



**PRODUCTION OF BIOACTIVE PEPTIDES FROM BOVINE COLOSTRUM WHEY USING ENZYMATIC HYDROLYSIS**

**PRODUCCIÓN DE PEPTIDOS BIOACTIVOS A PARTIR DE SUERO DE CALOSTRO BOVINO POR HIDRÓLISIS ENZIMÁTICA**

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**Abstract**

Bovine colostrum is an essential source of nutritional and immunological factors that are vital for the early development and protection of the newborn. The aim of this study was to evaluate the antioxidant, mineral-binding (iron and calcium), and angiotensin-converting enzyme (ACE) inhibitory properties of the peptides generated through the enzymatic hydrolysis of bovine colostrum whey proteins. Whey proteins were hydrolyzed with pepsin at two pH values (1.3 and 2) in a sequential hydrolysis system with pancreatin at pH 7.5. Three different molecular weight fractions (<10, 10 to 30 and >30 kDa) were obtained by ultrafiltration. The antioxidant and calcium-binding activities increased with the degree of hydrolysis of the proteins and the highest values were obtained in the fractions with less than 10 kDa. The fractions with molecular weights higher than 30 kDa had the best iron-binding capacity. A high positive correlation ( $R=0.9669$ ) was obtained between the degree of hydrolysis and the ACE inhibitory activity. These results indicate that the hydrolysis of bovine colostrum whey proteins with pepsin and pancreatin generated peptides with enhanced antioxidant, mineral-binding, and ACE inhibitory capacities.

**Keywords:** bioactive peptides, bovine colostrum whey, antioxidant capacity, mineral binding capacity, ACE inhibition.

**Resumen**

El calostro bovino es una importante fuente de factores inmunológicos y nutricionales que son vitales para el desarrollo y protección de la cría. El objetivo de este estudio fue evaluar las propiedades antioxidantes, de quelación de minerales (hierro y calcio) e inhibidora de la enzima convertidora de angiotensina (ECA) de los péptidos generados por hidrólisis de las proteínas de suero de calostro bovino. En el presente estudio se prepararon hidrolizados de proteína de suero de calostro bovino usando pepsina a dos valores de pH (1.3 y 2) y también en hidrólisis secuencial con pancreatina a pH 7.5. Los hidrolizados se separaron por ultrafiltración para obtener 3 fracciones de distintos pesos moleculares (<10, 10 a 30 y >30 kDa). Se observó un aumento en la actividad antioxidante y quelante de calcio de los hidrolizados, principalmente en las fracciones de peso molecular <10 kDa. En la actividad quelante de hierro las fracciones que presentaron mayor actividad fueron las de peso molecular >30kDa. Se observó una alta correlación positiva ( $R=0.9669$ ) entre el grado de hidrólisis y la actividad inhibidora de la ECA. Estos resultados indican que la hidrólisis de las proteínas de suero de calostro bovino con pepsina y pancreatina generan péptidos con capacidades antioxidante, quelante de minerales e inhibitoria de la ECA mejoradas.

**Palabras clave:** péptidos bioactivos, suero de calostro bovino, capacidad antioxidante, capacidad quelante de minerales, inhibición de la ECA.

**1 Introduction**

Bovine colostrum can be defined as the milk produced during the first 3 or 4 days after parturition. This secretion product has a unique nutritional and

immunological profile different from that of the mature milk (Playford *et al.*, 2000). Bovine colostrum is a yellowish liquid product which is thicker than milk and has a distinctive odor. It cannot be used in the dairy industry since it does not meet the quality and sanitary specifications and its pasteurization is difficult since it induces the denaturation of 14% of the IgG (Elizondo-

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Salazar *et al.*, 2008). It is then important to perform studies to be able to use it in the food industry (Chiang and Chang, 2005; Chen *et al.*, 2013).

