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Utilization of the Weed Cichorium Pumilum, Jacq as Vegetable Protease Enzymes for Whey Protein Hydrolysate Production

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Abstract: *This study was conduction to find a suitable and cheap plant as a source for extracting vegetable protease enzymes and investigate the effect of these enzymes for producing whey protein hydrolysate. The extract prepared from the composite weed cichorium pumilum had a satisfactory protease activity, each ml of this extract prepared from 0.1 gm dried cichorium weed stems.An alkaline and acidic cichorium protease enzymes were purified by precipitated with ammonium sulfate, followed by dialysis and gel filtration.Enzymes activity greatly increased after purification with gel filtration.*

Heated whey protein solution (100ml solution of 10 % protein) was incubated with each of 1, 2,3, 4,and 5 ml extract (CCPE treatments)or each of 0.1, 0. 2, 0. 3, 0. 4 and 0. 5 ml cichorium alkaline protease enzyme (PKCPE treatments) or 0. 1, 0. 2, 0. 3, 0. 4 and 0. 5 ml cichorium acidic protease enzyme (PACPE treatments) for 30, 60, 90, 120, 150, 180, 210 and 240 min at the optimum temperature and pH of each enzyme type.

Cichorium crude protease extract (CCPE) at volume of 3ml and incubation period 240 min. had ability to produce good hydrolysate without bitter taste,.the corresponding volumes of PKCPE& PACPE were 0. 3ml and 0. 4 with same incubation time, respectively.

1. INTRODUCTION

One of whey proteins most beneficial uses is as important supplement used in bodybuilding or weight training.

Whey protein contains four main protein fractions named : beta lacto globulin (approx 65%), alpha lactalbumin (approx 25%), serum albumin (approx 8 %) and immunoglobulin, (Walzem et. al., 2002)

The active component in the whey protein play an important role in bone formation and a potential therapeutic role in osteoporosis by activating osteoblasts (American Dairy Science Association 2009).

Enzymatic hydrolysis of whey protein can yield a variety of new peptides that may offer many physiological benefits for humans (otte et al, 1997, Madureira et.al. (2010).

Whey protein hydrolysates are being extensively prepared and used to nutritionally support human patients with various physiological insufficiencies and anomalies (Halken and Host, 1997)

Several peptides with considerable biological role have been identified in the enzymatic hydrolysated of whey protein for example, opiod peptide (Meisel and Fetzgerald, (2000) angiotensin I converting enzyme inhibitory peptide (Gobbetti et al, 2004), antithrombotic peptide (Chabance et al, 1995), immunomidulatory peptide (Mecier et al, 2004), anti carcinogenic peptide (Marshall, 2004), mineral carrier peptide (Kim and Lim, 2004), lactoferrin a peptide which have iron binding capacity Wakabayashi et al, 2003) such peptides can help in the prevention of anemia.Whey protein hydroysate had some of amino acid peptide binds broken enzymatically into shorter chains of amino acids.This leads to better absorption in the intestine.

Enzymatic hydrolysis of whey offers a practical way to reduce its antigenic protein fractions (Heyman 1999, Kim et al 2007 and Hai et al 2008), however BLG can induce milk allergy in human infants because of the underdeveloped infantile gastrointestinal tract (Zeiger et al, 1986, Kim et al 2007).

Enzymes used for hydrolysis of whey protein costs the manufacturer extra money and also these enzymes have high activity that may cause more hydrolysis of whey protein which will taste more bitter whey protein hydrolysate.

This research is carried out as contribution to reduce the cost of whey protein hydrolysate production through use protease enzyme of plant source instead of microbial one for whey protein hydrolysate production.

The composite weed cichorium pumilium is selected for this purpose, this weed grows naturally associated with clover fields during fall, winter and spring seasons. It is preferable and eaten by the Egyptian farmers with cheese as a common Egyptian meal.

2. MATERIALS AND METHODS

2.1. Preparation of Crude Cichorium Protease Extract(CCPE)

Plant stems were cut into small pieces, then the plant crude protease extract was prepared as described by Silva et al (2006)via maceration 10 gm of stem pieces in 100 ml water using an electric mixer. The extract filtered and centrifuged at 30000 rpm for 30 min. The supernatant was referred as crude cichorium protease extract (CCPE).

