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Sara S. Faiad\*;

Ethar Z. Naji<sup>1</sup>;

Jadooda M. Hajeej<sup>1</sup>

<sup>1</sup> Department of Food Science,  
College of Agriculture,  
University of Tikrit, Tikrit,  
Iraq

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## Optimization for Extraction Proteins from Pulp of Watermelon Seed

### ABSTRACT

The study was carried out in the laboratories of the Faculty of Agriculture / University of Tikrit to the period 2017/11/7 - 1/5/2018. The study of watermelon seed pulp the local variety, to obtain protein isolates of the graft after removal of fat. The results showed chemical composition of the imported and local variety the percentage of moisture was 2.9%, 2.5%, ash 4.2%, 3.8%, fat 41%, 40.2%, carbohydrates 19.4%, 18.5% respectively. The result show that the percentage at important and locally were 32.5% and 35%, respectively. The watermelon seeds (imported and local) showed that the moisture 4.2% and 4%, ash 1.9% and 2.5%, fat 23.5%, 21.8%, carbohydrates 49.2%, 48.4%, protein 21.2%, 23.3% respectively. The results of this study showed that the best conditions in the preparation of protein extract from defatted pulp of watermelon seed, were by extraction with distilled water after equalized pH to 12 with ratio bran: water of 1:10 (w:v) for 60 min ,at agitation speed of 500 rpm/min, followed by sedimentation at the isoelectric point .

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## INTRODUCTION:

Watermelon is one of the important crops belonging to the family of Cucurbitaceae. It is usually used in direct consumption as traditional, refreshing and highly nutritious food especially in the summer, and in the production of juices, nectar, fruit cocktail and some other products. As a result, large quantities of waste, such as husks and seeds, are left behind (Hussein et al.,2011) . It is cultivated in order to obtain the ripe fruit that is eaten for its sweet taste and as a refreshing, food and therapeutic, and it is grown in all areas of Iraq, the cultivated area of the yield in Iraq for the year 2006 more than 50 thousand hectares, while the total area planted in the rest of the Arab countries reached 240.81 thousand hectares and the productivity was 13400 kg / ha of the total Arab production rate of 22693 kg / ha, while the total production amounted to 670 thousand tons of total Production Arab states of 5.46463 million tons (Arab Organization for Development and Agriculture, 2007).The production of one plant fruit of the fruit was 5.02 kg / plant for the season (2008) and 4.40 kg / plant for the season (2009) (ALdouri, 2010), and the number of seeds in the fruit 150.44 seeds, seed production is related to the variety and size of fruits , Large fruits were superior to small fruits in the quantity and quality of seeds (Saied, 2008). One of the most common problems in food processing is the disposal of waste from consumption and industrialization (Otutu et al.,2015). The waste causes many environmental problems, including the spread of insects and rodents, as well as the economic burden of transporting transport to waste collection depots. An attempt to take advantage of these residues in the production of some materials required in the food processing industry, as the edible parts are processed and

\* Corresponding author: E-mail: [Sarah282sara@gmail.com](mailto:Sarah282sara@gmail.com)

converted to products such as mashed, canned slices, and the production of juices or jams or pickles, while the seeds are usually neglected as waste if not they are now exploited for commercial purposes (Mello et al., 2000). Consumer demand for protein-rich foods has risen due to concerns about diabetes, obesity, coronary heart disease, high blood pressure and cholesterol reduction (Zucco et al., 2011). The advantage of eating large amounts of protein is a stronger and longer-lasting feeling compared to carbohydrate-based foods with a similar calorie value (Wani et al., 2015). The production of protein-rich foods has been reduced by the low availability of cheap proteins in a number of developing countries, and this has raised interest in the search for inexpensive, non-traditional protein sources of completely unused crops. The origins of the plant family are in the tropics of Africa they are grown as a source of food and edible oil their seeds are good sources of protein and fat. They have been used effectively in oil production fat grains can also be used as a protein supplement in human nutrition watermelon seeds contain high levels of proteins (Wani et al., 2006). Its proteins also contain important amino acids, arginine, glutamic acid, aspartic acid and leucine, the predominant amino acids (Mabaleha et al., 2007). However, the successful use of plant proteins as food additives depends to a great extent on the desired properties they add to the food, so it is necessary to check the quality of the protein and the functional properties it gives (Mello et al., 2000). On food when added to them, as these proteins should have the high nutritional value and desirable functional characteristics, which include the characteristics in which the behavior of protein within the diet is affected during the processing, storage and preparation, Surface properties of proteins own unique due to the large size of the molecule, and the characteristics of a bilateral deal (Amphiphilic properties) (Otutu et al., 2015).

