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ALPHA- LACTALBUMIN ISOLATION, PURIFICATION AND CHARACTERIZATION FROM IRAQI CAMEL MILK

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ABSTRACT

alpha-lactalbumin (α-Lac) is a crucial protein in several biological procedures like a strong antioxidant and prospective cancer therapy, This study was aimed to isolate, pacificate, and characterize α-lactalbumin α-Lac from Iraqi camel milk sourced from new local camel farms in Samawah Govermanate, southern Iraq. purified step done by ion exchange chromatography through DEAE-cellulose column and gel filtration chromatography through Sephadex G-100 column, the purification fold and yield were (58.20 time and 82%) respectively. The purity of α-Lac to homogeneity was examined by polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE) with a single bond The molecular weight was determined by two methods and it was 14.40kDa and 14.23kDa, The quantitative and qualitative analysis using HPLC technique was without significant differences and the used of HPLC assay as an additional confirm purification step.

Keywords: Camel milk, α -lactalbumin, Isolation, Purification, Characterization.

عزل وتنقية وتوصيف الفالكتالبومين من شرش حليب الابل العراقية

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الخلاصة

الفالكتالبومين احد اهم بروتين شرش الحليب ويدخل في العديد من العمليات البيولوجية والحيوية كمضاد الأكسدة وله فعل علاجي للسرطان، تهدف هذه الدراسة إلى عزل وتنقية وتوصيف بروتين الفاكتالبومين من شرش حليب ابل محلية تم الحصل على الحليب من مزارع ابل محلية في محافظة السماوة جنوبي العراق. تمت خطوة التنقية بواسطة كروماتوغرافيا التبادل الأيوني من خلال عمود السليلوز DEAE وكروماتوغرافيا الترشيح الهلامي من خلال عمود Sephadex G-100، وكانت حصيلة التنقية ووالتركيز (58.20 مرة و 82٪). تم تاكيد نقاوة α-Lac بواسطة الفصل الكهربائي للهلام متعدد الأكريلاميد مع العوامل الماسخة (SDS-PAGE) حتى الحصول على حزمة واحدة وتم تحديد الوزن الجزيئي بطريقتين مختلفة وكان 14.40 كيلو دالتون و 14.23 كيلو دالتون، تم التحليل الكمي والنوعي من خلال استخدام تقنية HPLC كخطوة إضافية لتأكيد للتنقية.

الكلمات المفتاحية: حليب الابل، الفالكتالبومين، عزل، تنقية، توصيف.

INTRODUCTION

Camel milk is a key part of the staple food, especially in the semi-arid and deserts area in world. α -lactalbumin, lactoferrin, lactoactive peptides, and mono- and polyunsaturated fatty acids andHigh amount of vitamins are among the nutrients contained in camel milk that are health-promoting **(Seifu *et al.*, 2023)**. Important human ailments including TB, asthma, gastrointestinal disorders, and jaundice can all be helped by these compounds **(Hussein, 2015)**. Camel milk when compared to cow's milk is more varied. The most significant factors affecting milk production in camels are feed, reproduction, age, and lactation stage. Region and season have a significant effect on the chemical ratio in camel milk **(Khatoon & Najam 2017**; **Roy *et al.*, 2020**). Whey protein of Camel's contains natural protease, which like chymotrypsintype (A) and cathepsin (D), as well as a large amount of solubly protein. These whey proteins are unique in their chemical, physical, physiological, functional, and technological characteristics, which make them useful for use in food preparation **(Al-taie *et al.*, 1989)**, besides has a high nutritional value bioactive peptides are produced by hydrolyzing camel's milk proteins, and these peptides have an effect on the body's major organ systems and supply them with physiological functions. The peptides in camel milk that inhibit the Angiotensin-converting enzymes (ACE), which also has anti-diabetic, antibacterial, antioxidant, and anti-cholesterol characteristics **(Wafaa *et al.*, 2022; Abdulsalam, 2012; Katab & Doosh 2017).**

