Purification and characterization of β-lactoglobulin protein from Iraqi buffalo milk whey and its use as a stabilizer in the dairy ice cream industry.

Ali Khudhiar Jaber Al-Rikabi Mohammed Jabbar Hafiz AL-Wahili

Department of Food Sciences - College of Agriculture - University of Basrah

Abstract

Iraqi buffalo whey was separated by acid method, then its proteins were precipitated using ammonium sulfate and the percentage of proteins was estimated after lyophilization. Beta-lactoglobulin protein was separated and purified by gel filtration technique using a column containing Sephadex G-50. Beta-lactoglobulin protein was separated using electrophoresis technique using polyacrylamide gel in the presence of teratogenic agents.Then prepare a gel of protein purified by the acid and salt method. The properties of the prepared jelly were studied by measuring its strength, porosity and Water-holding capacity . The prepared jelly was used in the milky ice cream industry as a stabilizer. The diffusion and fusion susceptibility of the manufactured mixture was measured and a sensory evaluation of the product was conducted. It was clear from the results that the percentage of separated, purified and lyophilized whey protein was 70%. The electrophoresis technique demonstrated the purity of β-lactoglobulin, separated by gel filtration technique, with a molecular weight of 18.197 kDa. The gel produced using calcium chloride at a concentration of 13 mM and a protein concentration of 12% was superior in strength, porosity, and Water-holding capacity compared to the gel prepared using sodium chloride, glucono-delta-lactone (GDL) and citric acid.The milky ice cream mixture with a higher concentration of jelly excelled in dispersibility and lower melting point compared to less concentrated treatments.

Keywords: β-Lactoglobulin, whey proteins, stabilizer, Ice Cream, Gel filtration

تنقية وتشخيص بروتين β-Lactoglobulin من شرش حليب الجاموس العراقي واستعماله كمثبت في صناعة المثلجات اللبنية

محمد جبار حافظ الوحيلي علي خضير جابر الركابي

قسم علوم الأغذية- كلية الزراعة- جامعة البصرة

المستخلص

فُصل شرش حليب الجاموس العراقي بالطريقة الحامضية ثم رسبت بروتيناته باستعمال كبريتات الامونيوم وقدرت نسبة البروتينات بعد التجفيد، فُصل ونقي بروتين البيتالاكتوغلوبيولين بتقنية الترشيح الهلامي باستعمال عمود يحتوي على Sephadex G-50 شخص بروتين البيتالاكتوغلوبيولين باستعمال تقنية الترحيل الكهربائي باستعمال هلام متعدد الاكريل امايد بوجود العوامل الماسخة ثم حضر هلام من البروتين المنقى بالطريقة الحامضية والملحية .درست خواص الهلام المحضر بقياس قوته ومساميته وقابليته على ربط الماء، استعمل الهلام المحضر في صناعة المثلجات اللبنية كمثبت وقيست قابلية الانتشار والانصهار للخليط المصنع واجري تقييما حسيا للمنتج . اتضح من النتائج ان النسبة المئوية لبروتين الشرش المفصول والمنقى والمجفد بلغت 70 %. اثبتت تقنية الترحيل الكهربائي نقاوة بروتين البيتالاكتوغلوبيولين المفصول بتقنية الترشيح الهلامي وبلغ وزنه الجزيئي 18.197 كيلو دالتون وتفوق الهلام المنتج باستعمال كلوريد الكالسيوم بتركيز 13 ملي مولاري وتركيز بروتين 12% بقوته ومساميته وقابليته على ربط الماء مقارنة بالهلام المحضر باستعمال كلوريد الصوديوم وحامض كلوكونو-دلتا-لاكتون ((GDL وحامض الستريك تفوقت خلطة المثلجات اللبنية ذات التركيز الاعلى من الهلام بقابلية اتنشار ودرجة انصهار أقل مقارنة بالمعاملات الاقل تركيزا.

الكلمات المفتاحية: **β-Lactoglobulin, whey proteins, stabilizer, Ice Cream, Gel filtration**

Introduction:

Whey is the accidental product that is obtained after separating casein or when making cheese. It is considered a surplus product in most dairy factories, so it is thrown into sewage, which causes environmental pollution and therefore must be exploited to prevent this damage because of its great economic feasibility, the proportion of lactose sugar is about 75% as solids. The rest contains whey proteins, and whey proteins represent 20% of milk proteins. Whey proteins are of great importance for their biological and functional benefits, and due to the large number of studies on these proteins, we decided to study the gel produced from β-lactoglobulin because it constitutes a large proportion of whey proteins, as it constitutes 50-60% of the total whey proteins.and due to the lack of a local study on this protein, this protein is considered the first milk protein that was crystallized in 1934, and it is the main protein in whey [1].β-lactoglobulin is one of the globular proteins. These proteins have a well-defined dense structure that determines their function in living organisms.

Globular proteins are usually found in aqueous solutions in the form of monmers. Heating or pressure or salts and acids may change the structural structure and make the protein chain more mobile and as a result parts of different molecules may interact through non-covalent interactions or by forming hydrogen bonds, provided that the appropriate concentration of the protein is reached, which leads to self-assembly or condensation (aggregation).and the production of gelatinous materials that have many uses in the food industry as an emulsifier and stabilizer and an increase in water retention preserves the texture and texture of the food and thus the gel has high moisture content and a three-dimensional network that resists pressure and maintains the external shape [2].

Materials and methods:

1- Material

Milk source

Iraqi buffalo milk was obtained from animal breeders in Al-Madina District / Basrah province.

