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Partition of Proteins from In-Nature Goat's Milk Whey Using a Natural Polysaccharide

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Abstract-The present study provides an evaluation of the application of a natural polysaccharide (cashew gum) in the partition of α-lactoalbumin and β-lactoglobulin proteins from goat's milk whey in-nature utilizing aqueous aqueous two-phase systems constituting of polymer - polysaccharide. The cashew gum was obtained after purification of the resin extracted from the cashew trees (*Anacardium occidentale* L.). The influence of the polymer type, of the polymer molecular weight, and the polymer mass percentage on the partition coefficient of these proteins was assessed. Among the analyzed polymers, the PPG 425 and PEG 1500 provided better results.

Keywords- *Cashew Tree, Aqueous Two-Phase Systems, Goat Milk Whey, α-Lactoalbumin, β-Lactoglobulin*

I. INTRODUCTION

Interest in healthy eating has increased in recent years, and the main way to get nutrients naturally is through the intake of safe and healthy food. Proteins are macronutrients which play an important role in the human organism. The proteins from milk are known to possess several functional properties and in addition to this their added economic value has been recognized. Besides milk, other byproducts from dairy industries can be source of proteins. During cheese production or in casein production, the aqueous material which remains and is not used by the industry is usually discarded. This material, called whey, has high nutritional value and retains about 55% of milk nutrients [1]. Among the milk proteins with high bioactivity, α-lactoalbumin (α-La) and β-lactoglobulin (β-Lg) are found in large quantities in milk whey [2]. The importance of these proteins (α-La and β-Lg) is related to their nutritional, functional, physiological, and therapeutic action and high availability of essential amino acids [3,4].

Due to the importance of milk proteins, the recovery of these bio-molecules has been performed using different processes such as separation by membranes, adsorption, crystallization, lyophilization, and liquid-liquid extraction, while aqueous two-phase system (ATS) is one of the most commonly utilized techniques [5]. The protein partition using liquid-liquid extraction in ATS is one of the most applied separation processes due to its several advantages when compared to other technique, these being as easy structuring of the operating system, easy transposition in the scaling up, quick mass transfer, change of variables for optimization in separation and selectivity increase, and obtaining high purity and yield grade [6,7]. An ATS is constituted of mixing two hydrophilic polymers, or a polymer and an organic or inorganic salt [6-8].

The search for efficient, economically feasible ATSs that preserve the biological activity of the molecule has become more prevalent every day. Alongside this, new materials have been considered in ATSs to try to improve the separation and minimize the costs of the process. The ATS formed by polymers and natural polysaccharides is innovative systems with its potential being increasingly evaluated in the protein partition. In this regard, one of the new approaches is to use natural polysaccharides as a component of ATS, and cashew gum has been studied for this purpose. Cashew tree (*Anacardium occidentale* L.) grows in a tropical environment. In Brazil, the cashew plantation area is mainly found in the Northeast, where the states of Rio Grande do Norte, Ceará and Piauí are responsible to 95% of the Northeastern production [9]. Cashew gum is a water-soluble branched heteropolysaccharide, which varies in color from yellow to brown. The substitution of Arabic gum by cashew gum was first suggested by Rosenthal [10] and reaffirmed by Owusu et al [11] due to the structural similarity of both. Cashew gum is used in i) the pharmaceutical industry as a binder of capsules and tablets, ii) the food industries as a juice stabilizer, in beers and ice creams, as well as being used for the preservation of flavor in industrialized foods, clarification of juices, such as in the manufacture of clarified cashew juice, and in the manufacture of cashew wine [12-14], and iii) other industrial segments due to its gelling, thickening, emulsifying, flocculating, clarifying, encapsulating and calorie controlling qualities [15], such as in the manufacture of paints and varnishes [12]; in addition, some studies have shown that it can also be used as a component of liquid-liquid extraction systems in the purification of high value-added biomolecules [16-18] and can be used as an alternative technique to traditional biomolecule recovery processes.

