Eclética Química
ISSN: 0100-4670
ISSN: 1678-4618
ecletica@journal.iq.unesp.br
Universidade Estadual Paulista Júlio de Mesquita Filho
Brasil

Ramirez-Perez, Javier Christian; Wiliam Janes, Harry
Impact of salinity on the kinetics of CO2 fixation by *Spirulina platensis* cultivated in semi-continuous photobioreactors
Eclética Química, vol. 46, no. 1, 2021, pp. 21-34
Universidade Estadual Paulista Júlio de Mesquita Filho
Brasil

DOI: https://doi.org/10.26850/1678-4618eqj.v46.1.2021.p21-34

Available in: https://www.redalyc.org/articulo.oa?id=42965151003

Eclética Química Journal

Original article

iq.unesp.br/ecletica

| Vol. 46 | n. 1 | 2021 |

Zinc fractionation in cow, goat, sheep and soybean milk samples using gel-electrophoresis and determination by electrothermal atomic absorption spectrometry (ETAAS)

Carla Maíra Bossu[1,2+], Rodrigo Giglioti[3], Marcia Cristina de Sena Oliveira[1], Pedro Vitoriano de Oliveira[4], Ana Rita de Araujo Nogueira[1]

1.Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil
2.Federal University of São Carlos, Chemical Department, São Carlos, São Paulo, Brazil
3.Zootechnics Institute of the São Paulo State Government, Nova Odessa, São Paulo, Brazil
4.University of São Paulo, Institute of Chemistry, Department of Fundamental Chemistry, São Paulo, São Paulo, Brazil

+Corresponding author: Carla Maíra Bossu, **Phone:** +55 16 3411-5724, **Email address:** carla.bossu@gmail.com

ARTICLE INFO

Article history:
Received: October 07, 2019
Accepted: June 02, 2020
Published: January 01, 2021

Keywords
1. zinc
2. milk
3. proteins
4. fractionation

ABSTRACT: A screening method for zinc levels in different milk samples (raw cow, raw sheep, UHT cow, UHT goat and soybean milk base) was performed to establish the Zn levels' differences in protein samples. The samples were digested in a cavity microwave oven and the total Zn levels in the extracts were determined by flame absorption atomic spectrometry (FAAS). The protein separation was performed by urea polyacrylamide gel electrophoresis (UREA-PAGE). Protein bands were digested in the cavity microwave oven and Zn-protein analysis was further conducted by electrothermal atomic absorption spectrometry (ETAAS). The results showed that Zn is mainly bound to 32 kDa (β-casein) protein (17.0 ± 2.0%) in UHT cow and 24 kDa (α-casein) protein (9.0 ± 0.6%) in raw sheep milk. This method provided quantitative information regarding Zn species present in the protein fractions of the milk samples. The accuracy was evaluated using certified reference material (whole milk powder, NIST 8435) with statistically equivalent concentrations (Student's *t*-test) for total Zn and by addition and recovery experiments applied to measure Zn-protein. The recovered values were in the 92-110% range.

1. Introduction

Zinc (Zn) is a trace element essential in human and animal nutrition and participates in critical biological processes, such as the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids. It is present in food in two forms: bound with organic molecules and in inorganic salts[1]. Milk is one of the main sources of zinc in the human diet, responsible for about 25% of the total recommended daily intake of this metal[1,2]. Milk is a complete source of nutrients, containing minerals, vitamins, carbohydrates, lipids, water and proteins, which are especially crucial for newborns' growth and development[3].

As humans continue to consume milk during childhood and adulthood, highlighting the expressive consumption of cow's milk, it is essential to understand better the chemical composition of milk, including the mineral levels and their physiological influence on human and animal nutrition, as well as trace elements toxicity[2].

Studies about different chromatographic methods have indicated differences in the association between Zn and proteins in human and cow's milk. In cow's milk, the complex Zn-casein-Ca-P is the predominant form, while in human milk, Zn-citrate species seem to be predominant[4]. In the environmental, biological, medical and biochemical research areas, studies about metal-protein interaction are necessary to improve the knowledge about the toxicity, bioavailability, transport and physicochemical properties of an element[5].

Nutrient fractionation in food is necessary to understand availability and absorption prediction. Fractionation is defined as the classification process of an analyte or an analyte group in a specific sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties[6]. One relatively simple procedure to fractionate proteins from food samples is the polyacrylamide gel electrophoresis, which shows high selectivity and consists in the separation of molecules loaded in a particular way under the influence of an electric potential difference[7]. One of the main uses of the gel electrophoresis technique containing urea is the characterization of proteins in various types of milk[8].

Studies about metal-protein interaction using polyacrylamide gel electrophoresis, followed by different analytical techniques for metals determination, have been developed for several kinds of application[9-16]. These works belong to an important field of science called metallomic, which has allowed for the integration of analytical studies with inorganic and biochemical studies. Therefore, there is a growing demand for accurate and selective procedures that allow the quantification and speciation of trace elements, especially in foods, to obtain relevant information about metal-protein binding, which is intimately related to the nutrients bioavailability[7,17].