2- working methods

Separating buffalo milk whey by the acid method

The fat was defatted using a centrifuge at 5000 rpm for half an hour at 4°C. The casein was then precipitated from the defatted milk in a glass beaker on a magnetic stirrer at 25°C.Until a pH of 4.6 was reached by gradual addition of 1 N HCl, the decrease in acidity was followed up by means of a pH-meter Then the casein was separated by filtering the milk with a dull cloth for the purpose of obtaining whey and then filtering the whey again with a special cloth to obtain a more clear whey. [3]

Deposition of whey protein

The whey proteins were separated by adding 291gm of ammonium sulfate (NH4)2SO4 per 1 liter of whey to obtain a 50% saturation while stirring for 10-15 minutes.

Then centrifugation was carried out at a speed of 10000 rpm for 30 minutes, then the precipitate containing whey proteins with ammonium sulfate was taken.Then the precipitate was dissolved in Tris-NaOH buffer solution at a concentration of 0.02 M, the membrane leaching process was conducted for 18 hours and the solution was replaced every 6 hours to increase the separation efficiency to get rid of ammonium sulfate using a dialysis bag with a molecular weight of 3500 Da.Then whey proteins were concentrated in a German-made RE 300 DB type evaporator rotary evaporator,

then dried by a Freeze dryer of a German-made ALPHA 1-2 LD type [4].

Protein percentage estimation

The total nitrogen percentage of lyophilized and dried whey proteins was estimated using an Automatic nitrogen analyzer according to Semi-Micro-Kjeldahl method and multiplying the result by the GMCF 6.38 to get the percentage of protein [5].

Gel filtration using Sephadex G-50

Prepare Sephadex (G-50) gel according to the instructions of the supplying company (Pharmacia fine chemicals).by suspending 54 g of Cephadex in 450 ml of distilled water, the mixture was heated in a water bath at a temperature of 90 ° C for three hours, and degassing was conducted. It was filled into the column to give a gel with dimensions (2.5-90) cm.The equilibration process was carried out with phosphate buffer solution at a concentration of 0.02 molarity and pH 8.6 and a flow rate of (1 ml/min), then the dead volume (Void volume) of the column was calculated by injecting Blue dextran 2000 dye with a molecular weight of 2 million Daltons at a concentration of 5 mg/5 ml distilled water. The tubes were collected from the beginning of their injection of 2 ml per tube until they were completely removed from the column, and the absorbance was measured at a wavelength of 600 nm.Then concentrated whey proteins were injected by lyophilization at a concentration of (200 mg/5 ml) and the separated portions were collected 2 ml for each tube and the absorbance was read at a wavelength of 280 nm. [4]

Diagnosis of β-lactoglobulin by polyacrylamide gel electrophoresis in the presence of SDS teratogens

Beta-lactoglobulin protein was characterized using polyacrylamide gel electrophoresis technique and its molecular weight was determined by Slab polyacryl amide gel electrophoresis under denaturated condition SDS-PAGE [6].

Preparation of β-lactoglobulin protein polymer for saline method

A polymer of β-Lg solution was formed in concentrations (12,9,6)% with deionized water, sodium azide was added at 02.0 mg/ml and mixed on a magnetic stirrer for two hours, and the pH function was adjusted to 7 with 1 M NaOH solution.The solutions were kept in a glass beaker covered with thermal paper in the refrigerator, and on the next day a heat treatment was conducted in a water bath at a temperature of 80 °C for two hours, and the tubes were placed directly in a basin containing cold water for the purpose of reducing the temperature of

the polymer solution to 22 °C at this temperature. Salt was added at concentrations of 1 mo of sodium chloride and 13 mM of calcium chloride, then the tubes were put back on a magnetic stirrer for (1-3) minutes with heat treatment at 60°C. The tubes were kept upside down in the refrigerator and the next day the base of the tubes was cut for the purpose of removing the gel [7].

Preparation of β-lactoglobulin . protein gel

The gel was prepared by two methods, saline and acid, as follows:

Salt method:

1- Using NaCl . Sodium Chloride

The polymer formed in the above paragraph was taken and salt was added at a concentration of 1 mo at a temperature of 22 °C and placed on a thermomagnetic stirrer at a temperature of 60 °C for (1-3) minutes. The gel formed was filled into tubes of 10 ml, and the height of the gel in the tube was 1.5 cm and kept upside down in the refrigerator until the next day, after which the best concentration of protein was selected by performing functional tests for the gel [7].

2- Using calcium chloride CaCl2

In the same way as before, salt was added at a concentration of 13 mM at a temperature of 22 °C, and the solution was placed on a thermomagnetic stirrer at 60 °C for (1-3) minutes.The formed gel was placed in tubes of 10 ml and kept upside down in the refrigerator until the next day, after which the best concentration of protein was selected by conducting the necessary tests [7].

Acid method

1- Using GDL . clucono-delta-lactone acid

A polymer of β-Lg solution was formed in concentrations (12,9,6)% and dissolved in deionized distilled water. The tubes were placed in a water bath at a temperature of 68 °C for 20 minutes, and the pH function was adjusted to 7.2, after which they were kept in the refrigerator for 24 hours.On the next day, the polymer temperature was raised to 22°C, and GDL in powder form was added to the β-Lg solutions to induce cold gel formation at ambient temperature until the pH decreased to 5.3 which is the IP electrophoresis (IP) of β-protein. Lg.Then, sodium azide was added at a ratio of 02.0 mg/ml, and the gel was filled into 10ml tubes, kept upside down, and incubated at 40°C. Then, the best concentration of protein and protein in the gel production was selected by conducting the gel tests [8].