CCPE solution was divided into three portions.one was kept to use as crude cichorium protease extract One was used for preparing crude cichorium alkaline protease enzyme and the other was used for preparing the crude acidic one as described by Kalpana et al (2008), Vadde and Ramakrishna (2005).,respectively.

2.2. Alkaline Protease Enzyme Purification

2.2.1. Ammomonium Sulfate Precipitation

From the preliminary studies alkaline proteases were collected from their crude supernatant by precipitation with 40-60 % saturated ammonium sulfate. After agitation for 30 min at room temperature at 25 ± 2 °c, the resulting precipitates was allowed to settle for 24 h at 4 °c. The supernatant were discarded and the precipitates dissolved in 0.05 M Tris HCl buffer, pH 7.8.

2.2.2. Dialysis

The resulted fraction was dialyzed against 0.05 M Tris HCl buffer of pH 7.8 at 4 °c for 24 h. The buffer was changed twice and the dialyzed enzyme was kept in brown bottles (partially purified alkaline protease) and stored at -20 °c for further purification.

2.2.3. Gel filtration

The dialyzed enzyme was fractionated on sephadex G-100 column of 1X50 cm. The column was equilibrated and eluted with 0.05 M Tris HCl buffer pH 7.8. Elution was carried out a flow rate of 20 ml / h and 3 ml fractions were collected. The major active fractions were collected and stored at -20 °c for further analysis.

2.3. Acidic Protease Enzyme Purification

2.3.1. Ammonium Sulfate Precipitation:

For preliminary assay 50 ml of enzyme extract were used to determine the appropriate ammonium sulfate concentration for partial purification.

The ammonium sulfate at saturation of (40-60%) which gave the highest purification fold and specific activity was used to purify the remaining initial extract through two steps : First, the solution was brought to 40 % concentration then centrifuged at 10,000 rpm for 10 min and the precipitate discarded. Secondly, the enzyme solution was brought to 60 % saturation and the enzyme precipitate retained and the remainder discarded. The resulting extract was dialyzed and defined as partially purified acidic protease and dissolved in 0.02 M acetate buffer at pH 5.0. It was kept in brown glass bottles and stored at 20 °c for further purification.

From the preliminary study, acidic protease was collected from the crude acidic supernatant by precipitation with 40-60 % saturation of ammonium sulfate. It was mixed for 30 min, then allowed to settle for 24h at 4 °c. The supernatant was discarded and the precipitate was dissolved in 0.02 M acetate buffer of pH 5.

2.3.2. Dialysis

The enzyme produced from the previous steps was dialyzed against the same buffer (0.02 M acetate of pH 5.0) for 24h at 4 °c and the buffer was changed twice.

2.3.3. Gel filtration

The dialyzed enzyme was fractionated on a sephadex G- 100 column of 1 X 50 cm. The column was equilibrated and eluted with 0.02 M acetate of pH 5.0.

Elution was carried out at a flow rate of 20 ml / h and 3 ml fraction was collected manually. Enzyme activity was measured in all fractions using spectrophotometer (Bausch and Lomb spectronic 2000). The major active fractions were collected and stored at - 20 °c for further analysis.

2.4. Assay of Unpurified And Purified Alkaline Protease Enzyme Activity

Enzyme activity was determined using N- benzoyl- - arginine-p-nitroanilide (BAPNA) as substrate (Erlanger et al 1961). BAPNA (43.5 mg) was desolved in 1 ml dimethylsulfoxide, and then brought to 100 ml with 0.05 M Tris – HCl buffer (pH 7.8) containing 0.02 M $CaCl_2$.

One BAPNA unit is defined as (Δ A 410nm / min X 1000 X 3) / 8800 where 8800 is the extinction coefficient of p-nitroanilide.

Enzyme activity was measured at 280 nm in cuvets of 1 cm path length using a spectrophotometer (Backman Du 7400, USA).

2.5. Assay of Unpurified and Purified Acidic Protease Enzyme Activity

It was determined as described by Abson and Mirsky (1932) using 2 % hemoglobin at pH 2 and 30 °c. The pepsin unit (PU) were calculated from the initial slope of the A 280 nm line course of the TCA soluble product, as the amount of enzyme that produces an A 280 nm increase of 0.0042 per min per ml of reaction mixture.

