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Evaluation Of Antioxidant And Metal Chelating Activities Of Protein Hydrolysates Produced From Leather Waste By Alkaline And Enzymatic Hydrolysis.

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ABSTRACT

The objective of this study was to evaluate antioxidant and metal chelating activities of leather protein hydrolysates (LPHs). The hydrolysates were produced from chrome-containing leather waste (CCLW) by alkaline hydrolysis (CaO or KOH) and enzymatic hydrolysis (Protease or trypsin). Degree of hydrolysis (DH), total amino acid content, amino acid composition, DPPH radical scavenging and iron and copper chelating activities of each hydrolysate were determined. Results showed that the highest DH percentage was recorded with LPH by CaO treatment (46.86%) and followed by KOH treatment (31.81%) then protease treatment (1.04%) and trypsin treatment (0.57%). The LPH obtained by CaO treatment contained the highest concentration of free amino acids (246.65 mg/g waste) and the highest Fe^{+2} chelating activity (85.54% at concentration 0.20 mg/ml) compared with the other treatments. The LPH obtained by enzymatic hydrolysis possessed the higher DPPH scavenging and Cu^{2+} chelating activities than LPH obtained by alkaline hydrolysis. It was concluded that the alkaline hydrolysis is a suitable and economically beneficial method to produce LPH. The LPH is an economic and natural source of amino acids and good antioxidant and metal chelating agents in plant and animal nutrition.

Keywords: leather waste, protein hydrolysates, amino acids, antioxidant, metal chelating.

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INTRODUCTION

Environmental pollution is a difficult problem for world leather industry [1, 2]. In past decades, a lot of effort has been made to study the solid collagenous wastes, including isolation of protein products from chromium-containing leather waste (CCLW). Unfortunately, most of these processes reported bring about new residues during treatment [3]. Improper disposal of these leather wastes causes environmental pollution; therefore, proper optimized utilization of these wastes into valuable end products will be a promising solution [4-6].

Leather industry provides the necessities, such as leather shoes and garments, while using the by-products of the meat industry. However, the leather-making process, in turn, generates by-products and wastes [3]. In the leather industry, it is accepted that only 30–35% of wet salted hides are converted into leather, 25% result in chromium containing leather waste (CCLW), and the remaining is non-tanned waste or contaminated wastewater [7, 8].

Solid wastes of chromium-tanned leather generated in the leather industry derive mainly from the treatment of chromium tanned leathers by mechanical processes such as shaving, buffing, splitting and trimming [9-11]. These solid leather wastes are composed of a large amount of structural fibrous proteins called collagen and contain 3-6% chromium III. These wastes require special attention because chromium III can oxidize to toxic chromium VI which may endanger ecological life and human health [12]. Solid leather wastes have become an important problem for the leather industry in the last few years because of the increase in dumping charges, the difficulty of finding new landfills, and environmental concerns [13-18].

Protein hydrolysate is a value added product that is derived from animal and plant sources via hydrolysis of protein utilizing acid, alkaline, enzymatic, and fermentation treatments. A variety of enzymes such as alcalase, bromelain, flavourzyme, nutrase, pepsin, trypsin and papain have been applied to prepare protein hydrolysates with enhanced functional and antioxidant properties [19, 20]. In general, the derived antioxidant protein hydrolysates are widely used by different food, beverage and pharmaceutical industries. These protein hydrolysates are finding their high value as nutritional supplements, functional ingredients, and flavor enhancers in different food preparations [21].

Between 1970 and 1993, a lot of publications and patents concentrated on hydrolyzing CCLW to recycle amino acids and peptides for use in feeds and fertilizers [22-26]. [27] did systematical laboratory [28-31] and pilot scale [32-34] studies on the treatment of CCLW in the past 10 years. The initial one-step process developed by them involved the use of alkaline proteolytic enzymes to isolate a chrome-free, hydrolysate product that can be used as feed or fertilizer [3, 13, 25].

The present study was focused on production of leather protein hydrolysates from CCLW by alkaline and enzymatic hydrolysis and evaluated their antioxidant and metal chelating potential.

EXPERIMENTAL

Materials

Chrome-Containing Leather Waste (CCLW)

Chrome-containing leather waste (CCLW) was obtained from a commercial leather tannery. Leather was kept at 5°C in refrigerator for further analysis.