1- Using citric acid

The method mentioned by [9] was adopted with some modifications, where the protein

solution was used with concentrations (12.9.6)% and it was dissolved in distilled, deionized water on a magnetic stirrer for two hours at a temperature of 80 °C and the acidity function was modified to 10 .Then the sample was incubated in the refrigerator for 12 hours, then the temperature of the solution was raised to 22 °C by means of a water bath and citric acid was added at a concentration of 011 mmol on a magnetic stirrer for 2-3 minutes. Sodium azide was added at 02.0 mg/ml, then the gel formed was filled into 10ml tubes inverted at 40°C for 96 hours, after which the best concentration of protein in the gel production was selected by conducting special tests for the gel.

Study of the functional properties of the gel produced from β-lactoglobulin

Gel strength measurement

The gel strength was measured using a BTI-FR2 Texture Analyzer. 5TN.D14 by removing the gel from the tube and measuring the dimensions of the gel piece (1.5 cm height and 1 cm diameter)It was placed on the base of the device and pressure was applied to it at a speed of 8 mm/min, then the mechanical strength reading was taken by the graph, which contains two axes, the y-axis represents the force measured in newtons, and the x-axis represents flexibility as a percentage [10].

Porosity measurement

The porosity of the freeze-dried gel was measured by a Freeze dryer

100 mg of gel was immersed in 1.25 ml of 99% ethanol alcohol in a volumetric flask covered with an aluminum plate.

It was kept in the refrigerator for 24 hours, then the gel piece was weighed after drying its surface with filter paper and the porosity ratio was calculated based on the equation mentioned below (11).

$$porosity = \left(\frac{mh - md}{pv}\right) \times 100$$

Mh: weight of the gel before ethanol immersion, Md: weight of the gel after ethanol immersion ,P: ethanol density V: gel volume before lyophilization

Water-holding capacity measurements

The water-holding capacity was estimated as 150 mg of lyophilized gel was dissolved in 1.5 ml of distilled water in a 10 ml tube.Put on the Vortex mixer for 1 minute and leave for 1 minute at room temperature to homogenize the gel with water and centrifuge at 3000 rpm for 10

minutes.The weight of the liberated water and the water forming the gel, and the holding capacity of the water were calculated based on the equation mentioned below [12].

$$WHC = \left(\frac{wt - wr}{wt}\right) \times 100$$

Wt: Amount of water added Wr: Amount of water released from the gel during centrifugation (Leachate) ,WHC: water binding capacity

The use of β-lactoglobulin gel as a stabilizer and thickener in the manufacture of milk ice cream:

It followed the method mentioned by him,[13] with some modifications to make ice cream,As it mixed 17 g of Nido milk powder, 31 g of sugar, and 1 g of stabilizer (Guar gum) for comparison and different concentrations of β-Lg salt gel by the method of calcium chloride as a stabilizer (A, B, C) with an amount of (1,0.7,0.5) g, respectively. With the stability of the rest of the materials and the solids were mixed well, then 125 ml of warm water was added to the mixture at a temperature of 45 ° C.

Then the mixture was heated at 85 °C for 5 minutes, then the mixture was cooled to 50 °C and mixed using a high-speed electric mixer for 5 minutes and poured into plastic containers of 500 ml and kept in the refrigerator until the next day for the purpose of aging. The next day, it was poured into a French Comfort ice cream maker at -30°C for 30 minutes under constant stirring.

Spread ability analysis

A cube of ice cream with dimensions 4 x 22 cm was placed after freezing in the refrigerator for 3 hours and placed on a metal ruler graduated to 30 cm. A plastic slice was placed on top of the ice cream cube and a metal weight of 100 g was placed on top of it. This was done at a temperature of 22 ° C and a picture was taken. Every two minutes for 10 minutes five times and the diffusion area in millimeters was taken with a ruler [14].

Melting time

Weigh 50 g of milk ice and put it on a glass funnel covered with a metal clip at a temperature of 18°C. The funnel was placed and the sample on it on a sensitive scale. The weight was recorded from the first drop and the weight was read every 10 minutes until the sample melted completely [14] and the flow rate was calculated From the following equation:

Flow rate = {dissolved weight of sample – sample weight / sample weight} x 100

Sensory evaluation

Sensory evaluation of the milky ice cream mixtures was carried out by 10 assessors of the department's professors and graduate students in the Department of Food Sciences / College of Agriculture / University of Basra, according to the form used by [13] [with some modifications.

Table (1) Sensory evaluation form

Rejected 1	Unacceptable 3	Acceptable 3	Good 4	Excellent 5	Degree
general acceptance	Odor	Taste and flavor	texture	appearance	sensory traits
					Control
					A
					B
					C

Statistical analysis

Statistical analysis of the results was conducted through analysis of variance using the Completely Randomized Design (CRD) with one or two factors, and the significant differences between the means were compared using the least significant difference (LSD) test at the 0.05 probability level and using the SPSS ver statistical program. 23.

