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PREPARATION OF PEPTIDE HYDROLYSATES FROM BETA CASEIN ISOLATED AND PURIFIED FROM IRAQI CAMEL MILK

Sharaf. Ali. Hadi. Al-Shaikh*[1], Kifah. Saed. Doosh[2]
*[1]Department of Food Science, College of Agriculture, University of Kufa, Iraq
[2] Department of Food Science, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq

ABSTRACT

The current study aimed to isolate and purify the Beta Casein (β-CN) protein from Iraqi Camel milk by urea, and ion exchange chromatography using a DEAE-Cellulose column, and gel filtration by using a Sephadex-G100 column, determining the molecular weight (M.wt) of protein isolate by polyacrylamide gel electrophoresis in the presence of the denaturant Sodium Dodecyl Sulphate (SDS). Enzymatic hydrolyzates were prepared using both pepsin and trypsin enzymes and their mixture (1:1), then the degree of hydrolysis (DH) was estimated after incubation for 8 h. and compared with bovine protein and standard proteins, the elution of β-CN using a DEAE-Cellulose column showed the appearance of two separate peaks, and their molecular weight was 24 KDa and 20 KDa Respectively. The Sephadex-G100 gel filtration technique also showed the appearance of a single peak whose molecular weight was 24 KDa compared to β-CN, which weighed 23 KDa. The results showed that the highest degree of decomposition was 52.32% by using a mixture of pepsin and trypsin. The results indicate that the protein β-CN separated from camel milk can be highly purified using ion exchange chromatography and gel filtration techniques, and peptide hydrolysates can be prepared from it.

Key words: active peptides, casein, enzymatic hydrolysates, gel filtration, ion exchange
***Part of Ph.D. Dissertation for the 1st author**

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INTRODUCTION

Camel milk is a major source of important nutrients necessary for growth and human health, especially in hot and arid desert areas, camel milk has high-value and unique physical and nutritional properties compared to cow's milk and other mammals (Ho *et al.*, 2022). Many studies have indicated that camel milk has many health and medical benefits both compared to cow's milk, the reason is that camel milk contains a high percentage of beneficial and effective compounds such as biologically active proteins, vitamins and mineral elements that are important for enhancing human health, Camel milk also has the advantage which that contains a low percentage of compounds harmful to health (Gaytan *et al.*, 2019; Swelum *et al.*, 2021). Milk proteins in general are an important food source for humans because they contain important essential amino acids, the amount of protein in camel milk ranges between 21.5-49 g/liter. With an average of 31 g/liter of Milk caseinates are precipitated by the acid method at the isoelectric point (PI) of 4.6 and 4.3 for cow's and camel's milk, respectively, (Liang and, Luo. 2020; Mati *et al.*, 2017). Many studies have proven that camel milk proteins contain many biologically active peptides with therapeutic and nutritional properties, which have a high ability to be antioxidants, anti-inflammatory and anti-bacterial, as well as

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anti-diabetic, anti-obesity, anti-cancer, lower blood pressure, and an effective treatment for kidney patients (Abbes *et al.*, 2021; Hoseini *et al.*, 2020; Wang *et al*., 2020). Caseins are irregularly structured proteins, which makes them sensitive to proteolysis and resistant to heat treatments. camel milk caseinates are phosphorylated proteins and the main and most abundant component in milk, whose percentage ranges between 61.8-88% of the total different camel proteins. Casein consists of four types of the caseins α_{S1}-CN, α_{S2}-CN, β-CN, and K-CN, in approximately 22, 9.5, 65, and 3.5%, respectively, structurally; these caseins are aggregated together (Kappeler *et al.*, 1998; Kim *et al*., 2020; *Lajnaf et al*., 2022). Camel milk is similar to human milk in its high content of β-CN protein. It is also characterized by the absence of β-lactoglobulin compared to cow's milk, β-lactoglobulin is the source of allergies in newborns, and the high percentage of β-CN and the absence of β-lactoglobulin in camel milk is possible, it reflects an increase in the rate of digestibility and greatly reduces the incidence of allergies in the intestines of children who drink camel milk (El-Agamy, 2009) Kappeler *et al*., (1998) indicated that β-CN is the main protein in camel milk and that its concentration ranges between 12-15 g/L of milk and represents the largest percentage of total caseinate, amounting to 65%, and its molecular weight is 24.65 KDa and contains 217 amino acids. While some studies indicated that the percentage of β-CN in camel milk casein is 53.4%, 44.8% (Felfoul *et al*., 2017; Lajnaf *et al*., 2022). Many studies have proven that camel milk β-CN possesses a number of low molecular weight bioactive peptides that have high efficacy and perform many important biological functions that have the potential to treat some diseases such as obesity, type 1 and type 2 diabetes, and reducing high blood pressure and hypertension, blood cholesterol, angiotensin-converting enzyme (ACE-I) inhibition, anti-cancer and antioxidant, and reduces the risk of heart disease (Ganzorig *et al*., 2020; Lajnaf *et al*., 2021; Redha *et al*., 2022). The β-CN protein is not limited to being a nutritional protein only, but it has a high biological value and has many distinct roles, and studies continue to add and confirm its nutritional and therapeutic importance, Therefore, our current studies aimed to isolate, purify and characterize the β-CN protein from camel milk and study the effect of the digestive enzymes' pepsin and trypsin on the protein by estimating the degrees of degradation up to eight hours.

MATERIALS AND METHODS

Milk source camel`s camel milk was prepared and collected from the farms of Badia, Samawah Governorate, and Badia, Najaf Governorate, from the local Iraqi breed, and the samples were transported in a sterile and refrigerated manner to the laboratories of the Food Sciences Department / College of Agriculture / University of Baghdad.