α -lactalbumin, serumalbumin, immunoglobulins, lactophorin and lactoferrins are the primary components of camel milk whey proteins. Concentration α -lactalbumin, serumalbumin, and lactoferrins in Camel Milk was assessed using capillary electrophoresis. These numbers were respectively 2.01, 0.40, and 1.74 mg/mL **(Ho *et al.*, 2022)**, Camel milk, same human milk, lacks ß-lac as globulins but contains between 25 and 35 percent of a protein termed a-La, which is the major protein of whey, shares 80 percent of its aminoacid sequences like bovine α -La. There are a lot of studies on a-lcatalbumin protein by many different solation and purification methods can be divided into several groups, such as using two separation steps using help of chromatography and gel-filtration columns **(Neyestani *et al.*, 2003)** and separation also using several methods of precipitation , aggregation and hydrolysis, in addition to separation methods that relied on membrane filtration, hydrolysis, and enzymatic digestion**(Cheang *et al.*,2003) ,** There are more sensitive and accurate affinity chromatography methods **(Al-Saadi ,2002).**

This updated study attempts to better isolate and purify α -Lac protein from Iraqi camel milk, as well as examine some of its features, with the goal of conducting future studies to learn more about its health and nutritional advantages.

MATERIALS AND METHODS

The milk has sourced from new local farms in Samawah Govermanate, southern Iraq. The sample was obtained at the height of the lactation phase and transferred under conventional sterile and refrigerated settings. Camel milk was tested at Baghdad University Department of Food Sciences, College of Agricultural and Engineering Sciences. skimmed milk produced in the manner indicated by **(Doosh *et al.*,2015)**. α-Lac was purified from skimmed milk using centrifugation in an Eppendorf - Germany centrifug 5000 g/min - 6 min 4°C. as **(Neyestani *et al.*, 2003)**, with some modifications. whey was` prepared by precipitation part of casein from the skim milk in acidic- condition with using a grad addition

HCl 1N until 4.3 pH, Centrifugation was used to extract the precipitated casein for 10 min at 4 °C. The supernatant (whey) was filtered through Whatman filter paper № 1, Then precipitation by salting out %45 with (NH4)2SO4 overnight at 4℃ The Remainder supernatant was dialyzed against 20mM phosphate-buffer and 35mM EDTA, pH 7.6 for 24 hr, stored at -17℃ until to use.

Isolation and Purification of α-Lac procedures as **(Neyestani *et al.*,2003)** was employed for isolation and purification. Anion-exchange chromatography through a DEAE-cellulose column was used, as well as gel-filtration chromatography with Sephadex-G 100.

Protein Determination

Protein concentration was determined using methods **(Bradford, 1976).**

Purity Test

The polyacrylamide gel-electrophoresis under denaturing conditions (SDS-PAGE) technique was used likewise **(Laemmli, 1970)**, used HPLC assay as an additional confirm purification step and to study congruence in properties **(Rotkāja *et al.*,2016).**

Molecular Weight Determination by Electrophoresis (SDS-PAGE)

(Doosh *et al.*,2015) describe the technique. Standard protein with molecular weights ranging from 9000 to 170000 Dalton was utilized. By graphing the connection between relative mobility and conventional protein molecular weight, the -Lac molecular weight was derived. logarithms.

RESULTS AND DISCUSSION

Isolation and Purification of a-Lactalbumin from Camel Milk Whey Ion-exchange chromatography and gel filtration were used in succession to separate a-lactalbumin from camel milk whey.Ion-Exchange Chromatography by DEAE-Cellulose

