



Physical and Functional Properties of Extracted Gum from Fenugreek Seeds

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Abstract: The physical properties of extracted gum from Fenugreek seeds *Trigonella foenum graecum* L. were determined and compared with both Arabic and Acacia gums. The gum was extracted using water and precipitated with ethanol. There were a number of differences among gums regarding the pH value, density, refractive index and the wavelength. The pH value of the Fenugreek gum was 6.69, while the lowest value was 5.02 in the Arabic gum. In contrast, the density of 1% Fenugreek gum was found to be 1.025 g.ml⁻¹ compared with Arabic gum 1.0133 g.ml⁻¹ and Acacia gum 1.0134 g ml⁻¹. The refractive index values were 1.3340, 1.3335 and 1.3336 for Fenugreek gum, Arabic gum, and Acacia gum, respectively. Moreover, the maximum wavelength of the Fenugreek seeds gum was at 330 nm, while the results observed that both Arabic gum and acacia gum had the same wave length (300 nm). The functional properties of extracted Fenugreek gum were studied; the value of swelling Index was 90.90% compared to both Arabic gum (0.20%) and Acacia gum (0.20 %). The solubility of the Fenugreek gum was 40% and 50% at 25°C, and 80°C, respectively. The results also showed that Fenugreek gum had high oil holding capacity value of 0.88% compared with the Arabic gum and Acacia gum. The Fenugreek gum was succeeded to form gels at 4°C using concentration 1, 2, 3 and 4%, while the Arabic gum and Acacia gum did not form gel at different concentrations.

Keywords: Fenugeek seeds gum, The physical parameters, Solubility, gel, Swelling index.

Introduction

Fenuqreek gum was produced from Fenuqreek seeds of *Tnigonella Foenum graecum* L. (Family: Lequminoella) and was cultivated in warm climates such as India. The Mediterranean weather may be successfully enabled to grow crops due to comparable daylight exposure (Robert *et al.*, 2012). It is an old cultivated spice bean crop in India, Middle East, Southern Europ, North

Africa, and North America (Mundhe *et al.*, 2012); it is one of the oldest cultivated plants and used in many applications such as food additives and traditional medicines. Fenugreek seeds contain a high percentage of gum which is mainly composed of galactomannan. The galactomannan is polysaccharides composed of linear β -(1-4)-D- mannan backbone with varying amounts

of single D-galactose units attached to the main backbone by α -(1-6) glycosides bond (Repin, 2016). It does not partially soluble in water and form a viscous tacky mass when exposed to fluids (Kumar *et al.*, 2009); the gum is most commonly used in various pharmaceutical properties such as binding, gelling agent, emulsifying and suspending agent. In addition, it was used in baking, bread making, graries and soup (Kay, 2016; Repin, 2016). It has been extensively used in the pharmaceutical industry as binder, gelling agent, emulsifier and suspending (Mundhe *et al.*, 2012) .

The aim of this research was to study the physical and functional properties of extracted gum from fenugreek seeds regarding the pH, refractive index, wavelength, density, swelling index, solubility, gelation and oil holding capacity.

Material & Methods

The seeds were purchased from local market of Basrah province. The seeds were gringing using a coffee grinder. Arabic gum and Acacia were obtained from the chemical BDH company, England. All the other solvent reagents and chemical used were analytical grade.

Extraction of gum

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

Physico-chemical characterization of the Fenugreek seed gum compared with Arabic and Acacia gums.

pH

One gram of gum was weighed in a beaker and mixed with 100 ml of distilled water. The suspension was stirred for 5 minutes and the pH was measured using pH meter at 25°C according to the method described by Farooq *et al.* (2015).

Determination of density and specific gravity

The density of 1% of gums solutions was determined at 25°C according to Yusuf (2011).

Refractive index

The refractive index of 1% of the gums solutions was measured at 25°C using Abbe refractometer according to the method described by Gashua *et al.* (2013).

