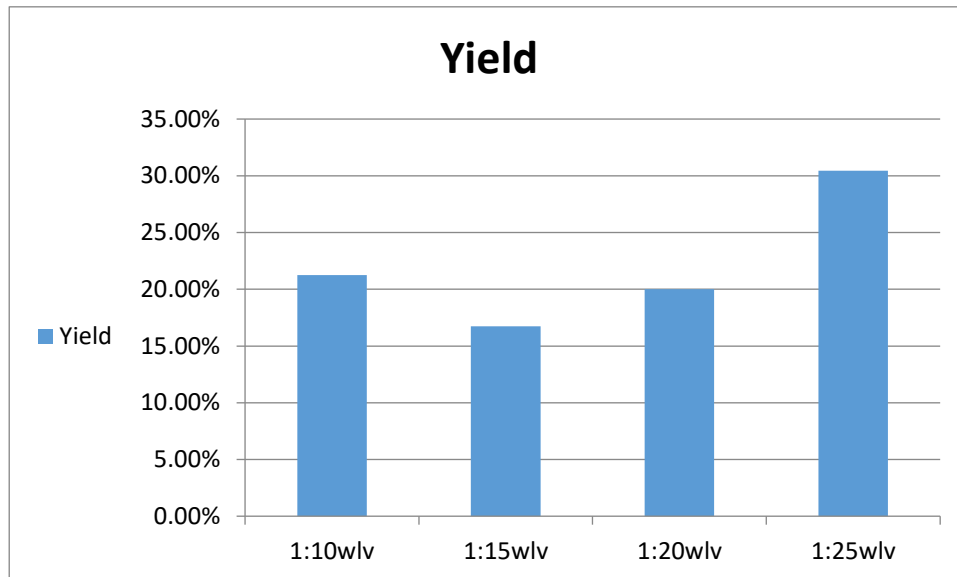


Fig1. Effect ratio of powder fenugreek seed: water on the yield.

The results are presented in Figure 2 indicate the effect of different temperature levels 40, 60, and 80 C° on the yield content of fenugreek gum. Fenugreek gum shows a higher yield at 40 C° (30.44%) while the lower yield at 80 C° (21.84)% which clearly shows that temperature has a significant effect on the yield content, increase of temperature has broken the chain of gum

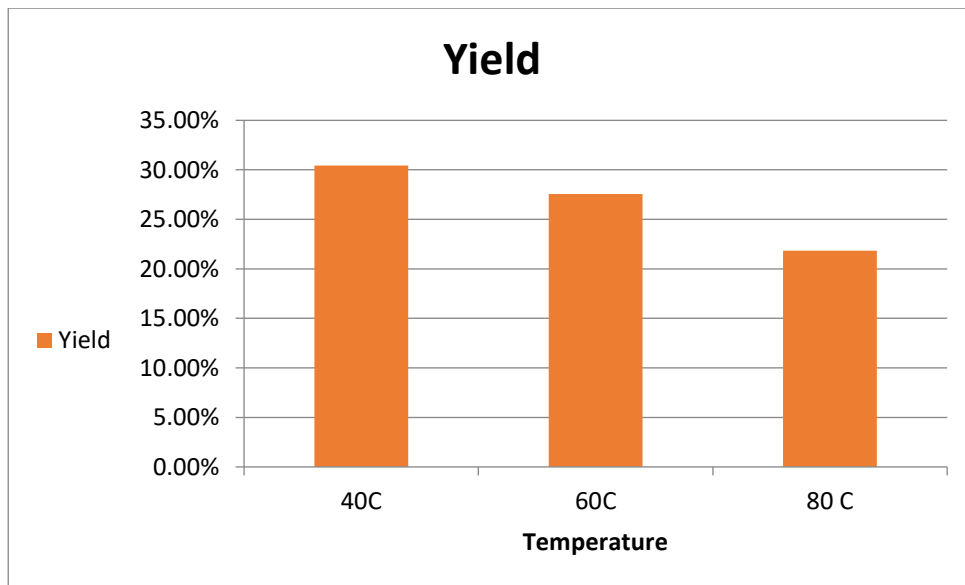


Fig2. Effect of different temperatures on the yield.