In this context, the present work aimed at investigating the separation of proteins in different kinds of milk samples, by urea polyacrylamide gel electrophoresis (UREA-PAGE), and to establish Zn(II) concentrations in each protein determined by electrothermal atomic absorption spectrometry (ETAAS). The Zn distribution results in protein bands were quantitative and provided information about Zn levels present in the milk samples' protein fractions.

2. Experimental

2.1 Instrumentation

A Varian model SpectrAA-800 graphite furnace atomic absorption spectrometer (Mulgrave, Victoria, Australia) equipped with a graphite furnace atomizer, GTA 100 autosampler, was used for zinc quantification in the proteins bands. Background correction by Zeeman-effect was employed to correct for non-specific absorbance. Pyrocoated graphite tubes (Part Number 63-100011-00, Varian) furnace with longitudinal heating was throughout. All signals were measured as integrated absorbance. A zinc hollow cathode lamp (λ = 213.9 nm, slit = 1.0 nm) operating in 5 mA was employed as radiation source. Argon 99.998% (Air liquid Sao Paulo, Brazil) was used as purge gas. The heating programs used after the optimization of pyrolysis and atomization temperatures are shown in Tab. 1.

The total Zn was carried out using a flame atomic absorption spectrometer (model SpectrAA 250 plus, Varian, Mulgrave, Australia) equipped with a Zn hollow cathode lamp (λ = 213.9 nm, slit = 1.0 nm) and 5 mA lamp current were employed as primary radiation sources. Air/acetylene flame was used at 13.4 L min^{-1} and 2 L min^{-1} air and acetylene flows, respectively.

The Mini-gel (Vertical Electrophoresis System Mini, BioAmerica Inc. Equipments, Miami, USA) with 8.5 cm (height) x 10.0 cm (width) and a centrifuge (5417R, Eppendorf, Hamburg, Germany) were used for gel-electrophoresis.

The milk samples were microwave digested assisted (Multiwave, Anton Paar, Graz, Austria) with 50 mL PFA vessels. The total protein amount was determined by spectrophotometry (Spectrophotometer model 432, Femto, Sao Paulo, Brazil).

Eclética Química Journal, vol. 46, n. 1, 2021, 12-20
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v46.1.2021.p12-20

Table 1. GF AAS heating program used in the zinc protein bands.

Step	Temperature / ºC	Hold Time / s	Ar Flow / L min^{-1}	Reading
Drying	85	2.0	3.0	No
Drying	95	8.0	3.0	No
Drying	120	10	3.0	No
Pyrolysis	1000	5.0	3.0	No
Pyrolysis	1000	1.5	3.0	No
Atomization	1600	0.8	0	Yes
Atomization	1600	2.9	0	Yes
Cleaning	2500	2.9	3.0	No

2.2 Reagents and materials

All solutions were prepared using deionized water (resistivity > 18.2 MΩ cm) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Nitric acid (HNO_3) (Synth, Diadema, SP, Brazil) previously purified using a sub-boiling distillation system (Milestone, Sorisole, Italy), and hydrogen peroxide (H_2O_2) 30 % w v^{-1} (Sigma Aldrich, Switzerland) were used to digest the samples.

A solution of 1.78% (m/v) $Mg(NO_3)_2$ (Merck, Darmstadt, Germany) was used as a chemical modifier. Analytical reference solutions between 0.2 and 1.0 mg L^{-1} of Zn were prepared by successive dilutions of a stock solution containing 1000 mg L^{-1} Zn (Tec-Lab, Hexis, Jundiai, SP, Brazil).

The analytical curve for Zn determination in protein bands was obtained using analytical solutions with concentrations between 0.5 and 3.0 µg L^{-1} Zn in blank gel band previously microwave-assisted digested with HNO_3 1.0% (v/v).

2.3 Samples

Fresh milk samples from Holstein cows aged between 3 to 4 years, weighing an average of 450 kg and raised in a semi-intensive system, and sheep from Santa Inês were collected at the Embrapa Pecuária Sudeste, located in São Carlos, SP, Brazil. Samples of ultra-high temperature (UHT) cow milk, UHT goat milk and soybean milk base were purchased in the local market of São Carlos, SP, Brazil. Samples were stored in a fridge at – 4 ºC before analysis.

Certified reference milk sample (NIST 8435 - Whole Milk Powder) from the National Institute of Science and Technology (NIST, Gaithersburg, MD, USA) was used to check the methods' accuracy for the measurement of total Zn. Addition and recovery experiments were performed to evaluate the species of Zn.

2.4 Procedures

2.4.1 Evaluation of pyrolysis and atomization temperature

The pyrolysis and atomization temperature curves were constructed with 18 µL of the standard or sample solutions in the presence of a chemical modifier, 5 µL of $Mg(NO_3)_2$ 1.78% (w/v). The temperatures were evaluated in a range of 700-1800 °C using increments of 100 ºC. Solutions of 1.0 µg L^{-1} Zn were prepared for this evaluation using the blank sample, a piece of polyacrylamide gel without protein band, in acid digested (HNO_3 1.0% v/v).