The maximum wavelength

The maximum wavelength of gum was determined using an ultraviolet –visible spectrophotometer at range of 200-800 nm according to the method described by Al-Janabi & Al- Abdullah (2013).

Swelling index

The Swelling index was determined according to the method described by Verma *et al.* (2014). One gram of fenugreek gum powder was weighed and transferred in to the plastic centrifuge tube. The mixture was shaken thoroughly every 10 min for 1 hr and then allowed to stand for 24 hrs at the room temperature.

The Swelling index of the gum was calculated using the equation

$$\text{Swelling Index (\%)} = \frac{\text{Final Volume} - \text{Initial volume}}{\text{final volume}}$$

Solubility

Solubility was determined according to Amid & Mirhosseini (2012). 1.0 g of gum powder was added to 100 ml of distilled water with stirring at room temperature, then the temperature was raised to 80° C for 30 min. After that, the gum solution was centrifuged at 6000 g for 30 min to remove the insoluble material. The settled portion was then transferred to a petri dish and dried at 105° C for 24 hrs.

The following equation was used to determine the solubility.

$$\text{Solubility (\%)} = C_1/C_2 \times 100$$

Where C₁ is the supernatant concentration (mg) C₂ is the Initial concentration (mg).

Determination of Gelation

The dry gum 1-10 gm dissolved in 100 ml of water distilled and then the solutions were kept overnight at 4°C. The formation of gel and their quality was assessed by visual inspection according to Chidewe (2004).

Effect of temperature

The gums 1-10 % were incubated in a water bath at temperature range 4-100° C. The Vials were allowed to stand overnight at 4°C.

Effect of PH on the formation of the gel.

$$\text{Oil Absorption (g oil.g gum}^{-1}\text{)} = \frac{\text{oil absorbed sample weight} - \text{Dry sample weight}}{\text{Dry sample weight}}$$

Statistical Analysis

All the data of physical and functional properties with three replicates were analyzed using SPSS 2012. The comparative analysis between parameters was carried out using L.S.D. with probability (P < 0.05).

Citrate buffer was used between pH 3 and 6, while phosphate buffer was used at pH 7 and Tris-HCl was used at pH 10. The dry gum 1-10 g was dissolved in 10ml of buffer and the mixtures were allowed to stand overnight at 4°C before assessment of gel Formation.

Effect of Calcium Chloride on the formation of the gel.

The gum 1-10% was dispersed in the solution containing calcium chloride at concentration 0.5 – 20Mm. The Suspension was allowed to stand overnight at 4°C before assessment of gel

Effect of EDTA on the formation of the gel.

EDTA was dissolved in distilled water to make solutions of concentration 10-50 mM. The gum 1-10% was Suspensions were allowed to be stand overnight at 4°C before assessment of gel formation.

Oil – holding capacity

The oil holding Capacity was determined according to Thanatcha & Pranee, (2011). 0.5 g of the gum was dispersed in 10 ml of sunflower oil and mixed using vortex Stirrer for 1 min, then kept at room temperature for 30 min and centrifuged at 10000 g for 30 mins. The oil gum was weight and calculated by the following equation:

Results & Discussion

The results showed in Table 1 indicate that the pH of 1% fenugreek gum was 6.69 which was neutral because it contained neutral sugars, while the Arabic gum and Acacia were 5.02 and 5.19, respectively. The acidity of gum was due to uronic acids in their structure (Yusf, 2011).

Table (1): The physical properties of gums.

Para meter	Fenugreek seeds gum	Arabic gum	Acacia gum
pH	6.69 a	5.02 b	5.19 b
Density (g.ml ⁻¹)	1.025 a	1.0133 b	1.0134 b
Specific gravity	1.0373 a	1.0255 b	1.0256b
Refractive Index	1.3340 a	1.3335 b	1.3336 b