Results and discussion:

Separation of whey from Iraqi buffalo milk by acid method:

The liquid whey of Iraqi buffalo milk was separated by the acidic method because of the ability of this method to obtain soluble protein, as well as obtaining complete precipitation of casein by 100%, and thus obtaining a clear whey,This was done by using hydrochloric acid, which leads to the transformation of calcium ions bound to caseins and colloidal calcium phosphates involved in bonding caseins with each other to an ionized state and in a dissolved form, making them separate from proteins that in turn precipitate due to reaching the isoelectric point. Then the whey proteins were precipitated with ammonium sulfate and centrifugation was carried out to reduce the quantity and get rid of the casein residues. The precipitate was taken and diluted with a buffer solution. The process of membrane perfusion was conducted to get rid

of the ammonium sulfate, then the process of concentration and lyophilization was conducted to obtain the whey proteins as a solid material. The protein ratio of whey proteins was estimated by Kjeldahl method and found that it is equal to 70%

Gel filtration using Cevadex G-50:

This technique was conducted to separate β-Lg protein from lyophilized whey proteins after being injected with a Sephadex G-50 column of molecular weight (5000_30000).Where this type of cevadex is considered a means of separation and purification at the same time because of its molecular weight, as all whey proteins have molecular weights higher than 30,000 Daltons, so they come out with the washing solution without entering the holes of the gel That is, it is considered within the dead volume of the gel, except for beta-lactoglobulin, alpha-lactoalbumin and lysozyme, which are within the molecular weight range of Cevadex G-50.The results shown in Figure (1) showed the presence of three protein peaks, where the first peak represents whey proteins that came out with the washer solution, the second peak represented β-Lg protein, and the third peak was supposed to represent valactoalbumin protein. The portions of the second tip of the tube (60-90) were taken, concentrated, then dried by alyophilization device, then the protein content was estimated by Kjeldahl β-Lg method and found to be 81%.

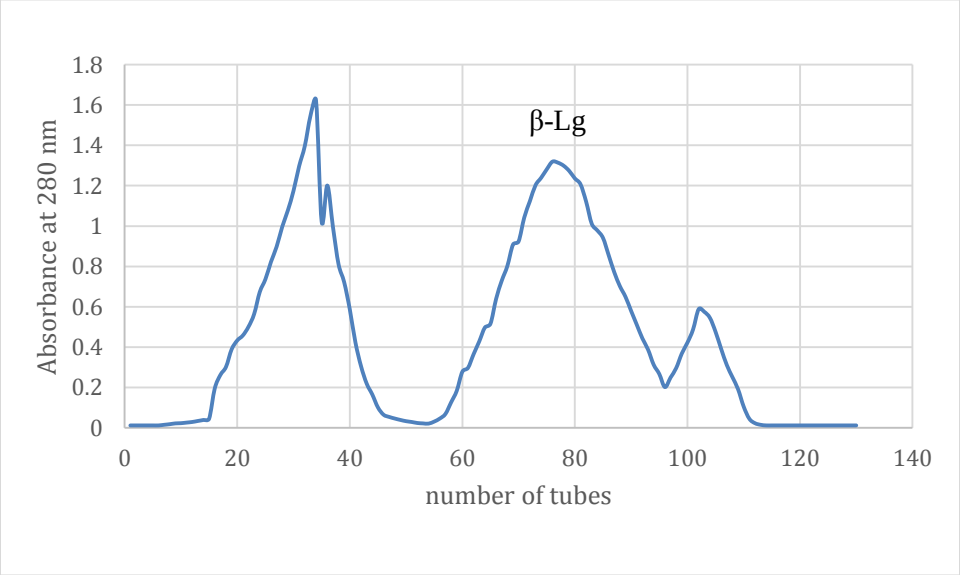

Figure (1) Separation of β-Lg protein by gel filtration chromatography technique using Cevadex G-50

Diagnosis of β-lactoglobulin by polyacrylamide gel electrophoresis technique in the presence of teratogenic agents:

Where it works to open the introversion of the globular protein and transform it into the initial structure of the protein in the form of a sequential line of amino acids for the protein with a negative charge and is separated on the basis of molecular weight.The larger the protein, the slower its movement during its movement towards the positive electrode of the electrophoresis device, and a standard protein of β-Lg was used to infer the separated protein by gel filtration, as shown in Figure (2).

β-Lg protein was characterized by gel electrophoresis at a concentration of 15% with the presence of SDS . teratogenic agents