2.4.2 Sample preparation for total Zn and species determination

Zinc's total mass fractions were determined in the milk samples using a microwave-assisted acid digestion. In brief, 2.5 mL of milk samples or 250 mg of the certified reference material was directly weighted in the microwave vessel. Then, 1.0 mL of H_2O_2 30% (w/v), 1 mL of H_2O and 1.0 mL of HNO_3 65% (v/v) were added and the mixture submitted to the following microwave heating program: 5 min from 0 to 100 W, 5 min at 600 W, 5 min at 1000 W followed by 15 min of cooling. After digestion and cooling, the digests were transferred to volumetric flasks and the volume was made up to 20 mL with deionized water.

Protein bands (8 mm wide by 3 to 5 mm in height) of the milk samples and the blank (gel region without the protein) obtained by electrophoresis were cut, washed with deionized water and dried on filter paper for 15 min in the laminar flow cabinet before weighting (each band was approximately 50 mg). The bands were placed in PTFE mini-bottles, and 75 µL 65% (v/v) HNO_3 plus 75 µL 30% (w/v) H_2O_2 were added.

The mini bottles were placed into the microwave PFA vials containing 2 mL deionized water. Figure 1

Eclética Química Journal, vol. 46, n. 1, 2021, 12-20
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v46.1.2021.p12-20

shows the container configuration used. The microwave heating program was run as follows: 1 min from 0 to 250 W, 1 min at 0 W, 5 min at 250 W, 5 min at 400 W, 10 min at 750 W followed by 10 min of cooling. After digestion and cooling, the digests were transferred to volumetric flasks and the volume was made up to 3.5 mL with deionized water.

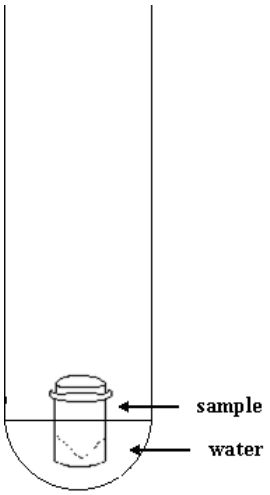

Figure 1. Sample container into the vial of the microwave cavity vessel.

2.4.3 Gel Electrophoresis

Urea-polyacrylamide gel electrophoresis (UREA-PAGE) was carried out using a separation gel composed of acrylamide (4% for stacking gel and 10% for separation gel), as described by Egito *et al.*[18], with modifications.

The separation gel was composed of 2.25 mL acrylamide/Bis (40 g acrylamide (Invitrogen, USA) and 2 g N,N-methylene bisacrylamide (BIS) (Sigma, Germany) in 100 mL solution) and 7.10 mL separation gel buffer (6.43 g of TRIS (Synth, SP, Brazil), 38.57 g urea (J.T. Baker, Germany), 0.572 mL HCl (Aldrich Chemical, Milwaukee, WI, USA), with pH adjusted to 8.9 in 100 mL solution).

The stacking gel was prepared using 0.39 mL acrylamide/Bis and 3.52 mL stacking gel buffer (0.830 g TRIS, 30 g urea, 0.44 mL HCl, with pH adjusted to 7.6 in 100 mL solution). Both gels also contained N,N,N',N'-tetramethylethylenediamine (TEMED) (J.T. Baker, Germany) and 10% (m/v) ammonium persulfate (Sigma, Germany). Finally, Tris-glycine buffer solution (3.75 g TRIS and 18.25 g glycine (Sigma, Germany) in 250 mL of solution, diluted 5 times) was used in the reservoir.

To sample preparation, 30 µL of samples and standard curve of purified protein markers (GE Healthcare) were dissolved in 1.0 mL of buffer solution, composed by 0.375 g TRIS, 24.5 g urea, 0.2 mL HCl, 0.35 mL β-mercaptoethanol (Inlab, Brazil) and 0.075 g bromophenol blue (Merck) diluted to 50 mL with water. After dissolution, the samples were immersed in a water bath at 40 °C for 1 h and centrifuged at 5 °C for 10 min at 10000×g. Then, 30 µL of supernatant was applied to a single slot. The initial and final currents were 23 and 24 mA and the voltage was set at 90 V for 4.5 h. After the protein migration, protein bands were stained overnight with a solution containing 0.5 g of Coomassie brilliant blue G-250 (Sigma, Germany) and 3.75 mL of H_2SO_4 (Quemis, Brazil) in 500 mL solution. This solution was then filtered using filter paper and mixed to 55.6 mL of 10 mol L^{-1} KOH and 66.67 mL of 12% (w/v) trichloroacetic acid (Synth, Brazil). Finally, the gels were washed with deionized water and stored in 10% (v/v) glycerol 10% (v/v) methanol solution (Proquímios, Brazil).

The molecular weight proteins were estimated according to standard purified protein markers, including phosphorylase b (97.0 kDa), albumin (66.0 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (30.0 kDa), trypsin inhibitor (20.1 kDa) and α–Lactalbumin (14.4 kDa). The gel electrophoresis UREA-PAGE was performed in triplicate for each sample and separately to avoid contamination.