Dairy proteins are among the most important raw materials to generate bioactive peptides for the elaboration of functional foods. These peptides have significant bioactivities such as antioxidant, antihypertensive, mineral binding, immunomodulatory, and antimicrobial capacities (Power *et al.*, 2013; Korhonen, 2009). Whey proteins have been used as a source of bioactive peptides and they are still a barely used byproduct of the cheese and Greek yogurt industries. The proteins in cheese and colostrum wheys are very similar although the concentration in the last product is higher. Lactoferrin is one of the most important proteins in these two products since it has free radical scavenging and iron-binding capacities making it very valuable for the food and pharmaceutical industries. Lactoferrin is an iron binding protein which has been reported to show antimicrobial, mineral binding, antioxidant, anticancer and immunomodulatory activities. Its concentration in bovine colostrum ranges from 1.5 to 5 mg.mL<sup>-1</sup>, being about 30-fold higher than that in milk (Chiang and Chang, 2005). The sequences for many bioactive peptides are already inside the structure of many whey proteins, and the action of proteolytic enzymes is enough to release them (Brandelli *et al.*, 2015; Tovar-Jiménez *et al.*, 2017).

As a consequence of normal metabolism, free radicals, which alter the concentration of enzymes such as superoxide dismutase, catalase, and peroxidase in the organism, are generated. These alterations may cause lethal and irreversible damage to the cell membrane and DNA in the tissues and organs. This oxidative stress has a definitive role in the development of different chronic degenerative diseases such as type-2 diabetes, cancer, atherosclerosis, and others (Gómez-Ruiz *et al.*, 2008). On the other hand, free radicals are also involved in lipid oxidation which causes rancidity in foods reducing notably their shelf life (Peng *et al.*, 2009). The antioxidant activity of some dairy peptides has been related to the presence of phosphoserine residues which can act as metal ion binders and free radical scavengers (Power *et al.*, 2013). These peptides are involved in the binding of calcium, iron, and copper (Zou *et al.*, 2016). Iron is an essential mineral for human beings since it is involved in different biochemical processes such as electron transfer, oxygen binding and transportation, and cell differentiation. Calcium is also essential for bone structure and many reactions including muscle

contraction, protein structure, and many enzyme reactions. Its deficiency results in diseases such as osteoporosis and some types of cancer (Huang *et al.*, 2015). It has been observed that stearic acid and some peptides formed during protein hydrolysis can enhance the bioavailability of several divalent cations (Torres-Fuentes *et al.*, 2012). It has been reported that the gastrointestinal digestion of fermented milks releases different kinds of peptides in hypertensive rats. These peptides are usually very small (such as VPP and IPP) and are inhibitors of the Angiotensin Converting Enzyme (ACE). They are effective against the ACE enzymes from the aorta and lungs. Some enzymes (prolyl endopeptidases) generate proline-containing peptides which are also ACE inhibitors (Aluko, 2015). Considering the above facts, the aim of this work was to evaluate the generation of antioxidant, metal binding, and antihypertensive activities from the enzymatic hydrolysis of the proteins of bovine colostrum whey.

## 2 Materials and methods

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### 2.1 Materials

Colostrum (a mixture of the first two milkings after parturition) was sampled from clinically healthy Holstein cows in a farm in Cuautitlán Izcalli, Mexico. It was centrifuged at 10,000Xg at 4°C for 20 min to remove the fat and its pH adjusted to 4.6 with acetic acid and centrifuged again to eliminate the caseins. The resulting product was labeled as colostrum whey (CW) and frozen and maintained at -20°C until used.

### 2.2 Colostrum analysis and processing

Protein concentration was determined by the Bradford (1976) method adapted for the Nanodrop 2000c spectrophotometer (Thermoscientific, Waltham, USA) following the procedure indicated by the equipment manual. The peptides were generated by enzymatic hydrolysis as follows.

a) H1. This peptide fraction was obtained through a simulation of the conditions of the gastrointestinal tract as described by Montoya-Rodríguez *et al.* (2014) with minor modifications. Colostrum whey was first hydrolyzed with pepsin (620 units/mg Sigma-Aldrich) using an enzyme: substrate ratio of 1:25 w/w at pH 2 (adjusted with 1N HCl) for 1 h. The resulting products were adjusted to pH 7.5 with 0.5 M ammonium

bicarbonate and treated with pancreatin (8X USP Sigma-Aldrich) using an enzyme:substrate ratio of 1:20 w/w for 3 h in Erlenmeyer flasks at 37°C and 250 rpm orbital agitation.

b) H2. Colostrum whey was first hydrolyzed with pepsin (620 units/mg Sigma-Aldrich) using an enzyme:substrate ratio of 1:25 w/w at pH 2 for 1 h at 37°C and 250 rpm orbital agitation.

c) H3. Colostrum whey was first hydrolyzed with pepsin (620 units/mg Sigma-Aldrich) using an enzyme:substrate ratio of 1:25 w/w at pH 1.3 for 1 h at 37°C and 250 rpm orbital agitation.