Chemicals

Amino acid standards were purchased from Sigma chemical co., Switzerland. Picryl sulfonic acid solution 5% (w/v) in H_2O and 3-(2-pyridyl)-5,6-diphenyl-1, 2, 4-triazine-p,p'-disulfonic acid monosodium salt hydrate – 97% reagent were obtained from Sigma chemical co., USA. Pyrocatechol violet was obtained from Aldrich chemical co. Ltd. Alkaline protease was obtained from Cisme, Italy. Trypsin was obtained from Loba Chemie, India. Ninhydrin was obtained from Research-Lab Fine Chemical Industries, India. All other chemicals were of analytical reagent grade.

Methods

Proximate chemical analysis of leather waste

Moisture content was determined according to [35]. Ash content was determined according to [36]. Protein content was determined according to [37]. Crude fat content was determined according to [38].

Production of leather protein hydrolysates (LPHs)

Alkaline hydrolysis

Alkaline hydrolysis of CCLW was carried out according to the method described by [3] as follows: Fifty grams of CCLW were shaken in 500 ml of distilled water and 5 g CaO or KOH into digestion flask. The hydrolysis was done at 98°C for 24 h, then the hydrolysates were filtered through whatman No.1 filter paper at room temperature. Each filtrate was stored at 4°C for analysis.

Enzymatic hydrolysis

Enzymatic hydrolysis of CCLW by alkaline protease or trypsin was carried out according to the method described by [3]. An accurate weight of CCLW (50 g) was shaken in 500 ml of distilled water and 1.5 g MgO at room temperature for 4 h. This pretreatment step is necessary to obtain the optimal pH for the enzymatic digestion. 1g of alkaline protease or trypsin was added and the mixture was incubated at 38°C for 24 h. The hydrolysates were filtered through whatman No.1 filter paper at room temperature. Each filtrate was stored at 4°C for analysis.

Determination of the degree of hydrolysis

The degree of hydrolysis (DH) of LPH was carried out using the TNBS method as described by [39].

Determination of total free amino acids

Total free amino acids were determined using the ninhydrin method according of [40].

Separation of amino acids by TLC

Amino acids were carried out according to the method described [41]. Analysis of amino acids by TLC was performed on silica gel covered aluminum plates using solvent system: n-butanol/acetic acid/water (80:20:20, v/v). Leather protein hydrolysates and standard solution (1 mg/ml containing 10% n-propanol by volume) of glycine, arginine, aspartic acid, glutamic acid and proline were spotted on TLC plate. The run was carried out in glass jar, covered with glass covered at room temperature. The chromatogram was dried then developed by spraying with ninhydrin reagent (0.5% w/v in acetone). After spraying, the chromatogram was dried immediately by air drier. The R_f values of all spots were measured.

Determination of amino acid composition

Amino acid composition of four leather protein hydrolysates obtained by different treatments was analyzed by automatic amino acid analyzer (AAA 400 INGOS Ltd., Czech Republic).

Assay of DPPH radical scavenging activity

Antioxidant activity of waste hydrolysates was determined according to the method of [42] as described by [43]. One milliliter of each hydrolysate was added to 1 ml of a DPPH solution (0.2 m*M* in ethanol). After a 30 min of reaction at room temperature in the dark place, the absorbance of the solution was measured at 517 nm. Control was prepared by the same procedure without hydrolysate. Ascorbic acid (0.03%, w/v) was used as a positive control. Radical scavenging activity (%) was calculated from the following equation:

$$\text{Scavenging activity (\%)} = [(\text{A control} - \text{A sample}) / \text{A control}] \times 100$$

Assay of Fe^{+2} chelating activity

Fe^{+2} chelating activity was determined according to the method of [44] as described by [45]. In clean test tubes, 0.5 ml of each hydrolysate was mixed with 0.5 ml of $FeSO_4$ (0.12 m*M*) and 0.5 ml of ferrozine (0.6 m*M*). The mixtures were allowed to stand for 10 min at room temperature. The absorbance was determined at 562 nm. Control was prepared by the same procedure without hydrolysate. Na_2-EDTA (0.01*M*) was used as positive control. The ability of the hydrolysate to chelate ferrous ion was calculated using the formula:
Chelating activity (%) = [(A control − A sample) / A control] ×100