2.6. Determination of Enzymes Optimum Temperature

The effect of temperature on the activity of the acidic protease was determined at various temperature intervals (10-80 °c).

2.7. Determination of Enzymes Optimum pH

The pH optimum of the cichorium protease enzymes was determined by preparing the hemoglobin substrate in various buffer solutions (0.2 M HCl – KCl buffer pH 2.0)

2.8. Preparation of Hydrolysates

1700 ml of 10 % (wt. / vol.) heated whey protein (HWP) solution on a protein equivalent basis was prepared using an electric mixer. This solution was defatted by ultracentrifugation at 12,000 rpm for 20 min.

The solution was then divided into 17equal portions each of 100 ml and each of them received one of the following additions:

- One portion received no addition (control reaction mixture).
- Eight portions adjusted to pH 8 to 6 of them purified alkaline cichorium protease enzyme (PKCPE) was added at volume of 0. 1, 0. 2, 0. 3. 0. 4, 0. 5or 0. 6 ml, respectively. To the tow reminder alkaline reaction mixture commercial trypsin enzyme at a rate of 0.002, 0.004 % was added, respectively. These commercial enzymes treatments used for comparable propose)
- Eight portions adjusted to pH 2.5 to 6 of them purified acidic cichorium protease enzyme (PACPE) was added at volume of 0. 1, 0. 2, 0. 3. 0. 4, 0. 5or 0. 6 ml, respectively. To the tow reminder acidic reaction mixture commercial pepsin enzyme at a rate of 0.002, 0.004 % was added, respectively. These commercial enzymes treatments used for comparable propose

The pH of each reaction was maintained at the constant value using a suitable buffer for each reaction, and samples of every portion was incubated at its enzyme optima temperature, during incubation period samples were withdrawn after 30,60,90,120,150,180,210 and240 min and the enzymes were inactivated by heating the reaction mixture for 10 min. at 90c.The supernatants were taken as HWP hydrolysates and the precipitates were discarded.

The HWP hydrolysates were stored at -20c for subsequent estimation.

2.9. Determination of Degree Of Hydrolysis (DH) and Non Protein Nitrogen

The DH OF HWP by cichorium crude extract, cichorium purified protease enzymes, trypsin and pepsin for the different incubation times were determined according to Adler Nissen (1979),and the NPN concentrations of the same samples were estimated by using the method of Lowry et. al. (1951).

Ten replications were carried out at each step of the experiment data.

3. SDS-PAGE

Sodium dodecyl sulfate-PAGE was used to estimate the DH of HWP incubated with either commercial or cichorium extract& purified enzymes c for different time durations and their α-LA, ß-LG, and BSA concentrations, as described by Laemmli (1970). The separating gel 14% (wt/vol) acrylamide, having pH 8.8, and the stacking gel 3% (wt/vol) acrylamide, with pH 6.8, were used. Gels were stained with 0.2% (wt/vol) Coomassie Brilliant Blue R-250 (Sigma Co., St. Louis, MO) in an acetic acid:methanol:H_2O solution (1:1:5, by vol.) and were destained in an acetic acid:methanol:H_2O solution (1:3:6, by vol.).

3.1. Determination Acceptability of Cichorium Hydrolysate

The sensory evaluation for bitter taste and over all acceptability of HWP hydrolysate was subjected by apanel of eleven judges.

4. Results and Discussion

4.1. pH optima

(Figure 1) showed that the crude protease extract CCPE had its optimal activity at pH 6 and increasing or decreasing pH far from pH 6 cause a significant reduction of the CCPE activities that it lose approximately 50 % of its activity at pH 3 or 9.

The activity of purified alkaline protease (table 3) increased with increasing pH and reached its maximum at pH 8 and then decreased with further increase of pH, Simpson et al (1990) stated that Atlantic cod trypsin was most active at pH 8.