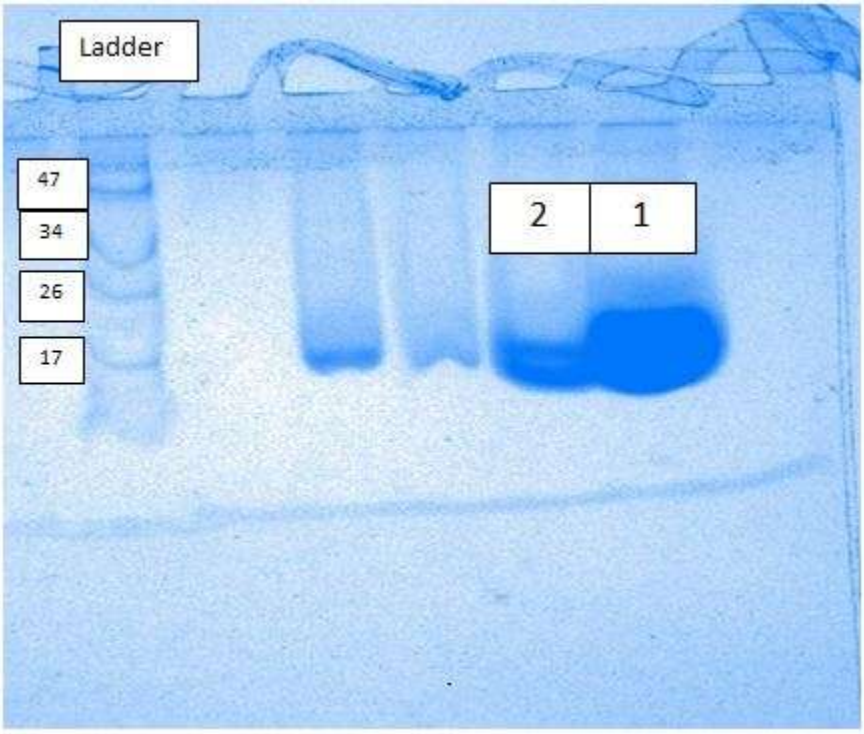

Figure 2 Electrophoresis of β-Lg protein. 1 = standard β-Lg protein, 2 = β-Lg protein sample separated from the second peak gel filtration, (47,34,26,17) are the molecular weights of standard proteins.

Determination of the molecular weight of β-lactoglobulin protein separated from Iraqi buffalo milk whey:

The standard curve method was used to estimate the molecular weight of the separated protein by comparing the relative motion with the standard protein,

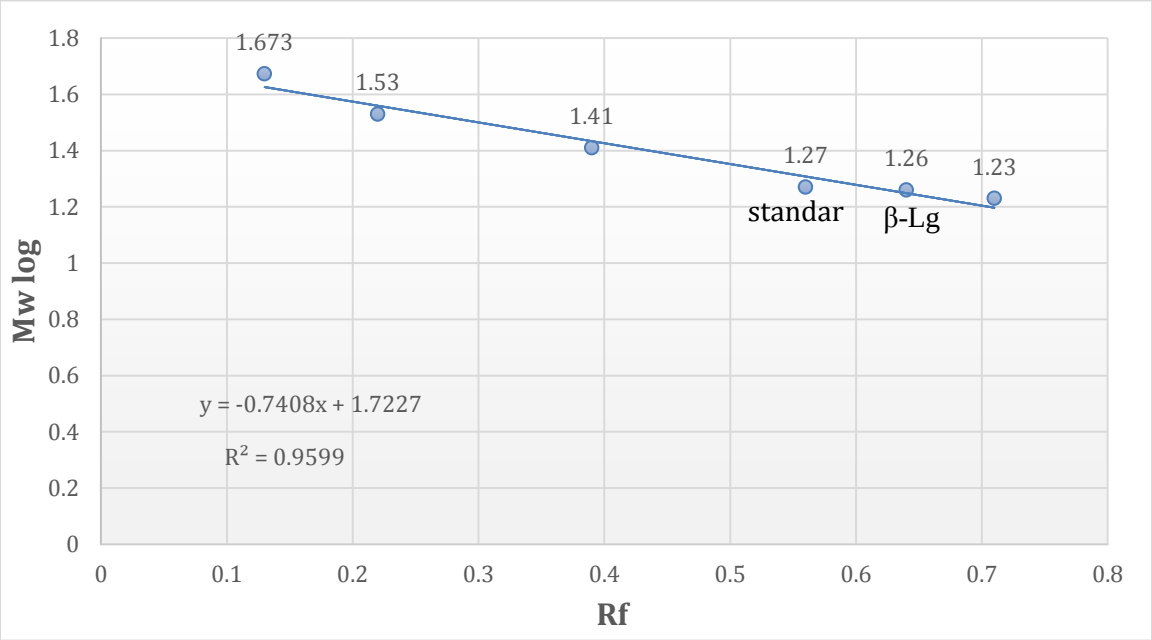

Figure (3) shows the molecular weights of the proteins, and the molecular weight of β-Lg protein separated from Iraqi buffalo milk whey was 18.197 Kda

This result is close to what was found by [4] and the molecular weight of the standard protein was 18.400 KDa. Figure 3 Standard curve for determination of the molecular weight of β-Lg . protein

Production of β-lactoglobulin protein gel

The gel consisting of β-Lg protein was characterized by a difference in its functional properties. The reason for this discrepancy is due to the different concentrations used of the protein as well as the difference in the type of salt and acid. The best concentration of protein and the best method of gelation were selected by studying the functional properties of the resulting gel.Studies have shown that the thermal treatment of whey proteins leads to the opening of the introversion, which makes the thiol group free and effective. When treating β-Lg protein at a temperature of 80 °C and above, it leads to protein aggregation (non-reversible reaction) and the formation of secondary units

(Dimer) and contains Each monomer of this protein contains 5 molecules of the amino acid cysteine distributed at positions 160,121,119,106,66.One sulfhydryl group (SH) is linked at site 106, and two groups of double sulfur bonds, the first linking sites 160 and 66, and the second linking sites 106 and 119, leaving a free thiol group at site 121 leading to covalent bonding between molecules

This helps to form disulfide bonds. Cross-linking bridges help in the cohesion and stability of the polymer, and therefore the higher the protein concentration, the stronger the gel [15].