2.4.4 Determination of Total Protein concentration

The total protein concentration was determined through the Bradford method[19] by adding 1.25 mL of the Bradford reagent (Coomassie Brilliant Blue G-250) and 100 µL of bovine serum albumin (BSA) (Sigma, USA) standard solutions or the samples and measuring the absorbance after 5 min at 595 nm, in triplicate. The analytical curve was constructed by using standard solutions of BSA at concentrations between 50 and 250 mg L^{-1}, prepared by successive dilutions of a stock solution containing 1000 mg L^{-1} of BSA.

3. Results and discussion

3.1 Gel Electrophoresis

Figure 2 shows the gels electrophoresis obtained for each milk sample, where columns 2 to 6 correspond to the protein bands of each sample, and column 1 shows

Eclética Química Journal, vol. 46, n. 1, 2021, 12-20
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v46.1.2021.p12-20

the low molecular weight (LMW) standard protein (14.4 to 97 kDa). The dashed rectangles represent the bands studied with the approximate molecular weight, as this is a qualitative analysis. The protein molecular mass values, found from top to bottom, were 52 and 39 kDa for the soybean milk base (column 2), 32 and 24 kDa for raw sheep (column 3), 32 and 24 kDa for UHT cow (column 4), 32 kDa for UHT goat (column 5) and 32 and 24 kDa raw cow (column 6).

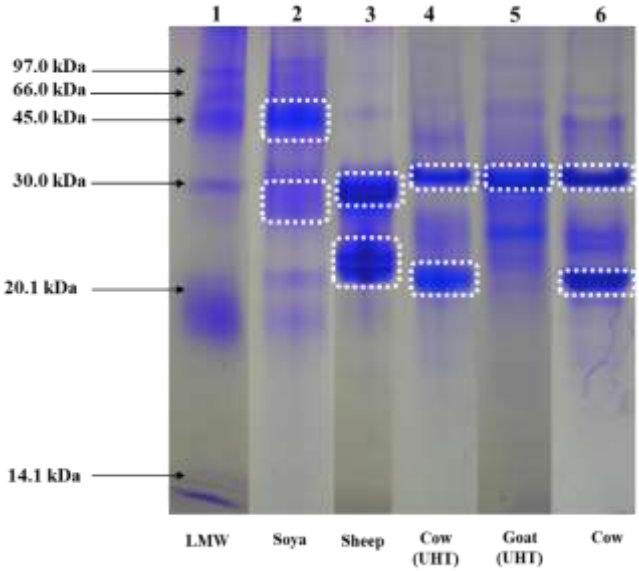

Figure 2. Zinc protein bands of milk samples run in polyacrylamide gel (10%) with urea.

Casein is the major component of milk protein. In this work, α-casein, β-casein and soybean proteins showed profiles similar to other studies[18,20,21] with approximately 24 kDa and 32 kDa for α-casein and β-casein, respectively. Bands of higher intensity for raw sheep, UHT cow, raw cow and UHT goat were in the molar mass range of 32 kDa. For the soybean milk base, the bands 52 and 39 kDa were assigned to β-conglycine subunits and glycine, respectively[20,22].

Naqvi *et al*.[23] reported that it was also possible to observe that Ca, Fe, Mg and Zn were mainly associated with colloidal calcium phosphate in casein micelles. For soybeans, 80% of the extracted proteins are β-conglycine (7S) and glycine (11S). Denaturation by heating occurs when there are interactions between these proteins, which can form basic subunits, such as (11S), β-subunits (7S), and α-,α'-subunits (7S)[20,22].

3.2 Total Zn and species determination

Among the micronutrients present in milk, Zn has the highest concentration and is important in human and animal nutrition, associated with organic molecules and present as inorganic salts. Concerning amino acids such as histidine and methionine and phosphates and organic acids, it enables the bioavailability of this element[1]. It is also associated with one of the most important milk proteins, casein, and in the bovine milk, Zn is present as Zn-casein-Ca-P complex[4].

The total zinc levels obtained in the samples are shown in Tab. 2 and agree with those reported in the literature[24,25]. No significant difference (Student's *t*-test 95% level, p>0.05) was found between the certified reference material NIST 8435 (28 ± 3 mg kg^{-1}) and the measured value of certified reference material (26 ± 1 mg kg^{-1}) for Zn. Therefore, this study can confirm the accuracy of the procedure. The slope and correlation coefficient (r) of the FAAS calibration curve prepared in the presence of 1.0% (v/v) of HNO_3 were 0.4688 and 0.9994, respectively. Moreover, limits of detection (LOD) calculated as three times the standard deviation of the blank (n=10)/slope was 0.02 mg L^{-1} and quantification (LOQ) (3 × LOD) was 0.03 mg L^{-1} for Zn, which are appropriate for Zn determination in the evaluated milk samples[26].

The pyrolysis and atomization temperature were evaluated to find the Zn measurement's best conditions in proteins by ETAAS (Fig. 3). The drying and cleaning temperatures were used according to Bossu *et al*.[27]. Pyrolysis temperature was set at 1000 °C and the atomization temperature was set at 1600 ºC. In this condition, the absorbance analytical signals' best profiles and the smallest deviations between the measurements were observed. The coefficient of variation was around 3.0% (n = 3) and the graphite furnace heating program used to Zn determination in the protein bands of different milk samples is shown in Tab. 1.

