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المجلة العراقية لبحوث السوق
وحماية المستهلك

# PREPARATION OF COACERVATES COMPLEX FROM ISOLATE FENUGREEK SEED PROTEIN AND ITS GUM FOR CAPSULE SHELL PRODUCTION.

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## ABSTRACT

**The isolate of fenugreek protein and fenugreek gum were extract previously obtained using steps conducted from Fenugreek seed. The fenugreek seed protein isolate (FPI) and Fenugreek seed gum (FG) were prepared as a coacervates complex as well as they were studied its characteristics. The best proportion of FPI-FG were optimized to obtain the highest production of (capsule shell) coacervates complex support by estimating of zeta potential and turbidity values. It was found the pH (4.0), FPI- to-FG proportion(6 protein to1 gum) had the best yield for complex production .The freeze-drying dried technique was ultilized to obtain the coacervates complex had the most stability particles by reducing its humidity. Therefore, the purpose of cross-linked formation between FPI-to-FG complex coacervates was obtained as a cover to protect against the sensitive effects of oxygen and heat factors whether food or medical supplies**.

**Keyword:** fenugreek seed, complex coacervates, zeta potential, freeze dried.

## تحضير complex coacervates من معزول بروتين بذور الحلبة وصمغ بذور الحلبة لعمل غلاف كبسولة

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### الخلاصة

**تم عزل وتجهيز معزول بروتين الحلبة ومستخلص صمغ الحلبة من بذور الحلبة مسبقاً في المختبر المركزي التابع لكلية علوم الهندسة الزراعية – جامعة بغداد. استخلص معزول بروتين بذور الحلبة (FPI) وصمغ بذور الحلبة (FG) وحضر منهما *Complex coacervates* وتمت دراسة صفاته. وقد درست النسبة الامثل لمعقد البروتين وصمغ الحلبة للحصول على أعلى إنتاج من (مادة الغلاف) معقد بروتين وصمغ الحلبة *Complex coacervates* من خلال تقدير قيمة زيتا والعكارة. حيث وجد ان الاس الهيدروجيني 4.0 ونسبة خلط بروتين : صمغ 1:6 أعطت افضل حصيلة لانتاج المعقد، استخدمت تقنية التجفيد للحصول على مادة اكثر ثباتًا بتقليل نسبة رطوبتها . ان الغاية من تكوين هذا المعقد *Complex coacervates* بين معزول بروتين الحلبة وصمغ الحلبة هو الحصول على غلاف للحماية من تأثيرات الأوكسجين والحرارة لمواد حساسة لهذه العوامل، سواء كانت مواد غذائية أو طبية.**

**الكلمات المفتاحية**: بذور الحلبة ، معقد complex coacervates ،تقنية زيتا ، التجفيد.

[*] **The article is taken from the doctoral thesis of the first researcher.**

## INTRODUCTION

Fenugreek *(Trigonella foenum gracum* L.) is legume cereale belonging to Leguminosae or Fabaceae family. fenugreek is a pretty source of protein (27.5%) **(Tavakoly *et al*., 2018), (Aziz *et al*, 2011)** which was used to employed as health food in pharmaceutical and nutraceutical domain. **(Dhull *et al*., 2021)**. Due to its abundance of many nutritious components, including proteins, lipids, and carbohydrates, fenugreek (*Trigonella foenum-graecum* L.) has been a staple in most regions around the world for centuries. minerals, vitamins, carbs, and other nutrients **(Yaseen *et al*., 2011)**, there is a great interest in finding novel sources of plant proteins because of the paucity of animal proteins with equivalent nutritional and functional advantages in food systems **(Al-Saddi *et al*., 2009)** Ingredients-wise, the seeds also have a lot of pharmacological and medical ingredients **(AL-hadwany, 2004).**

Fenugreek gum is component of galactomannans, its molecular structure is composed of a (1-4)-ß-D-mannan vertebral to single α-D-galactopyranosyl groups. The polymer is rich in hydroxyl groups and have hydrogen bonds with water to viscous solution. **(Salarbashi *et al*.,2019)**. The research has focused on the characterizations of fenugreek gum of its potential applications in food, food products, pharma and different industrial uses **(Yaseen, 2011), (Inas *et al.,* 2013).**

The type of polymers utilized, their ratio, concentration, and charge density all have an impact on the complex coacervation process. The complex coacervates process are affected in other factors such as pH, temperature, ionic strength, an emulsification technique **(Siow *et al*., 2013).** For all mentioned reasons, the aforementioned components interact in vital to the coacervation process's optimization Between FPI and FG, respectively.