Study of the functional properties of the gel produced from β-lactoglobulin

Gel strength measurement

Studying the strength of the gel by measuring its strength with a Texture Analyzer is one of the basic tests to determine its quality, where the structural properties of the gel differ through the variation of the strength of the gel caused by the protein concentration, concentration and type of salt and acid used, temperature and pH function. However, in this study, the gel was produced with a constant temperature, acidity function, and salt or acid concentration with the change in protein concentration and type of salt and acid only.Table (2) shows the methods of gel production by the two salty and acidic methods. The results showed that the saline method using calcium chloride with a protein concentration of 12% gave the best value for the strength of the gel, reaching 2.229.This is consistent with [7] in terms of preference, which is the highest value in all gel formation methods, where the result of the statistical analysis confirmed the existence of significant differences at the probability level of $P<0.05$ between the methods of gel formation, either the acid method gave the highest value 1.303 using Glucono Delta Lactone acid (GDL) with a protein concentration of 12% as well. The results showed that the increase in the protein concentration is accompanied by an increase in the gel strength due to the increase in the cross-bridges of the disulfide bonds.

Table (2) Strength of β-lactoglobulin gel using a tissue analyzer

Gel production methods	Concentrations		
	6%	**9%**	**12%**
NaCl	0.215	0.523	1.177
$CaCl_2$	0.501	1.491	2.229
GDL	0.332	1.021	1.303
Citric acid	0.125	0.341	0.902

L.S.D ($p<0.05$) =3.122

Gel porosity measurement

Porosity means the distances between inside and outside the material. The porosity assay is one of the important tests for hydrogels because it determines the ability of the gel to retain water. Table (3) shows the porosity ratios of the gel produced from β-Lg protein at 12%. Neglecting the rest of the ratios due to giving 12% the highest gel strength for all methods of gel formation by examining the gel strength, and therefore it is logical that it gives higher porosity ratios than the rest of the ratios due to the presence of cross-linking bridges) more than the rest of the ratios and between [11].The more cross-bridges that make up the triple network, the smaller the pores, and thus the greater the water retention capacity, because the large pores allow water to permeate through them. The results of the statistical analysis showed that there were significant differences at the probability level of $P<0.05$ between the methods of gel formation, where the gel was superior to the saline method using calcium chloride and recorded the highest porosity rate, which amounted to 81.9%. While the percentage of porosity by a saline method using sodium chloride for the same percentage of protein (12%) is 62.2 and this is due to the fact that the percentage of protein gathering using divalent salts such as calcium chloride increases more than monovalent salts by forming bridges between protein molecules or by reducing repulsion Charges between proteins [7] The lowest porosity ratio was recorded for the acid method using citric acid, which amounted to 55%, and these results were close to what was found [16] using several concentrations of hydrogels.

Table (3) Percentage of gel porosity for β-lactoglobulin

Gel production method	**Porosity**%
Nacl	62.2
CaCl2	81.9
GDL	60.5
Citric acid	55

L.S.D ($p<0.05$) =2.008

Water-holding capacity measurements

Water-holding capacity is the ability of a substance to retain water between its molecules and measuring this property of the gel, gives an idea of the extent of the cohesion of this gel and its ability to retain water and thus determines the structural functions of the gel.The results of the statistical analysis showed that there were significant differences at the probability level of $P<0.05$ between the methods of gel formation Table (4) shows the superiority of the saline method using calcium chloride in its ability to bind water, which amounted to 78.5%, and these results are close to what was found [12].

Table (4) Water-binding ratio of β-lactoglobulin protein gel

Gel production method	Water-holding capacity
NaCl	56
CaCl2	78.5
GDL	50
Citric acid	25

L.S.D ($p<0.05$) =1.860

As we mentioned previously, the temperature, type and concentration of salt and acid, the acidic function and the concentration of protein play a big role in the synthesis of the gel, as the concentration of protein has an apparent effect on the synthesis of gel particles.It was found [17] that the thermally modified protein at a temperature of 120 m/min has a water-holding capacity five times the non-thermal modified protein due to the formation of multiple peptides, and this indicates the significant effect of temperature on gel production.

Study of the use of β-lactoglobulin gel as a stabilizer in the manufacture of milk ice cream diffusion

The diffusionability of milk ice is one of the important physical characteristics that must be studied when manufacturing it in order to reach the most appropriate diffusion ability with the lowest possible concentration of the fixative, as the spreadability depends on the type and quantity of the fixative used. With Guar gum industrial fixative.The results of the statistical analysis showed that there were no significant differences between the comparison sample and the A sample at the probability level of P<0.05, where the comparison sample recorded the lowest spreading distance, which was 31 mm at a time of 10 minutes, while the spreadability of the A sample (highest concentration) was close to the control sample, which amounted to 33 mm. At a time of 10 minutes, either sample B. It gave a greater diffusion ability than sample A as well as sample C gave greater diffusion than sample B and the reason for this difference in diffusion is the concentration of the stabilizer. Spreadability The results did not agree with what was found [14].