H1, H2 and H3 were then ultrafiltered through Amicon Ultra centrifugal filter units (Millipore) to obtain additional fractions with molecular weights of <10, 10 to 30, and >30 kDa following the indications of the supplier.

### 2.3 Degree of hydrolysis

The methodology described by Adler-Nissen (1979) was followed, using the reagent 2,4,6-Trinitrobenzenesulfonic acid (TNBS) in 5% w/v solution (Sigma-Aldrich). Briefly, 125  $\mu\text{L}$  of the colostrum whey sample or its hydrolysates were used with 1 mL of 0.2125 M phosphate buffer, pH 8.2 and 100  $\mu\text{L}$  of the 0.1% prepared reagent; the reaction mixture was stirred and incubated at 50 °C for 60 min. The reaction was stopped by adding 2 mL of 0.1 N HCl to each tube, these tubes were allowed to cool and were read at 340 nm using a GENESYS 10S Vis spectrophotometer (Thermo Scientific, USA). L-Leucine (0-2 mM) was used to make a standard curve and to calculate the concentration of free amino groups in the samples (Adler-Nissen, 1979).

The degree of hydrolysis was determined for the sample of colostrum whey (CW), the hydrolysate with pepsin-pancreatin (H1), pepsin at pH 2 (H2) and pepsin at pH 1.3 (H3). Tests were carried out to determine antioxidant activity (FRAP, ABTS, and TAC), iron and calcium-binding, as well as ACE inhibitory activity.

### 2.4 Determination of antioxidant activity

The antioxidant activity was determined according to the following methodologies:

#### 2.4.1 Ferric ion reducing antioxidant power (FRAP)

The FRAP technique was carried out according to the methodology proposed by Benzie and Strain (1996).

An aliquot of 20  $\mu\text{L}$  of the sample and 180  $\mu\text{L}$  of distilled water were mixed with 2mL of FRAP reagent, prepared at the time of use. The FRAP reagent is composed of TPTZ (ferric tripyridyltriazine) 10 mM  $\text{L}^{-1}$  in 40 mM of HCl, 20 mM  $\text{L}^{-1}$   $\text{FeCl}_3$ , and 0.3 mM acetate buffer (pH 3.6). The sample was incubated at 37 °C. The absorbance at 595 was read after 60 minutes. The results were expressed as trolox equivalent antioxidant capacity (TEAC) values by constructing a standard curve using TROLOX as an antioxidant (Fluka Chemie, Switzerland) (Martínez-Ruíz *et al.*, 2018).

#### 2.4.2 ABTS

The quantification of the decrease in absorbance of the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical ( $\text{ABTS}^{\bullet+}$ ) was determined following the methodology reported by Re *et al.* (1999) and López-Hernández *et al.* (2018) with the modifications made by Gómez *et al.* (2008). The ABTS radical solution (Sigma-Aldrich) was prepared by reacting 7 mM ABTS in water with 2.45 mM potassium persulfate in water. This mixture was allowed to stand in the dark at room temperature (25 °C) for 12-16 hours before use. The working solution was prepared by diluting the radical with 0.01 M phosphate buffer, pH 7.4 until obtaining an absorbance value of 0.7 ( $\pm 0.02$ ) at 734 nm. The synthetic reference antioxidant compound known as Trolox (final concentration 0-600  $\mu\text{M}$ ) was used, as well as samples of hydrolysate fractions in each reaction tube. In the evaluation, 10  $\mu\text{L}$  of sample and 990  $\mu\text{L}$  of the  $\text{ABTS}^{\bullet+}$  radical solution were used. After 15 min of reaction at room temperature and in the dark, the change in absorbance was read with respect to the reagent and solvent blank. This blank consists of a solution of the radical  $\text{ABTS}^{\bullet+}$  with the solvent of the sample. The results are expressed as TEAC values by the construction of a standard curve using TROLOX as an antioxidant (Servín de la Mora-López *et al.*, 2018).