This study attempts to better isolate protein and gum from fenugreek seed, as well as examine some of its features, The goal of conducting future studies due to learn more about the health and nutritional advantages about the complex coacervation technique in Department of Food Science, College of Agriculture Engin. Science University of Baghdad, Iraq.

## MATERIALS AND METHODS

Fenugreek seeds were obtained from the Agricultural Research Department / Ministry of Agriculture, Iraq, and they were cleaned of impurities, purified, ground, sieved, and stored in opaque polyethylene bags in the refrigerator.

The isolate protein prepares from defatted fenugreek seed the method used with some modifications **(Ayoub *et al*., 2023)**. The isolate protein dried by freeze-drying method. The gum prepares from defatted fenugreek seed the method used **(Ayoub *et al*., 2023) (shukla *et al*., 2022)** and dried by air oven at 50C°

**1- Preparation the Transglutaminase enzyme solution**

Dissolve 1 g of transglutaminase enzyme (Turkish origin) in 50 mL of sterile distilled water and mix.

**2- Preparation sodium azide solution**

To prepared 0.2% sodium azide solution (weight: volume) added to both protein and gum solutions to protect them from the growth of microbes, **(Capitani *et al*, 2013).**

**3-Preparation the protein solution**

In the first step the protein isolate from fenugreek seed were prepared by dissolving 25 g of protein isolate powder in 500 mL distilled water at pH8 and mixing in shaker at a speed of 100 rpm for one hour at ambient temperature. The solution was kept in refrigerator at 4°C until

the subsequent steps were completed.Then it was left for overnight to ensure the moisturizing and melting process has been completed **(Ayoub *et al*., 2023), (Oomah *et al*.,1994).**

**4- Prepartion the gum solution**

In the sconed the gum solution from fenugreek seed was prepared by dissolving 2.6 g of gum extract powder in 500 ml of distilled water at pH=7. Then, the solution was mixed using the shaker at speed around 100 rpm for one hour at room temperature. The solution was stored at refrigerator temperature for overnight until all steps were ended **(Pratibha, 2016).** The sodium azide solution was added to both of solutions (the protein and glue solution) at a concentration 0.2% (W/V) from the volume of the prepared solution. When, the protein and glue solution were mixed, the transglutaminase enzyme solution was added at a concentration 2%, with an addition rate around 5% of the total solution volume.

**5- Determine the optimal pH and ratio of protein isolate to gum**

The complex coacervates was prepared by mix 300 ml of 0.1% protein isolate solution (weight: volume) at speed 1000 rpm using a magnetic stirrer for 2 minutes. The prepared gum solution is 50 mL at a concentration of 0.1% (weight: volume). Add it slowly to the protein solution while mixing on the mixer, take the solution to settle for 2 h at room temperature. Add the 5% Transglutaminase enzyme solution (weight: volume) prepared with distilled water and a temperature of 5°C. The protein-gum solution was added to the bonding process and took place after 2 hours at ambient temperature **(Xiao *et al*., 2013).**

**6- Effect of pH on zeta potential**

The zeta potential was measured in the laboratories of the Ministry of Science and Technology The zeta potential is estimated according to the method **(Timilsena *et al*., 2016)** from the protein-gum solution diluted using distilled water containing 1 mg/mL protein, and the same to the gum. 0.2M citric acid or 1M NaOH was added to measure the pH values. Zeta potential for pH values were 5-1. The different proportion of protein-gum solutions was prepared from 2:1,1:1,1:2,4:1,6:1 and 8:1. The zeta potential was estimated using the scattered light technique at 25°C.

**7- Optical density (Turbidity) measurement**

Optical density is measured according to the method followed **(Timilsena *et al*., 2016)** for the protein-gum solution at a concentration of 0.1% (W/V) for both prepared solutions and based on the pH estimated in method 2-6 using a UV spectrometer with a wavelength of 600nm.

**8- The yield of protein-gum solution measurement**

The protein-gum solution was prepared in the proportions ranging between 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 8:1. 10:1 and 12:1, respectively. It was left for 2 hours to stabilize at ambit temperature. The separation process was carried out using centrifugation, then it was dried at 105°C until the weight was stabled **(Timilsena *et al*., 2016).**

The yield for different pH was obtained in the protein-gum solution at a ratio of 1:6. This ratio was found to be the result of linking the protein-gum solution (complex solution) with the highest ratio. Calculating by equation below:

$cy\%=(m_i)/m_0 \times 100\%$

CY = Yield of coacervates %

$m_i$ and $m_0$ mass(mg) of dried coacervates and total mass of both FPI, FG.