Chemical analysis of milk

The components of raw camel milk were estimated using a programmed electronic milk components estimator, Lacto Flash, of German origin, according to the company's instructions. The pH was estimated using a pH meter, model 211, type HANNA, of Romanian origin. The acidity was estimated by titrating the raw milk with a 0.1N NaOH solution in the presence of phenolphthalein, according to what was stated in A.O.A.C, (2016).

Milk fat separation

Whole camel milk fat was separated using a German refrigerated centrifuge at 2400 x g for 20 minutes (AlKhalidy, and Dosh, 2023).

Acidic casein preparation

Acid casein was prepared according to what was mentioned by Haddad and Doosh, (2023), with some modifications. Casein was precipitated from the milk using hydrochloric acid (1 M) until the pH of 4.3 was reached, which is the electrical neutralization point for camel milk casein. Then the precipitated casein was separated using centrifugation at a speed of 3000 x g for 20 minutes and at a temperature of 4 °C. The casein was filtered through Whatman filter paper No 4 under vacuum using a Buchner funnel, the precipitated casein was washed with distilled water three times and dissolved in distilled water with the addition of sodium hydroxide (1 M) to raise the pH to 7. Then the casein was

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precipitated again using 0.1 M hydrochloric acid. The precipitate was separated by centrifugation and the washing process was repeated by dissolving and precipitating the casein again, the precipitate was dried by lyophilization and preserved by freezing until preparation for subsequent tests.

Primary β-Casein purification: Primary β-casein was purified from total acidic casein according to the method described by Ptiček Siročić *et al*., (2016) . 30 g of acidic casein was dissolved in 700 ml of 3.3 M urea solution and the pH was adjusted to 7.5 by adding 1 M sodium hydroxide and the volume was brought to 900 ml of distilled water, then the pH of the solution was reduced to 4.6 using 1 M hydrochloric acid, and left until a precipitate formed, which was eliminated by filtration using Whatman filter paper No 4. The pH was adjusted to 4.9 for the remaining filtrate. Two liters of distilled water were added to the filtrate and incubated at 30°C for 24 h. The white precipitate formed was collected by filtration using Whatman filter papers No. 4 a Buchner funnel. The white precipitate was taken and dissolved in 400 ml of 3.3 M urea solution and the pH was adjusted to 7.5 using 1 M sodium hydroxide solution and reduced the pH was set to 4.6 and left at a temperature of 30 °C until the precipitate formed. The filtration process was carried out to get rid of the precipitate. The filtrate with a pH of 4.9 was taken and 800 ml of distilled water was added to it and incubated at a temperature of 30 °C for 24 hours. Then the precipitate formed by filtration, which represents β-casein, was collected, washed with distilled water several times, and dried by lyophilization.

β-Casein purification by ion exchange chromatography: β-Casein was purified by ion exchange chromatography according to the method mentioned by AlKhalidy and Dosh, (2022) , where 0.5 g of lyophilized β-casein was dissolved in 100 ml of a 0.1 M solution of phosphate buffer, pH 7.4, containing urea. (3.3 molar) and Mercaptoethanol (0.010 molar). The mixture was mixed with Cellulose DEAE treated with the same buffer used to dissolve the mixture for 15 minutes at 4 °C. The ion exchange mixture was poured into a column with dimensions x 3.515 cm. The column was washed with the buffer solution to remove unbound proteins. Proteins bound to the β-CN protein were removed using a graded salt solution (0.1, 0.175, 0.2 molar) with the buffer solution, at a flow speed of 50 ml/hour and 5 ml/tube. The optical absorption of the β-casein protein solutions was read at a wavelength of 280 nm with a spectrometer UV-1900i. The tubes containing β-casein were collected and treated with membrane osmosis. The lyophilization process was performed.

β-Casein purification by gel filtration

β-Casein was purified according to the method described by Al-easawi *et al*., (2020), with some modifications. Sephadex G-100 gel was used to prepare the gel filtration column according to the instructions of the supplying company, Pharmacia Fine Chemicals. 0.2 g of lyophilized β-casein, previously purified using the DEAE-Cellulose ion exchange technique, was dissolved in 5 ml 0.005 of phosphate buffer solution, and passed on a Sephadex G-100 column, with dimensions (63 x 1.5 cm). Then the parts were recovered using a balancing buffer, where the flow rate was adjusted at 15 ml/hour, 3 ml per tube. The absorbance intensity of each tube was measured with a UV-1900i spectrophotometer at a wavelength of 280 nm. The tubes containing the pure protein were collected, treated with membrane osmosis against distilled water for 24 hours at refrigeration temperature, and concentrated using Crystalline Sugar. The total protein concentration was estimated by the Bradford method, and then dried by lyophilization.

Protein concentration assay

The concentration of the β-casein protein recovered from the ion exchange column and gel filtration was measured by the Bradford method, as shown in Figure (1), according to (Maehre *et al*., 2018).

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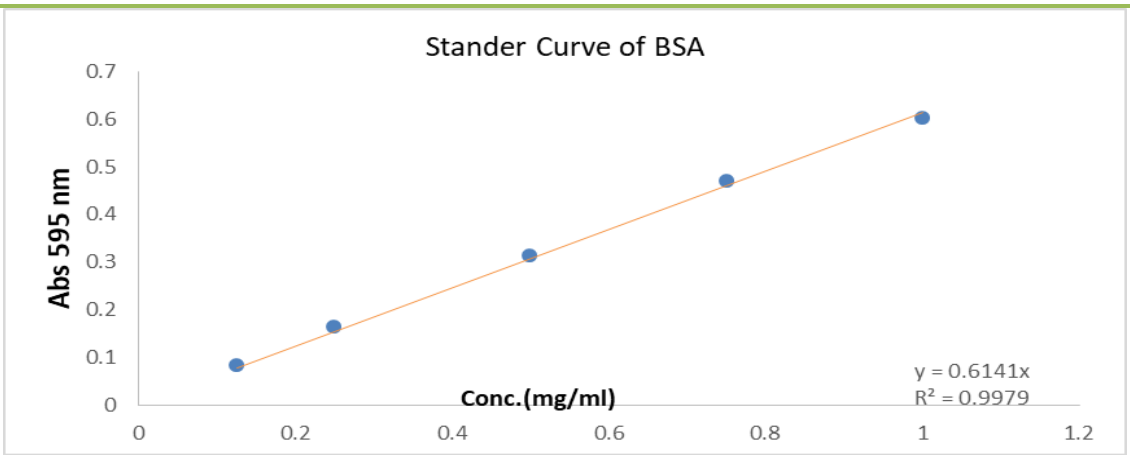