Protein is one of the important ingredients involved in food processing and product development because it has some desirable properties which affect the consumer's acceptance of these food products, physical and nutritional properties, including protein solubility, water and fat binding ability, foam composition and stabilization, Composition and persistence of emulsification, viscosity, which is a functional characteristic that affects the quality of protein and affects its use in food products. The objective of this study is designed to take advantage of these wastes by conserving the environment and reducing waste, as well as taking advantage of the proteins of these seeds as non-traditional sources, as well as the importance of seeds when consumed directly.

## **MATERIALS AND METHODS:**

### **Collection and Sample Pretreatment:**

Two types of watermelon (imported and local) were obtained from the local markets of Salah Al-Din. The seeds were isolated from the fruits and washed and published for drying. Then dry the samples (Kernel and seeds) at room temperature by spreading them in a hot, dry place with stirring to speed up the drying process until complete dehydration is achieved. The samples were crushed using an electric grinder and sifted through a 2 mm sieve to obtain a homogeneous powder, and kept in polyethylene bags and stored in refrigerator until uses.

### **Chemical composition**

#### **Determination of Moisture Content:**

The percentage of moisture was estimated according to the method described in AOAC (2005) by using the electric oven for drying at a temperature of 105 Co until the weight stable.

$$\text{Moisture content \%} = \frac{\text{Weightloss} * 100}{\text{Weightofsample(g)}}$$

#### **Determination of Ash Content:**

Determination of the percentage of ash as described in the method described in the AOAC (2005) by using the incinerator furnace at a temperature of 550 Co until white ash is obtained.

$$\text{Ash Content \%} = \frac{\text{Ash Content} * 100}{\text{Weightofsample(g)}}$$

**Determination of Crude Oil:**

The percentage of oil was estimated by using the AOAC (2005) method by using petroleum ether (40-60) as a solvent for 3 -5 hours, after obtaining the solvent and the fat and then eliminating the solvent using the rotary evaporator.

$$\text{Crude Fat \%} = \frac{\text{Weight of oil extracted(g)} * 100}{\text{Original weight of sample}}$$

**Determination of protein:**

The crude protein was determined using the micro Kjeldah method, sample 0.2 g was weighed into a Kjeldah flask. Catalyst containing sodium sulphate, selenium, and copper was added to the sample along with 10 ml of concentrated sulphuric acid in order to speed up the rate of digestion. The flask was swirled and gently clamped in an inclined position and heated electrically in a fume cupboard. This was heated until a clear solution was obtained. The clear solution was cooled and transferred into a 100 ml volumetric flask and made up to mark with distilled water. The resulting mixture (10 ml) was measured into the distillation set through the funnel. About 5 ml of 2% boric acid was transferred into a 100 ml conical flask containing 2 drops of screened methyl orange and placed at the receiving end of the distillation apparatus. Sodium hydroxide (40%) was used to liberate ammonia from the digest under alkaline condition during distillation. As soon as the contents became alkaline, the pink colour changed to green showing sodium hydroxide to be in excess. Steam was generated into the distillation set and ammonia was trapped in the boric acid solution and about 50 ml of the solution collected into the conical flask. The solution in the flask was titrated against 0.1 M HCl until the first permanent pink colour change was observed. A blank sample was carried through the procedure and the titre value of the blank was used to correct the titre of the samples. The nitrogen content was determined using the equation.

$$\%N = \frac{\text{Molarity of HCL} * 0.014 * \text{titre} * \text{dilution factor} * 100}{\text{Weight of sample used}}$$

The percentage of nitrogen was converted to crude protein by multiplying with 6.25.