Important qualities, such as ease of preparation and the potential of reusing after reactivation **(Neyestani *et al.*,2003)**, led to the application of anion exchanger in the purification of camel milk whey. One protein peak, likely representing unbounded proteins like lactoferrin and cation proteins, was observed during the washing step, and another protein peak was observed during elution using a gradients of 0.0-0.9M NaCl, as shown in Fig. 1 the equilibration buffer was a 0.05 M phosphate buffer pH7.6 solution. The α-Lac carry a tepid -23 charge. The results showed that the washing peak emerged in fractions numbered between 1 and 46, whereas the elution peak, which belonged to a-La, appeared in fractions numbered between 68 and 93. The peak was eluted in 0.2M NaCl, as in fig. 1, and then concentrated by sucrose after being desalted by overnight dialyzing against distilled water. using the Bradford test's standard curve of bovine serum albumin, we found that the concentration of α-La after this purification step was around 2.4 mg/mL (table 1).This study's findings are consistent with those of prior research **(Lajnaf *et al*.,2022)**.Purifying a-La from cow milk through ion exchange chromatography with a DEAE-cellulose column and a Sephadex G-100 column yielded two peaks, one of which corresponded to a-LA and the other to ß-La. Similar results were obtained by **(Kamau *et al*.,2010),**which purified a-La from goat milk in a single step using affinity chromatography with an octal-Sepharose column, and by **(Pettersson *et al* .,2006; Noppe *et al.*,1999)**, which purified -La from bovine whey and colostrums in a single step using phenyl-Sepharose chromatography. They used expanded bed chromatography and size exclusion

chromatography to purify the α-La from bovin and goat milk, and SDS-PAGE confirmed that both proteins had the same molecular weight of 14.2 kDa.

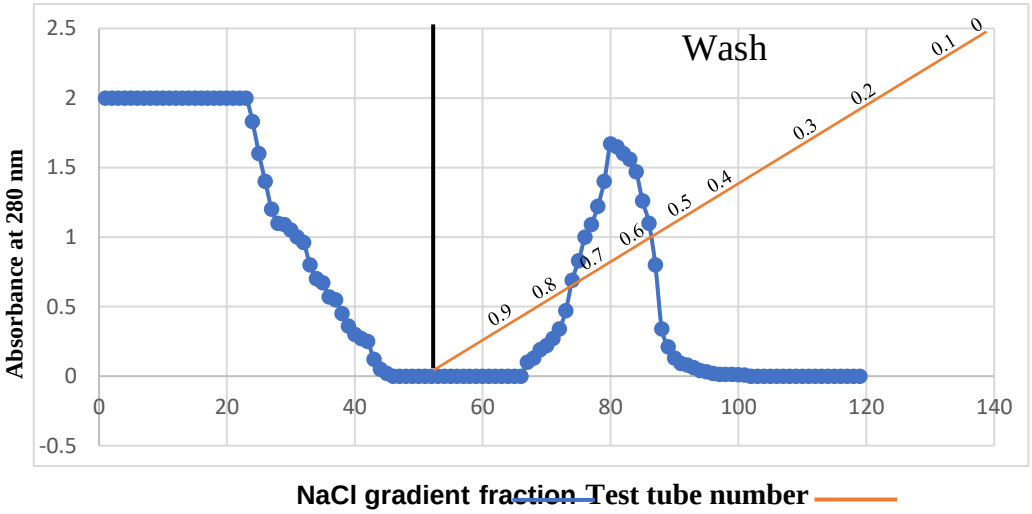

Figure (1): Ion exchange chromatography for purification α-Lac by using DEAE-cellulose column (2×25 cm) equilibrated with 0.9M phosphate buffer pH7.6 eluted with same buffer and NaCl gradient 0.1 and 0.5 M in flow rate 30 ml/hr 5ml for each fraction.

Gel Filtration Chromatography

Following ion-exchange purification, α-Lac-representing elution fractions were collected, dialyzed against distilled water, and concentrated using sucrose before being applied to gel filtration chromatography using Sephadex G-100 column Fig. 2**.**

Gel-filtration, an additional purification step commonly utilized in addition to ion-exchange to gain the cleanest form of protein possible, was widely used in investigations of α -La purification, and Sephadex G-100 was the resin of choice **(Uversky *et al.*, 2016)** used a Sephadex G-50 column for isolating α -La from camel whey, while a Sephadex G-100 column was used to isolate a-La from camel milk (**Lajnaf *et al.*,2023)**.When **(Doosh *et al.*, 2015)** purified α -La from sheep and cow milk, they employed sephadex G-50 and checked for purity with SDS-PAGE, where they detected a single band. According to Bradford test, the concentration of α -Lac after this purification stage was around 2.1 mg/mL. Concentration and yield of recovered α -Lac were shown in **Table (1**) for each individual separation phase. The α -Lac concentration in whey camel milk decreased by a startingout point of 2.7 mg/ml to 2.4 mg/ml after the ion exchange step and 2.1 mg/ml after the gel filtering step. contrasted with a final protein recovery of 82% and a purification factor of 58.20. The concentration and purity of the purified α -Lac in this study were both excellent. In addition to being quick and easy, the aforementioned technique also has the potential to yield extremely pure α -Lac Fig. 3. This study's results were consistent with those found in **(Ibrahim& Masoud, 2023**).