Figure 1. The standard curve for bovine serum albumin proteins using the Bradford method, which represents the relationship between the concentration of proteins and absorbance at a wavelength of 595 nm

Confirm purity and molecular weight estimation: The purity of β-casein was confirmed and the molecular weight was determined according to the method of Al Khalidy and Dosh, (2022) with some modifications, as the polyacrylamide gel electrophoresis method was used in the presence of the denaturant (SDS) Sodium Dodecyl Sulphate and using a vertical electrophoresis device and compared with standard proteins and bovine β-CN protein.

Confirm the purity of β-CN using RP-HPLC: The purity of β-CN was confirmed according to the method described by (Maehre *et al*.,2018), where a reversed-phase RP-HPLC device with a C18-OSD separation column with dimensions (150 × 4.6 mm × 5 µm) equipped by Cecil company was used. Adept U.K., 50 mg of freeze-dried β-CN was dissolved in 0.250 ml of deionized water with pH 7. The mixture was filtered using a Millipore filter (0.2 micron). The mobile phase consists of two solutions, the first (A) consists of deionized water. ions with 0.1 formic acid, while the second solution (B) consists of 0.1% formic acid with 5% acetonitrile, and the separation conditions included a liquid phase with a 5:95 (volume: volume) solution of solution A: B, which was filtered using a Millipore filter (0.45 microns), 50 microliters of sample was injected into the device at a temperature of 50°C, and the flow rate was 0.5 ml/min. Readings were recorded at a wavelength of 214 nm.

Hydrolysis of β-casein protein by proteolytic enzymes: β-CN Hydrolysis was performed according to what was mentioned to Al-Shaikh, and Doosh, (2024) by dissolving 0.1 g of β-CN in 10 ml of deionized water and the pH was adjusted to 2 for the pepsin enzyme, while the trypsin enzyme adjusted the pH to 8, and the synergistic was with a mixture of Pepsin and trypsin enzyme in a ratio of 1:1, and the pH was changed using NaOH and HCl. Pepsin and trypsin were added to the reaction mixture in the amount of 20 and 18 enzyme units, respectively, then the digested samples were taken after 8 hours of digestion, then the enzyme reaction was stopped by exposing the mixture to a temperature of 95 °C for 5 minutes. Centrifugation was performed at 12,000 × g for 15 minutes. The filtrate was collected, lyophilized and stored at -18°C.

Protein degree hydrolysis determination (DH): The degree of decomposition was estimated according to the method described by Kumar *et al*., (2016), where the degree of decomposition was estimated by dissolving 100 mg of β-CN protein and dissolving it in 10 ml of deionized water, then transferring 0.250 of the samples under study for all time periods and mixing with 2 ml of buffer of phosphate NaPO4 with a concentration of 0.2125 M and pH 8.2 and 2 ml of TNBS solution and incubate in a shaking water bath for one hour at a temperature of 50C⁰ in isolation from light. The reaction was stopped by adding 4 ml of 0.1 M HCl and the tubes were left at room temperature at 30 ± 2 ° C for 30 minutes. Then

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the absorbance was measured with a UV-1900i spectrophotometer at a wavelength of 340 nm, and the degree of DH decomposition was estimated by calculating the terminal NH3 groups (concentration of released peptides) by entering the equation below into the UV-PROBE 2.1 program.

$$DH = [(Lt-L0)/(Lmax - L0)]\times 100$$

Where:

Lt = free amino groups per time (1-8 hours)

L0 = amount of amino acids present in the original β-CN sample without any treatment

LMAX = the amount of total amino acids in the sample not hydrolyzed by enzymes that could be obtained after acid hydrolysis using 6 M hydrochloric acid at a temperature of 120 °C for 24 hours.

RESULTS AND DISCUSSION

Chemical composition of camel milk : Table (1) shows the physicochemical characteristics of the Iraqi camel milk under study. The results showed the percentage rates of moisture, fat, protein, lactose, total solids, and non-fat solids for raw, full-fat camel milk, which amounted to 87.43, 3.42, 3.49, 4.87, 12.57, and 9. 07%, respectively, and this result agreed with what was found by Abd El-Aziz et al., (2022), and the results were close to what was found by Karaman *et al*., (2021). The results also showed that the pH ratios and the percentage of acidity expressed as lactic acid and the specific gravity was 6.64, 0.17, and 1.30%, respectively, and the percentages of this study agree with many other studies conducted on camel milk, including the findings of (Abdullahi, 2019; Alhaj *et al*., 2022, Khalil, and Lafta, 2023), and these percentages are considered among Natural limits for camel milk.

Table 1. Chemical composition of raw, full-fat camel milk

Milk ingredients	Percentage%
Moisture	87.43
Fat	3.42
Protein	3.49
Lactose	4.87
Ash	0.79
Total solid	12.57
Non-fat Solid	9.08
Titratable acidity (as lactic acid)	0.17
pH	6.64
Specific weight	1.030

***Each number in the table represents an average of three replicates**

The approximate chemical composition of camel milk is affected by environmental changes resulting from genetic and non-genetic factors (Mohamed *et al*, 2022), and many studies conducted to evaluate the chemical composition of milk in the United Arab Emirates indicated that the average percentages of the main camel milk components range between 2.15-3.5% protein, 2.58-4.3% fat, 4.19-5.8% lactose, 10.49-16.06% total solids (Mohamed *et al*., 2021).