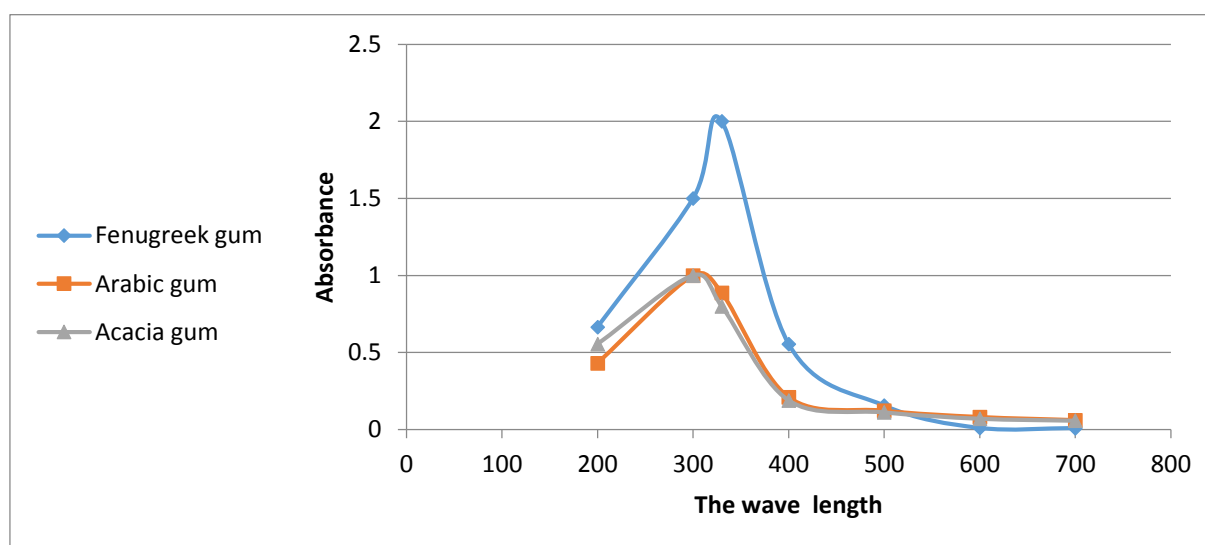
Means within the same parameter having different letters are significantly different ($P < 0.05$)

The result was in agreement with Kumar *et al.* (2014). The Fenugreek gum exhibited a high refractive index (1.3340) compared to the Arabic gum (1.3335) and Acacia (1.3336). The results were not in agreement with Gashua *et al.* (2013). The result also revealed that the Fenugreek gum had a higher density 1.025 g.ml⁻¹ and specific gravity of 1.0373 than the density and specific gravity of the Arabic gum and acacia gum of 1.0133, 1.0134 g.ml⁻¹ and 1.0255, 1.0256, respectively.

Fig. (1) represents the maximum wave length of the Fenugreek seeds gum, Arabic gum and acacia gum. The results showed that the

maximum wave length of 330 nm for the Fenugreek gum, while the wave length of Acacia and Arabic gum were 300 and 300nm, respectively. These differences may attribute to the nature and chemical composition of gums (Al-Janabi & Al-Abdullah , 2013).

According to the statistical analyses, the swelling index value of Fenugreek gum was significant ($P < 0.05$) compared to a standard Arabic gum and Acacia (Fig. 2). The Fenugreek gum had high swelling index, the swelling coefficient depends on the size and distribution of the gum particles.