The purified acidic protease had the highest activity at pH 3 and its activity decreased with increasing of pH (Figure1), more than 50% of the relative activity was lost over pH 5 and no activity was detected at pH 9. The optimum pH for the hydrolysis of hemoglobin by partially purified polar cod pepsin was found to be 3.2 at 30 °c and 2.5 at 5 °c (Haard et al, 1982) also crude pepsin had a broad optimum of pH 2-3 at 28 °c

4.2. Temperature Optima

Figure 2 illustrates the influence of different temperatures on the CCPE, PKCPE and PACPE activities. It was appeared that relative activity increased with increasing the temperature from 10 to 30, 40and 30 for CCPE, PKCPE and PACPE, respectively then it decreased gradually as temperature degreased. However no activity was detected at 70 °c for cichorium protease extract and enzymes.

4.3. Degree of Hydrolysis (DH)

Heated whey protein rapidly degraded within 90 min of incubation with CCPE, PKCPE and PACPE at all enzyme concentrations used and steadily increased thereafter Table (1,2,3). The DH achieved during a hydrolysis reaction is expected to be related to the activity and specificity of the enzymes (Smyth and filzgerald, 1998).

According the overall acceptability score, especially the appearance of bitter taste, it was found that the DH value of CCPE hydrolysate at which the bitter taste appeared was 21.5, consequently, the sensory analysis for bitter taste of hydrolysate support the DH values. Upon the previous mentioned, the crude extract CCPE had satisfactory ability to produce hydrolysate when it used at volume of ≥ 3 to 5ml /100ml of protein solution for a time ranging from120 to 240(time reduced as amount of enzymes increase) (table 1).

Results in Table 1 also show that heated whey protein received 0.3 ml CCPE had degree of hydrolysis values of 2.9, 6.1, 8.0, 9.3, 10.1,11.8, and 12.5 at incubation time of 30, 60, 120, 150, 180, 210 and 240 days, respectively. However, the corresponding values when CCPE used at volumes of 0.6 % were 9.4,15.3, 17.4, 18.7, 19.8,20.6, 21.2,and 21.5 at the same incubation times mentioned above. The sensory evaluation for bitter taste appeared when CCPE used at volume of 0.6ml and incubation time of 150 min. Sukan and Andrews (1982) mentioned that the high DH indicated that the enzyme used contains several different proteinases with different specificities. This property may be found in cichorium crude protease extract CCPE, resulted of its satisfactory degree of hydrolysis.

Table (2) revealed that purified acidic cichorium protease enzyme PACPE showed typical hydrolysis profiles as commercial enzymes pepsin. However the concentration of PACPE used in our study was ten time than that used of commercial pepsin. The values of DH of PACPE were lower than the corresponding values of PKCPE. The panelists reported the limited volume of acidic enzyme solution PACPE to avoid bitter taste were higher than that of the alkaline enzyme PKCPE it was 0.6ml for incubation period of 210 min. As regard to the DH results and sensory evaluation it was found that

concentration and incubation time needed for producing good hydrolysate without any excise hydrolysis were ≥ 0. 4 to ≤ 0. 6 ml/100ml of protein solution for incubation times ranging from120 to210 min(time reduced as amount of enzymes increase).

The rate of hydrolysis occurred by PACPE when used at volume of 0. 6 was approximately equal to that of trypsin when it used at concentration of 0.004%.

Bitter taste appeared in pepsin treatments at degree of hydrolysis lower than that of PACPE treatments (DH: 18.7 for pepsin vie 21.9 for PACPE).

Table (3) showed more extensive hydrolysis of heated whey protein (higher DH values) when PKCPE used as compared with PACPE. Sensory qualities revealed that the limited volume of PKCPE to avoid bitter taste of the resulting hydrolysate were between the range of ≥ 0.03 and ≤ 0.05ml for incubation times between 60 and 240 min as(the time reduced as amount of enzymes increase) min, respectively. The DH values at volume of 0.03 ml of PKCPE were 6.1, 10.9, 12.5, 14.7, 15.5, 16.7, 17.2, and 18.0 at incubation times of 30, 60, 90, 120, 150, 180, 210, and 240 min, respectively. The corresponding DH values at volume of 0.05 % were 9.4, 18.9, 20.9, 21.4, 22.9, 23.2, 23.6, and 23.2.

We observed that the high degradation within, incubation began latter (within60 min) with compared with trypsin(within 30 min.),but it followed the same pattern. The rate of hydrolysis occurred by PKCPE when used at cvolume of 0.04ml was approximately equal to that of trypsin when it used at concentration of 0.004%.