The slope and correlation coefficient (r) of ETAAS analytical curve in the presence of blank sample media (gel without protein) were 0.1457 and 0.995, respectively. The method's accuracy was evaluated by the addition and recovery procedure. Recoveries of 110 ± 1%, 92 ± 3%, 99 ± 4%, 95 ± 8% were obtained after adding 1.5 µg L^{-1} of Zn to proteins bands 32 kDa UHT cow, 32 kDa UHT goat, 39 kDa soybean and 24 kDa raw sheep, respectively. These recovery values are in the range established according to the quantified concentration level[28,29]. LOD and LOQ for Zn determination by ETAAS, using the optimized conditions, were 0.20 µg L^{-1} and 0.66 µg L^{-1}, respectively.

The results for Zn distribution in protein bands were quantitative and provide information about the relative

Eclética Química Journal, vol. 46, n. 1, 2021, 12-20
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v46.1.2021.p12-20

Zn concentration in each protein band from milk samples, as well as its relationship with the total levels of Zn found in milk samples (Tab. 2). Zinc is present predominantly in 32 kDa protein in UHT cow milk, followed by 32 kDa raw cow milk and 24 kDa raw sheep milk, which showed similar Zn levels.

Table 2. Zinc mass fractions (%) obtained for protein bands (Zn-protein) by ETAAS and Total Zinc (mg L^{-1}) obtained for milk samples (ZnT) by FAAS, n = 3.

Concentration/% ± standard uncertainty[a], Concentration/mg L^{-1} ± standard uncertainty[b] and coefficient of variation (CV) / %				
Samples / kDa	Zn-protein / %[a]	CV / %	Total Zinc / mg L^{-1} [b]	CV / %
Soybean milk-based / 52	n.d	n.d	2.50 ± 0.07	2.8
Soybean milk-based / 39	4.4 ± 0.6	12.6		
UHT Cow / 32	17.0 ± 2.0	9.4	3.20 ± 0.02	0.6
UHT Cow / 24	8.0 ± 1.0	17.6		
UHT Goat / 32	5.2 ± 0.9	16.3	2.75 ± 0.01	0.4
Cow / 32	8.0 ± 1.0	8.3	3.44 ± 0.05	1.5
Cow / 24	n.d	n.d		
Sheep / 32	1.7 ± 0.2	9.7	2.76 ± 0.01	0.4
Sheep / 24	9.0 ± 0.6	6.8		

n.d = not detected.

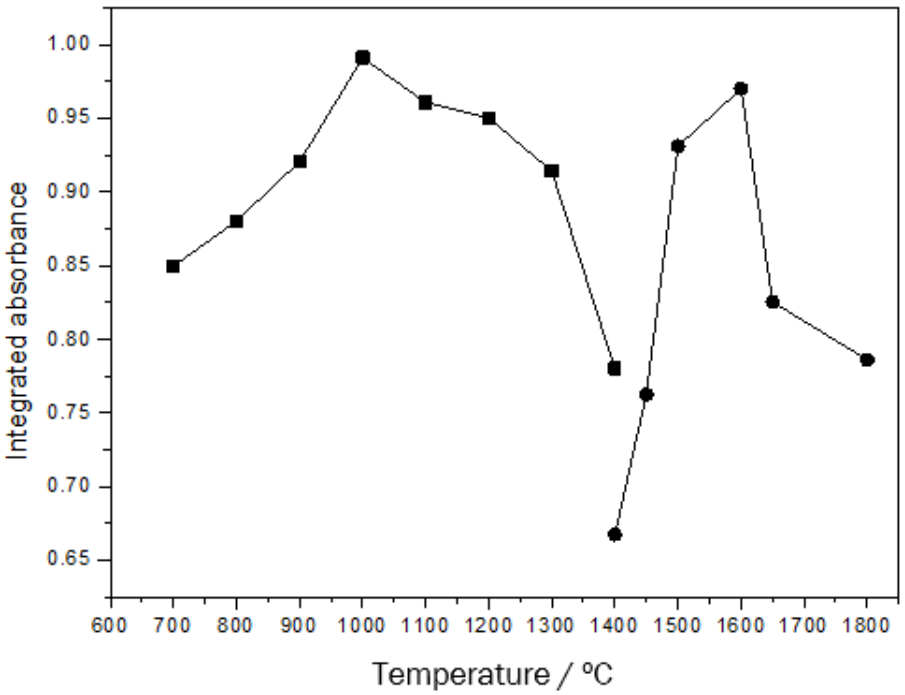

Figure 3. Pyrolysis (■) and atomization (●) temperature curves for 1.0 µg L^{-1} Zn in the blank (absence of band gel) in 1.0% v/v HNO_3 with 1.78% w/v $Mg(NO_3)_2$ of chemical modifiers.