Oliveira [19] evaluated the partition of pure trypsin in ATS formed by PEG and cashew gum and found that the protein was predominantly present in the gum-rich phase. The same author analyzed the influence of the molecular mass of the

PEG, the pH, the addition of NaCl and the temperature in the trypsin partition. The results showed that the increase of the molecular mass caused a decrease in the partition coefficient of these proteins, while an increase in temperature (27 and 40 ° C) and pH variation (6.0, 7.0 and 8.0) did not change the binodal. The presence of NaCl in the PEG 4000 and 8000 systems caused an increase in the partition coefficient, whereas for the PEG 1500 the opposite was observed. Finally, the author concluded that the system composed of PEG and cashew gum was viable in the trypsin partition. Sarubbo et al [13] evaluated the partition of the pure bovine serum albumin protein using ATS formed by PEG and cashew gum. In the ATS formed by 13% PEG 1500 and 21% gum with pH 7.0, the largest albumin partition was obtained; while in the system formed by 13.8% PEG 1500 and 22.5% gum and in the system formed by 11% PEG 4000 and 20% gum, both with pH 6.0, albumin did not participate. However, in all systems and conditions studied, albumin was predominantly concentrated in the lower phase, rich in gum, with partition coefficients less than 1 being obtained.

Most studies of protein partition in ATS make use of standard solutions containing pure proteins obtained commercially [20-24]. However, studies using milk whey in-nature and goat milk whey are still scarce [25,26]. In this scenario, the objective of this study was to evaluate the partition of α-La and β-Lg from goat milk whey in-nature in ATSs using different polymer-cashew gum systems.

II. Material and Methods

A. Materials and Reagents

The goat milk whey (GMW), containing 0.65 ± 0.02 mg/mL of α-La and 3.19 ± 0.09 mg/mL of β-Lg (A+B), was supplied by Association of Small Ranchers of the Angicos wilderness (APASA). The polymers utilized were PEG of molecular weight 1500 Daltons (Impex - Lot 35263-D), 4000 (Synth - Lot 152264), 8000 (Sigma - Lot 120M0004V), PPG of molecular weight 425 (Sigma – Lots 01817PUV), 2000 (Sigma - Lots BCBJ2891V), 4000 (Sigma – Lot MKBK1954K) and PVP of molecular weight 3500 (Acros Organisc - Lot A0316503), 10000 (Sigma - Lot SLBH8882V), 40000 (Sigma – Lot SLBF0853V). Cashew gum, natural polysaccharide, was collected as a natural exudate of trees from different cashew trees in Natal, Rio Grande do Norte, and for the isolation of the polysaccharide, ethanol (Exodus, AE10700RA, 99.5%) was used as the solvent.

B. Purification of the Cashew Gum

The polysaccharides of gums are found in mixtures of inorganic salts, proteins, lignins and nucleic acids and need to be separated [27,28]. The polysaccharide isolation of cashew gum was carried out based on the methods described by Torquato et al [29] and Landim [12] with some modifications. First, excess bark of the crude resin from the cashew trees was removed and ground into a mill (Tecnal, TE-631/2, Brazil). Then, a mortar and a pestle were used to improve the grinding and afterwards the material was sieved in a 212 μm mesh. A solution containing 50 g of the sieved gum and 500 mL of distilled water was prepared. This solution was kept under stirring for 2 hours and then the filtration was carried out using gauze and cotton with the aid of a vacuum pump (Solab, SL61, Brazil). Ethanol was added to the filtrate in the ratio of 3: 1 (ethanol: filtrate) to precipitate the polysaccharide. This mixture was kept at rest and refrigerated for 12 hours, and after that period, the precipitate was centrifuged at 3600 rpm for 5 min. The obtained precipitate was placed in a polyethylene tray where it remained for 24 hours for the evaporation of the ethanol. The dried material was ground, using mortar and pestle, and sieved again in a 212 μm mesh. The yield of the cashew gum purified (CGum) was calculated considering the ratio of the mass of the gum after the purification and the mass of the gum before the purification.

C. Partition of the Proteins

For the partition of the proteins, aqueous two-phase systems were prepared using a polymer and a polysaccharide to form the system. The aqueous biphasic systems were prepared according to the methodology described by Sarubbo [16]. Initially, stocks solutions of polymer and the CGum were prepared. The systems components masses were defined by weighing their stock solutions until obtaining the desired concentration. For the partition, the materials were inserted into the equilibrium cells for the formation of the aqueous two-phase systems using a ratio of 1:40 (w/w) of GMW:ATS. A factorial design with 33 + 2 central points was used, considering the type of polymer, molecular weight, and percentage of the polymer as variables of the process. In all systems, deionized water was used. The samples were filtered through cellulose acetate membrane with a porosity of 0.45 μm using acetonitrile (Sigma, Lot SHBD1824V, Purity 99.9%) and trifluoroacetic acid (Sigma-Lot BCBM0756V, Purity 99%). Equilibrium condition of the ATSs was carried out at 25˚C with pH 7.0.