**Fig. (1): The maximum wave length of plant gums.**

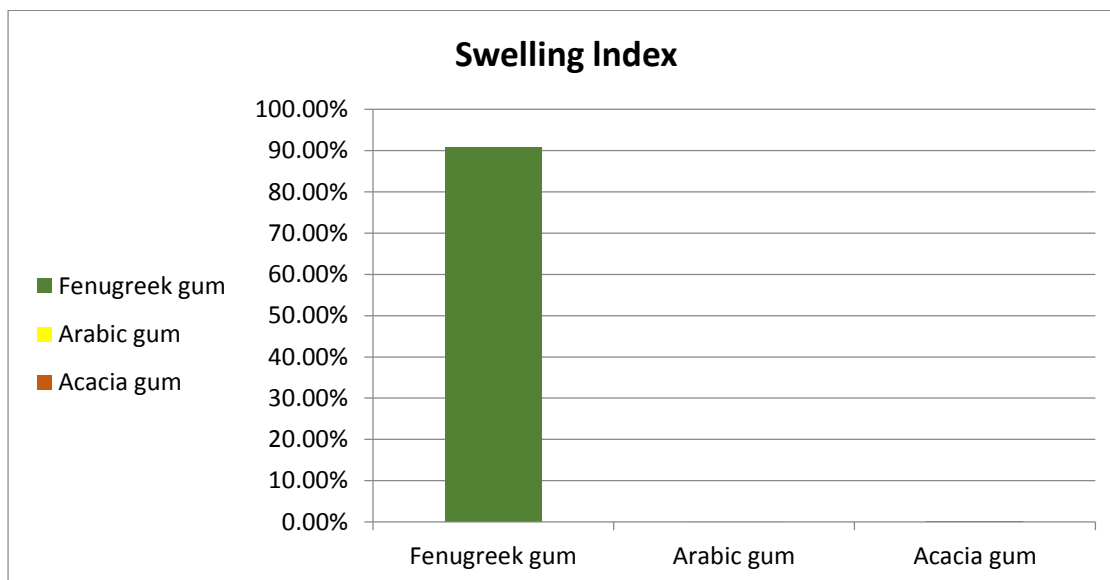


Fig. (2): The swelling index of three different plant gum ($P < 0.05$).

The gum, which has a soft structural with many small pores, produces a high swelling coefficient and greater water retention compared to the gum containing large pores such as Arabic gum and Acacia, another reason was attributed to the non-polar group in crude gum causing a high swelling index (Amid & Mirhossieni, 2012). In the research, the Fenugreek gum may have a finer uniform gum structure than standard Arabic gum and Acacia. The result was in agreement with Verma *et al.* (2014).

The results presented in Figs. (3 & 4) indicate the solubility of gums increased as the temperature values increase at 25°C and 80°C. The solubility of Fenugreek gum was significantly ($P < 0.05$) lower at 25°C with an average of 40%, whereas the highest solubility at 80°C was 50%. The solubility values for each of the Arabic gum and Acacia were recorded 90% at 25°C, and were increased to 100% at 80°C. The solubility is affected by several factors not only the content of galactose but also the type and

content of the impurities, as well as the size and weight of molecules. The low solubility of crude gum is associated with the presence of the impurities and high molecular weight of molecules and insoluble matter. The solubility is affected by the ratio of mannose to galactose and galactose distribution along the straight mannose chains, when reduced the content of galactose increased solubility at high temperature and the solubility was decreased at low temperature, and on the other hand; break the hydrogen bond between the polysaccharide chains and the emergence of hydroxyl groups to water at high temperature, leading to improved solubility (Sciaroni *et al.*, 2009; Amid & Mirhossieni, 2012). A similar observation was made by Bilal *et al.* (2015) indicating the solubility of raw and refined Arabic gum was increased as the temperature values increased from 25°C to 100°C. Amid & Mirhossieni (2012) reported the increasing solubility of the raw and refined durian gum as the temperature was increased.