Bitter taste appeared in trysin treatments at degree of drolysis lower than that of PKCPE treatments (DH:19.6 for trypsin vie 21.8 for PKCPE).

4.4. The Non Protein Nitrogen Content (NPN) of HWP Hydrolysate

The NPN followed the hydrolysis pattern of DH. It was also highly increased within 90 min 0f incubation with each of CCPE,PKCPE and PASPE, thereafter it steadily increased with increasing incubation periods (table 4,5and 6). The highest NPN was noticed after 240 min of incubation with all levels of enzyme. As the concentration of enzyme increase the NPN content increase at each incubation times. Similar results were reported previously by Kim et. al. (2007). Non protein nitrogen WPH represents the small peptides, amino acids and soluble ammonia nitrogen (Leonil et. al.,1997).

The NPN contents of cichorium purified enzymes hydrolysates followed the hydrolytic pattern of pepsin and trypsin hydrolysates.

4.5. Electrophoresis Analysis (Antigenic Fractions)

Electrophoretic patterns Figure 3,4,5 (Sodium dodecyl sulfate-PAGES) revealed complete removal of ß-LG, α-LA, and BSA in all hydrolysates of HWP at 240 min of incubation, but patterns of crud cichorium extract hydrolysate indicated a few remaining traces of both ß-LG and α-LA even after 240 min. The major ß-LG, α-LA HWP degraded within 90 min of its incubation with either commercial or cichorium enzymes. The relation between antigenicity (the ability of a substance to bind to specific antibodies) and allergenicity (its ability to elicit an allergic reaction and an immune response) implies that the antigenic peptides of ß-LG can also be allergenic. Van Beresteiin et al.,1994 and Kananen et al.,2000 have indicated a positive relation between ß-LG concentration and whey antigenicity. Complete enzymatic degradation of this fraction to low molecular weight could lower the antigencity and allergenicity of whey.

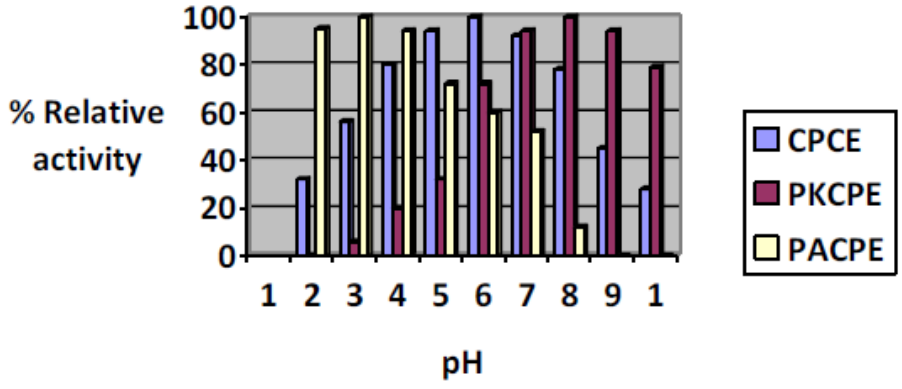


Figure1. *Effect of pH on the activity of cichorium extract and enzymes*

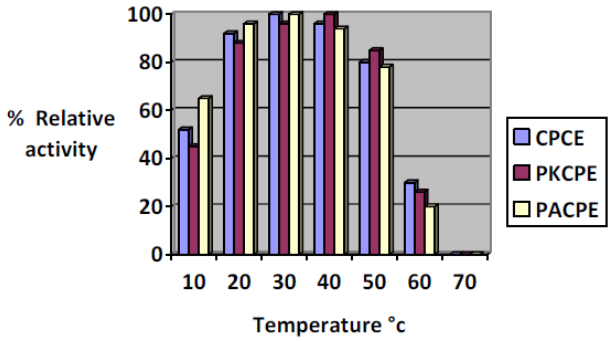


Figure2. *Effect of temperature on the activity of cochorium extract and anzymes*

Table1. *Degree of hydrolysis of heated whey protein treated by crude protease cichorium extract CCPE, the sensory evaluation for bitter taste included.*