D. Quantification of α-La and β-Lg Proteins

The quantification of α-La and β-Lg protein present in the superior phase, rich in polymer, after the partition was done by high performance liquid chromatography (Shimadzu, Prominence, Kyoto, Japan series) containing a ternary pumping system (LC-20AT), detector per diode array (SPD-M20A), column oven (CTO-20A), autosampler (SIL-20AHT) and interface (CBM-20A). LC solution data were analyzed using an acquisition software, version 1.25 and Rigaku data treatment. The chromatographic separation happened in reversed phase, the column and parameters used were similar to those presented in Buffoni [16], a Jupiter C4 column (2504.6 mm, 300 Å pore size, 5 μm particle size, Phenomenex) was used, the oven temperature was 30˚C, injection volume equal to 20 μL, mobile phase flow equal to 1 mL/min, and the eluent was monitored using a detector per diode array at 205 nm. The eluents solutions and elution gradient utilized were similar to those described by Enne [17], where the mobile phase (A) was composed of water of HPLC grade containing 0.1% of trifluoroacetic acid, and mobile phase (B) was composed of Acetonitrile containing 0.1% of trifluoroacetic acid. The elution gradient was 35% B from 0 to 1 min, 35% - 38% B from 1 to 8 min, 38% - 42% B from 8 to 16 min, 42% - 46% B from 16 to 22 min, 46% - 90% B from 22 to 24 minutes, 90%

B from 24 to 25 min, 90% - 35% B from 25 to 30 min, and 35% B from 30 to 35 min. Due to the high viscosity of the gum-rich phase, the concentrations of α-La and β-Lg proteins at this stage were determined through mass balance.

E. Separation Process Analysis

The partition process of the proteins was evaluated in terms of partition coefficient, selectivity, yield and purity, which were determined through as the methods described below.

a) The partition coefficient (*Ki*) was determined considering the ratio between the protein concentration in the upper phase (CUP) and the concentration of the same protein in the lower phase (CLP), according to Eq (1):

$$K_i = \frac{C_{UP}}{C_{LP}} \quad (1)$$

b) The selectivity (*S*) was calculated using the ratio between the partition coefficients of α-La and β-Lg in the two equilibrium phases:

$$S = \frac{K_{\alpha La}}{K_{\beta Lg}} \quad (2)$$

c) The yield and the purity of proteins were calculated according to the following equations [23]:

$$Y_{\alpha La,UP} = \frac{100}{\left(1+\left(\frac{1}{V_r}\times\frac{1}{K_{\alpha La}}\right)\right)} \quad (3)$$

$$Y_{\beta Lg,LP} = \frac{100}{\left(1+\left(V_r\times K_{\beta Lg}\right)\right)} \quad (4)$$

where $Y_{\alpha La,UP}$ and $Y_{\beta Lg,LP}$ correspond to α-La yield in the upper phase and β-Lg yield in the lower phase, respectively; Vr corresponds to the volume ratio between the phases.

Then, for the α-La and β-Lg proteins purity:

$$P_{\alpha La,UP} = 100 \times Y_{\alpha La,UP} \times \frac{0.7}{\left(Y_{\alpha La,UP}\times 0.7\right)+\left(100-Y_{\alpha La,UP}\right)\times 3} \quad (5)$$

$$P_{\beta Lg,LP} = 100 \times Y_{\beta Lg,LP} \times \frac{3}{\left(100-Y_{\beta Lg,LP}\right)\times 0.7+\left(Y_{\beta Lg,LP}\times 3\right)} \quad (6)$$

where PαLa,UP and PβLg,LP correspond to the purity percentage of α-La proteins in the upper phase and β-Lg in the lower phase, respectively.

III. Results and Discussion

For the application of ATS using polymer-polysaccharide blends, first, the cashew gum purification process was performed. Fig. 1 shows the crude and treated cashew gum used in this work. The cashew gum purification process proved to be viable with about 73% yield, better than that obtained by Rodrigues et al [15], which obtained 50% yield.