#### 2.4.3 Total Antioxidant Activity (TAC)

This activity was determined using the Total Antioxidant Capacity Assay kit (MAK187 Sigma-Aldrich), which evaluates the antioxidant capacity of both proteins and small molecules, where the  $\text{Cu}^{2+}$  ion is reduced to the  $\text{Cu}^+$  ion that can be detected spectrophotometrically, with a maximum of absorbance at 570 nm, which is proportional to the total antioxidant capacity (Apak *et al.*, 2004).

This antioxidant capacity is given in TEAC, using a calibration line with Trolox (4-24 nmol/well). The assay was performed according to the instructions of the manufacturer without using the protein mask solution provided.

### 2.5 Determination of mineral-binding activity

For the iron-fixing activity, the methodology reported by Hwang *et al.* (2001) was followed. Briefly, 0.1 mL of the sample (diluted 1/10 with deionized water) was mixed with 0.6 mL of deionized water. Thereafter, 0.1 mL of 0.2 mM FeCl<sub>2</sub>·4 H<sub>2</sub>O (Sigma-Aldrich) was added, and incubated at room temperature, after 30 seconds, 0.2 mL of 1 mM ferrozine (Sigma-Aldrich) was added, the reaction mixture was stirred and incubated 10 minutes at room temperature. The absorbance was measured at 562 nm and the unbound iron concentration was calculated from a control without sample and compared with EDTA (Sodium salt, Mallinckrodt Baker, Xalostoc, Mexico) (Hwang *et al.*, 2001). For the determination of calcium bound by hydrolyzed samples, the technique reported by Jung *et al.* (2006) with some modifications was used. 2 mL of calcium chloride (5 mM, prepared in deionized water) was mixed with 1 mL of the diluted sample (1:10 in deionized water). The mixture was incubated at 25 ° C for 30 minutes under constant agitation. This was filtered through Whatman 1 filter paper and the content of the supernatants was determined by the calcium selective electrode of the Horiba brand (B-751 LAQUAtwin Compact Calcium Ion Meter) (Jung and Kim, 2007).

The bound calcium was determined with the following equation:

$$\text{Bound Ca} = C_0 - C_1 \quad (1)$$

Where  $C_0$  = total calcium concentration of the blank (calcium chloride solution plus deionized water) and  $C_1$  = unbound calcium concentration (with samples and after incubation).

### 2.6 ACE inhibitory activity

The methodology reported by Cushman and Cheung (1971) was followed. The pH of the sample was adjusted to 8.3, which is optimal for the reaction. An aliquot of 80  $\mu$ L of hydrolysate (50% diluted) was mixed with 200  $\mu$ L of 5 mM Hippuryl-Histidyl-Leucine substrate (Sigma-Aldrich) dissolved in 0.1 M borate buffer at pH 8.3 with 0.3 M NaCl, which is

prepared fresh before being used. The mixture was pre-incubated at 37 °C for 3 minutes and the reaction was started by adding 20  $\mu$ L of the ACE (0.2 U/mL) in 0.1 M phosphate buffer at pH 8.3 with 0.4 M NaCl, shaken for 30 seconds and then incubated at 37 °C for 30 minutes; the reaction was stopped by adding 250  $\mu$ L of 1N HCl. Ethyl acetate (1.7 mL) was added to the tubes to extract the hippuric acid by gently shaking them, then these tubes were centrifuged briefly to take 400  $\mu$ L of the organic phase and lastly, they were heated in a water bath at 92 °C for 30 minutes to evaporate the ethyl acetate. The content was resuspended in 1 ml of distilled water just before reading at 230 nm (Cushman and Cheung, 1971).

The inhibition percentage was calculated with the equation

$$IA(\%) = \frac{(B - A)100}{B - C} \quad (2)$$

Where IA is inhibition activity, A = absorbance of the sample with hydrolysates, B = absorbance of the sample without hydrolysates (100% activity) and C = absorbance of the reaction mixture without ACE (0% activity).

### 2.7 Statistical analysis

A one-way analysis of variance (ANOVA) was performed and the difference between groups was evaluated by means of the Tukey HSD (Honestly Significant Difference) test ( $\alpha = 0.05$ ) using the SPSS software (IBM SPSS Statistics, version 24, USA).

