Characterization of Hydrolysates Produced by Enzymatic hydrolysis of Camel Casein and Protein Isolates of Al-Ban (*Moringa peregrina*) and Karkade (*Hibiscus sabderiffa*) Seeds

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ABSTRACT: Camel casein and protein isolates of Al-Ban and Karkade were enzymatically hydrolyzed , and functional properties were evaluated. The degree of hydrolysis by pepsin followed by pancreatin was higher than that by trypsin + chymotrypsin. Amino acid compositions of the proteins investigated and their hydrolysates were determined also. The overall amino acid composition of the hydrolysates was similar to that of the starting materials except minor changes. The levels of essential amino acids of the unhydrolyzed proteins and their hydrolysates met the amino acid requirements compared to the reference pattern except for lysine in Al-Ban protein and lysine, threonine and isoleucine in Karkade protein. Solubility of the three hydrolysates was increased compared to their untreated ones. Camel casein and Karkade hydrolysates showed decreased in turbidity, while Al-Ban showed high turbidity values. Casein and Karkade hydrolysates produced by pepsin followed by pancreatin hydrolysis showed significant ($p< 0.05$) increase in water holding capacity compared to nuhydrolyzed proteins. On the other hand, Al-Ban and Karkade hydrolysates produced by trypsin + chymotrypsin hydrolysates showed significantly higher oil absorption capacities compared to their unhydrolysed proteins.

Key Words: Enzyme hydrolysates, Camel casein, Al-Ban seeds, Karkade seeds, Functional properties

INTRODUCTION

Enzymatic hydrolysis of dietary proteins is an ancient food practice. According to Lahl and Braun (1994), hydrolysis of the proteins is carried out to improve nutritional characteristics, modify functional properties, remove off-flavors and odors and eliminate toxic or inhibitory ingredients.

Protein hydrolysated-based products include formulas for infants with allergy to intact protein or inborn error of metabolism (e.g. PKU) and elemental diets for patients with impaired gastrointestinal function (Mahmoud, 1994).

Cow's milk protein is the most important protein source used in the development of protein hydrolysates. Recently, plant protein sources have been investigated for the production of hydrolysates that used as functional foods, flavor inhancers and medical foods (Clemente, 2000).

Camel milk is abundant in Saudi Arabia, the chemical composition and nutritional quality of camel milk (Najdi breed) was reported by Sawaya et al. (1984). The nutritive value of camel milk casein was reported by Pant and Chandra (1981). These studies showed that camel milk contained 3% protein, a balanced amino acid

composition and that camel casein had PER value higher than cow milk due to the relatively higher value of sulphur amino acids in camel milk.

The protein and oil components of Al-Ban (*Moringa peregrina*) and karkade (*Hibiscus sabderiffa*) seeds, although not used in food applications, represent promising sources of protein and oil from these plants. The protein content of Al-Ban seed was found to be 28.2% (Al-Housein and Abu-Tarboush, 1997) and that of karkade seed was 25.2% (Abu-Tarboush and Ahmed, 1996). A high protein content, and low levels of protease inhibitors and polyphenols facilitate and improve the production of plant protein hydrolysates (Clemente, 2000). Functional properties of Al-Ban protein concentrate (64.6% protein) and Al-Ban protein isolate (97.8% protein) were investigated by Al-Kahtani and Abou-Arab (1993). Some nutritional and functional properties of karkade seed protein concentrate and protein isolate were investigated by Abu-Tarboush et al. (1997).

In this work, camel casein, Al-Ban and karkade seeds protein isolates were investigated for production of protein hydrolysates using pepsin followed by pancreatin and trypsin + chymotrypsin. Amino acid composition and some functional properties of the produced hydrolysates and their substrate proteins were also investigated.

MATERIALS AND METHODS

Materials:

Karkade seeds (*H. sabdariffa*) were obtained from the western region of Sudan. The seeds after cleaning were ground to fine powder, the resultant whole seed flour was kept in a refrigerator.

Seeds of Al-Ban (*M. peregrina*) were brought from Al-Ola region of northwest Saudi Arabia. The seeds were cleaned, hand cracked, dehulled and ground with a Waring commercial blender.

Camel milk samples were collected from four females at Dormma Area, Riyadh district, Saudi Arabia. The pooled batch of milk samples was immediately refrigerated at 4°C until used.