The results are presented in Figure 2 indicate the effect of different temperature levels 40, 60, and 80 C° on the yield content of fenugreek gum. Fenugreek gum shows a higher yield at 40 C° (30.44%) while the lower yield at 80 C° (21.84)% which clearly shows that temperature has a significant effect on the yield content, increase of temperature has broken the chain of gum

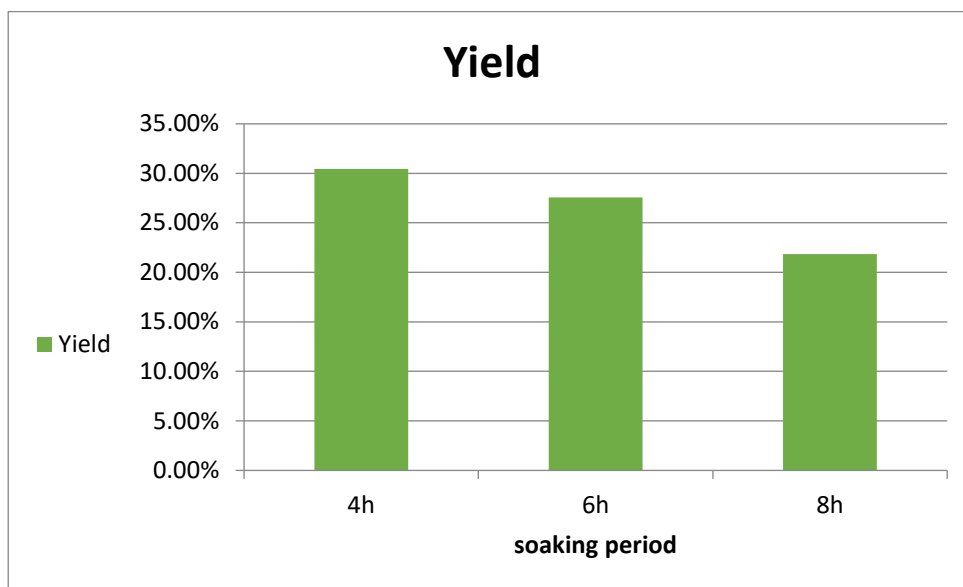


Fig3. Effect of soaking period on the yield.

The results are shown in Figure 3 The effect of soaking period on yield, it is clear that the fenugreek gum the highest yield at 4 h 30.44% and then decreased gradually at 8 h 22.42%. This may high-temperature increase in macromolecular motion and solubilization of gum solution.

Phytochemical test

As shown in Table 1 the isolated gum gave positive test for carbohydrates , protein , fat, and ruthenium red and negative test for phenol compound ,alkaloids, flavonoids, starch, sterols glycosides and resins because

the fenugreek gum was raw, while Arabic gum and Acacia were gave positive for carbohydrate and negative test for protein, fat, sterols, flavonoids, starch, glycosides and resins. The difference could be attributed to purity of Arabic gum and Acacia.

Table 1: Phytochemical tests of gums

Test for phytoconstituents	Fenugreek gum	Arabic gum	Acacia gum
Carbohydrate			
• Molisch's test	+	+	+
• Barfoeds test	+	+	+
• Benedicts test	+	+	+
• Iodine test	-	-	-
Ruthenium red test	+	+	+
Protein and Amino acid	+	-	-
Alkaloid	-	-	-
Glycoside	-	-	-
Flavonoid	-	-	-
Phenolic compounds test	-	-	-
Resins test	-	-	-
Sterols	-	-	-
Fixed Oil	+	-	-

The results are showed in Table 2, indicate the fenugreek had 9.4% moisture this indicates that the gum is hygroscopic in nature compared with the moisture content in the Arabic gum and Acacia (5.44, 5.56%). The protein and fat content of fenugreek gum were

found to be 2.49, 0.9 % respectively, that is higher than the protein and fat in Arabic gum (0, 0%) and Acacia (0, 0%). The difference may be attributed to the fenugreek gum was raw.

Table 2: chemical composition of gums

Parameter	Fenugreek seeds gum	Arabic gum	Acacia gum
Moisture (%)	9.4a	5.44b	5.56b
Crude fat (%)	0.9 a	0b	0 b
Protein (%)	2.49a	0 b	0b
Ash (%)	2.34 a	3.47 b	2.97 b
Carbohydrate	84.75 a	90.80b	91.25 b

Means within the same parameter having different letters are significantly different ($P < 0.05$)

Ash value of isolated gum was found 2.34% that was lower than the ash content in Arabic gum 3.74% and Acacia 2.97%, the low value of ash show that fenugreek gum has a good quality of mineral content with a low level of contamination (Adeyanju et al., 2015). The fenugreek seed gum contains about 84.75% of polysaccharides slightly lower than the results obtained for the polysaccharides of Arabic gum 90.80% and Acacia 91.25%, the ash content in the fenugreek gum in percent study that is higher than 1.02% that was reported by Verma *et al.*, (2014).