It can be concluded that Zn is present in these samples in metal-binding proteins. There were no differences between the Zn-binding proteins' estimated values in UHT cow milk (8.0 ± 1.0%) and raw cow milk (8.0 ± 1.0%), which presented the highest Zn amount in 24 and 32 kDa, respectively. However, the largest proportion of Zn found in the 32 kDa protein was in UHT cow milk (17.0 ± 2.0%) and 24 kDa protein (9.0 ± 0.6%) in raw sheep milk. Studies were conducted to determine Zn bound to casein in breast milk, in which processed milk samples presented a change in the Zn distribution, with a decrease in the serum fraction and an increase in the fat fraction. There was no significant difference in the Zn percentage regarding casein. However, there was a trend towards increasing in processed samples[30], as observed in this study.

Eclética Química Journal, vol. 46, n. 1, 2021, 12-20
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v46.1.2021.p12-20

Previous studies[9,19,31,32] showed that zinc could also be linked to the casein fraction, α-casein and β-casein in bovine milk. The complex fractions of α-k-casein and α-β-casein showed significant zinc bound amounts, as can be demonstrated in this work.

Gaucheron *et al*.[31] estimated that cations are bound to α-casein and β-casein in the following affinity order: Fe > Zn > Ca. Zinc was found with 3 CPP (casein phosphopeptide) and the fraction containing α-casein and the 3 CPPs containing amino acids (glutamic acid, serine and phosphoserine), showing the Zn-complexes formation[23,32]. Moreover, the Zn distribution between casein, whey and other components may be affected by pH, heat and other cations present in the diet. Thus, the proteins are denatured and their ability to bind to Zn is probably reduced[33-35]. This reduction can be explained by the Zn percentage found in some proteins such as soybean (52 kDa), raw cow (24 kDa) and raw sheep (32 kDa).

Although contamination or species conversion problems cannot be ignored, gel electrophoresis is an important tool for the fractionation or separation of compounds, such as caseins, and Zn distribution can provide valuable information about the proteins' activity and their associated components.

3.3 Determination of total protein

Lipid extraction is not necessary during the Bradford method, so total protein determination was made directly in the diluted milk[36]. The aim was to observe if the ultra-high temperature (UHT) process caused changes in the milk total protein concentration when compared with the unprocessed samples. Results obtained for raw sheep, raw cow, UHT cow, UHT goat and soybean were 34 ± 1 g L^{-1}, 21 ± 1 g L^{-1}, 23 ± 1 g L^{-1}, 18 ± 1 g L^{-1} and 16 ± 1 g L^{-1}, respectively. No significant difference was observed in the results that compared processed and not processed milk, in agreement with a previous work[30].

Soybean is a protein supplier food composed by saturated and unsaturated fatty acids, vitamins and polyphenolic compounds, such as isoflavones[35]. It may be prepared to have the same protein content as bovine milk[34,35], which can be confirmed in this work (16 ± 1 g L^{-1}). However, the biological value of soybean proteins is lower than bovine milk or eggs proteins[35].

Proteins found in goat milk showed results comparable to bovine milk. The highest total protein concentration was found in sheep's milk, in agreement with the results reported by Raynal-Ljutovaca *et al*.[24] and was similar to bovine milk.

4. Conclusions

This study evaluated a fractionation method for Zn-proteins measurement in different milk samples by ETAAS. A method for total Zn quantification in different milk samples was also studied.

The Zn distribution results in protein bands were quantitative and could provide information about the relative Zn concentration in milk proteins. It was also possible to define the most appropriate polyacrylamide gels preparation procedure, the milk sample preparation and Zn determination. No differences were observed between the estimated values of Zn-binding proteins in UHT cow milk and in raw cow milk, which presented the highest Zn concentrations in 24 and 32 kDa, respectively. Total protein content in processed (UHT) and non-processed (raw) milk samples were also remarkably similar. The results showed that Zn is mainly bound to 32 kDa (β-casein) protein in UHT cow and 24 kDa (α-casein) protein in raw sheep milk. The use of fractionation has been demonstrated as a complementary analytical tool for the characterization of Zn species present in milk.

Acknowledgments

This study was supported by the São Paulo Research Foundation (FAPESP, process 06/59537-0), CAPES (001) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 409852/2018-0, 3091878/2018-1).

References

[1] Scheplyagina, L. A., Impact of the mother's zinc deficiency on the women's and newborn's health status, Journal of Trace Elements in Medicine and Biology 19 (1) (2005) 29-35. https://doi.org/10.1016/j.jtemb.2005.07.008.

[2] Ballard, O., Morrow, A. L. Human Milk Composition: Nutrients and Bioactive Factors, Pediatric Clinics of North America 60 (1) (2013) 49-74. https://doi.org/10.1016/j.pcl.2012.10.002.

[3] Hodgkinson, A. J., Wallace, O. A. M., Smolenski, G., Prosser, C. G., Gastric digestion of cow and goat milk: Peptides derived from simulated conditions of infant digestion, Food Chemistry 276 (2019) 619-625. https://doi.org/10.1016/j.foodchem.2018.10.065.

[4] Michalke, B., Element speciation definitions, analytical methodology, and some examples, Ecotoxicology and Environmental Safety 56 (1) (2003) 122-139. https://doi.org/10.1016/S0147-6513(03)00056-3.