Assay of Cu^{+2} chelating activity

Copper chelating activity was determined according to the procedure described by [46], with some modifications of [47]. One milliliter of each hydrolysate was mixed with 1 ml of the copper sulfate solution (0.01% in 50 m*M* sodium acetate buffer, pH 6.0). 250 µl of pyrocatechol violet (0.3 m*M* in acetate buffer) were added then mixed well and the absorbance was measured at 626 nm. Control was prepared by the same procedure without hydrolysate. Na_2-EDTA (0.01*M*) was used as positive control. Chelating activity was calculated using the following formula:

Chelating activity (%) = [(A control − A sample) / A control] ×100

Statistical analysis

The results were analyzed by an analysis of variance (P<0.05) and the means separated by Duncan's multiple range test. The results were processed by CoStat computer program (1986).

RESULTS AND DISCUSSION

Proximate analysis of CCLW

The proximate chemical analysis of chrome-containing leather waste (CCLW) is shown in Table 1. The obtained results revealed that the CCLW composed of 40.16% moisture, 10.76% ash, 73.75% protein, 5.79% crude fat and 9.7% other components base on dry basis. The results revealed that the protein was the major component in CCLW; therefore, the objective of research depends on protein hydrolysate. The results obtained are in agreement with those of [48] who found that the chromium containing leather waste contains 74.3% protein, 10.4% ash, 1.3% ether extract and 14% non-fibrous carbohydrates (based on dry weight). Whilst [3, 49] mentioned that the average percent composition of chrome leather waste: 5-10% moisture, 16.5-18.4% total nitrogen (about 80% protein), 11.5-13% ash and 2% fat.

Table 1. Proximate chemical composition (g/100 g dry weight) of chrome-containing leather waste (CCLW)

Parameter	CCLW
Moisture	40.16
Ash	10.76
Crude protein	73.75
Crude fat	5.79
Other components	9.70

-Values are means of three replicates

Degree of hydrolysis

The degree of hydrolysis (DH) measures the progress of hydrolysis of protein. DH has been defined as the percent ratio of the number of peptide bonds cleaved to the total number of peptide bonds in the substrate studied [50]. It is the proportion of cleaved peptide bonds in a protein hydrolysate [51].The DH percentages of leather protein hydrolysates (LPH) obtained by alkaline hydrolysis (CaO or KOH) and enzymatic hydrolysis (Protease or trypsin) are shown in Table 2. The obtained results revealed that the DH percentage was higher in LPH obtained by alkaline hydrolysis than LPH obtained by enzymatic hydrolysis. The highest DH

percentage was recorded with LPH by CaO treatment (46.86%) and followed by KOH treatment (31.81%) then protease treatment (1.04%) and trypsin treatment (0.57%). These results are in agreement with those of [3] who found that the basifiers, i.e., CaO, MgO and NaOH, are more efficient and easier to filter than the enzymes when hydrolyzing the CCLW. The CaO as a source of alkalinity had advantages over MgO and NaOH.

Table 2. The degree of hydrolysis (%) of leather protein hydrolysates (LPH) obtained by alkaline hydrolysis (CaO or KOH) and enzymatic hydrolysis (protease or trypsin)

Treatment		Degree of hydrolysis (%)
Alkaline hydrolysis	LPH/CaO	46.86^{a}±1.18
	LPH/KOH	31.81^{b}±0.44
Enzymatic hydrolysis	LPH/protease	1.04^{c}±0.04
	LPH /trypsin	0.57^{c}±0.09
	L.S.D	2.046

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05.

Total free amino acids content

Free amino acids content was colorimetrically determined in leather protein hydrolysates obtained by alkaline and enzymatic hydrolysis. As demonstrated previously, the obtained results (Table 3) showed that LPH obtained by CaO treatment contained the highest concentration of free amino acids (246.65 mg/g waste) whilst LPH obtained by trypsin contained the lowest concentration of free amino acids (2.04 mg/g waste).