## 3 Results and discussion

### 3.1 Determination of the degree of hydrolysis

The highest degree of hydrolysis was obtained in the H1 (0.3867 meq Leucine/g), followed by H2 (0.3312 meq Leucine/g), H3 (0.2942 meq Leucine/g) and finally CW (0.1712 meq Leucine/g). The results were as expected considering that H1 involves a complete hydrolysis equivalent to that carried out in the gastrointestinal tract.

### 3.2 Determination of antioxidant activity

The sample with the highest antioxidant activity was H1 for FRAP (1469 TEAC mM) and for ABTS (4568 TEAC mM), and H3 for TAC (9.56 TEAC mM) as shown in Figure 1.

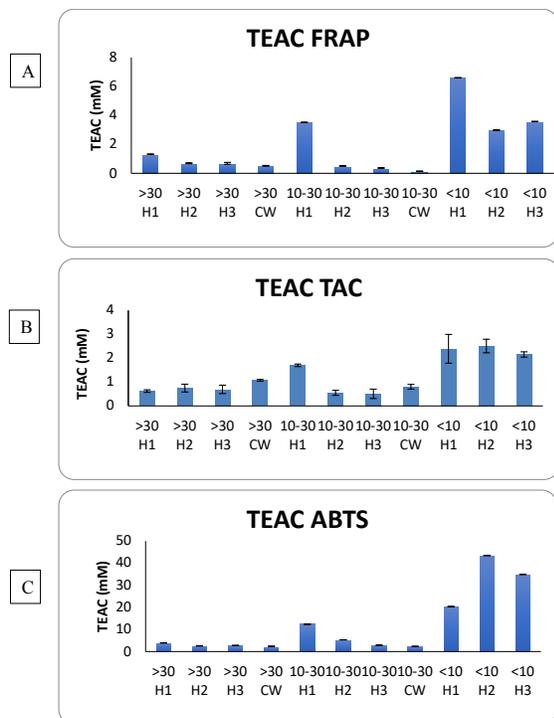


Fig. 1. Antioxidant capacity (Trolox equivalents or TEAC) of the different molecular weight fractions of the H1, H2 and H3 hydrolysates. A: FRAP antioxidant capacity in TEAC (mM). B: ABTS antioxidant capacity in TEAC (mM). C: Total antioxidant capacity (TAC) in TEAC (mM). All the analyses were performed in triplicate.

These results were higher than the antioxidant activity of whey protein hydrolyzed with Flavourzyme, corolase and alcalase carried out by Mann *et al.* (2015), who obtained the highest antioxidant activity for a beverage added with the hydrolysate, containing 2% alcalase or corolase (2.58 and 2.57 mM TEAC, respectively). In this study, the addition of 1% or 2% of the hydrolysates with the three enzymes to flavored milks was tested, finding a significant increase in the antioxidant activity of these beverages compared to the control (Mann *et al.*, 2015). In 2009, Peng *et al.* found an antioxidant activity close to 0.6 mM TEAC in hydrolyzed whey with alcalase at 30 minutes and close to 1.2 after 8 hours (Peng *et al.*, 2009). The antioxidant capacity of the hydrolysates was significantly higher to CW for FRAP and ABTS. In the determination of TAC, H3 was the one with the highest antioxidant capacity, followed by CW. In this test the reagent “protein mask” included in the kit was not used, so it was concluded that the protein fractions of higher

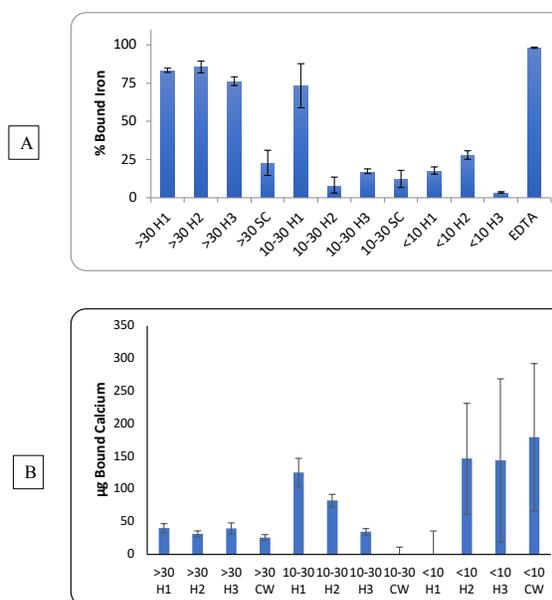


Fig. 2. Mineral binding capacity of the different molecular weight (kDa) fractions of the H1, H2 and H3 hydrolysates. A: Percentage of bound iron compared with EDTA (10 mg/mL). B: Amount of bound calcium ( $\mu\text{g}$ ). All the analyses were performed in triplicate.