Acidic casein preparation

The amount of dried casein obtained was 19.2 g/L of fresh camel milk. This quantity is within the natural limits of camel milk protein, and this result was consistent with what was found by El-Agamy, (2009), as the amount of casein was found to be 20.6 g/L of camel milk. While this result does not agree with what other researchers found, as they found an amount of 10.9 g/L (Akindykova *et al*., 2019). This variation may be due to several factors, the most important of which are the geographical region, breed, feeding conditions, physiological stages of the animal, and environmental conditions (Darani *et al*., 2023).

Primary β- casein purification

The primary β-CN protein was separated and purified from total Camel milk caseinate using the urea fractionation method according to the method of Ptiček *et al*., (2016), which depends on dissolving the acidic casein in a 3.3 M urea solution and lowering the pH of the solution to 4.6. Under such conditions, the total caseinate precipitated, except for β-CN remains dissolved, which is obtained by diluting the urea solution to 1 M with distilled water. β-CN was obtained in the form of a white precipitate

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and was lyophilized and then preserved by freezing. Then it was purified using ion-exchange chromatography, and the concentration of β-CN was 12.35% of caseinate. Overall, this result is consistent with what was found by Lajnaf *et al*., (2022), who indicated that the concentration of β-casein in camel milk ranges between 12.8-15 g/L, which represents 65% of the total casein, and this confirms that beta-casein is the main casein in Camel milk.

Primary crude β- casein purification of by anion exchanger: The lyophilized acidic β-CN was purified using a Freeze Drier device and ion-exchange chromatography. It was passed over a DEAE-Cellulose ion-exchanger column, as this substance binds a negative charge under separation conditions, and these proteins can be dissociated from this substance using different salt concentrations of sodium chloride NaCl in order to separate these proteins from the column material. Figure (2) shows the presence of two peaks. The first is a small peak, which represents the area in which a 0.100 M sodium chloride solution was added in the void volume, which is included the tubes (5-11), as this concentration works to remove the contaminating of β-CN (K-CN) from DEAD-Cellulose column. The results also showed that this concentration of sodium chloride solution is suitable for the recovery of K-CN protein, as for the second peak, it appeared in tubes (15-28) after using a salt concentration of 0.175 M of NaCl, which represents β-CN. It is also noted that no peak appeared in the washing area. The appearance of peaks in the recovery area at the first and second concentrations of sodium chloride indicates the absence of any positively charged proteins, if they were present, they would have appeared in the wash area due to the protein charge repulsing the column charge and not being connected. This result is consistent with what was found by Al Khalidy and Dosh, (2022), they found two protein peaks when the αs-CN protein was separated by DEAD-Cellulose.

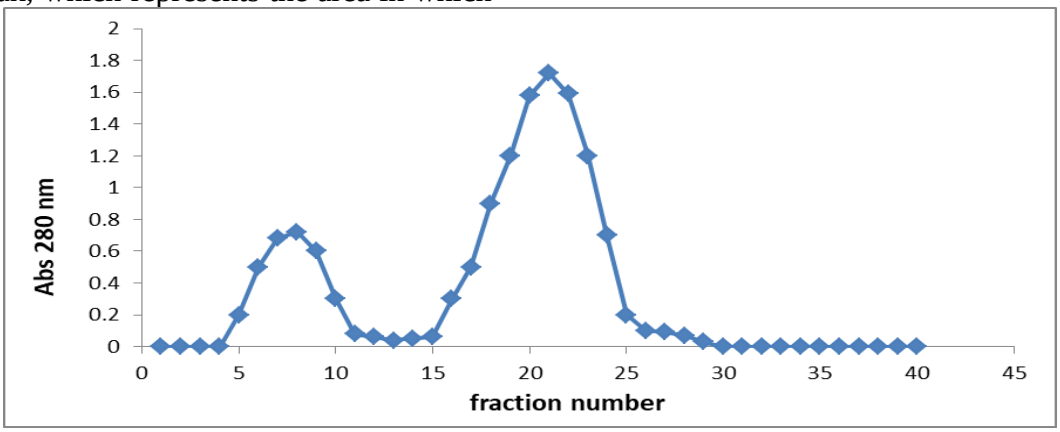

Figure 2. Shows the purification of camel milk β-CN on a DEAE-Cellulose column with dimensions of 3.5 * 15 cm, using 0.1 M phosphate buffer with a pH of 7.4

Confirm the purity of β-CN by SDS-PAGE Figure (3) shows the results of purification by SDS-PAGE, where a main protein band clearly appeared, in addition to the presence of another band, which are traces of κ-CN his result is consistent with what was mentioned by Ellouze *et al*., (2021), that the beta casein prepared with this method is contaminated with κ-CN When comparing the visible bands with the bands of standard proteins and the standard β-CN protein of cow's milk, it was noted that the first band was for the β-CN protein, as it traveled the same distance as the standard β-CN protein, with a very slight difference. The partial weight was estimated to be approximately 24 kDa. Based on the distance traveled by the standard cow protein, which traveled a slightly longer distance than the camel milk β-CN protein, this indicates that its molecular weight is relatively lower than the camel milk protein. As for the ability of the camel milk β-CN protein to move, it was slower compared to its counterpart cow's milk, and this is consistent with what was mentioned by Lajnaf *et al*., (2022).