The partition of the α-La and β-Lg proteins in ATS formed by polymer-cashew gum was evaluated using in-nature goat's milk serum and the results are presented below. Table 1 indicates the partition coefficients (Ki) of in-nature goat whey proteins (α-La and β-Lg) for systems composed of polymer-cashew gum.

(a) (b)

Figure 1. Cashew gum (a) crude, (b) after purification

TABLE I. Partition coefficients of in-nature goat whey proteins α-La (Kα-La) and β-Lg (Kβ-Lg) for systems composed of polymer - cashew gum (CGum)

Type	Polymer (%)	CGum (%)	Water (%)	V_r	$K_{\alpha\text{-La}}$	$K_{\beta\text{-Lg}}$
PPG 425	13.17	21.09	66.16	***	***	***
	16.68	24.89	58.08	0.7 ± 0.1	0.033	0.024
	20.28	22.87	56.76	1.5 ± 0.2	0.007	0.001
PPG 2000	13.03	20.98	65.98	0.2 ± 0.0	**	**
	20.03	20.96	59.01	0.5 ± 0.1	**	**
	24.99	21.01	54.01	5.3 ± 1.1	**	**
PPG 4000	12.9	20.99	66.11	0.3 ± 0.0	**	**
	20.03	21.91	58.06	0.5 ± 0.1	**	**
	22.95	23.11	53.93	0.9 ± 0.1	**	**
PEG 1500	13.16	21.02	66.49	2.3 ± 0.3	0.019	0.012
	14.06	22.41	63.73	1.9 ± 0.2	0.005	0.001
	14.45	24.05	62.31	1.5 ± 0.2	0.002	*
PEG 4000	9.17	18.02	73.13	4.1 ± 0.6	0.005	0.004
	11.14	20.07	70.89	2.3 ± 0.3	0.0037	0.00125
	12.99	21.92	64.84	1.8 ± 0.2	*	*
PEG 8000	9.3	16.00	75.21	3.1 ± 0.4	0.008	0.002
	10.97	18.15	71.35	2.1 ± 0.2	0.004	0.001
	13.52	19.92	67.3	1.9 ± 0.2	0.003	0.002
PVP 3500	8.21	25.07	66.72	***	***	***
	12.95	21.01	66.72	***	***	***
	23.35	12.25	64.40	***	***	***
PVP 10000	13.05	20.89	66.06	***	***	***
	14.95	21.03	64.02	***	***	***
	24.92	15.05	60.03	***	***	***
PVP 40000	13.17	20.82	66.01	***	***	***
	14.81	14.84	70.35	***	***	***
	19.86	18.08	62.05	***	***	***

s.d. = 0.002 for $K_{\alpha\text{-La}}$ and 0.0001 for $K\beta\text{-Lg}$.
(*) The α-La and /or β-Lg proteins did not partition, both were in the lower phase, rich in CGum.
(**) It was not possible to carry out the analysis due to the high viscosity of the solutions in both phases.
(***) The systems in the compositions studied did not form two phases.

In the systems containing PPG 425, PEG (1500, 4000, 8000), the partition coefficients of the proteins (α-La and β-Lg) from the in-nature goat whey in all samples were lower than 1,

therefore both proteins were concentrated in the lower phase, rich in gum, with the best result obtained from the system formed by 16.68% PPG 425 and 24.89% CGum. Sarubbo et al [13] evaluated the partition of pure albumin from bovine serum using the same system and also obtained partition coefficients less than 1 in all systems studied, showing that albumin was also concentrated in the lower phase, rich in gum.

In relation to the polymer-type effect, it was observed that the systems formed by PPG 425-CGum were capable of separating both proteins, with the exception of the system consisting of 13.17% PPG 425 and 21.09% CGum, which did not form two phases. In the systems formed by PPG 2000 and 4000 it was not possible to carry out the analyzes, since the solutions of the two phases presented high viscosity. In the systems formed by PEG 1500, 4000 and 8000- CGum, it was possible to partition the proteins, except for the system formed by 14.45% PEG and 24.05% CGum, where β-Lg remained in the lower phase, rich in gum, and in the system composed of 12.99% PEG 4000 and 21.92% CGum, where both proteins were concentrated in the gum rich phase. However, the systems formed by PVP (3500, 10000 and 400000) and gum in the compositions studied did not form two phases. Nonetheless, this does not mean that the use of the PVP-CGum mixture at any of the concentrations may lead to the separation of the proteins, since for the system PPG425-CGum the change in concentrations makes it possible to separate such proteins.