**Determination of Carbohydrate:**

The percentage of carbohydrates is calculated by the method described by (Eshun et al, 2013) by subtracting the total percentages of moisture, oil, protein and ash from 100 .

$$\text{Carbohydrates\%} = 100 - (\% \text{ moisture} + \% \text{ oil} + \% \text{ protein} + \text{ash\%})$$

**Determination of optimal conditions for protein extraction:****Determine the optimum pH for extraction:**

The effect of pH on the ability to extract protein from the of watermelon seeds was investigated using distilled water with different hydrogens (2,4,6,8,10,12) using 1N Hydrochloric acid or 1N Sodium hydroxide, as needed, and then calculated the proportion of protein from Biuret after a central sample rejection, and then read absorption by using the optical spectrometer at a wavelength of 540 nm (Plummer,1988).

**Determination of optimum mixing ratio for extraction:**

After selecting the optimal pH for extraction, the protein from the sample under study was extracted with different mixing ratios (1:5, 1:10, 1:15, 1:20) (w:v) and then the protein content in the filtrate was determined by the Biuret method(Plummer,1988).

**Specify the optimum time for extraction:**

The protein was extracted at different times (30, 60, 90, 120) minutes with optimum conditions determined from the previous paragraphs, after which the protein ratio was estimated in a Biuret method (Plummer, 1988) to determine the best time for extraction.

**Determine the best mixing speed for extraction:**

The protein was extracted at a different speed (300, 500, 700, 900) (rpm/ min) using the predefined optimal conditions and then the protein content in the filtrate was determined by the Biuret method (Plummer, 1988).

**RESULTS AND DISCUSSION:****Chemical composition of whole seeds and watermelon:**

Table (1) shows the chemical composition of the whole seeds and Kernel and for the imported melon and local watermelon pulp, showing that the moisture content was higher in the watermelon seeds of the imported and local varieties, with the whole seeds reaching 4.2%, 4% respectively, moisture content of kernel was found to be 2.5%, 2.9% respectively. On the other hand, the value obtained was lower than the Gladvin et al (2016), Jacob et al (2015), who indicated in their study that the moisture content of whole seeds is 6.14% and 7.1% respectively, Abiodun and Adeleke (2010) reported that the moisture in full watermelon seeds ranges from 5.21-4.78%, ratio that is higher than his grandfather and (Egbebi, 2014). It was found that the percentage of moisture in the whole watermelon seeds ranging from 1.41\_1.55%. The percentage of ash in the imported seeds was 1.9%, which is lower than the local, which reached 2.5%. The table showed crude ash content of higher for the imported and local varieties watermelon seed pulp, 4.2% and 3.8% respectively. Oyeleke et al. (2012) reported that the percentage of ash in red watermelon seeds was 4.1%. Wani et al. (2008), when studying the chemical composition of watermelon seeds and seeds Kernel, reported that the ratio was 3.71% and 2.48% respectively. The ash reached 2.70% in whole watermelon seeds by Jacob et al (2015), as Rekha and Rose (2016) pointed out that the ash percentage in watermelon seeds was 2.6%. The percentage of fat in whole seeds, as shown by the same table, was 23.5% for the imported variety, which is higher than the local category of 21.8%. In the pulp of the red watermelon seeds, the fat ratio of the imported and local varieties was higher than that of the whole seeds, And 40.2%, 41% respectively. Wani et al. (2008) reported that the percentage of fat in pulp and whole grains was 33.88% and 21.93%, respectively, Mehra et al (2015) reported that the percentage of fat in whole seeds was 31.99%, Egbebi (2014) showed that the percentage of fat ranged from 44.0 to 55.0% in whole seeds. Oyeleke et al. (2012) and Milovanović (2005) found that the percentage of fat in the whole seeds are 47.9% and 53.5%, respectively. The percentage of protein in the whole seeds was lower than the pulp, where it reached the imported and local varieties 23.5% and 21.8% respectively. The pulp of the watermelon seeds is the high protein content of the imported and local varieties, 32.5% and 35%, respectively, as the percentage of protein in the pulp was higher than the whole seed. Oyeleke et al. (2012) and Arise et al. (2016), when studying the chemical composition of red melon seeds, reached a protein ratio of 27.4% and 30.36-43.60% respectively, and the proportions were lower than those reported by Loukou et al (2007) showed that the protein content was 36%. Wani et al. (2008) reported that the percentage of protein in pulp and whole grains was 40.46% and 16.34%, respectively, when studying their chemical composition. The table shows that the percentage of carbohydrates in the whole waterseeds of the imported and local varieties was 49.2% and 48.4%, respectively, and the pulp of the two varieties was 19.4% and 18.5%, respectively. The results differed with those found by Wani et al. (2008) and Mehra et al. (2015) with 26.31% and 26.57% respectively. Oyeleke et al (2012) found that the proportion of carbohydrate in whole seeds was 9.9%. Tabiri et al (2016) indicated that the percentage of carbohydrate ranged between 9.55 - 15.32%.