[5] Arruda, M. A. Z., Azevedo, R. A., Metallomics and chemical speciation: towards a better understanding of metal-induced stress in plants, Annals of Applied Biology 155 (2009) 301-307. https://doi.org/10.1111/j.1744-7348.2009.00371.x.

[6] Templeton, D. M., Ariese, F., Cornelis, R., Danielsson, L.-G., Muntau, H., van Leeuwen, H. P. V., Lobinski, R., Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects and methodological approaches (IUPAC Recommendations 2000), Pure and Applied Chemistry 72 (8) (2000) 1453-1470. https://doi.org/10.1351/pac200072081453.

[7] Shi, W., Chance, M. R., Metalloproteomics: forward and reverse approaches in metalloprotein structural and functional characterization, Current Opinion in Chemical Biology 15 (1) (2011) 144-148. https://doi.org/10.1016/j.cbpa.2010.11.004.

[8] Dupont, D., Croguennec, T., Pochet, S., Milk Proteins – Analytical Methods, Reference Module in Food Science (2018) 1-14. https://doi.org/10.1016/B978-0-08-100596-5.22616-4.

[9] Pozzi, C. M. C., Braga, C. P., Vieira, J. C. S., Cavecci, B., Queiroz, J. V., Barbosa, H. S., Arruda, M. A. Z., Gozzo, F. C., Padilha, P. M., Metal ions bound to the human milk immunoglobulin A: Metalloproteomic approach, Food Chemistry 166 (2015) 492-497. https://doi.org/10.1016/j.foodchem.2014.06.040.

[10] Aslebagh, R., Channaveerappa, D., Arcaro, K. F., Darie, C. C., Comparative two dimensional polyacrylamide gel electrophoresis (2D-PAGE) of human milk to identify dysregulated proteins in breast cancer, Electrophoresis 39 (14) (2018) 1723-1734. https://doi.org/10.1002/elps.201800025.

[11] Vacchina, V., Oguey, S., Ionescu, C., Bravo, D., Lobinski, R., Characterization of metal glycinate complexes by electrospray Q-TOF-MS/MS and their determination by capillary electrophoresis–ICP-MS: application to premix samples, Analytical and Bioanalytical Chemistry 398 (2010) 435-449. https://doi.org/10.1007/s00216-010-3907-1.

[12] Santos, F. A., Lima, P. M., Neves, R. C. F., Moraes, P. M., Pérez, C. A., Silva, M. O. A., Arruda, M. A. Z., Castro, G. R., Padilha, P. M., Metallomic study on plasma samples from Nile tilapia using SR-XRF and GFAAS after separation by 2D PAGE: initial results, Microchimica Acta 173 (2011) 43-49. https://doi.org/10.1007/s00604-010-0522-y.

[13] Neves, R. C. F., Lima, P. M., Baldassini, W. A., Santos, F. A., Moraes, P. M., Castro, G. R., Padilha P. M., Fracionamento de cobre em proteínas do plasma, músculo e fígado de tilápia do Nilo. Química Nova 35 (3) (2012) 493-498. https://doi.org/10.1590/S0100-40422012000300010.

[14] Wang, Q., Bernhard Michalke (Ed.): Metallomics: analytical techniques and speciation methods, Analytical and Bioanalytical Chemistry 409 (2017) 5617–5618. https://doi.org/10.1007/s00216-017-0524-2.

[15] Magalhães, C. S., Arruda, M. A. Z., Sample preparation for metalloprotein analysis: A case study using horse chestnuts, Talanta 71 (5) (2007) 1958-1963. https://doi.org/10.1016/j.talanta.2006.08.039.

[16] Wang, Q., Xiong, Y. L., Zinc-binding behavior of hemp protein hydrolysates: Soluble versus insoluble zinc-peptide complexes, Journal of Functional Foods 49 (2018) 105-112. https://doi.org/10.1016/j.jff.2018.08.019.

[17] Silva, M. S., Sele, V., Sloth, J. J., Araujo, P., Amlund, H., Speciation of zinc in fish feed by size exclusion chromatography coupled to inductively coupled plasma mass spectrometry using fractional factorial design for method optimisation and mild extraction conditions, Journal of Chromatography B 1104 (2019) 262-268, https://doi.org/10.1016/j.jchromb.2018.11.010.

[18] Egito, A. S., Rosinha, G. M. S., Laguna, L. E., Miclo, L., Girardet, J. M., Gaillard, J. L., Método eletroforético rápido para detecção da adulteração do leite caprino com leite bovino, Arquivo Brasileiro de Medicina Veterinária e Zootecnia 58 (5) (2006) 932-939. https://doi.org/10.1590/S0102-09352006000500032.

[19] Gomez, B. G., Perez-Corona, M. T., Madrid, Y., Availability of zinc from infant formula by in vitro methods (solubility and dialyzability) and size-exclusion chromatography coupled to inductively coupled plasma-mass spectrometry, Journal of Dairy Science 99 (12) (2016) 9405-9414. https://doi.org/10.3168/jds.2016-11405.

[20] Liu, W., Lou, H., Ritzoulis, C., Chen, X., Shen, P., Lu, Y., Wu, K., Dong, L., Zhu, H., Han, J., Structural characterization of soybean milk particles during *in vitro* digestive/non-digestive simulation, LWT 108 (2019) 326-331. https://doi.org/10.1016/j.lwt.2019.03.086.