In order to evaluate the effect of the molecular weight of the polymer, the systems formed by PEG and cashew gum were considered as a basis. In these, it was observed that there was a decrease in the partition coefficient of the proteins with the increase of the molecular mass, these results being consistent with that observed in the literature [26].

Furthermore, analyzing Table 1, and using as a basis of analysis the systems formed by PEG and gum, it can be observed that the increase of the percentage of the polymer in all the systems studied caused a decrease in the coefficient of partition of the α-La proteins and β-Lg from in-nature goat's milk. This effect is related to the increase in the volume excluded, in general one molecule in a solution tends to exclude all others from the volume it occupies, with the increase of polymer concentration [32]. The same occurred in the partition of pure bovine serum albumin protein in the study described by Sarubbo et al [13].

Besides the value of the partition coefficients, other factors must be taken into account in a separation process, such as selectivity, yield and the purity of proteins. Table 2 presents these values for the ATSs in which it was possible to partition α-La and β-Lg.

From Table 2, it can be observed that although the best values of partition coefficients were obtained for the system 16.68% PPG 425 and 24.89% CGum, the higher value of selectivity of α-La in relation to β-Lg was observed for the system 20.28 % PPG 425 and 22.87% CGum (S = 7.00). The higher yield of α-La in the upper phase (YαLa,UP = 4.19) was obtained for the system 13.16 % PEG 1500 and 21.02 % CGum. These results show that, different possibilities can be used to separate the α-La protein from the goat's milk whey. On the other hand, for β-Lg, all ATSs presented higher values of YαLa,UP and YβLg,LP. This is coherent with the results presented in Table 1, since Kβ-Lg <<< Kα-La indicating that this protein prefers to remain in the lower phase, rich in gum.

TABLE II. SELECTIVITY, YIELD AND PURITY OF α-LA ($K_{\alpha\text{-}LA}$) AND β-LG ($K_{\beta\text{-}LG}$) IN THE ATSS COMPOSED OF POLYMER - CASHEW GUM (CGUM)

Type	Polymer (%)	CGum (%)	Water (%)	S	$Y_{\alpha La,UP}$	$Y_{\beta Lg,LP}$	$P_{\alpha La,UP}$	$Y_{\beta Lg,LP}$
PPG425	16.68	24.89	58.08	1.4	2.26	98.3	0.54	99.6
	20.28	22.87	56.76	7.0	1.04	99.9	0.24	100.0
PEG1500	13.16	21.02	66.49	1.6	4.19	97.3	1.01	99.4
	14.06	22.41	63.73	5.0	0.94	99.8	0.22	100.0
PEG4000	9.17	18.02	73.13	1.3	2.01	98.4	0.48	99.6
	11.14	20.07	70.89	3.0	0.84	99.7	0.20	99.9
PEG8000	9.3	16.00	75.21	4.0	2.42	99.4	0.58	99.9
	10.97	18.15	71.35	4.0	0.83	99.8	0.20	100.0
	13.52	19.92	67.30	1.5	0.57	99.6	0.13	99.9

IV. CONCLUSIONS

This study had the aim to evaluate the use of ATSs formed by polymer - cashew gum-water in the partition of proteins (α-La and β-Lg) from goat's milk whey in nature. The results were coherent with the literature which used similar ATSs and other kinds of proteins (pure trypsin [19] and pure bovine serum albumin [13]). Even if the partition coefficient values had been low, this study would still provide important information about the applicability of cashew gum as a natural polysaccharide to be used in ATSs in the partition of bio-molecules. Goat whey is a byproduct with high added value that is rarely used by the dairy industry and many times is wasted by the food industry. Efforts to try to recover the important proteins from this material at low cost and using natural materials are to be supported. There is a great deal to be discovered and understood, but the importance of this study lies in trying to find new natural alternatives to help in the recovery of important bioactive compounds which have previously been wasted by the industry, be it for technological or economic reasons. The study also indicated that the best results were obtained by ATSs formed by PPG 425- cashew gum-water and PEG 1500- cashew gum-water.