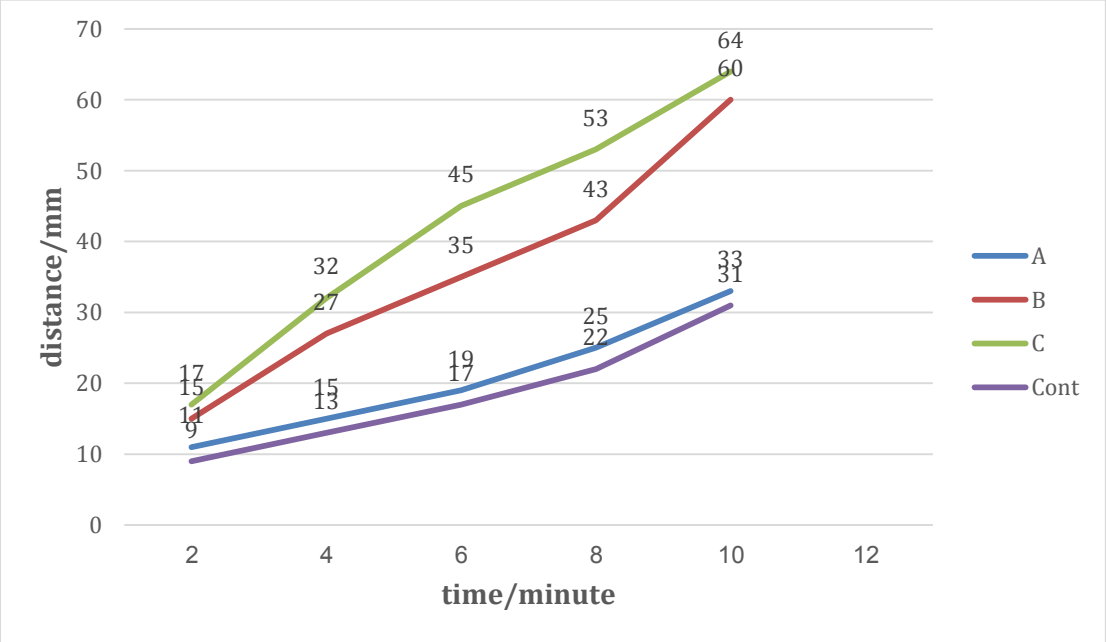

Figure (4) The ability to spread milky ice cream mixtures

melting time

The ability of milk ice to maintain its external shape and consistency is one of the important and required qualities in the manufacture of milk ice cream, so these products must be characterized by the lowest possible melting average. Industrial Guar gum excelled by the lowest melting average.Where

the results of the statistical analysis showed that there were significant differences at the probability level of P<0.05, and sample A was close to the comparison sample, and the reason for this may be due to its containing the highest percentage of a protein gel.Because the gel has the ability to preserve water, it gave a lower melting rate than sample B, and the latter gave a lower melting average than sample C, where the higher the concentration, the lower the melting average. This is what was clarified by the results in Figure (5), which shows the flow average of the milky ice cream mixtures, and this is consistent with [14] where the melting time was estimated for six samples of traditional ice cream.

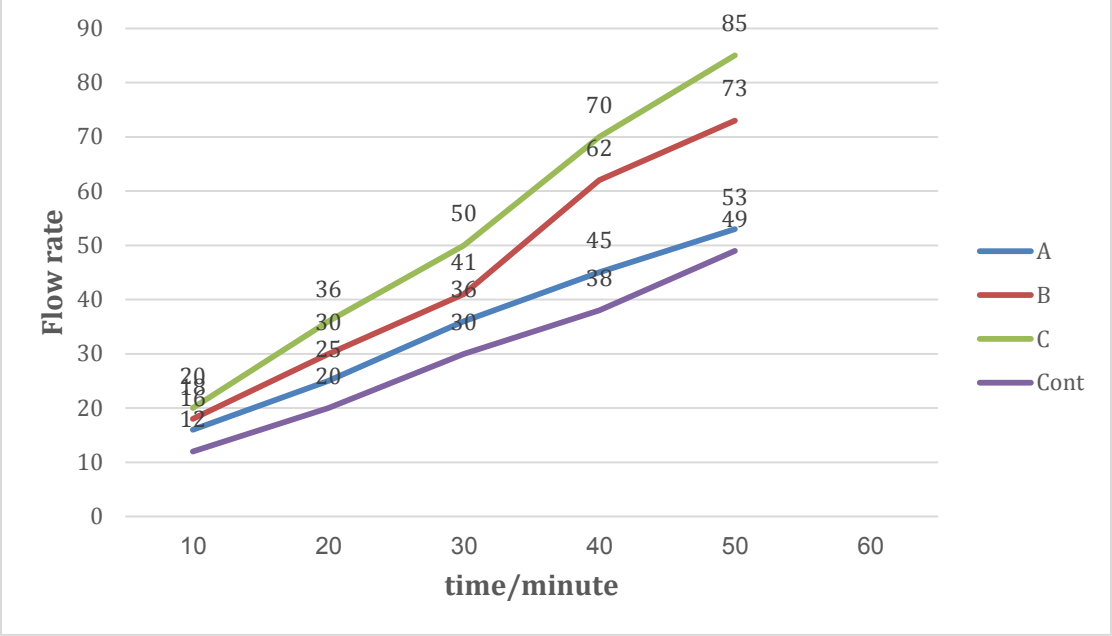

Figure (5) Flow rate of milky ice cream mixtures

sensory calendar

Table (5) shows the results of the sensory evaluation of the milk ice cream mixtures, where the results of the statistical analysis showed that there were no significant differences between the control sample and sample A in all characteristics, with a significant difference between them and sample B except for texture, where the results did not show any significant difference. This is due to the low concentration of protein gel in sample B and less in sample C. As for the texture of the comparison sample and sample A, the result of the statistical analysis was similar to the statistical analysis of appearance.Where appearance is related to texture from a physical point of view, the comparison sample excelled in all treatments, and sample A was similar to

the comparison sample in terms of general acceptance and identical to it in terms of taste, flavor and smell due to the different concentration of protein gel, where it was found [18] that whey proteins act as an emulsifier and this works to bind flavoring materials.