**9- Drying of the protein-gum complex solution**

The freeze-drying technique was used to dry the protein-gum complex solution using a (CHRIST freeze-drying) at a temperature of -81°C.

**10- Fourier transform infrared spectroscopy (FTIR)**

The FTIR fourier transform infrared spectroscopy device to diagnose the protein-gum complex by making tablets using a mortar with KBr, where 40 mg of the sample is weighed with 120 mg of KBr. After 10 min, weigh 40 mg of the mixture and compress it under a pressure 8 bar for 60 seconds with the compressor of the IR device. The tablets drying in an oven at 80°C for 16 h and analyzed with an FTIR device at a frequency of 650-4000 $cm^{-1}$ **(Timilsena *et al.*, 2016)**.

## RESULTS AND DISCUSSION

**Isolate protein - gum ratio and effect of pH on zeta potential**

pH plays the most important role in the process of cohesion of complex coacervates between the fenugreek gum solution and the fenugreek isolate protein solution because it controls the degree of functional ionization (amine groups in the protein with carboxyl groups in the polysaccharide of the gum) and the strength of the electrostatic interaction between the charged parts. Bonding strength is important because the physical, mechanical, and thermal properties of the complex coacervates are governed by the extent of interaction between the shell polymers and the degree of cross-linking **(Huang *et al.*, 2012).**

The zeta potential of the gum solutions and the fenugreek isolate protein was estimated within a pH range between 1-8. We note from Figure (1) that the dispersion of the gum solution is a result of the weak negative charge in the pH range due to the carboxyl group in the uronic acid, and at pH 2 it became dispersion. The gum solution is positively charged, but as for the protein isolate at pH 4, the negative charge began to decrease as the pH decreased, reaching the Iso electric point at which the charge becomes neutral. Below that, the charge is positive because the protein isolate contains an amine group ($NH_2$). The carboxyl group (COOH) is below the electrical neutrality point, as the number of amine groups ($NH_3$) increase of carboxyl groups($COO^-$), the charge is positive, and exactly the opposite at a pH higher than the electrical neutralization point**.( Amirafshar *et al.*, 2021).**

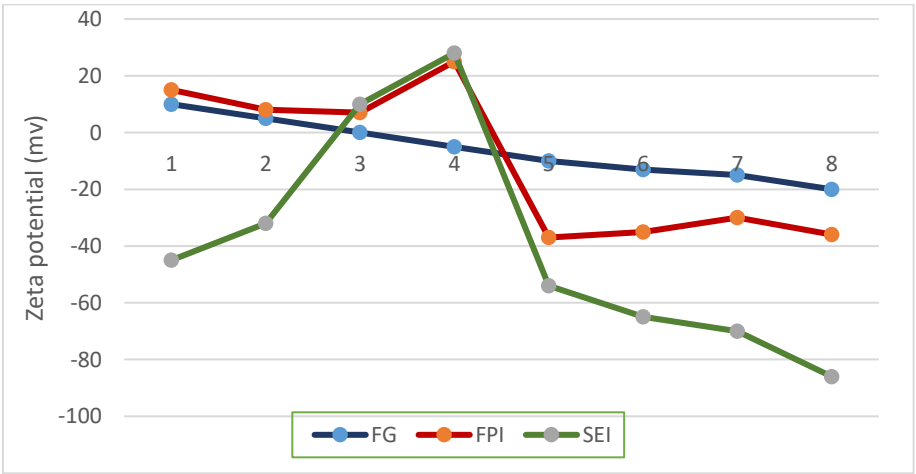


**Figure (1):** Effect of pH on zeta potential to complex coacervates between the fenugreek gum solution (FG), the fenugreek isolate protein solution (FPI) and Strength Electrostatic Interaction (SEI).

**Optical density (Turbidity) measurement**

Figure (2) shows the variation in optical density (turbidity) of complex coacervates between the fenugreek gum solution and the fenugreek isolate protein solution with different proportions of them. As the figure shows, the turbidity increases gradually with an increase in the mixing ratio between the gum solution and the protein isolate solution, reaching the ratio of 6:1 protein: gum, while when the proportion of protein isolate is increased to the gum, a mixing ratio of 8:1 led to a significant decrease in turbidity. The reason for the decrease in turbidity is attributed to the inability of the excess protein to bind to the gum (polysaccharide), withe amount of free protein in the solution **(Timilsena *et al.*, 2016)**, who conducted a similar study on chia seeds It was concluded that the best mixing ratio of protein isolate and gum is 6:1.