Table 3. Total free amino acids content of leather protein hydrolysates (LPH) obtained by alkaline hydrolysis (CaO or KOH) and enzymatic hydrolysis (protease or trypsin)

Treatment		Total free amino acids (mg/g waste)
Alkaline hydrolysis	LPH/CaO	246.65^{a}±5.95
	LPH/KOH	36.44^{b}±0.61
Enzymatic hydrolysis	LPH /protease	3.10^{c}±0.15
	LPH /trypsin	2.04^{c}±0.04
	L.S.D	9.753

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05.

Identification of amino acids by thin layer chromatography

Since each LPH contained considerable amounts of amino acids, it is important to identify the types of amino acids in each LPH by a simplest method (i.e. thin layer chromatography). The leather protein hydrolysates and amino acid standards [arginine (Arg), proline (Pro), glycine (Gly), aspartic acid (Asp) and glutamic acid (Glu)] were subjected to TLC separation. After separation and visualization processes, spots were marked with a pencil. The results obtained of R_f value of each spot are given in Table (4). TLC separation of amino acid standards (Arg, Pro, Gly, Asp and Glu) revealed only one spot with R_f values of 0.185, 0.296 0.309, 0.315 and 0.358, respectively. From the results in Table (4), it could be revealed that both leather protein hydrolysates obtained by alkaline hydrolysis (CaO or KOH) contained the same compounds (amino acids); hence the R_f values of separated spots were much closed. Moreover, both alkaline hydrolysates contained Pro, Gly, Asp and Glu, hence the R_f values of spots of each hydrolysate were much closed to the R_f values of pure standard amino acids except proline was detected only in CaO treatment. According to [41], bands (I, VI and VII) in LPH obtained by CaO and bands (I, V and VI) in LPH obtained by KOH treatment may be histidine, tyrosine and tryptophan. On the other hand, TLC revealed that both leather protein hydrolysates obtained by enzymatic hydrolysis (protease or trypsin) did not detect any spot of amino acids.

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Table 4. R_f values of amino acids of leather protein hydrolysates (LPH) obtained by alkaline hydrolysis (CaO or KOH) and enzymatic hydrolysis (protease or trypsin) separated by thin layer chromatography.

Treatment		Spot No.	R_f	Identification
Alkaline hydrolysis	LPH/CaO	I	0.135	Unknown
		II	0.265	Proline
		III	0.302	Glycine
		IV	0.320	Aspartic acid
		V	0.382	Glutamic acid
		VI	0.506	Unknown
		VII	0.604	Unknown
	LPH/KOH	I	0.135	Unknown
		II	0.302	Glycine
		III	0.320	Aspartic acid
		IV	0.382	Glutamic acid
		V	0.506	Unknown
		VI	0.604	Unknown
Enzymatic hydrolysis	LPH/protease	-	-	N.D.
	LPH/trypsin	-	-	N.D.

N.D.: non detectable

Amino acid composition

The leather protein hydrolysates obtained by alkaline hydrolysis (CaO or KOH) and enzymatic hydrolysis (protease or trypsin) were analyzed for amino acid composition and the results are presented in Table (5). In general, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine were all found in leather protein hydrolysates obtained by alkaline hydrolysis (CaO or KOH). Whilst the amino acid composition of leather protein hydrolysates obtained by enzymatic hydrolysis was characterized by phenylalanine, lysine and arginine. In addition, aspartic acid and methionine were found only in LPH obtained by CaO, whilst proline was found only in LPH obtained by KOH. Also, leucine and tyrosine were found in LPH obtained by protease treatment but did not found in LPH obtained by trypsin treatment. The obtained results are similar to great extent with previous results of TLC. Certain essential amino acids enumerated in table 5, such as lysine, valine, phenylalanine, leucine, isoleucine and histidine occur in the composition of hydrolysates especially obtained by alkaline hydrolysis. This is an important point in selection leather protein hydrolysates charges for preparation active formulations to be used as plant growth biosimulators and animal feed additive. Many investigations supported the amino acid composition of LPHs obtained by alkaline or enzymatic hydrolysis [3, 52, 53].