Degree of Hydrolysis						
Extract volume Reaction Time in min.	ml extract*/10gm protein					
	0.1	0.2	0.3	0.4	0.5	0.6
30	0.5	1.1	2.9	4.2	6.2	9.4
60	2.1	3.9	6.1	8.4	12.1	15.3
90	3.9	5.1	8.0	10.5	13.5	17.4
120	4.5	6.4	9.3	12.2	14.2	18.7
150	5.2	7.2	10.1	13.0	14.8	19.8
180	5.9	8.0	10.9	14.2	15.5	**20.6
210	6.4	8.9	12.6	15.8	15.9	**21.2
240	7.1	10.8	13.1	16.3	16.6	**21.5

**Every ml of cichorium extract prepared from 0.1 gm cichorium weed*

*** Appearance of bitter taste*

Table2. *Comparing of degree of hydrolysis of heated whey protein treated by purified acidic protease cichorium enzyme and pepsin, the sensory evaluation for bitter taste appearance included.*

Degree of hydrolysis								
	Pepsin concentration%		ml purified acidic cichorium protease enzyme/ 10gm protein					
	0.002	0.004	0.01	0.02	0.03	0.04	0.05	0.6
30	6.2	9.4	0.8	2.6	3.1	5.3	6.8	8.4
60	8.5	12.2	2.5	4.9	6.2	8.2	10.2	14.3
90	10.0	15.8	3.7	5.3	8.8	9.8	12.8	17.1
120	11.4	17.2	4.5	6.2	9.5	10.5	13.9	19.5
150	11.9	**18.7	4.8	6.9	10.3	11.8	15.1	20.4
180	12.5	**19.5	6.4	7.6	11.1	13.0	16.8	21.1
210	12.8	**20.0	6.1	8.2	11.9	14.9	17.4	**21.9
240	13.1	**20.2	6.9	8.9	12.4	15.6	18.8	**22.4

**Appearance of bitter taste.

Table3. *comparing between degree of hydrolysis of heated whey protein concentrate treated by purified alkaline cichorium enzyme PKCPE and trypsin, the sensory evaluation for bitter taste appearance included.*

Degree of Hydrolysis							
	Trypsin Concentration%		ml purified acidic cichorium protease enzyme/ 10gm protein				
	0.002	0.004	0.01	0.02	0.03	0.04	0.05
30	7.8	11.5	2.9	5.3	6.1	8.2	9.4
60	9.2	15.9	5.8	9.7	10.9	16.5	18.9
90	9.8	18.8	6.8	10.6	12.5	18.1	20.9
120	10.2	*19.6	7.5	11.8	14.7	19.2	21.4
150	10.6	*20.1	8.6	12.1	15.5	20.8	**22.9
180	11.0	*21.5	9.8	12.4	16.7	21.4	**23.24
210	11.2	*21.8	10.5	12.5	17.2	21.6	**23.4
240	11.4	*22.3	11.3	12.7	18.0	**21.8	**23.7

** Appearance of bitter taste*

Table4. *NPN fractions in hydrolysate produced by curde cichorium protease enzymes extract.*

Absorbance at 540 nm					
Extract volume	ml extract/10gm protein				
Reaction time in Min.	0.1	0.2	0.3	0.4	0.5
30	0.00	0.12	0.12	0.28	0.32
60	0.00	0.24	0.32	0.38	0.48
90	0.16	0.32	0.40	0.42	0.50
120	0.20	0.36	0.42	0.42	0.52
150	0.24	0.38	0.42	0.44	0.56
180	0.24	0.38	0.46	0.48	0.56
210	0.26	0.40	0.46	0.50	0.60
240	0.26	0.44	0.48	0.54	0.62

Table5. *Comparing NPN fractions in hydrolysate produce by purified acidic cichorium protease enzyme (PAPCE) and pepsin.*