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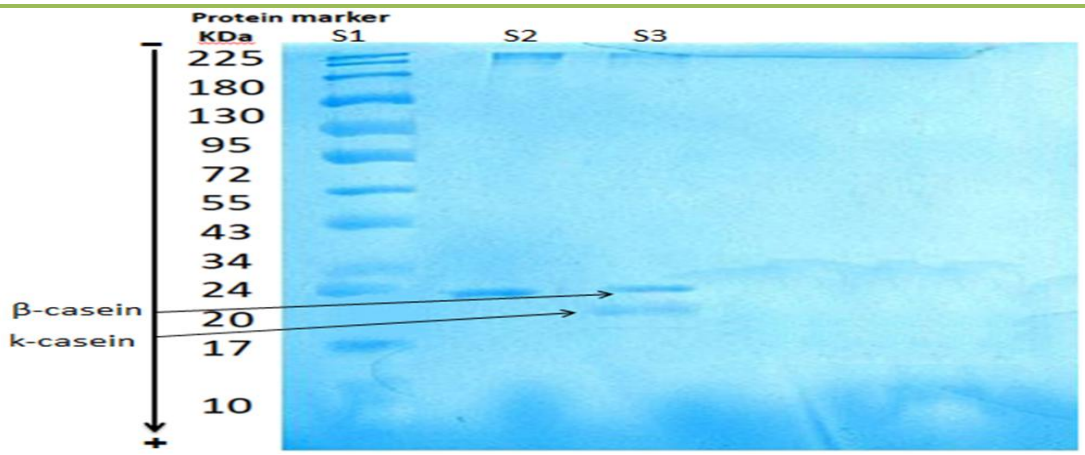

Figure 3. Shows β-CN protein purification by SDS-PAGE, where S1 = Ladder protein, S2 = β-CN resulting from purification by ion exchange, S3 = β -CN standard

As for the other protein band that was accompanying the β-CN protein, it may be traces of κ-CN , the molecular weight of which was estimated to be equivalent to 22 kDa, noting that these molecular weights are consistent with what was found by (El-Agamy, 2009; Ellouze *et al*., 2021), and this result does not agree with what was found by Saliha *et al.*, (2013), which indicated the appearance of two bands of caseins, the first was β-CN, and its molecular weight was 32 kDa, and the second band was αS-CN, a casein whose the molecular weight is approximately 22 KDa. On the other hand, no other band was observed indicating the presence of contaminants from other caseins such as αS-CN casein. In order to confirm the bands that appeared by the electrophoresis method, an HPLC examination was conducted of the sample protein precipitated and purified by the ion exchange method with the standard bovine protein. The results in Figure (4) showed the identification and appearance of two peaks, one of which was large and very clear and appeared at the time. It appeared on RT at 2:43 and when compared with the results of standard bovine HPLC, it turned out that it belonged to the beta-casein of camel milk. A small peak, which is traces of proteins bound to the beta-casein, appeared at 0.44 minutes. This result is consistent with what was found by Lajnaf *et al*., (2022).

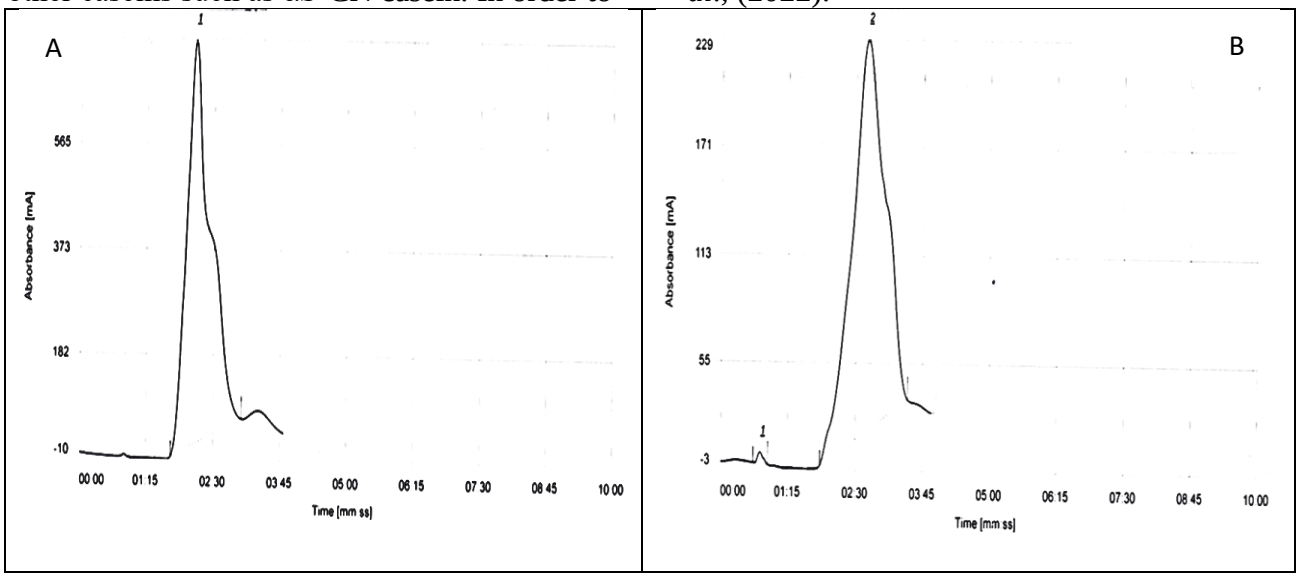

Figure 4. Shows the analysis (RP-HPLC) where A = standard bovine β-CN / B = camel milk β-CN purified by ion exchange.