**Table (1)** Chemical composition of whole seeds and kernel of watermelon seeds under study

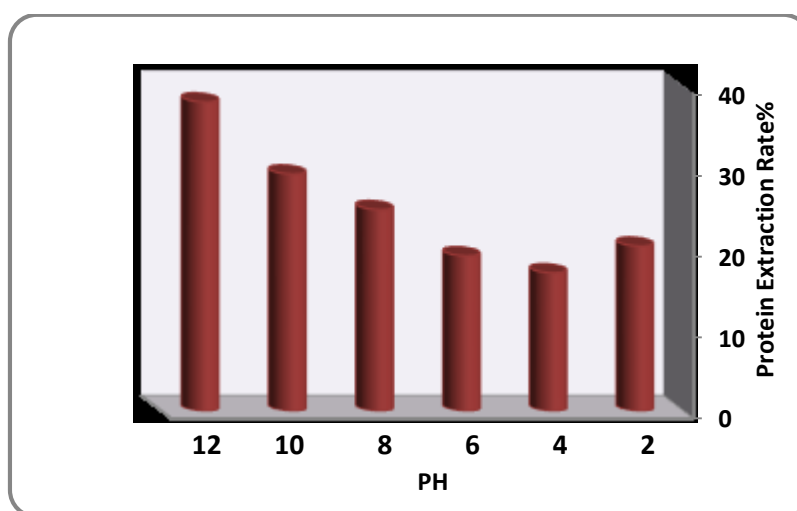
Watermelon seed		Kernel seed watermelon		component
Local	importer	Local	importer	
4	4.2	2.5	2.9	Moisture%
2.5	1.9	3.8	4.2	Ash%
21.8	23.5	40.2	41	Fat%
23.3	21.2	35	32.5	Protein%
48.4	49.2	18.5	19.4	%Carbohydrate

### Optimal conditions for protein extraction

#### pH optimization for extraction: -

Figure (1) shows the effect of pH on the ratio of extracting protein from the pulp of watermelon seeds under study, there is fluctuation in the ratio of extracted protein there was an increase in the protein extraction ratio with high pH increase in the direction of basal numbers significantly compared to acid numbers. High extract at pH 12 reached 41%, while this was lower at lower pH 6, 8, 10, reached 23.5%, 24.9% and 29.3%, respectively. Give pH 4, which is approaching to the isoelectric point Extraction rate to 18%.

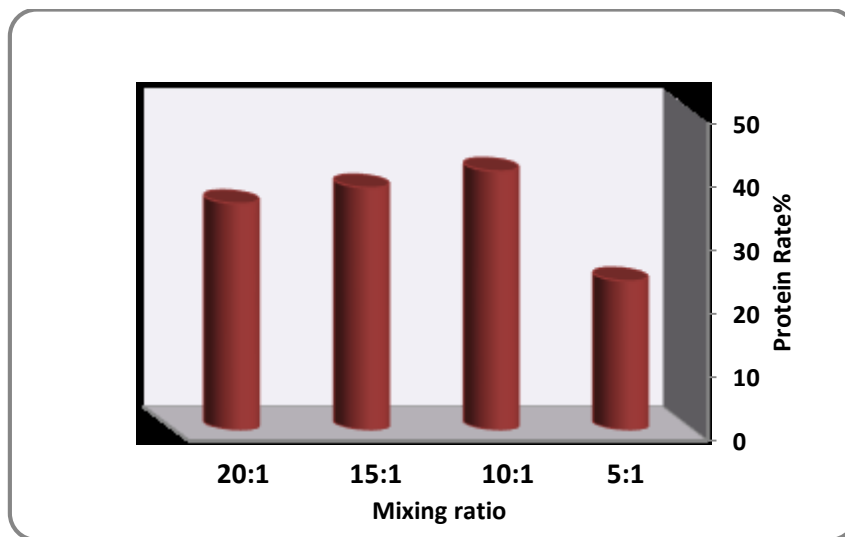
According to Kaul (2011), the ratio of protein extraction increases with the rise of pH toward basal numbers. Alashi et al. (2014) reported that the extraction ratio at pH 12 is the best, as Kaul (2011) PH 11 gave the highest extraction rate, and Alashi et al(2014) reported that the solubility of the protein increases with the rise of the pH to the base more than the acid numbers, Ogunwolu et al. (2009) and mao and hua (2012) note that the solubility of most proteins is almost identical and is lowest in pH 5-4 and increases with the increase or decrease in pH and stay away from the isoelectric point. This is because the total charge of proteins are a negative or positive charge when the pH changes to basal or acidic respectively, thus increasing its solubility and thus extracting it. It also increases toward basal numbers towards the acidic, because most plant proteins are acidic . Solubility gives a clear picture of the best way to extract proteins based on their solubility in different solutions that play a major role in food processing processes (Hrynets et al., 2011).