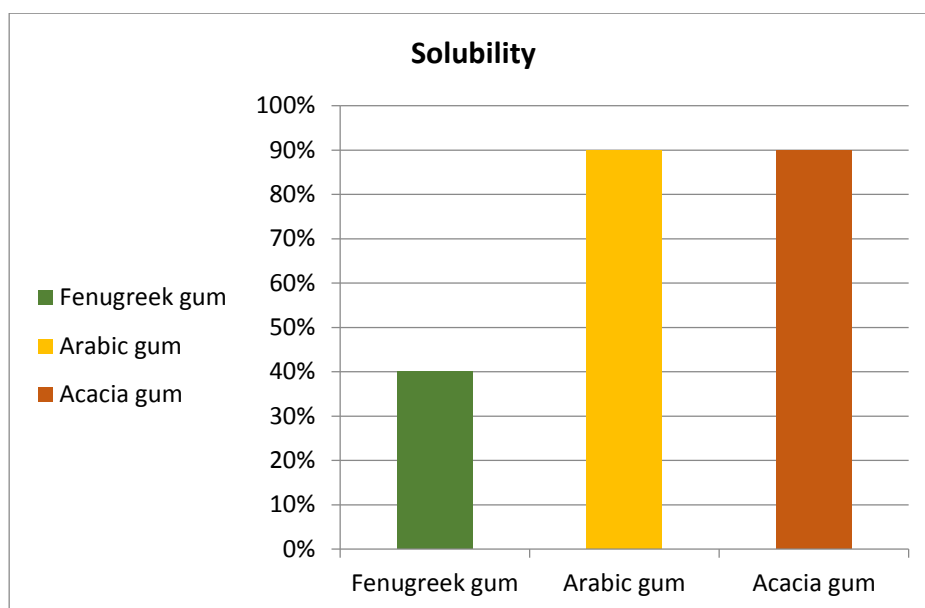


Fig. (3):The solubility of three different plant gum at 25°C ($P < 0.05$).

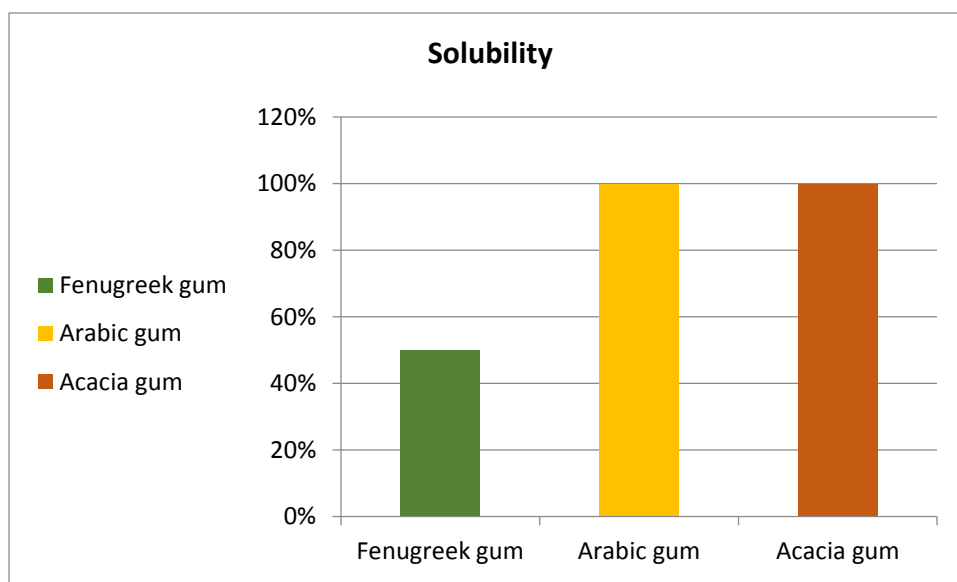


Fig. (4): The solubility of three different plant gum at 80°C ($P < 0.05$).

Effect of gums concentration on formation of gel

The results of table (2) indicated that the minimum concentration of Fenugreek gum which required to form a firm translucent gel was 1%, while the maximum concentration of gum (4%) was required to form a hard gel compared to Arabic gum and Acacia which did not form the gel. These differences may be due to the Fenugreek gum which had high swelling index to produce gels or highly viscous solutions (Amid & Mirhosseini, 2012)

compared to the Arabic gum and Acacia with lower swelling index (Masuelli, 2013).

Effect of temperature on the formation of gels

The results of table (3) indicated that the gels were formed at concentration 1, 2, 3 and 4% of Fenugreek gum, Gum formed a strong gel at 4°C, while the gels formation decreased at temperatures 20 -100°C. The decreasing of gels formation could be attributed to the impact of the temperature on the bonds responsible for maintaining the gel structure (Chidewe, 2004).