Trypsin (EC 3.4.21.4), α-chymotrypsin (EC 3.4.21.1), pepsin (EC 3.4.23.1), pancreatin, L-serine, DL-Dithiothreitol, O-phthaladialdehyde (OPA), and Bicinchoninic acid (BCA) kit were purchased from Sigma (Sigma Chemical, St. Louis, MO, USA). All other reagents were of analytical grade.

Preparation of seeds protein isolates and casein:

The oil was extracted from the whole flours of the seeds of karkade and Al-Ban by shaking with two volumes of n-hexane for two days at room temperature (Solvent to flour ratio 10:1), the resultant defatted flours were desolventized in open air at room temperature and were ground to pass 425 μm mesh and kept in a refrigerator until used. Seed protein isolates of karkade and Al-Ban were prepared from the defatted flour, according to the method of El-Tinay et al. (1988). Defatted flours were mixed with distilled water in a ratio 1:10, the pH of the mixture was adjusted to 10.0 using 1N and 0.1N NaOH, and the pH of the mixture was kept constant while stirring the mixture for two hours at room temperature. The soluble protein extract was obtained by centrifugation of the mixture at 4500 r.p.m for 20 minutes at 10°c. To coagulate the protein in the alkaline extract,

the pH was adjusted to 4.5 using 1N HCl and 0.1N HCl and the coagulated protein was recovered by centrifugation at 4500 r.p.m for 20 minutes at 10 °c. The pH of the resulted protein isolate was adjusted to 7.0 and then freeze-dried (Freeze Mobile 125 L , USA).

Camel casein was prepared according to AOAC methods # 927.03 (1995). Skimmed camel milk was mixed with distilled water in a ratio 1:1 (g/ml), the mixture heated to 35°C, a volume of acetic acid (10%) was added to the mixture (volume of diluted acetic acid was 0.1 of volume of distilled water added to casein). Stirring the mixture for 10 minutes followed by adding sodium acetate (1N). After filtration (Watman No. 40), the precipitated casein was washed several times with distilled water, the pH of casein raised to 7.0 and then freeze-dried.

Chemical composition:

Moisture, protein and ash contents (of camel milk and karkade and Al-Ban seed protein isolates) were determined using AOAC (1995) methods (# 930.15, 920.87 and 942.05, respectively)

Preparation of protein hydrolysates:

Enzymatic hydrolysis of the proteins by pepsin followed by pancreatin was carried out according to the method of Kim and Barbeau (1991) with minor modifications. Freeze-dried protein samples were dispersed in 0.1N HCl solution in a ratio of 1:100 (W/V). After incubation for 30 minutes at 37°C, pepsin was added to the dispersed protein solution in a ratio (enzyme:protein) of 2:100 (W/W). Hydrolysis proceeded for two hours at the end of which the pH of the reaction mixture was adjusted to 7.0 by addition of 0.2N NaOH. After the protein samples were re-equilibrated at 37°C for 30 minutes, pancreatin was added in a ratio of 4:100 enzyme:protein (W/W) and protein digestion was continued an additional 8 hours. At timed intervals during the pepsin / pancreatin digestion (0, 2, 6, 10 hr) aliquots were removed to determine degree of hydrolysis. Termination of hydrolysis of these aliquots, was carried out by heating the samples at 85°C for 30 minutes. Enzyme hydrolysis of the proteins by trypsin and chymotrypsin was carried out according to the method of Kim (lee) et al. (1990) with some modifications. Protein samples were dispersed in distilled water in a ratio of 1:100 (w/v). The pH of the protein mixture was adjusted to 8.0. After equilibration at 37°C for 30 minutes, trypsin (2:100 enzyme:protein) and chymotrypsin (4:100 enzyme:protein) were added. Digestion continued for 10 hours and aliquots were removed from the reaction mixture at 0, 2, 6, , 10 hrs to determine degree of hydrolysis. Termination of hydrolysis of these aliquots, was carried out by heating the samples at 85°C for 30 minutes. Protein sample hydrolyzed for 10 hrs (by pepsin followed by pancreatin and by trypsin + chymotrypsin) were freeze-dried.