**Figure 1:** Effect of pH in extracting the Kernel of melted watermelon seeds under study

#### 4: Optimal Mixing Ratio for Extraction

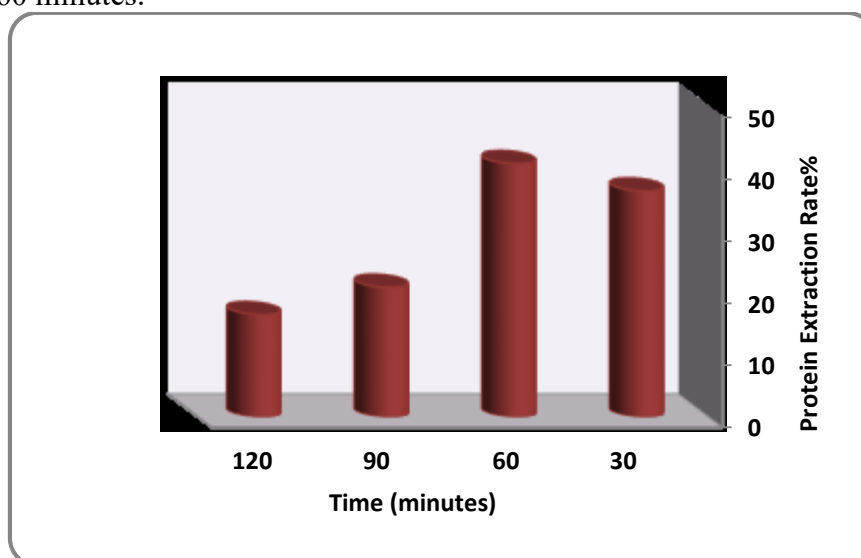
Figure (2) shows the effect of the mixing ratio on the ability to extract the protein from the pulp of the watermelon seeds under study. It is also noted that the ratio (1:10) (weight / volume) gave the highest extract rate of 40.8%, while 23.5% At a ratio of 1:5, and rose to 38.2% at 1:15 and then dropped slightly back to 1:20 to 35.7%. This result is in line with what Arise et al. (2016) indicated that the best mixing ratio for extracting protein from red melon seeds was 1:10 (weight / volume). Ogunbusola et al. (2013) found that the best ratio of protein extract from white watermelon seeds It was 1:20.



**Figure (2):** Effect of the mixing ratio on the ability to extract the Kernel of the melted watermelon seeds under study

#### Specify the optimum time for extraction

Figure (3) shows the effect of the time of extracting the protein from the pulp of the melted watermelon seeds under study. As noted in the table, the best extraction time was 60 min with 40.8% protein extraction. As it decreased to 20.9% and 16.5% at 90 min and 120 min, respectively. The decrease in extraction rate with time increase is due to the possibility of a protein reaction with increasing time. At 30 minutes, the extraction rate was 36.4%. 30 min to insufficient time to extract the protein. This result is consistent with Wani et al (2015) that the best time to extract protein from melon seeds is 60 minutes.