Table (2): Effect of gums concentrations on the formation of gels.

Concentration of gums (%)	Fenugreek seed gum	Arabic gum	Acacia gum
1	+	=	=
2	+	=	=
3	++	=	=
4	+++	=	=
5- 10	=	=	=

Table (3): Effect of temperature on the formation of gel.

	Concentration %	Temperature (° C)					
		4	20	50	70	90	100
Fenugreek gum	1	+	=	=	=	=	=
	2	+	=	=	=	=	=
	3	++	=	=	=	=	=
	4	+++	=	=	=	=	=
	5-10	=	=	=	=	=	=
Arabic gum	1-10	=	=	=	=	=	=
Acacia gum	1-10	=	=	=	=	=	=

The Arabic gum and Acacia did not formed the gel at all different temperature. These results were in agreement with Chidewe (2004).

Effect of pH

The results of table (4) indicated that the fenugreek seeds gum is not able to form a gel at pH values 3-5 . The can be attributed to the fracture of polysaccharide galactomannan. Therefore values of the below and the above of 7 do not have gel, and this is due to the possibility of partial depolymerization of the galactomannan loss of viscosity and weak gum susceptibility to gel formation (Norbrillinda *et al*, 2014).

The formation of firm gels increased when pH was raised from 6 to 7, while the gel formation decreased at pH 10; the gum molecules in

solution would be negatively charged and experiencing repulsive force against each other, such that the aggregation of the gum chains for gels formation would not be promoted (Chidewe, 2004); The Arabic gum and Acacia did not form the gel at all different values of pH because they had lower swelling index .This might be resulted high solubility 90% (Masuelli, 2013) .The results were in agreement with Chidewe (2004).

Effect of calcium chloride and EDTA

Table (5) represents that the decreasing of formation of gels fenugreek gum when calcium chloride was added at concentration 5-20 mM, Addition of calcium chloride at increasing concentrations to the gel forming suspensions of the gum , could have resulted

Table (4): Effect of pH on the formation of gels.

Material	Concentration %	pH				
		3	5	6	7	10
Fenugreek gum	1	-	-	-	+	-
	2	-	-	+	+	-
	3	-	-	+	++	-
	4	-	-	++	+++	-
	5-10	-	-	-	-	-
Arabic gum	1-10	-	-	-	-	-
Acacia gum	1-10	-	-	-	-	-

Table (5): Effect of calcium chloride on the gelation of aqueous suspensions of gums.

	Concentration %	Concentration of calcium chloride (mM)					
		0.0	0.5	1.0	5.0	10.0	20.0
Fenugreek gum	1	+	+	+	-	-	-
	2	+	+	+	-	-	-
	3	++	++	++	+	-	-
	4	+++	+++	+++	++	+	-
	5-10	-	-	-	-	-	-
Arabic gum	1-10	-	-	-	-	-	-
Acacia gum	1-10	-	-	-	-	-	-

in aggregation of the gum molecules, giving rise to the formation of precipitates with poor water binding capacity (Chidewe, 2004). The results of table (6) indicated that the gel formation was decreased when EDTA was added at concentrations 10-50 mM, the

decreasing of formation could be related to the salts which destroyed the hydrophobic bonds and hydrogen bonds which are responsible for maintaining the gel structure (Chang *et al.*, 2017), whereas Arabic gum and Acacia did not form the gel when calcium chloride and EDTA were added.

Table (6): Effect of EDTA on The gelation of aqueous suspensions of gums.