Final β-CN purification by gel filtration sephadex G-100: Final purification was carried out using gel filtration chromatography in order to finally purify the camel milk β-CN protein from traces of bound proteins that were recovered from the ion exchanger column and

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were not excluded based on the difference in charges. A Cevadex G-100 column was used for this purpose based on the differences in molecular weight between the different casein fractions (Ahmed, and Al-Mousawi, 2021), in order to obtain the β-CN protein in a pure form and free of any traces of contaminants from other proteins, as the β-CN was passed over a Sephadex G-100 column to obtain high proteins purity, and then verify its purity using electrophoresis. The results shown in Figure (5) showed that there was only one major peak, representing β-CN, which appeared after using a diphosphate solution containing 6.6 M urea and EDTA in tubes No. (20-36). It represents the β-CN protein, which was collected, then they were concentrated and dried. The appearance of the β-CN protein in only one peak indicates the ability and efficiency of separation and purification in getting rid of all other remaining trace protein. The process of separation and purification by gel filtration contributed to obtaining the β-CN protein with high purity. This result is consistent with what was found by (AlKhalidy, and Dosh, 2022; Maree et al., 2020), who confirmed that one main pure protein peak was obtained for the casein fractions in goats, cows, and sheep when using ion exchange purification followed by gel filtration purification using a Sephadex column. We can conclude from the above that it is possible to purify β-CN using the ion exchange method, followed by the gel filtration method, as purification with only one technique is not sufficient to obtain a highly pure protein (Saliha *et al*., 2013).

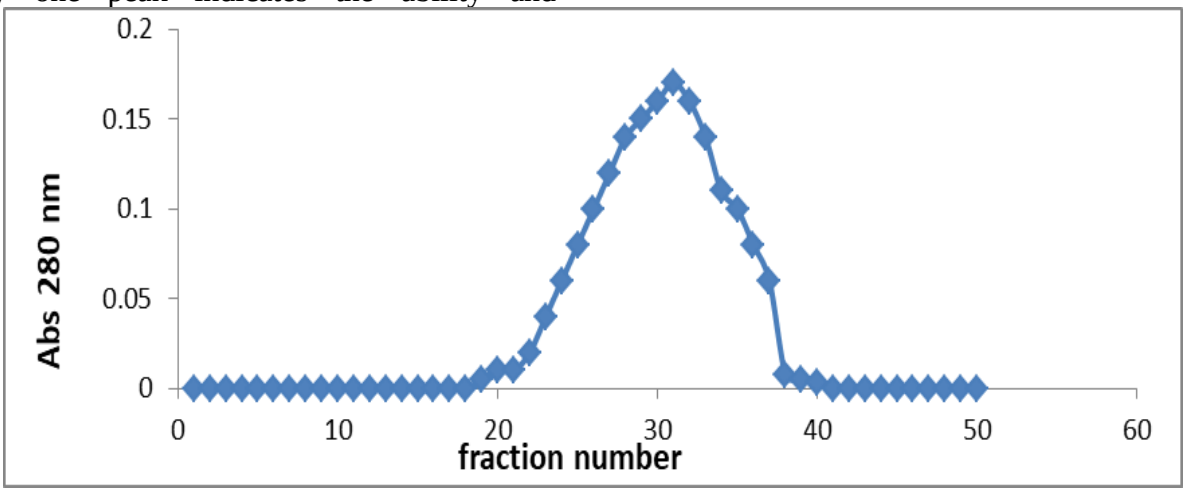

Figure 5. shows the purification of β-CN of camel milk by gel filtration on a Sephadex G-100 column

Confirm the purity of β-CN by SDS-PAGE

The β-CN peak obtained from gel filtration was migrated in SDS-PAGE. Figure (6) shows the appearance of one pure band individually in the multiple gels, which confirms the purity and absence of β-CN from any traces of other proteins. When compared with the standard bovine protein, we notice that it traveled a distance similar to that traveled by the standard protein, with a difference. The distance traveled by camel milk β-CN with the distance of the standard protein is very simple, as the distance for the cow β-CN protein was slightly longer, and this indicates that its molecular weight is relatively lower than that of camel milk protein. Also, no other bands appeared accompanying the purified package, and this confirms the purity of the β-CN protein, which is the main protein in cow's milk casein. The molecular weight was determined in comparison with standard proteins and the standard bovine β-CN protein. The weight of β-CN was 24 KDa, and this result is consistent with what was found by both (Ellouze *et al*., 2021; Kappeler *et al*., 1998), who found that the molecular weight for beta casein is 24.90 and 24.65 KDa, respectively.

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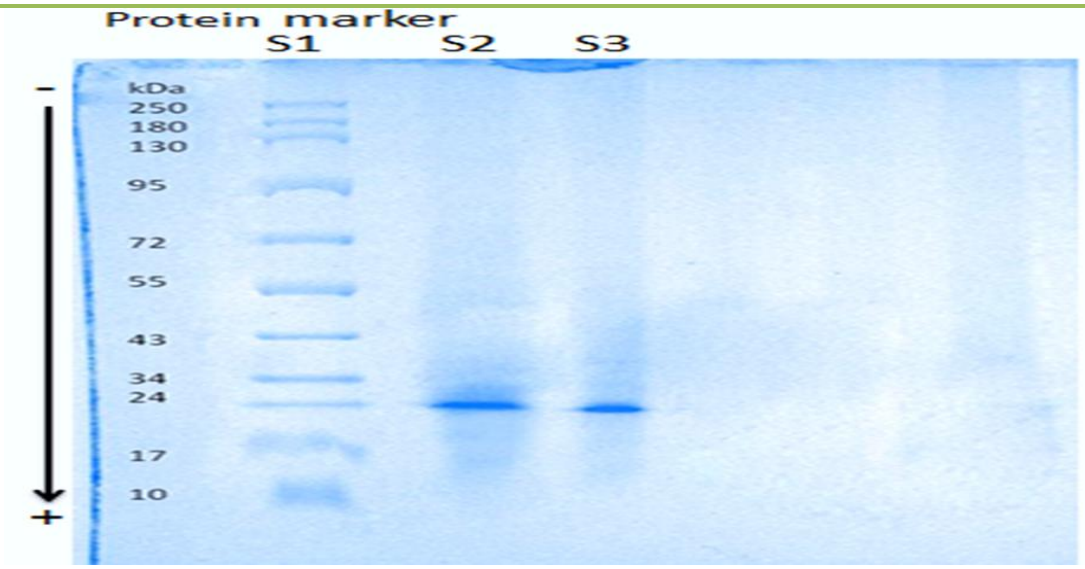

Figure 6. Shows the β-CN protein purified, standard proteins, and the β-CN protein by SDS-PAGE, where S1 = Ladder protein, S2 = β-CN precipitated resulting from the purification process by gel filtration, and S3 = standard β-CN.