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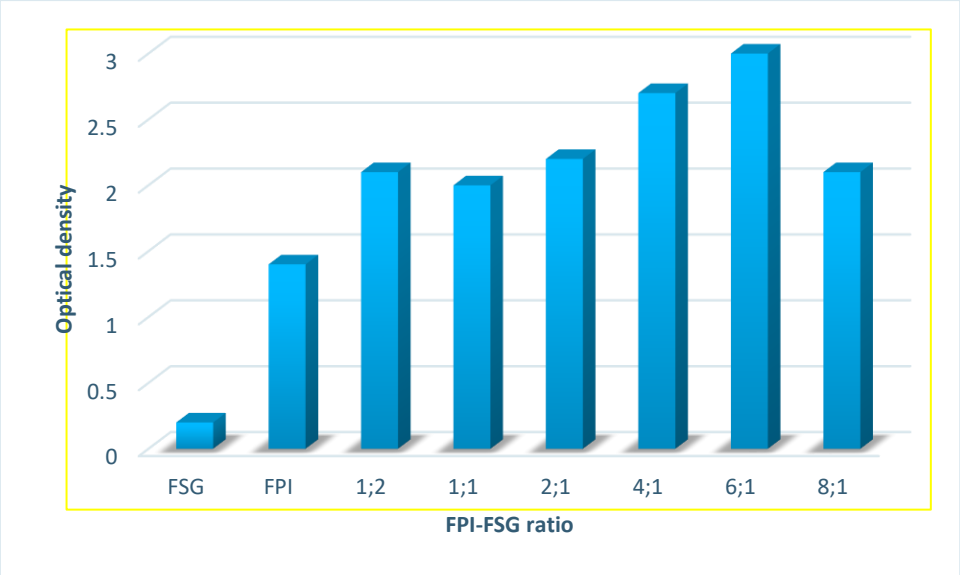


**Figure (2):** Optical density of complex coacervates between the fenugreek gum solution and the fenugreek isolate protein solution.

**Effect of pH and Mixing Ratio on the Yield of complex coacervates**

Estimating the yield depends on two factors: the mixing ratio of the gum and protein isolate and the pH. The yield was calculated based on different mixing ratios. It was found that the highest yield was at a ratio of 6:1 protein: gum, where the yield reached 90%, as the ratio of protein to polysaccharides affects in the mixture, the charge balance of the multiple ions and thus the method of complex formation **(Eratte *et al.*, 2014)** Figure (3) shows an increase in the yield values with increasing mixing ratios, reaching a ratio of 6:1, after which the yield decreases as the mixing ratio increased because more free protein solution not interaction with gum solution to bind to it and form the required complex coacervates, meaning that the process of balancing the proportions between the solutions led to a decrease in the yield after reaching a concentration higher than 6:1 protein :gum**(Su *et al.*,2022)**

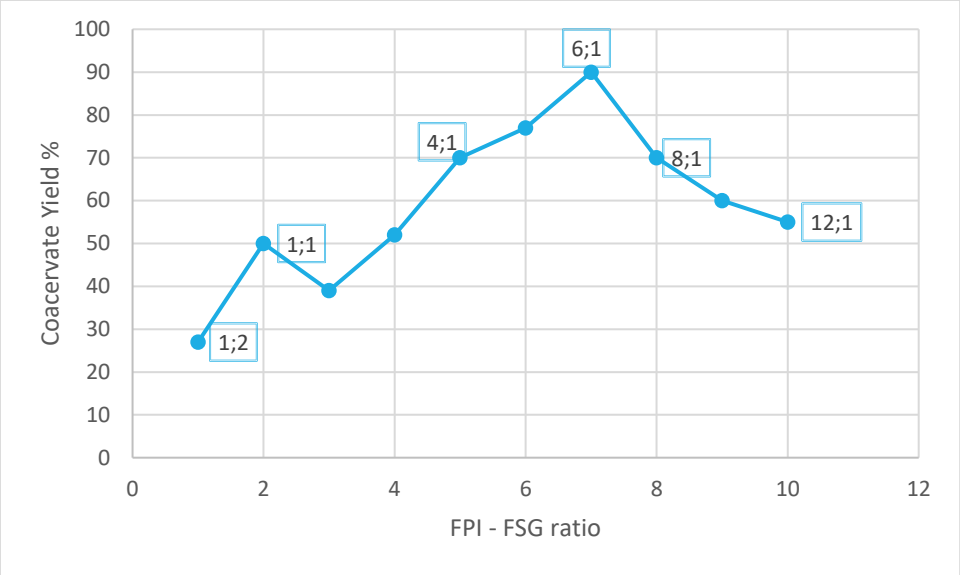


**Figure (3):** Yield of complex coacervates between the fenugreek gum solution and the fenugreek isolate protein solution ratio mixing.