Determination of degree of hydrolysie (DH):

Degree of hydrolysis (DH) was determinated according to the procedure described by Nielsen, et al. (2001) using OPA assay and L-serine as standard. DH is defined as the percentage of cleaved peptide bonds:

$$DH = h/htot\ 100\%$$

Where htot is the total number of peptide bonds per protein equivalent and h is the number of hydrolyzed bonds.

The following equation was derived from Nielsen, et al. (2001) equations for calculating DH:

$$DH = \frac{A \times 118.5}{A^{o} \times W \times P} - 5$$

Where:

A : absorbance of sample at 340 nm
A^{o} : absorbance of standard at 340 nm
W : weight (g) of the sample
P : protein content of the sample

Amino acid analysis:

Acid hydrolysis (6N HCl) for the freeze-dried protein (casein, Al-Ban Karkade and their enzymatic hydrolysates prepared after 10 hrs hydrolysis) samples was performed according to AOAC (1995) method (# 982.30 E), then amino acid analysis was performed on reverse phase-high pressure chromatography (Shimadzu LC-10 AD, Shimadzu Corporation, Kyoto, Japan). Samples were analyzed on Shimpack amino –Na type column (10 cm × 6.0 mm) obtained from Shimadzu corporation. The amino acids of samples were derivatized with O-phthadialdehyde (OPA) detected by Flourescent detector and data were integrated using an integrator model C-R7A (Shimadzu chromatopac data processor).

Functional properties:

Protein solubility:

The analysis of protein solubility followed the method of Bryant et al. (1988) with minor modification. Suspensions sample of freeze-dried protein containing 1% protein (W/V) were prepared at pH values ranging from 2.0 to 12.0 using NaOH or HCl. The suspension was stirred for 30 minutes at room temperature, then centrifuged at 4500 r.p.m for 20 minutes. Protein of the supernatant was determined using Bicinchoninic acid (BCA) assay (Vojdani, 1996).

Water absorption and oil absorption:

The centrifugation method of Lôpez et al. (1991) was followed with minor modifications. Protein suspensions were prepared at pH 4.0, 7.0 and 10.0 by mixing 0.5 g of freeze-dried protein with 5ml of distilled water or 5ml corn oil in a graduated centrifuge tube. The centrifuge tube was allowed to stand for 30 minutes at room temperature, then centrifuged at 4000 r.p.m for 25 minutes. The volume of free liquid was measured and the retained liquid was expressed as ml of water or oil absorped per gram of protein.

Clarity:

Clarity was performed according to the method reported by De La Barca et al. (2000). Protein suspensions 1% (W/V) in distilled water were adjusted to pH 4.0, 5.5 and 7.0 using either 2N HCl or NaOH. Optical clarity was assessed quantitatively by the measurements of the absorbance at 660 nm using uv / visible spectrophotometer (Utrospec II 4050, Pharmacia, Sweden). Double distilled water was used as the blank.

Statistical Analysis:

The statistical analyses of the data were performed with a SAS program version 6 (SAS, 1990). Three replicates were

performed in a completely randomized design. Results were expressed as the mean ± standard error. To ascertain the significance among means of the samples , Duncan's multiple range test was used (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Enzymatic Protein hydrolysis:

The protein contents of camel casein, Al-Ban and Karkade protein isolates on dry weight basis, as derived from table (1), were 73.21, 84.41 and 83.10%, respectively. With respect to their protein contents, the three protein sources may be considered as suitable starting materials for enzymatic hydrolysis.

The hydrolysis of camel casein and Al-Ban isolate (Figure 1) started by pepsin proceeded very rapidly after addition of pancreatin as evidenced by the remarkable increase in values of degree of hydrolysis (DH). Hydrolysis of karkade isolate by pepsin as shown in Figure (1) resulted in cleavage of the protein significantly higher ($P \leq 0.05$) than that of camel casein and Al-Ban isolate, this may show the susceptibility of karkade seed protein to the action of pepsin. After 10 hours of hydrolysis of camel casein, Al-Ban and karkade isolates by pepsin followed by pancreatin, values of DH of these hydrolysates were 31.6%, 29.9% and 42.5%, respectively (Figure 1). DH is considered the most practical means for controlling the hydrolysis process, it also the most widely used indicator for comparison among different protein hydrolysis, since DH is a principal determinant of protein hydrolysates properties (Mahmoud, 1994). The values of DH having a negative sign as shown in Figure 1 and Figure 2 indicate none enzyme hydrolysis, these values were expressed numerically as difference between part of the equation (for calculating DH) and a constant. Nielsen et al. (2001) found that unhydrolyzed soy protein isolate had DH equal to –1.53 when they used OPA assay.