Absorbance at 540 nm								
	pepsin concentration %		ml purified acidic cichorium protease enzyme/10gm protein					
	0.002	0.004	0.01	0.02	0.03	0.04	0.05	0.06
30	0.30	0.46	0.00	0.22	0.26	0.30	0.35	0.40
60	0.42	0.57	0.00	0.28	0.38	0.36	0.42	0.46
90	0.47	0.66	0.22	0.32	0.42	0.40	0.48	0.56
120	0.53	0.69	0.22	0.40	0.44	0.44	0.52	0.60
150	0.56	0.72	0.24	0.42	0.44	0.46	0.55	0.62
180	0.58	0.73	0.26	0.40	0.48	0.50	0.59	0.64
210	0.59	0.76	0.28	0.44	0.53	0.55	0.64	0.70
240	0.60	0,7 6	0.28	0.46	0.58	0.61	0.69	0.74

Table6. *NPN fractions in hydrolysate produced by purified alkaline cichorium enzyme PkCPE and trypsin.*

Absorbance at 540 nm							
	Trypsin		ml purified acidic cichorium protease enzyme/10gm protein Cichorium enzyme				
	0.002	0.004	0.01	0.02	0.03	0.04	0.05
30	0.36	0.52	0.00	0.22	0.36	0.38	0.38
60	0.48	0.68	0.00	0.28	0.42	0.42	0.48
90	0.52	0.74	0.14	0.32	0.54	0.58	0.58
120	0.56	0.80	0.26	0.40	0.58	0.60	0.62
150	0.58	0.82	0.32	0.42	0.60	0.60	0.65
180	0.58	0.84	0.36	0.45	0.61	0.63	0.67
210	0.58	0.87	0.40	0.46	0.62	0.65	0.68
240	0.58	0.0.88	0.42	0.44	0.64	0.62	0.68

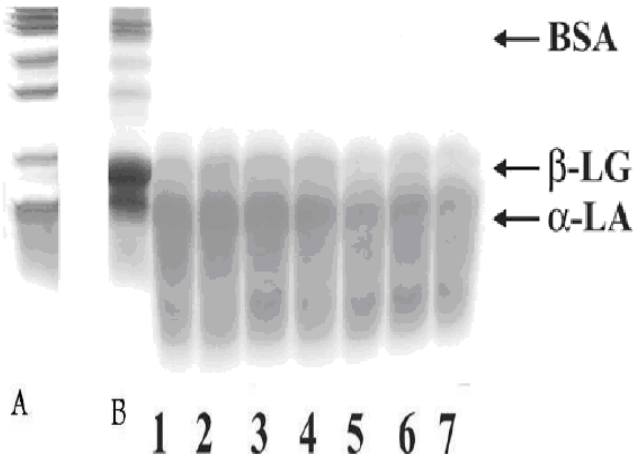


Figure3. *Sodium dodecyl sulfate -PAGE patterns of enzymatic hydrolysate*

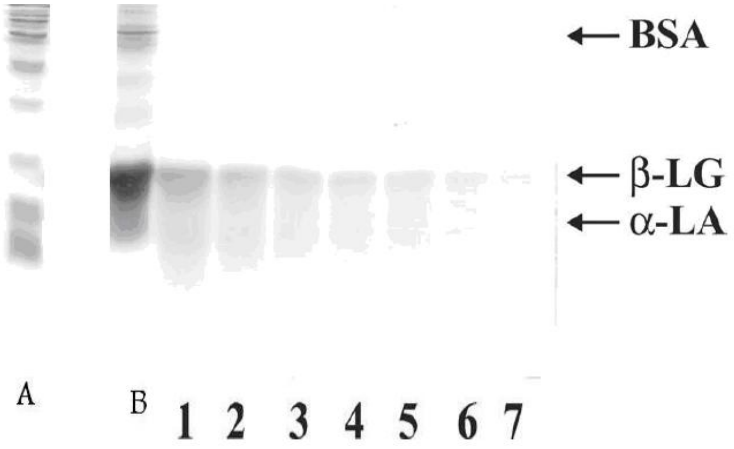
of heated whey protein treated by crude protease cichorium extract CCPE at concentration of 0.4%,and at incubation times of 60, 90, 120, 150, 180, 210, and 240 min, respectively.

A= standard broad-range marker

B=Heated whey protein

Lance 1 to 7 =heated whey protein hydrolysates produced at 60, 90,120,150,.180,210and240 min.of incubation, respectively.