An FTIR test was conducted for the fenugreek gum complex and the protein isolate, where the figure (4) shows the infrared absorption peaks, at the formation of the complex, the amine groups in the protein isolate interact with the carboxyl groups in the gum to a complex coacervates that contains an amide bond and includes the characteristic peaks of amides.and the first peak is Amide I (C=O, C-N) at 1600 $cm^{-1}$, Amide∏ (N-H, C-N) 1544 $cm^{-1}$. Amide III (C-N, N-H) at 1150 $cm^{-1}$ the FTIR of the protein isolate, we find that the frequency range between 1200 $cm^{-1}$ - 1350 $cm^{-1}$ represents the binding of NH with the C-N stretches in Amide II and it appears clearly at the frequency 1230 $cm^{-1}$ and the frequency 1300 $cm^{-1}$. Amide I and Amide II are specialized for the infrared spectrum of the protein **(Abdalah, 2011)**. The frequency is 2850 $cm^{-1}$ - 2950 $cm^{-1}$ associated with the extended bonds - CH, as the frequency 2850 $cm^{-1}$ falls within them. The frequency 1600 $cm^{-1}$ - 1700 $cm^{-1}$ is attributed to Amide I, as it is characterized by C=O and C-N groups and its vibration is stretched, and this explains the peak at 1684 cm. $^{-1}$, we find that the infrared spectrum in examining the complex between the gum and the protein made the readings move to the right because this type of transformation of the amides is a characteristic of distinguishing the random coil and the α-helix, as well as the lamellar structure of the protein, the β-sheet, which represents the structure of the protein, which is attributed to the high ratio of the complex components, i.e. the ratio of isolated protein: gum. Regarding the carboxyl group (C=O), we find that 1010 $cm^{-1}$, in the FTIR test of fenugreek gum previously that the bond 1155 $cm^{-1}$ refers to the carbonyl group C-O-C present in the rapinose ring (pyranose ring)**( Cerqueira, *et.al*, 2011)**, so the frequency 1050 cm-1 represents the C-O-C bond, which is Of the α 1-4 glycosidic bonds as well as the C-O-H bond that crosses the characteristic bonds of polysaccharides **(Fonseca *et al.*, 2011)**, it is worth noting that the strong bond bond at 1400 cm-$^{1}$ in the structure of the gum protein complex is similar to N. The infrared rays present in the glue test as well as the protein isolate test, which represents an indicator of the binding interaction during complex formation **(Timilsena *et al.*, 2016)**.

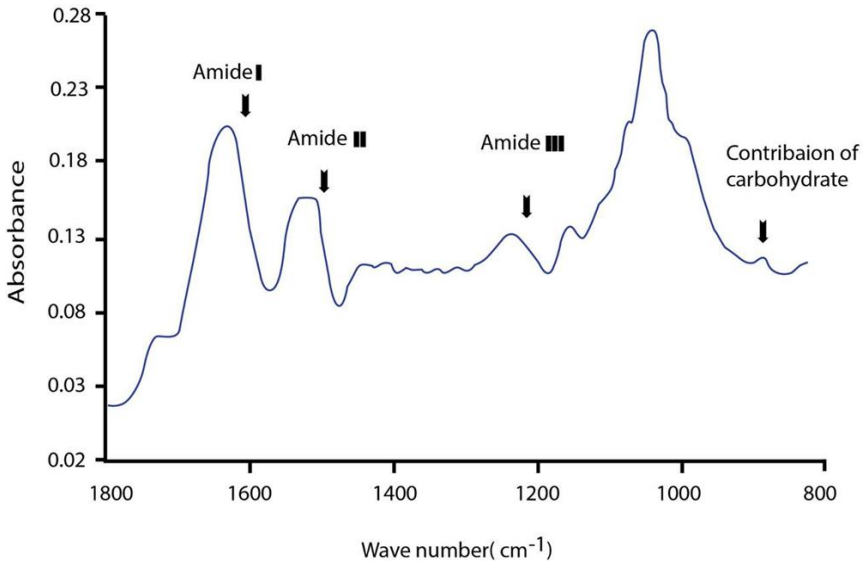


**Figure (4):** FTIR of complex coacervates between the fenugreek gum solution and the fenugreek isolate protein solution.

## CONCLUSIONS

1-Possibility of preparing complex materials used for micro capsulation for food packaging purposes.
2-The complex showed positive effects for preserving the fortified material in food
3- Preparing the complex from fenugreek protein and gum resulted in obtaining a natural coating material

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