The existence of differences in the molecular weights of the caseinate parts in the milk of different animals is due to several factors, the most important of which is the connection by disulfide bonds between the protein parts, the degree of phosphorylation of the casein types, and the effect of gel concentration.

Confirmation of the purity of β-CN separated by gel filtration using reversed-phase high-pressure chromatography (RP-HPLC): In order to confirm the purity of the camel milk β-CN protein, purification was carried out by passing the camel milk β-CN protein with the standard bovine protein in a reversed-phase RP-HPLC device. Figure (7) shows the appearance of a single peak for beta-casein in camel milk compared to the appearance of a single peak for the standard protein, the peaks were close to each other, and the slight difference in the time of appearance of the camel milk β-CN protein may be due to its molecular weight being relatively higher than the cow milk β-CN protein.

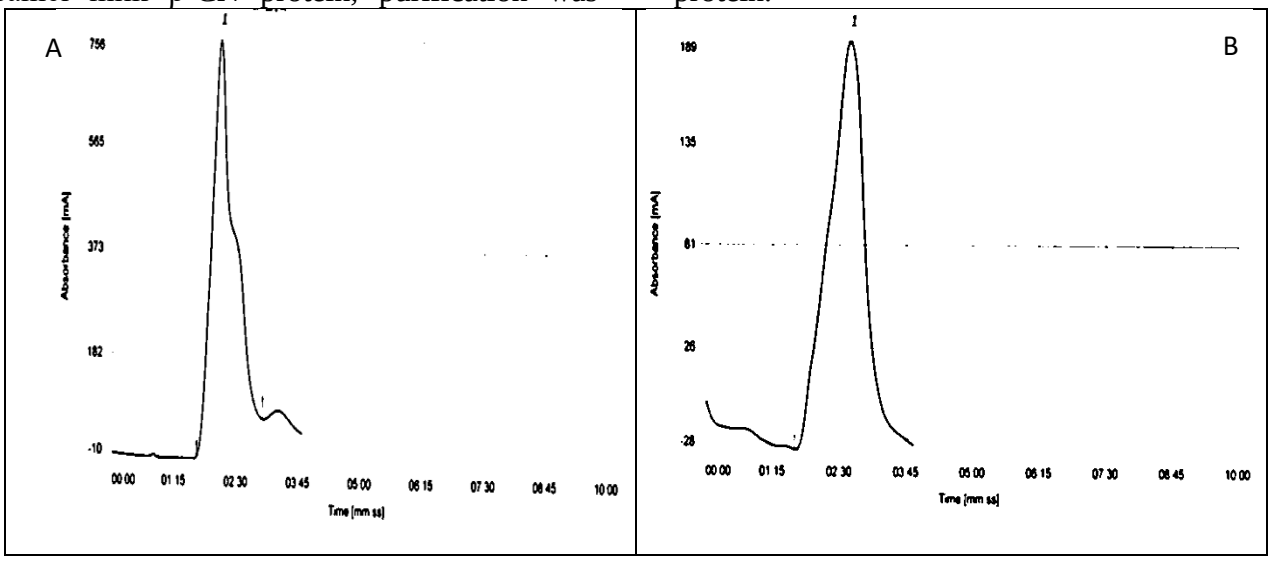

Figure 7. Shows confirmation of the purity of β-CN by high-performance chromatography (RP-HPLC), where A = standard bovine β-CN protein / B= camel milk β-CN protein for this study

β-CN hydrolysis by Pepsin, trypsin, and their mixture: The degree of degradation of the β-CN protein was estimated under the influence of pepsin, trypsin, and a mixture between the two enzymes at a ratio of 1:1 for 8 hours. Readings of the degree of hydrolysis