Table 5. Amino acid composition (%) of leather protein hydrolysates (LPH) obtained by alkaline hydrolysis (CaO or KOH) and enzymatic hydrolysis (protease or trypsin)

Amino acid	Alkaline hydrolysis		Enzymatic hydrolysis	
	LPH/CaO	LPH/KOH	LPH/protease	LPH/trypsin
Aspartic acid (Asp)	0.56	-	-	-
Proline (Pro)	-	1.22	-	-
Glycine (Gly)	6.45	53.82	-	-
Alanine (Ala)	13.63	2.55	-	-
Valine (Val)	14.95	9.52	-	-
Methionine (Met)	7.43	-	-	-
Isoleucine (Ile)	4.46	1.55	-	-
Leucine (Leu)	8.27	6.72	2.05	-
Tyrosine (Tyr)	2.57	4.19	6.63	-
Phenylalanine (Phe)	9.66	12.83	55.49	5.48
Hisitidine (His)	0.76	1.40	-	-
Lysine (Lys)	10.90	4.89	26.89	89.96
Arginine (Arg)	0.10	0.11	1.47	4.56

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DPPH radical-scavenging activity

The DPPH radicals have been widely used to investigate the scavenging activity of some natural compounds. DPPH is a stable free radical that shows maximum absorbance at 517 nm. When DPPH radical encounters a proton-donating substrate such as an antioxidant, the radicals are scavenged and the absorbance is reduced. Thus, the decrease in absorbance is taken as a measure for DPPH-scavenging activity. In the present data (Table 6), all leather protein hydrolysates under investigation showed DPPH radical scavenging activity. The results revealed that the scavenging activity (%) of each hydrolysate is increasing with increasing the concentration. The DPPH scavenging activity (%) of leather protein hydrolysates obtained by various treatments at higher concentration (50 mg/ml) and ascorbic acid as standard (0.3 mg/ml) were in the following decreasing order: LPH/protease (94.87%) > ascorbic acid (93.22%) > LPH/KOH (79.36%) > LPH/trypsin (73.91%) > LPH/CaO (51.91%).

Finally, the antioxidant activity of leather protein hydrolysates under investigation is attributed to their contents of amino acids or peptides [53]. The highest antioxidant activity of LPH obtained by protease in comparison with other treatments was associated with its content of bioactive peptides [54]. Generally, the ability of a peptide to stabilize free radicals is due to its ability to donate electrons to the free radical or absorb the free radicals electron in order to reduce its reactivity. The negatively charged amino acids, such as aspartic and glutamic acids, are reported to exhibit strong antioxidant properties as they have the ability to donate their excess electrons during free radical reactions [55, 56]. Another factor which has been observed to contribute to the potency of antioxidant peptides is the presence of tyrosine, methionine, histidine and lysine. Lysine and tyrosine are reported to act as hydrogen donors while histidine has been shown to possess strong radical scavenging activity as a result of the chelating, lipid trapping and decomposition of the imidazole ring and the ability of the cysteine to donate its hydrogen from the sulfhydril side chain (57-60, 55].

Table 6. DPPH radical scavenging activity (%) leather protein hydrolysates (LPH) obtained by alkaline hydrolysis (CaO or KOH) and enzymatic hydrolysis (protease or trypsin)

Treatment		Concentration (mg/ml)	Scavenging activity (%)
Alkaline hydrolysis	LPH/CaO	20	24.77^{h}±0.81
		35	43.28^{fg}±4.46
		50	51.91^{ef}±6.51
	LPH/KOH	20	32.44^{gh}±4.27
		35	64.67^{cd}±4.37
		50	79.36^{b}±0.53
Enzymatic hydrolysis	LPH/protease	20	48.40^{ef}±4.11
		35	74.02^{bc}±2.02
		50	94.87^{a}±0.36
	LPH/trypsin	20	57.67^{de}±4.31
		35	65.23^{cd}±5.16
		50	73.91^{bc}±3.54
Standard	Ascorbic acid	0.3	93.22^{a}±0.42
	L.S.D		10.889

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at $P<0.05$.

Fe^{+2} chelating activity

The results of the Fe^{++} chelating activity of leather protein hydrolysates obtained by alkaline and enzymatic hydrolysis are shown in Table 7. In this study, three concentrations from each hydrolysate were used (0.10, 0.15 and 0.20 mg/ml) and EDTA was used as positive control. The obtained results revealed that LPH obtained by CaO possessed the highest percentage of Fe^{2+} chelating activity (85.54% at concentration 0.20 mg/ml) compared with the other treatments. Whilst the leather protein hydrolysate obtained by trypsin possessed the lowest percentage of Fe^{2+} chelating activity (3.26% at concentration 0.20 mg/ml). The chelating activity was increasing with increasing the concentration of each hydrolysate.