- *Pepsin*



- *Acidic Cichorium Enzyme*

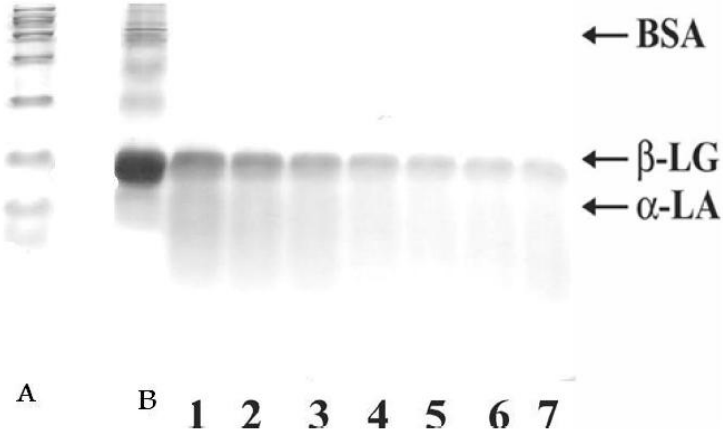
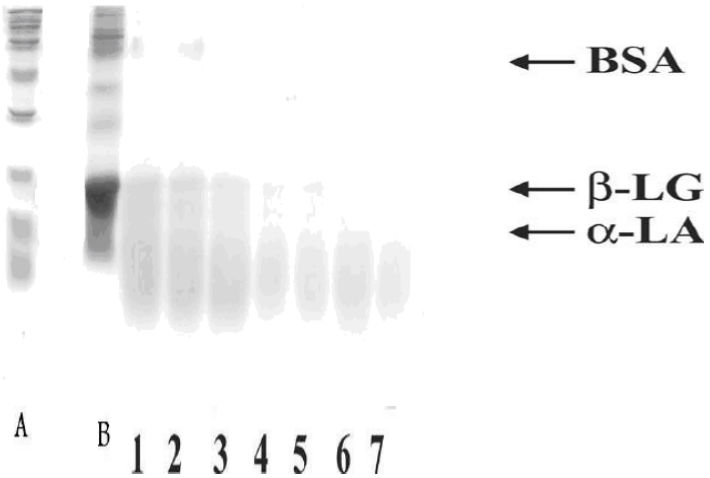


Figure4. *Sodium dodecyl sulfate- PAGE patterns of enzymatic hydrolysate of heated whey protein treated by purified acidic protease cichorium enzyme at concentration of 0.04 %, comparing with trypsin at concentration of 0.004% at incubation times of 60, 90, 120, 150, 180, 210, and 240 min, respectively*

- For more details see Figure 3

- *Trypsin*



Alkaline Cichorium Enzyme

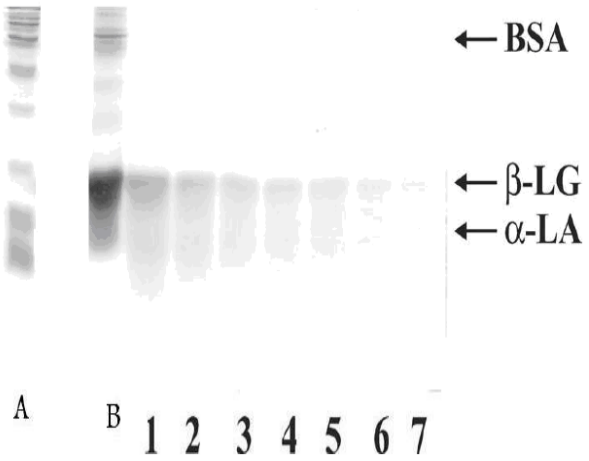


Figure5. *Sodium dodecyl sulfate -PAGE patterns of enzymatic hydrolysate of heated whey protein treated by purified alkaline cichorium enzyme PKCPE at concentration of 0.04%, comparing with trypsin at concentration of 0.004% at incubation times of 60, 90, 120, 150, 180, 210, and 240 min, respectively.*

- For more details see Figure 3

The plant enzymes protease from cichorium were extracted, purified and characterized. The results summarized in this paper illustrate the high potential of cichorium crude extract or its purified acidic and alkaline enzymes for whey protein hydrolysis, however the alkaline one showed the highest activity which capable it beside its thermal character to have great interest for future extend application in food industry.

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