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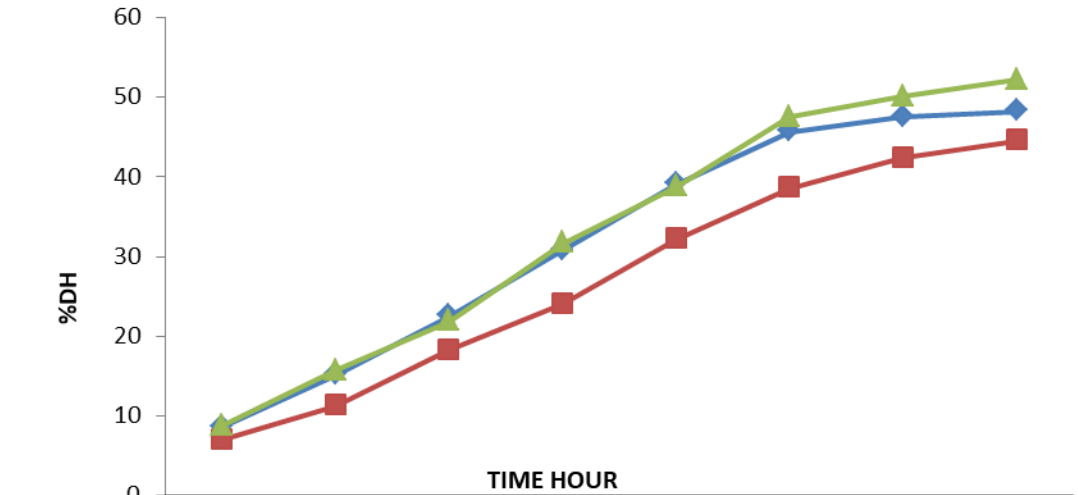
were taken every hour and compared with the standard curve for the amino acid leucine combined with the compound Trinitrobenzensulphoric acid (TNPS), as this compound combines with the amine group. The free terminal of the amino acids is attached to the alpha carbon atom to produce a very pale-yellow color that can be read at a wavelength of 340 nm. The intensity of the color is directly proportional to the concentration of the complex formed in the solution. The results in Figure (8) show that the degree of DH decomposition increases with increasing enzymatic hydrolysis time. This means that peptides are increasingly released as the incubation period progresses. The figure also shows the decomposition behavior of the three treatments by the enzyme pepsin and trypsin and their mixture from the first hour until the end of incubation at hour 8. The values of the degree of DH decomposition reached 48.267, 44.563, and 52.165%, respectively, and the results agree with what was found to (Taghipour *et al*., 2023; Vorob'ev, 2022). The results indicate the superiority of the synergistic action in the degradation of the β-CN protein, as the DH value after 8 hours reached 52.16. This result agreed with what was found by Akan, (2021) who reported that the DH values of the β-CN protein with the synergistic action of the enzyme pepsin and trypsin together are 53.06%, who stated that the peptides obtained from digested camel milk caseinates possess strong antioxidant and anti-diabetic properties. This result also agreed with what was reported by Al-Saleh *et al*., (2014), who used different enzymes to reach the values DH to 53.36%, and is close to what was found by Abderrahmane *et al*., (2015), who found an increase in the DH value of the β-CN protein with the passage of time, as it increased from 31.06% to 60.13% when using a mixture of enzymes, but this result is higher than what was found by Mudgil *et al*., (2023), as the degree of DH for beta casein protein ranged between 20-30%, and is lower than what was found by Tagliazucchi *et al*., (2016), which is 69.6%. This difference in the degree of decomposition can be attributed to the type of enzyme used, the rate of the enzyme reaction, and the type of substance.



	1	2	3	4	5	6	7	8
PEPSIN	8.583	15.089	22.458	30.767	39.076	45.661	47.483	48.267
TRYPSIN	6.937	11.248	18.225	24.026	32.178	38.606	42.368	44.563
MIX	8.741	15.6384	21.831	31.629	38.763	47.542	50.089	52.165

Figure 8. Percentages of decomposition of β-CN by pepsin, trypsin, and their mixture in a 1:1 ratio during incubation at 37°C for 8 hours

CONCLUSION

The results indicate that β-casein protein isolated from Iraqi camel milk can be effectively purified using ion-exchange chromatography (DEAE-Cellulose) and gel filtration (Sephadex-G100). The purified protein showed molecular weights around 20 and 24 kDa, and enzymatic hydrolysis using a

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mixture of pepsin and trypsin produced the highest degree of hydrolysis (52.32%), demonstrating the potential for preparing peptide hydrolysates from this protein.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, no figures or images that do not belong to us have been incorporated into the manuscript, and the required permissions for re-publication are included with the manuscript. -Author/s signature on the Ethical Approval Statement.

AUTHOR'S CONTRIBUTION STATEMENT

Sh. A. H. designed the project data collection, analysis, and paper writing. Author k.s.d review the article and participates in its design and analysis

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تحضير متحللات ببتيدية من البيتاكازين المعزول والمنقى من حليب الإبل العراقية

شرف علي هادي الشيخ كفاح سعيد دوش
[1]قسم علوم الأغذية/كلية الزراعة/جامعه الكوفة /العراق
[2]قسم علوم الأغذية /كلية علوم الهندسة الزراعية/جامعة بغداد/ العراق

المستخلص

هدفت الدراسة الحالية الى عزل وتنقية بروتين البيتا كازين β–CN من حليب الابل العراقي باستعمال اليوريا وتقنية كروماتوكرافي التبادل الايوني باستعمال عمود DEAE–Cellulose والترشيح الهلامي باستعمال عمود السيفادكس Sephadex–G100 وتحديد الوزن الجزيئي للمعزول البروتيني باستعمال طريقة الترحيل الكهربائي بهلام متعدد الاكريلامايد بوجود المادة الماسخة Sodium Dodecyl Sulphate (SDS) وتحضير المتحلل الأنزيمي باستعمال كل من انزيم البيسين والتربسين ومزيجهما بنسبة 1:1، وقدرت درجة التحلل المائي بعد الحضن لمدة 8 ساعات، ومقارنته مع البروتين القياسي البقري والبروتينات القياسية، اظهرت تنقية β–CN باستعمال عمود DEAE–Cellulose ظهور قمتين منفصلتين وكان مقدار الوزن الجزيئي لهما 24 KDa و 20 KDa على التوالي، كما اظهرت تقنية الترشيح الهلامي Sephadex–G100 ظهور قمة واحدة وكان وزنها الجزيئي 24 KDa مقارنة مع β–CN الذي وزنه 23 KDa، وبينت النتائج ان اعلى درجة تحلل بلغت 52.32% باستعمال خليط البيسين والتربسين، تشير النتائج الى ان بروتين β–CN المفصول من حليب الابل يمكن تنقيته بدرجة عالية باستعمال تقنية كروماتوكرافي التبادل الايوني والترشيح الهلامي كما يمكن تحضير متحللات ببتيدية منه.

الكلمات المفتاحية: ببتيدات فعالة، casein، متحللات انزيمية، الترشيح الهلامي، التبادل الايوني.

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