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Table 7. Ferrous chelating activity (%) of leather protein hydrolysates (LPH) obtained by alkaline hydrolysis (CaO or KOH) and enzymatic hydrolysis (protease or trypsin)

Treatment		Concentration (mg/ml)	Fe^{2+} chelating activity (%)
Alkaline hydrolysis	LPH/CaO	0.10	9.41^{cd}±0.99
		0.15	35.99^{b}±1.27
		0.20	85.54^{a}±6.80
	LPH/KOH	0.10	7.42^{cde}±0.11
		0.15	9.64^{cd}±0.23
		0.20	10.92^{c}±0.06
Enzymatic hydrolysis	LPH/protease	0.10	2.31^{de}±0.2
		0.15	2.98^{cde}±0.75
		0.20	5.09^{cde}±0.04
	LPH/trypsin	0.10	1.05^{e}±0.07
		0.15	1.63^{de}±0.38
		0.20	3.26^{cde}±0.26
Standard	EDTA	3.36	33.83^{b}±5.12
	L.S.D		7.028

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at $P<0.05$.

Cu^{+2} chelating activity

Data in Table (8) show the Cu^{2+} chelating activity of leather protein hydrolysates produced from different treatments (CaO, KOH, protease and trypsin). The results showed that all treatments possessed Cu^{2+} chelating activity in the presence of EDTA as positive control. The leather protein hydrolysates obtained by enzymatic hydrolysis (protease and trypsin) exhibited higher chelating activity than leather protein hydrolysates obtained by alkaline hydrolysis (CaO and KOH). At the highest concentration (35.56 mg/ml), The LPH obtained by trypsin recorded the highest chelating activity (70.86%) followed by LPH obtained by protease (69.88%) then LPH obtained by KOH (64.75%) and CaO (62.24%) compared with EDTA (96.62%, at concentration 3.36 mg/ml). Finally, it was noticed that the higher chelating activity of LPH obtained by alkaline hydrolysis for Fe^{2+} than Cu^{2+} and the reverse with LPH obtained by enzymatic hydrolysis. These results may be due to variation in their contents of amino acid and peptides or concentration of each metal which used or requirement of coordination sites to bind with metal. These possibilities were supported by many authors [46, 61, 62]. [63] reported that the thiol and carboxylic groups are considered high-affinity metal binding sites, through electrostatic and ionic interactions with copper. [64] stressed the importance of histidine in the chelation of Cu^{2+} and [65] related the decrease of Cu^{2+} binding capacity with the content of negatively charged amino acids, like aspartate and glutamic acid.

Table 8. Copper chelating activity (%) of leather protein hydrolysates (LPH) obtained by alkaline hydrolysis (CaO or KOH) and enzymatic hydrolysis (protease or trypsin)

Treatment		Concentration (mg/ml)	Cu^{2+} chelating activity (%)
Alkaline hydrolysis	LPH/CaO	17.78	62.28^{f}±0.42
		26.67	62.41^{f}±0.83
		35.56	62.24^{f}±0.25
	LPH/KOH	17.78	59.62^{g}±0.75
		26.67	61.78^{f}±0.17
		35.56	64.75^{e}±1.17
Enzymatic hydrolysis	LPH/protease	17.78	75.58^{b}±0.22
		26.67	74.72^{b}±0.61
		35.56	69.88^{cd}±0.87
	LPH/trypsin	17.78	55.19^{h}±0.44
		26.67	68.44^{d}±0.11
		35.56	70.86^{c}±0.72
Standard	EDTA	3.36	96.62^{a}±0.19
		L.S.D	1.776

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at $P<0.05$.

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CONCLUSION

Finally, it could be concluded that the alkaline hydrolysis is a suitable and economically beneficial method to produce leather protein hydrolysate. The leather protein hydrolysate is an economic and natural source of amino acids and good antioxidant and metal chelating agents in plant and animal nutrition.

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