molecular weight present in these two samples are responsible for these results.

The antioxidant capacity of the fractions (Figure 1) behaved similarly in the three tests and a greater antioxidant activity was obtained in the fractions of molecular weight of less than 10 kDa of the 3 hydrolysates by FRAP, ABTS and TAC, as shown in Figure 2. This may be due to the release of low molecular weight peptides by the digestive enzymes as shown in Figure 1. Gómez-Sampedro and Zapata-Montoya (2016) also found that the higher the degree of hydrolysis, the higher the antioxidant activity in the case of bovine plasma protein hydrolysates. Colbert and Decker (1991) observed that whey proteins with greater antioxidant activity were in a weight range between 500 and 5000 Da. However, other studies claim that proteins such as lactoferrin,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin have antioxidant activity. These proteins could be responsible for the activity observed by TAC assay in the CW sample (Colbert and Decker, 1991). It has been reported that antioxidant peptides with smaller molecular weights are easier to absorb and to show an antioxidant effect; some of the mechanisms described for this effect include oxygen inactivation, scavenging of free radicals and metal

ions chelation. This activity has also been related to hydrophobic and aromatic amino acids presence, mainly in the side chain or C-terminal and N-terminal. Some of the amino acids reported are Tyr and Trp, that can act as proton donors; others, as Asp or Glu, possess electron withdrawing effect; the presence of Leu in the N-terminal can enhance the interaction of peptides with fatty acids and may allow the capture of lipid free radicals; and some other amino acids by themselves can have an antioxidative activity (Cys, Met) and react directly with free radicals (Li and Yu, 2015).

### 3.3 Determination of mineral binding activity

The capacity of the hydrolysates and CW to bind iron and calcium was determined as well as their fractions of different molecular weights (Figure 2). In the binding capacity of iron for the hydrolyzed samples, it was observed that H1 and H2 showed a very good activity (84.14 and 83.27% respectively) while H3 and CW did not present this activity. The increase in the binding capacity of this mineral by hydrolysis of whey proteins had already been observed by Kim *et al.* (2007). They found a greater ability to chelate iron in whey protein samples hydrolyzed with different proteases; however, the fractions with the highest capacity were those with the highest molecular weight in their hydrolysates, which means that the peptides involved have a weight higher than 10 and even 30 kDa in the case of colostrum hydrolysates. This again is in agreement with the results reported by Kim *et al.* (2007), who found a positive correlation between the percentage of bound iron and the size of the peptides resulting from the hydrolysis of whey proteins. In the case of the hydrolysates, only fractions with a molecular weight greater than 30 kDa had similar activity to the non-fractionated hydrolysates. Lv *et al.* (2009) observed that peptide fractions of soy protein hydrolysate with a weight close to 14.4 kDa had a greater capacity to bind this mineral. The binding activity of iron was lower in the other fractions. These results contrast with those obtained by Torres-Fuentes *et al.* (2012), who obtained an iron-binding activity of up to 75% in the fractions of low MW (100 Da) for the case of chickpea protein hydrolysates.

In the case of the calcium-binding capacity, the lower molecular weight fractions of the hydrolysates showed the highest activity as shown in Figure 2. These results are similar to those obtained by Reyes-Méndez *et al.* (2015) in peptides obtained from soy and casein hydrolysis. Previous studies have

shown that whey proteins such as  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, and lactoferrin have calcium-binding sites (Huang *et al.*, 2015).