Table (5) Sensory evaluation of milky ice cream mixtures

Rejected 1	**Unacceptable 3**	**Acceptable 3**	**Good 4**	**Excellent 5**	**Degree**
general acceptance	**Odor**	**Taste and flavor**	**texture**	appearance	sensory traits
4.5	4.3	4.4	4.2	4.7	Control
4.3	4.3	4.4	4.5	4.2	A
4	3.9	4.1	4	4.1	B
3.5	3.4	3.6	3.3	3.6	C

L.S.D ($p<0.05$) =0.398 appearance , **L.S.D** ($p<0.05$) =0.269 Taste and flavor ,**L.S.D** ($p<0.05$) =0.535 Odor,**L.S.D** ($p<0.05$) =0.49 texture ,**L.S.D** ($p<0.05$) =0.582 general acceptance

Conclusions:

1- The possibility of obtaining β-Lg protein by purifying it from Iraqi buffalo milk whey by gel filtration method using Cevadex G-50.

2- The saline method using 13 mM calcium chloride and 12% protein concentration proved the best for gel production by studying the functional properties of the gel.

References

1-Liu, H. C.; Chen, W. L. & Mao, S. J. T. (2007). Antioxidant nature of bovine milk *β*-Lactoglobulin. *Journal of Dairy Science*, 90(2): 547-555.

2-Banerjee, S. &Bhattacharya, S. (2012). Food Gels*:* Gelling Process and New Applications. *Critical Reviews in Food Science and Nutrition*, 52(4): 334-346.

3- Al-Mashikhi, S. A. &Nakai, S. (1987). Isolation of bovine immunoglobulins and Lactoferrin from whey protein by gel filtrations techniques. *Journal of Dairy Science*, 70:2486-2492.

4- Neyestani, T. R.; Djalali, M.; & Pezeshki, M. (2003). Isolation of α-lactalbumin, β-lactoglobulin, and bovine serum albumin from cow's milk using gel filtration and anion-exchange chromatography including evaluation of their antigenicity. *Protein Expression and Purification*, 29(2), 202–208.

5- Edwards, A. H. (2007). The semi-micro Kjeldahl method for the determination of nitrogen in coal. *Journal of Applied Chemistry*, 4(6), 330–340.

6- Roy, v.k; Kumar, n.; Gurusubramanian, G. (2012) Proteins – structure, properties and their separation by SDS-polyacrylamide gel electrophoresis. *Science Vision,*16 (4): 170-181.

7- Ako, K.; Nicolai, T.; & Durand, D. (2010). Salt-Induced Gelation of Globular Protein Aggregates: Structure and Kinetics. *Biomacromolecules*, 11(4):864-871.

8- Alting, A. C.; Hamer, R. J.; de Kruif, C. G.; & Visschers, R. W. (2003). Cold-Set Globular Protein Gels: Interactions, Structure and Rheology as a Function of Protein Concentration. *Journal of Agricultural and Food Chemistry*, 51(10): 3150-3156.

9- Abaee, A.; Madadlou, A.; & Saboury, A. A. (2017). The formation of non-heat-treated whey protein cold-set hydrogels via non-toxic chemical cross-linking. *Food Hydrocolloids*, 63: 43–49.

10- Yang, S.; Park, K.; & Rocca, J. G. (2004). Semi-interpenetrating Polymer Network Superporous Hydrogels Based on Poly (3-Sulfopropyl Acrylate, Potassium Salt) and Poly (Vinyl Alcohol): Synthesis and Characterization.

Journal of Bioactive and Compatible Polymers, 19(2), 81–100.

11- Rashid, A.; Ahmad, M.; Tulain, R.U.; & Iqbal, F.M. (2015) Fabrication and Evaluation of 2-Hydroxyethyl Methacrylate-co-Acrylic Acid Hydrogels for Sustained Nicorandil Delivery. *Tropical Journal of Pharmaceutical Research*, 14 (7): 1121-1128.

12- Maltais, A.; Remondetto, G. E.; Gonzalez, R.; & Subirade, M. (2005). Formation of Soy Protein Isolate Cold-set Gels: Protein and Salt Effects. *Journal of Food Science*, 70(1), 67–73.

13- Vargas-Bello-Pérez, E.; Cancino-Padilla, N.; Geldsetzer-Mendoza, C.; Vyhmeister, S.; Morales, M. S.; Leskinen, H.; & Ibáñez, R. A. (2019). Effect of feeding cows with unsaturated fatty acid sources on milk production, milk composition, milk fatty acid profile, and physicochemical and sensory characteristics of ice cream. *Animals*, 9(8), 568.

14- Fiol, C.; Prado, D.; Romero, C.; Laburu, N.; Mora, M.; & Iñaki Alava, J. (2017). Introduction of a new family of ice creams. *International Journal of Gastronomy and Food Science*, 7, 5–10.

15- Hernández-Ledesma, B.; Recio, I. and Amigo, L. (2007). β-Lactoglobulin as source of bioactive peptides. *Amino Acids*, 35(2):257-265.

16- Ranjha, N.M. and Qureshi, U.F. (2014). Preparation and characterization of cross-linked acrylic acid/hydroxypropyl methyl cellulose hydrogels for drug delivery. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(4):400-410.

17- Kneifel, W. and Seiler, A. (1993). Water-holding properties of milk protein products-A review. *Food Structure*, 12(3): 3.

18- Batista, M. A.; Campos, N. C. A.; & Silvestre, M. P. C. (2018). Whey and protein derivatives: Applications in food products development, technological properties and functional effects on child health. *Cogent Food & Agriculture*, 4(1).