Evaluation of micromeretic studies of gums

The results are illustrated in Table 3 indicate The relative viscosity of a 1% solution of fenugreek seeds gum investigated at 25 C° was found to be 72.40 cp that is higher than the viscosity of Arabic gum 9.1 cp and Acacia 9.61 cp. The high viscosity of fenugreek seeds gum is due to a low branch, while branched binogalactan structure is responsible for the low viscosity of a solution of Arabic gum and Acacia gum (GmbH and KGaA, 2015).

Table 3: Micromeritic study data of gums

Parameter	Fenugreek seeds gum	Arabic gum	Acacia gum
Viscosity (C.P)	72.40 a	9.1 b	9.61 b
Bulk Density (1m/g)	0.37a	0.90b	0.5c
Tapped Density (ml/g)	0.58 a	1b	0.55 c
Hausner s Ratio (%)	1.56 a	1.11 b	1.1 b
Carr s index (%)	36.20 a	10b	9.09 b
mean particle size (M)	88 a	7.5 b	35c
Anqle of vepose (0)	34.02 a	21.80 b	19.79 c

Means within the same parameter having different letters are significantly different ($P < 0.05$)

Kumar *et al* (2014) reported the viscosity of 1% solution of fenugreek gum at 37 C° to be found 39 cp that is lower than is reported in this study. Bulk and tapped densities of powder fenugreek seeds were found to be 0.37 g/ml, 0.58 g/ml were low compared with Arabic gum 0.90, a 1 g/ml and Acacia 0.5, 0.55 g/ml, the higher swelling index of fenugreek gum might be due to this low bulk and tapped density values (Samy *et al*, 2015). The bulk and tapped densities values of powder fenugreek gum indicate that powder is heavy in nature, while the bulk and tapped densities values of powder Arabic gum and Acacia are light in nature. Bulk and tapped densities of the gum gave an insight into its flow properties packaging and arrangement of particles (Emeje *et al*, 2011). Hauser's ratio and compressibility of powder fenugreek gum around 1.56 and 36.20 % respectively that were higher than the Hauser's ratio and compressibility of Arabic gum 1.11, 10% and Acacia 1.1, 9.09%. The carr's index and Hauser's ratio values of fenugreek gum indicate that powder has poor flow character, while shows Arabic gum and Acacia excellent flow properties, Verma *et al.*, (2014) reported that the bulk density tapped density, Hauser's ratio, and compressibility of powder fenugreek were 0.66 g/ml, 0.94 g/ml, 1.39 and 28.2% that is higher than is results in this study. The obtained results show the bulk and tapped densities in powder fenugreek gum that is lower than 0.47 g/ml, 0.52 g/ml that was reported by Kumar, (2014). The results are showed irregular particle size was found to be 88 µm compared with the particle size of Arabic gum 7.5 µm and Acacia 35 µm. The angle of repose of the isolated gum was found to be 34.02. It found to be very high and this shows that fenugreek gum has more concessive properties than the Arabic gum and Acacia as the value of repose angle has been shown to be high powder is non-cohesive.

The effect of temperature, pH, and salt on the viscosity of gums.

Temperature effect

The results are illustrated in Figure 4 indicate The effect of an increase of temperature on viscosity of 1% of fenugreek seeds gum solution in the range 30 - 80 C°

compared with Arabic gum and Acacia solution. Fenugreek seeds gum had high thermal stability than Arabic gum and Acacia. The viscosity of Arabic gum and Acacia had been affected by the change of temperature. The result is in agreement with the reported by Norbrillinda *et al.* (2014). The viscosity of pineapple gum and locust bean gum decreased as the temperature increased from 10 to 70 C°. The decrease in viscosity of gums was due to the increase in macromolecular motion and solubilization of gum solution at a higher temperatures.

pH effect

The results are depicted in Figure 5 indicate the plant gums solution showed the highest viscosity at pH 7 and then decreased gradually at pH 3 and pH 10. The viscosity increased over the pH values from 3 to 7 and then decreased with increasing pH from 7 to 10. The result is in agreement with the reported by Norbrillinda *et al.* (2014). The viscosity of pineapple gum and locust bean gum decreased as pH 3 and pH10. The decrease in viscosity of gums was due to the galactomannan can be destroyed and lose its viscosity at high and low pH. Thanatcha and Pranee (2011) reported that increasing the pH and temperature led to a decrease of viscosity.