Hydrolysis of Al-Ban isolate by trypsin + chymotrypsin (Figure 2) showed low DH values. This could be due to the presence of trypsin (Al-Kahtani, 1995) and chymotrypsin inhibitors activities in Al-Ban seeds. Hydrolysis of camel casein by trypsin and chymotrypsin (Figure 2) resulted in higher DH values with significant difference ($P \geq 0.05$) compared to DH values of Al-Ban and karkade isolates. Dzwolak and Ziajke (1999) prepared hydrolysates from casein and whey proteins using trypsin and chymotrypsin. They stated that endopeptidases hydrolize protein to average DH values (≥20%). Kim et al. (1990) found that trypsin effectively decreased the molecular size of soy protein isolate more than α-chymotrypsin and other proteases used.

Comparison between the hydrolytic activity of pepsin followed by pancreatin and trypsin + chymotrypsin for the three proteins, was illustrated in Figure (3). Pepsin, trypsin and chymotrypsin used in the hydrolysis are endoproteases while pancreatin contains both endoprotease and exoprotease activities. Clemente (2000) stated that the initial use of endoproteases facilitates the action of exoproteases in a second step to achieve a more complete protein degradation. He referred to the hydrolysis of chickpea protein isolate obtained by sequential treatment of endoprotease (alcalase) and exprotease (Flavourzyme) enzymes, this hydrolysis resulted in DH values more than 50%.

Table 1: Moisture, protein and ash contents of the camel casein, Al-Ban and Karkade protein isolates[1]

Sample	Moisture (%)	Protein (%)	Ash (%)
Camel casein	8.25±0.026	67.17±0.033	7.63±0.171
Al-Ban	12.13±0.014	74.17±0.033	2.73±0.038
Karkade	2.14±0.098	81.33±0.133	3.72±0.007

[1] Mean± standard error of three determinations expressed as fresh weight. Crude protein for casein (N×6.38) and N×6.25 for the other two samples.

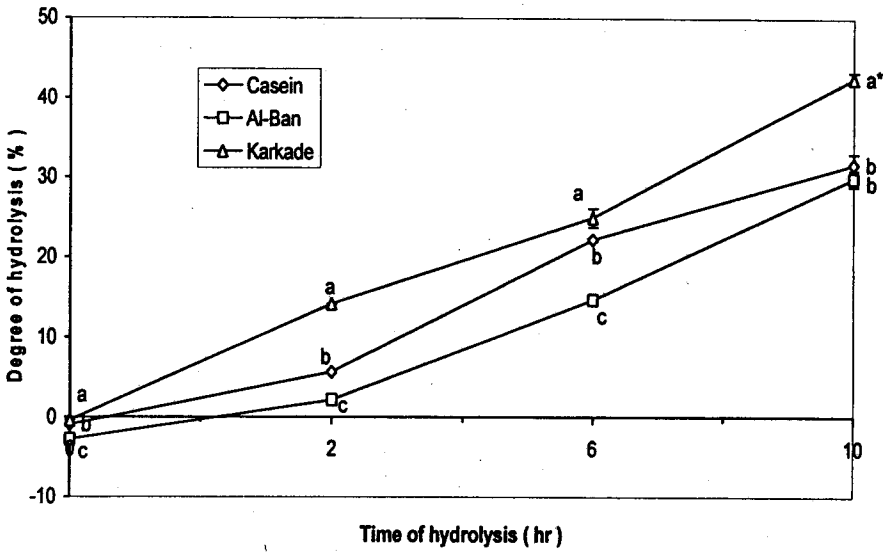

Fig. 1. Degree of hydrolysis (DH) of the protein isolates of Camel casein , Al-Ban and Karkade using pepsin followed by pancreatin hydrolysis .
* Unlike letters differ significantly (P<0.05)