Salami *et al.* (2010) observed that some amino acids (Trp, Phe, Tyr, His and Cys) are able to donate protons to free radicals, and others (Glu, Asp, Lys, Arg and His) have the capacity to bind metals. Their presence and sequence determine the antioxidant capacity of the proteins, as well as the significant differences observed between the hydrolysates and the whey. Additionally, they provide a better accessibility due to the structural changes resulting from the enzymatic hydrolysis. Among these structural changes are a lower molecular weight, exposure of hydrophobic groups and increase in charged groups.

Zou *et al.* (2016) observed that some changes in the molecular structure of proteins, such as a greater number of random coils instead of  $\beta$ -sheets or  $\alpha$ -helices, provide greater activity; however, in globular proteins it is observed that they lose their antioxidant capacity when hydrolyzed (Zou *et al.*, 2016).

### 3.4 Inhibitory activity of ACE

The inhibitory activity of the ACE was compared in CW, H1, H3 and also with that of milk whey (MW). The highest activity was observed in H1 (57.11%) and, in both hydrolysates, the activity was higher than in CW which can be attributed to the release of bioactive peptides. No significant differences were observed between H1 and H3. A correlation and regression analyses (Figure 3) were made between the ACE inhibitory activity and the amount of free amino groups, obtaining correlation (R) and regression coefficients (R<sup>2</sup>) of 0.9669 and 0.9349, respectively. On the other hand, milk whey (MW) with a lower concentration of  $\mu\text{eq}$  of Leucine ( $15.91 \pm 1.9 \mu\text{eq}$  Leucine) showed the lowest ACE inhibitory activity (0.39%). This result was similar to that obtained by Silva *et al.* (2014) where no ACE inhibitory activity was found for intact whey protein concentrates, indicating that a proteolytic treatment, which gives rise to small-size molecules, is required for the generation of this bioactive property. Chobert *et al.* (2005) reported the ACE-inhibitory activity of tryptic peptides released from ovine  $\beta$ -lactoglobulin. Sipola *et al.* (2002) found that two tetrapeptides ( $\alpha$ -lactorphine and  $\beta$ -lactorphine), derived from whey proteins, lowered the blood pressure of hypertensive rats. This would explain that H1 is more effective at inhibiting ACE, since it has been demonstrated that

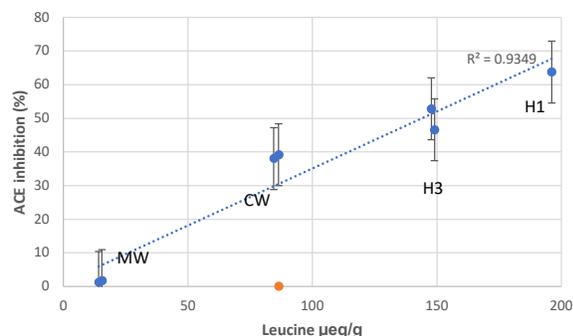


Fig. 3. Linear regression analysis between the inhibitory capacity of the ACE and the degree of hydrolysis measured by the concentration of leucine  $\mu\text{eq}$  in the samples H1, H3, colostrum whey (CW) and milk whey (MW) diluted 1:2 in phosphate buffer 0.1 M, pH 8.3. All the analyses were performed in triplicate.

peptides of milk origin with low molecular weight can have an important antihypertensive effect.

## Conclusions

It was observed that, in the case of the pepsin and pancreatin hydrolysates, a degree of hydrolysis that allows the generation of peptides and even free amino acids with high bioactivities was obtained. The antioxidant activity determined by FRAP, ABTS, and TAC of CW and its hydrolysates is higher than that reported for whey in other studies. In the determination by the commercial kit for TAC, a lower sensitivity was observed for the activity of the fractions with lower molecular weight. There was a significant increase in the antioxidant activity of the hydrolysates, being higher in H1 both in the determination by ABTS and by FRAP. Likewise, it was observed that the fractions with the lowest molecular weight were the ones with the highest activity.

It was observed that the hydrolysis using the pepsin-pancreatin system increases the capacity to bind minerals such as iron and calcium, in a higher degree than in the studies where other enzymes were used. The higher molecular weight fractions showed a greater capacity to bind iron whereas the activity to bind calcium was higher in the lower molecular weight fractions. A positive correlation was observed between the inhibitory capacity of the ACE and the degree of hydrolysis measured by the concentration of Leucine

$\mu\text{eqs}$  in the samples.

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