Salt effect

The results are presented in Figure 6, 7 the effect of NaCl and CaCl₂ at concentration 0.2-1 M on the viscosity of three plant gum solutions. It was observed that the viscosity of gums solution is decreased with increasing concentration of salt, positive ions by reducing the repulsive force in negatively charged molecules lead to a reduction in viscosity (Mozafari *et al*, 2016), CaCl₂ had a higher effect on viscosity of gums. The proximity of gums to metal ions depends on the ionic radius to charge ratio. It means that small ions with higher charge are stronger to link to the chain there for Ca⁺² = 2.1 and Na = 1 ratio. The interaction of gums is higher with Ca⁺² than Na (Mozafari *et al*, 2016). The result is in agreement with the reported by Chang *et al.* (2017). The viscosities of gum such as guar gum, locust Bean, tamarind, and flax seed gum decreased as the salt increase.

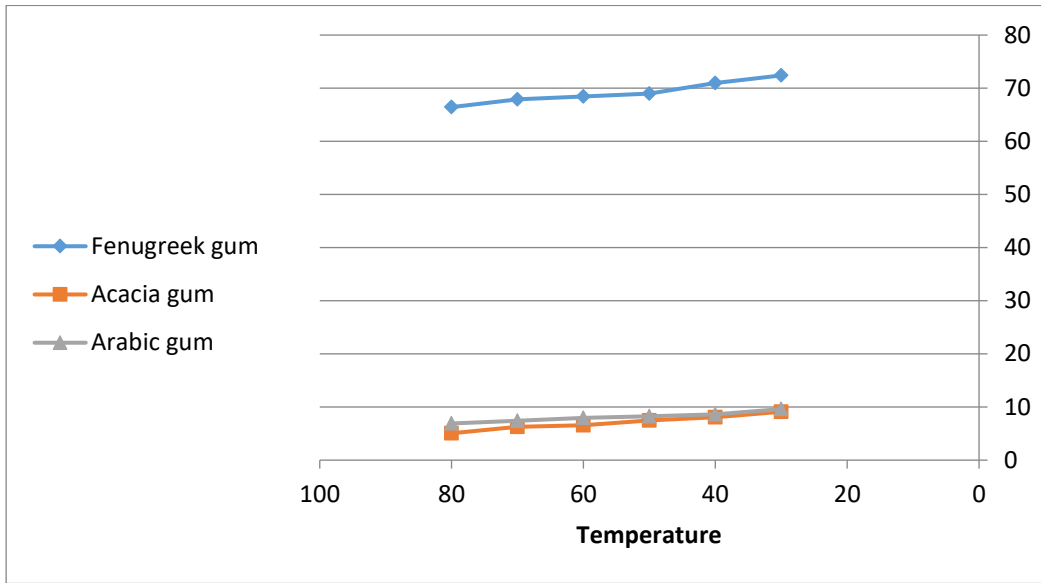


Fig 4. Effect of different temperatures on the viscosity of 1% gums solution.