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REFERENCES

[1] F.V. Kosikowski. Whey utilization and whey products. J Dairy Sci, 63 (7), pp. 1149-1160, 1979.

[2] C.V. Morr, E.Y.W. Ha. Whey protein concentrates and isolates: processing and functional properties. Crit. Rev. Food Sci. Nutr. 33, pp. 431-476, 1993.

[3] D.M. Mulvihill, J.E. Kinsella, J. E. Gelation characteristics of whey proteins and α-lactoglobulin. Food Technol., 41, pp. 102-111, 1987.

[4] N. Guirguis, C. Brosnahan, M.W. Hickey, M. W. Whey disposal: recovery of nutrients for animal feeding. Water Sci. Technol., 27, 149-157, 1993.

[5] P.F. Fox, P.L.H. McSweeney. Advanced Dairy Chemistry- 1 Proteins, 3rd Ed. Springer 2003.

[6] P.A. Albertsson. Partition of cell particles and macromolecules. NY John Wiley & Sons, 1986.

[7] A.D. Diamond, J.T. Hsu, Aqueous two-phase systems for biomolecule separation. Adv. Biochem. Eng. Biot., 47, pp. 89-135, 1992.

[8] S.C. Silverio, P.P. Madeira, O. Rodriguez, J.A. Teixeira, E.A. Macedo. ΔG(CH2) in PEG-Salt and Ucon-Salt Aqueous Two-Phase Systems. J. Chem. Eng. Data. 53, pp. 1622-1625, 2008.

[9] A.C. Lima, R.A. Santos, F.A.G. Almeida, C.T. Bandeira. Chemical stimulants in the extraction of cashew gum. cajueiro (*Anacardium occidentale* L.). Original title: Estimulantes químicos na extração da goma do cajueiro (*Anacardium occidentale* L.).Ciência Rural 31, pp. 409-415, 2001.

[10] F.R.T. Rosenthal. Cashew gum; Chemical and technological study. Ministry of Labor, Industry and Commerce. Institute of Technology, Brazil Original title: Goma do cajueiro; Estudo químico e tecnológico. Ministério do Trabalho, Indústria e Comércio. Instituto de Tecnologia, Brasil, 1951. 35p.

[11] J. Owusu, J.H, Oldham, I. Oduro, W.O. Ellis, J. Barimah, Viscosity studies of cashew gum. Trop. Science. 45, pp. 86-89, 2005.

[12] E.M.C. Landim, Obtaining, characterizing and evaluating the stability of microencapsulated natural pigments. Original Title: Obtenção, caracterização e avaliação da estabilidade de pigmentos naturais microencapsulados. 2008. 90f. Master Thesis. Universidade Federal do Ceará, Fortaleza.

[13] L.A. Sarubbo, G.M. Campos-Takaki, A.L.F. Porto, E.B. Tambourgi, L.A. Oliveira, Cashew gum (*Anacardium occidentale* L.) as an innovative liquid-liquid extraction system Original title: A goma do cajueiro (*Anacardium occidentale* L.) como sistema inovador de extração líquido-líquido. Exacta, 5(1), pp.145-154, 2007.

[14] P.F.A.P. Pessoa, C.T. Bandeira, Cashew Gum: New income alternative for Northeastern cashew. Original title: Goma do cajueiro: Nova alternativa de renda para a cajucultura nordestina. Embrapa, ano VI, n.1, 1993.

[15] J.F. Rodrigues, R.C.M. Paula, S.M.O. Costa, Methods of isolation of natural gums: comparison through cashew gum (*Anacardium occidentale* L.). Original title: Métodos de isolamento de gomas naturais: comparação através da goma do cajueiro (*Anacardium occidentale* L.). Polímeros: ciência e tecnologia, 1, pp. 31-36, 1993.

[16] L.A. Sarubbo, Characterization of a new aqueous biphasic system and application in protein extraction with column of rotary drilled discs . Original title: Caracterização de um novo sistema bifásico aquoso e aplicação em extração de proteínas com coluna de discos perfurados rotativos. 2000. 223 f. PhD Thesis. Programa de Pós-Graduação da Faculdade de Engenharia Química Universidade Estadual de Campinas. Campinas, São Paulo, Brazil.