[21] Maqsood, S., Al-Dowaila, A., Mudgil, P., Kamal, H., Jobe, B., Hassan, H. M., Comparative characterization of protein and lipid fractions from camel and cow milk, their functionality, antioxidant and antihypertensive properties upon simulated gastro-intestinal digestion, Food Chemistry 279 (2019) 328-338. https://doi.org/10.1016/j.foodchem.2018.12.011.

[22] Ren, C., Tang, L., Zhang, M., Guo, S., Interactions between whey soybean protein (WSP) and beta-conglycinin (7S) during the formation of protein particles at elevated temperatures, Food Hydrocolloids 23 (3) (2009) 936-941. https://doi.org/10.1016/j.foodhyd.2008.06.007.

[23] Naqvi, M. A., Irani, K. A., Katanishooshtari, M., Rousseau D., Disorder in Milk Proteins: Formation,

Structure, Function, Isolation and Applications of Casein Phosphopeptides, Current Protein & Peptide Science 17 (4) (2016) 368-379. https://doi.org/10.2174/1389203717666151201191658.

[24] Raynal-Ljutovac, K., Lagriffoul, G., Paccard, P., Guillet, I., Chilliard, Y., Composition of goat and sheep milk products: An update, Small Ruminant Research 79 (1) (2008) 57-72. https://doi.org/10.1016/j.smallrumres.2008.07.009.

[25] Güler, Z., Levels of 24 minerals in local goat milk, its strained yoghurt and salted yoghurt (tuzlu yoğurt), Small Ruminant Research 71 (1-3) (2007) 130. https://doi.org/10.1016/j.smallrumres.2006.05.011.

[26] Pereira Junior, J. B., Fernandes, K. G., Müller, R. C. S., Nóbrega, J. A., Palheta D. C., Determinação direta de Ca, Mg, Mn e Zn em amostras de leite de búfala da ilha de marajó por espectrometria de absorção atômica com chama (FAAS) Química Nova 32 (9) (2009) 2333-2335. https://doi.org/10.1590/S0100-40422009000900018.

[27] Bossu, C. M., Carioni, V. M. O., Naozuka, J., Oliveira, P. V., Nomura, C. S., Direct determination of arsenobetaine and total As in robalo fish liver and tuna fish candidate reference material by slurry sampling graphite furnace atomic absorption spectrometry (SLS-GF AAS), Eclética Química Journal 44 (2) (2019) 37-44. https://doi.org/10.26850/1678-4618eqj.v44.2.2019.p37-44.

[28] Taverniers, I., Loose, M., Van Bockstaele, E., Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance, TrAC Trends in Analytical Chemistry 23 (8) (2004) 535-552. https://doi.org/10.1016/j.trac.2004.04.001.

[29] Chudzinka, M., Debska, A., Baralkiewicz, D., Method validation for determination of 13 elements in honey samples by ICP-MS, Accreditation and Quality Assurance 17 (2012) 65-73, https://doi.org/10.1007/s00769-011-0812-z.

[30] Góes, H. C. A., Torrese, A. G., Donangelo, C. M., Trugo, N. M. F., Nutrient composition of banked human milk in Brazil and influence of processing on zinc distribution in milk fractions, Nutrition 18 (7-8) (2002) 590-594. https://doi.org/10.1016/S0899-9007(02)00813-4.

[31] Gaucheron, F., The minerals of milk, Reproduction Nutrition Development 45 (4) (2005) 473-483. https://doi.org/10.1051/rnd:2005030.

[32] Miquel, E., Farré, R., Effects and future trends of casein phosphopeptides on zinc bioavailability, Trends in Food Science & Technology 18 (3) (2007) 139-143. https://doi.org/10.1016/j.tifs.2006.11.004.

[33] Pabón, M. L., Lönnerdal, B., Bioavailability of zinc and its binding to casein in milks and formulas, Journal of Trace Elements in Medicine and Biology 14 (3) (2000) 146-153. https://doi.org/10.1016/S0946-672X(00)80003-6.

[34] Jung, S., Murphy, P. A., Sala I., Isoflavone profiles of soymilk as affected by high-pressure treatments of soymilk and soybeans, Food Chemistry 111 (3) (2008) 592-598. https://doi.org/10.1016/j.foodchem.2008.04.025.

[35] Zaheer, K., Akhtar, M. H. An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. Journal Critical Reviews in Food Science and Nutrition 57 (6) (2017) 1280-1293. https://doi.org/10.1080/10408398.2014.989958.

[36] Kamizake, N. K. K., Gonçalves, M. M., Zaia, C. T. B. V., Zaia, D. A. M., Determination of total proteins in cow milk powder samples: a comparative study between the Kjeldahl method and spectrophotometric methods, Journal of Food Composition and Analysis 16 (4) (2003) 507-516. https://doi.org/10.1016/S0889-1575(03)00004-8.

Eclética Química Journal, vol. 46, n. 1, 2021, 12-20
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v46.1.2021.p12-20