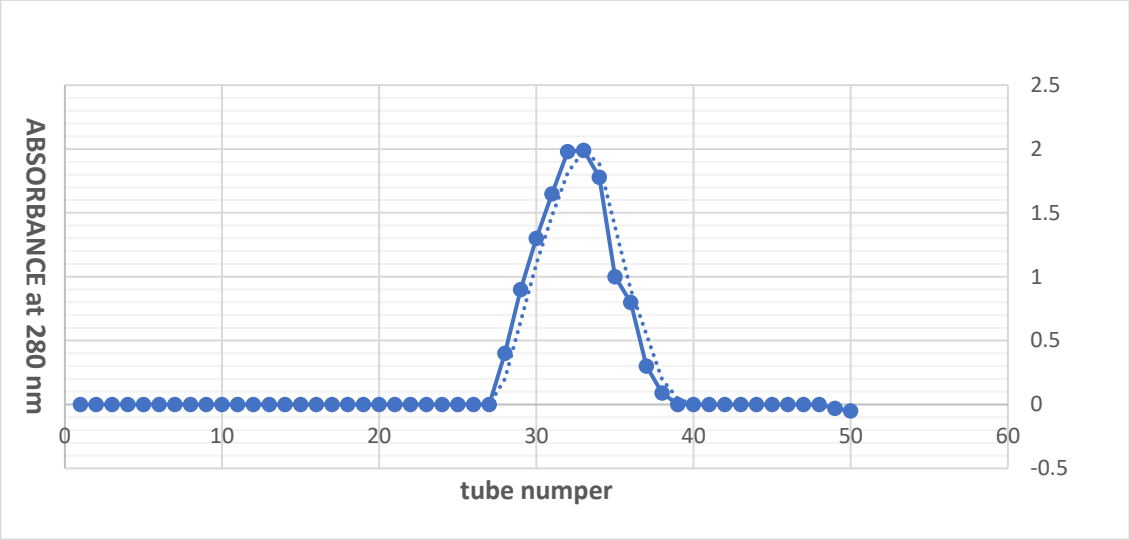

Figure (2): the purification α-Lac by Gel filtration chromatography for using Sephadex G-100 column (2×40 cm) equilibrated with 0.05M phosphate buffer pH7 eluted with same by a flowrate 30 ml / hr 5ml for all fraction.

Determination of a-Lactalbumin Purity by SDS-PAGE

Purity of α-Lac by SDS-PAGE Purified α -Lac appeared as a single band at the predicted M.W of 14.40kDa with 12% SDS-PAGE analysis, as illustrated in Fig. 3. Purification can be taken one step further with electrophoresis on polyacrylamide gel to evaluate the efficacy of the purification procedures. After extensive purification, α -Lac showed as a single band in the downstream region of a polyacrylamide gel Fig. 3. The fact that the isolated α -Lac from camel milk whey tested pure proves that the purification methods employed were effective. Bovine α -Lac was purified by HPLC, and when run on a 12.5% SDS-PAGE gel, a single band was observed. A single band was obtained using SDS-PAGE to verify the purity of isolated α -Lac from defatted bovine **(Neyestani *et al.*,2003; Krešić *et al.*, 2008)**. In addition to phenyl-sepharose chromatography, SDS-PAGE was employed to verify the purity of α -Lac recovered from cow's milk whey.

By extrapolating the linear relationship wich in molecular weight and relativemobility (Rm), the M.W of α -Lac purified has calculated until 14.40 kDa Fig. 4. A highly significant match was discovered between lane 5's standard a-La and the Sigma aldrich standard.