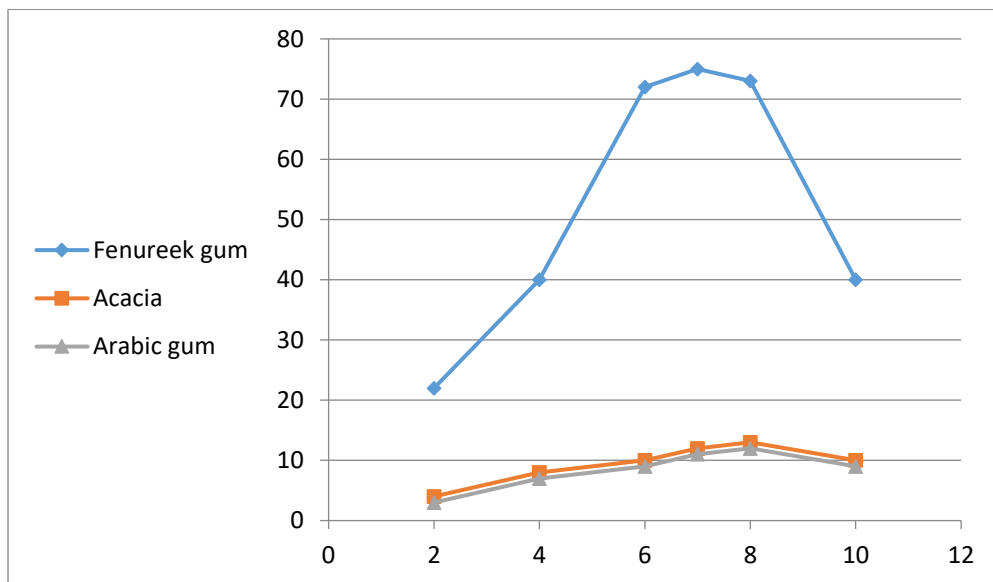


Fig 5. Effect of different levels of pH on the viscosity of 1% gums solution

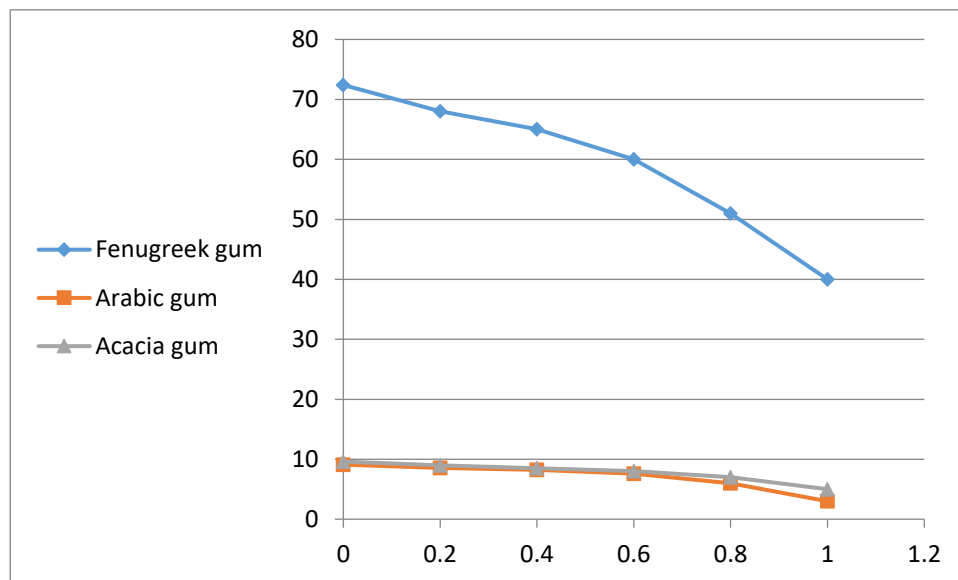


Fig 6. Effect of different concentration of NaCl on the viscosity of 1% gums solution.

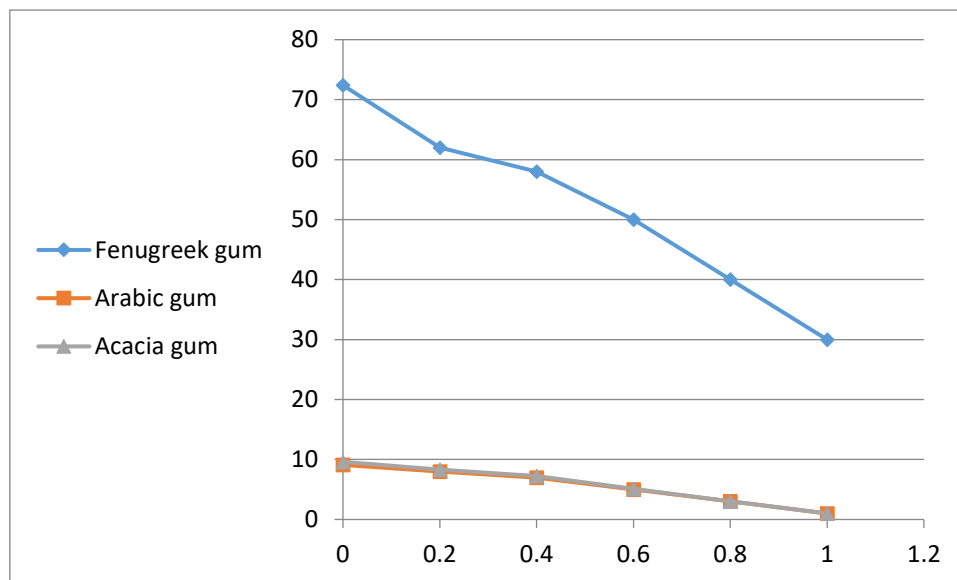


Fig7. Effect of different concentration of CaCl on the viscosity of 1% gums

Conclusions

The maximum yield of gum was 30.44% for the 1:25 dilution, soaking period for 4 h at 40 C°, the fenugreek seeds gum had the highest moisture, fat, protein, and viscosity than other gums, the temperature, pH, and salts affected the viscosity of gums. The viscosity decrease with increasing the temperature, pH, and concentration of salt. The study through some light on the understanding the gum potential applications in the food industry.

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