[17] L.A. Sarubbo, L.A. Oliveira, A.L.F. Porto, G.M. Campos-Takaki, E.B. Tambourgi, Partition of proteins in aqueous two-phase systems based on cashew-nut tree gum and poly(ethylene glycol). Braz. Arch. Biol. Techn., 47, pp. 85-691, 2004.

[18] L.A. Oliveira, L.A. Sarubbo, A.L.F. Porto, J.L.Lima-Filho, G.M. Campos-Takaki. Physical and rheological characterization of PEG-cashew-nut tree gum aqueous two-phase systems. J Chromat B, 766, pp.27-36, 2002.

[19] L.A. Oliveira, Characterization of the aqueous biphasic system Polyethylene Glycol-Gum of Cajueiro. Original title: Caracterização do sistema bifásico aquoso Polietilenoglicol-Goma do Cajueiro. 1999. 99f. Master Thesis. Faculdade de Engenharia Química, Universidade Estadual de Campinas, Brazil.

[20] L.H. Silva, A.J.A. Meirelles. Phase equilibrium and protein partitioning in aqueous mixtures of maltodextrin with polypropylene glycol. Carbohyd. Polymers 46, pp.267-274, 2001.

[21] A. Boaglio, G. Bassani, G. Picó, B. Nerli. Features of the milk whey protein partitioning in polyethyleneglycol-sodium citrate aqueous two-phase systems with the goal of isolating human alpha-1 antitrypsin expressed in bovine milk. J. Chromat B, 837, pp. 18-23, 2006.

[22] L.R. Rodrigues, A. Venâncio, J.A. Teixeira, Partitioning and separation of alfa-lactalbumin and beta-lactoglobulin in polyethylene glycol/ammonium sulphate aqueous two-phase systems. Biotechnol. Lett., 23, pp. 1893- 1897, 2001.

[23] J.P. Chen, Partitioning and separation of α- lactoalbumin e β-lactoglobulin in PEG/Potassium phosphate aqueous two-phase systems. J. Ferment. Bioeng, 73 (2), pp. 140, 1992.

[24] B. Mokhtarani, H.R. Mortaheb, M.Mafi, M.H. Amini. Partitioning of α-lactoalbumin e β-lactoglobulin in aqueous two-phase systems of polyvinylpyrrolidone and potassium phosphate. J. Chromatogr. B. 879, pp. 721-726, 2011.

[25] L.A.C. Freire, C.G. Pereira. Partition of α-lactoalbumin and β-lactoglobulin from the goat milk whey utilizing aqueous biphasic systems. Sep. Sci. Technol. 51, pp. 457-464, 2016.

[26] L.A.C. Freire, C.G. Pereira. Aqueous Two-phase Systems Applied to Partition Proteins from goat milk whey in-nature. Adv. Biosci. Biotechnol. 7, pp. 350-359, 2016.

[27] G.O. Aspinall, G. O. The Polysaccharides. New York: Academic Press, 1982. V. 1, Cap 2.

[28] J.F. Kennedy, C.A. White, Bioactive Carbohydrates. In: Chemistry, Biochemistry and Biology, Inglaterra: Ellis Horwood Ltda, 1983. Cap 4.

[29] D.S. Torquato, M.L. Ferreira, G.C. Sá, E.S. Brito, G.A.S. Pinto, E.H.F. Azevedo. Evaluation of antimicrobial activity of cashew tree gum. World J. Microbiol. Biotech. 20, pp. 505-507, 2004.

[30] J.N. Buffoni, I. Bonizzi, A. Pauciullo, L. Ramunno, M. Feligini, Characterization of the major whey proteins from milk of Mediterranean water buffalo (Bubalus bubalis). Food Chem., 127, pp. 1515-1520, 2011.

[31] G. Enne, D. Elez, F. Fondrini, I. Bonizzi, M. Feligini, R. Aleandri. R. High-performance liquid chromatography of governing liquid to detect illegal bovine milk's addition in water buffalo mozzarella: Comparison with results from raw milk and cheese matrix. J. Chromatogr., 1094, pp. 169-174, 2005.

[32] Albertsson, P. A. Partition of cell particles and macromolecules. 3. ed. New York: John Wiley & Sons, 1986.