Characterization of the Purified a-Lactalbumin from Camel Milk Estimation of *α*-Lactalbumin The molecular weight of the *α*-lac was measured using SDS-PAGE. owing to its small molecular weights, α -Lac was only represented by a single protein band in the gel's lower third. Figures 4 and 5 depict the relationship which relativemobility (Rm) of typical proteins and the logs of their M.W, which is used to determine Rm of α -Lac. The calculated value for a-La's M.W was around 14.4KDa. α -Lac bit varies in M.W**(Permyakov *et al.*,2020)** depending on the species of animal being studied. This study's α -Lac result was comparable to other research' results when the two sets of data were compared. Due to the protein's tiny size, most studies agree on its M.W, for example, finds the M.W of α -Lac to be at 14.43 kDa and that of goat and bovine to be around 14.20 kDa, both obtained by SDS-PAGE. Camel and cow

milk α -Lac had detectable M.W of 14.4 and 14.2 kDa, respectively, when analyzed by SDS-PAGE **(Lajnaf *et al.*, 2022*)***. Respectively SDS-PAGE estimates a M.Wt of 14,17kDa for α -Lac, whereas predicted a weight of 14,17kDa.

The M.W of α -Lac purified from camel milk was determined to be 14.40 kDa using gel filtration chromatography and a standard curve depicting the relationship wich in the log of M.W of standard proteins versus (Ve / Vo), as shown in Fig3**.** The M.W of a-La extracted from camel milk was 14.40 kDa, which is consistent with the results observed by **(Uversky *et al.*, 2016)**. Gel filtration [36] found the M.W of human a-La to be 14.18kDa, while another study reported a MW for Bovin a-La of 14.2. Isolated a-La from various sources had a M.W of 14.17-14.4 kDa. Sheep milk a-La was discovered to have a M.W of 14.20 kDa by researchers **(Redington *et al.*,2016)**, while α -Lac purified from human, bovine, caprine, and swine using a Sepharose -4B column had a M.W of 14.2kDa by the same researchers **(Pettersson *et al.*,2006).**

Table (1). Yield of Concentration and recovery α -Lac by every steps wich in separation process.

DET	WHEY	ION-EXCHANGE STEP	GEL FILTRATION STEP
CONCENTRATION a-lac (MG/ML)	2.7	2.4	2.1
RECOVERY WHEY SOLUTION RELATIVE%	100	93	82
PURIFICATIONS NUMBERS	--	46.75	58.20

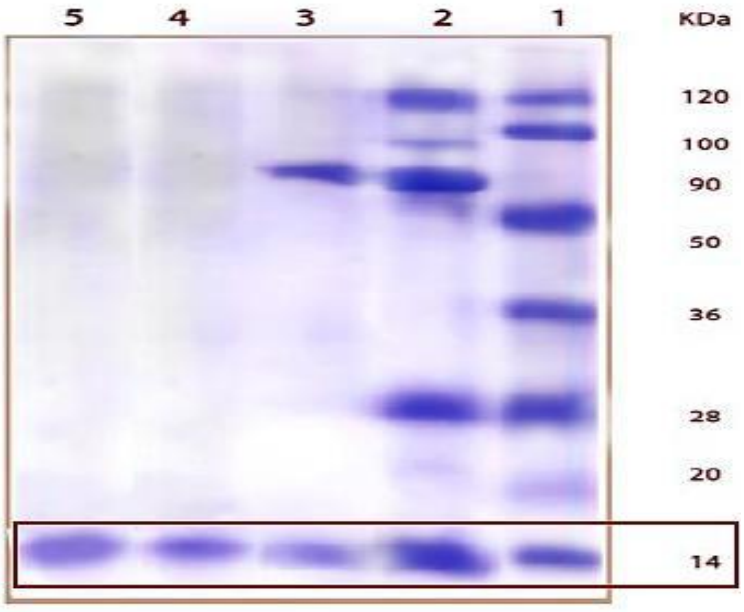

Figure (3): SDS-PAGE of purified camel α-La purification steps. -Lane 1 lader mass marker. -lane 2 whey camel milk after precipitation by 45% ammonium sulfate -lane 3 (ion-exchange) eluted fractions from DEAE–cellulose column - lane 4 purified α-a from Sephadex G100 column. - lane 5 purified stander α-La from sigma.