	Concentration %	Concentration of EDTA (mM)					
		0.0	10	20	30	40	50
Fenugreek gum	1	+	-	-	-	-	-
	2	+	+	-	-	-	-
	3	++	++	+	-	-	-
	4	+++	+++	++	+	-	-
	5-10	-	-	-	-	-	-
Arabic gum	1-10	-	-	-	-	-	-
Acacia gum	1-10	-	-	-	-	-	-

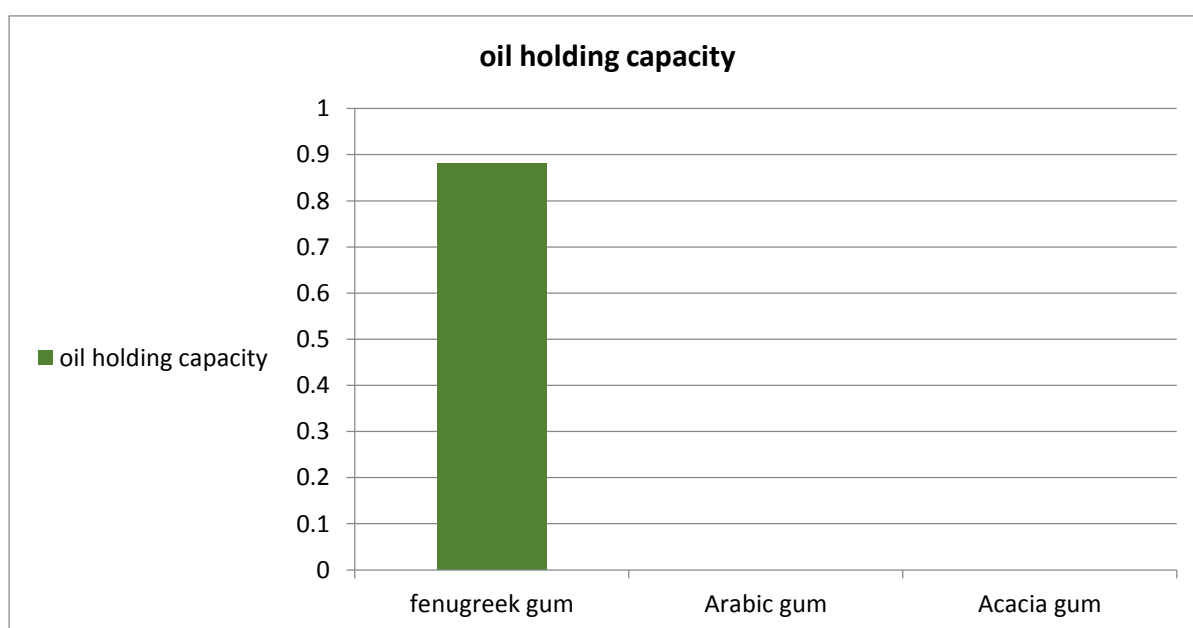
**Fig. (5): The oil holding capacity of three different plant gum P < 0.05.**

Fig. (5) results showed that the oil holding capacity of Fenugreek gum was higher than the oil holding capacity of Arabic gum and Acacia (0.0 g oil.g⁻¹ gum respectively) with average of 0.88 g oil .g⁻¹ gum. Fenugreek gum has high oil holding capacity because the presence of many nonpolar molecules in the crude gum such protein, that entrapment of the high amount of oil particles (Amid &

Mirhosseini, 2012) compared with Arabic gum and Acacia did not had oil holding capacity because they were refined, many nonpolar molecules of gum that can trap high amount of oil particles; this property has a significant effect on food texture; for example, the high oil absorption in a meat product could help to reduce the losses of flavor and oil during the cooking of the meat (Thanatcha & Pranee, 2011).

Conclusions

Fenugreek gum has pH value (6.69) which is close to the neutral pH value (7). Fenugreek gum is partially soluble in cold water at 25°C and in hot water at 80°C lower compared to the standard Arabic gum and Acacia gum and Acacia; According to the Comparison of functional properties of Fenugreek gum with standard Arabic gum and Acacia, the Fenugreek gum had high swelling index and oil holding capacity than the Arabic gum and Acacia. The concentration of the Fenugreek gum, temperature, pH and adding CaCl₂ and EDTA affected the gel formation of the fenugreeks gum, the increasing the gel formation with increasing of the gum concentration at 4% at 4°C, whereas the gel formation was decreased when calcium chloride and EDTA were added at 5-20 mM and 20-50 mM respectively.

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