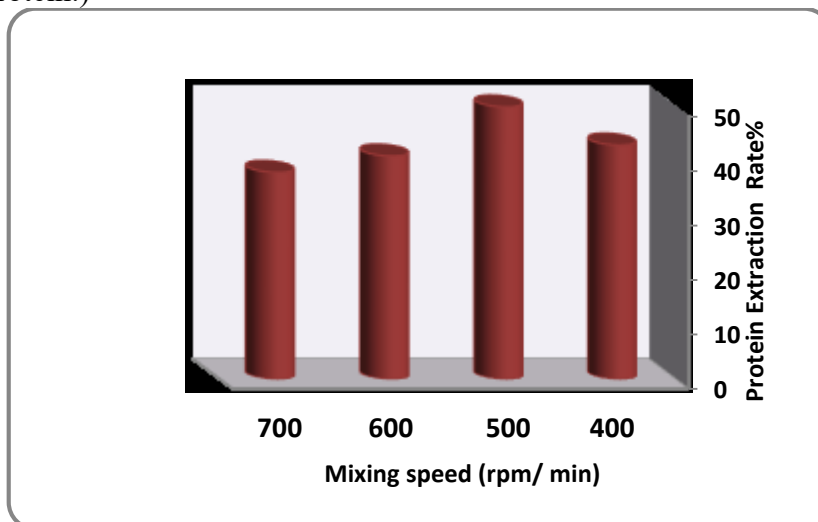


**Figure (3)** Effect of extraction time on protein extraction potential of melted watermelon seed Kernel under study



### Mixing speed is best for extraction

Figure 4 shows the effect of the mixing speed on the ability of extracting the protein from the pulp of the melted red melon seeds. It is also noted is an inconsistent relationship between the protein extraction rate and the mixing speed. The maximum value at the mixing speed was 500 rpm 50% and then decreased to 41% and 38% at the mixing speed of 600 and 700 rpm / min, respectively. This decrease in protein extraction rate when the acceleration is increased may be due to the high-speed denaturation of protein.)



**Figure (4)** Effect of mixing speed (rpm/min) in the ability to extract protein from the Kernel of watermelon seeds

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### الظروف المثلى لاستخلاص بروتينات لب بذور البطيخ الاحمر

ساره سليمان فياض علي وايتار زكي ناجي<sup>1</sup> وجدوع محمد هجيج<sup>1</sup>

<sup>1</sup>قسم علوم الأغذية \_ كلية الزراعة \_ جامعة تكريت

#### المستخلص

تمت هذه الدراسة في مختبرات كلية الزراعة/جامعة تكريت للفترة 7/11/2017 الى 1/5/2018 وتضمنت استخدام لب بذور البطيخ الاحمر للصنف المحلي ، للحصول على المعزول البروتيني من الكسبة بعد ازالة الدهن منها أوضحت نتائج تقدير التركيب الكيميائي لللب لـ صنف المستورد والمحلي أن نسبة كل من الرطوبة بلغت (2.9) و % (2.5، والرماد (4.2) و% (3.8) ، والدهن (41و40.2)% والكربوهيدرات (19.4) و% (18.5 على التوالي، في حين نسبة البروتين للـ صنف المستورد والمحلي والذي يمثل الهدف الرئيسي للدراسة (32.5 و 35 %) على التوالي، اما البذور الكاملة للصنفين (المستورد والمحلي) فان نسبة الرطوبة بلغت (4.2 و 4) %، والرماد (1.5 و 2.5 % )، والدهن (23.5 و 21.8 %) والكربوهيدرات ( 49.2 و 48.4%)، والبروتين (21.2 و 23.3) % على التوالي.

تم استخلاص وعزل البروتين من الكسبة منزوعة الدهن للـ لب بذور البطيخ الاحمر الصنف المحلي بطريقة الاستخلاص القلوي، بعد تحديد الظروف المثلى للاستخلاص وشملت الرقم الهيدروجيني ونسبة الخلط ووقت وسرعه الاستخلاص، اذ كان أفضل رقم هيدروجيني للاستخلاص 12، عند نسبة خلط 10:1 (وزن/حجم)، لمدة 60 دقيقة، عند السرعة 500 دورة /دقيقة، تبعها الترسيب عند نقطة التعادل الكهربائي والبالغة 5.0 .

**الكلمات المفتاحية:** استخلاص، بروتين، لب البذور، البطيخ الأحمر.