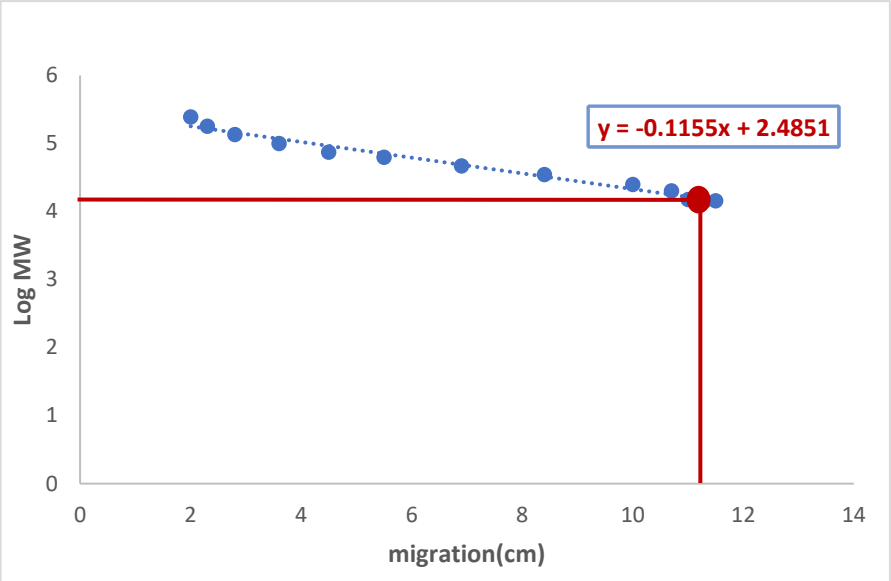

Figure (4): Standard curve definition α -Lac M.W by SDS-PAGE.

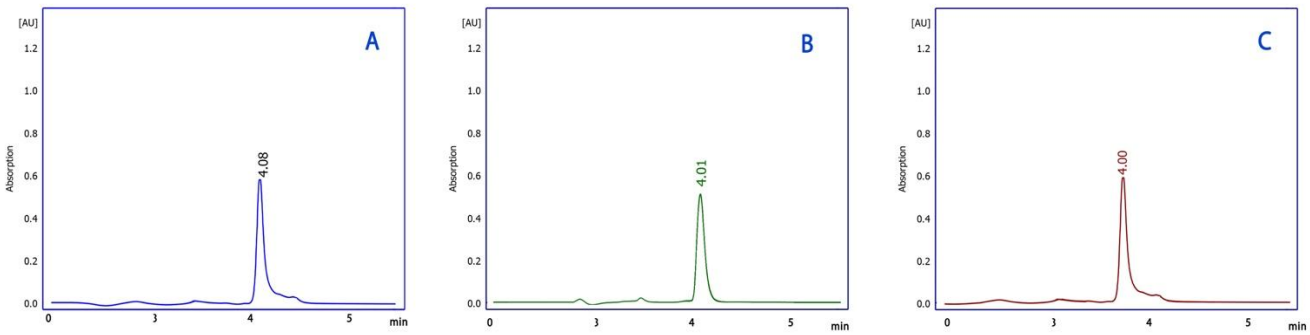

Figure (5): HPLC assay curve of stander α-La (A) and α-Lac frome Ion excheng using DEAE-cellulose column. (B) Gel filtration chromatography Sephadex G-100 column (C).

CONCLUSION

Our purification procedures were modified to obtain acceptable yields of high purity α -Lac from a iraqi camel milk sample was (>90%, as on total proteins) from camel whey using high purification agents. A useful protein was produced using a simple two-step chromatographic purification process. Higher pure of α -Lac samples was be helpful for future investigations such as assessing antioxidant and anticancer properties, as well as in vitro vital tests on experimental mice.

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