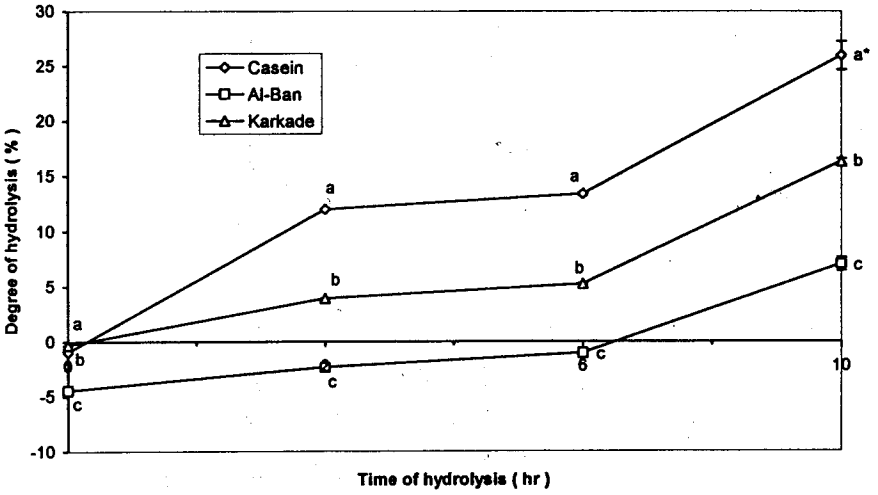

Fig.2. Degree of hydrolysis (DH) of the protein isolates of Camel casein , Al-Ban and Karkade using Trypsin + Chymotrypsin hydrolysis .

* Unlike letters differ significantly (P<0.05)

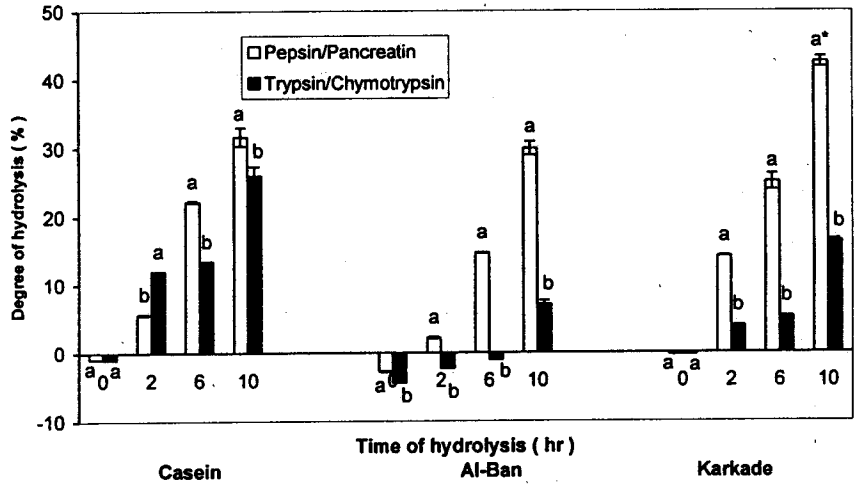

Fig.3. Comparison of the degree of hydrolysis (DH) for each protein isolate of Camel casein , Al-Ban and Karkade by pepsin followed by pancreatin and trypsin+chymotrypsin .

* Unlike letters differ significantly (P<0.05)

It is clear from Figure (3) that pepsin followed by pancreatin model was more effective than trypsin + chymotrypsin in producing hydrolysates (specially in case of plant proteins) with high DH values. This is due to combined action of endopreases and exoproteases as stated before. The expected end products of pepsin followed by pancreatin hydrolysis composed of free amino acids and low molecular weight peptides. The extensively hydrolyzed proteins are primarily used in hypoallergenic infant formulas (Mahmoud, 1994). The hydrolysates produced by trypsin + chymotrypsin action specially Al-Ban and karkade hydrolysates could be described as partially hydrolyzed proteins and can be used as stated by Mahmoud (1994) as nitrogen source in specialized adult nutritional products.

Amino acid composition:

Amino acid composition of camel casein and the effect of enzymatic treatment on the amino acid profile were shown in Table (2). Camel casein was found to contain most of the essential amino acids in high ratios, glutamic acid was the most abudant amino acid followed by leucine, lysine and aspartic acid. Camel milk is rich in sulphur amino acids (Sawaya et al., 1984). Methionine level (Table 2) alone exceeded, the level of methionine + cystine in the reference protein (FAO/WHO/UNU, 1985). This reference pattern proposed for children of preschool age is recommended to be used to evaluate dietary protein quality for all age groups, except infants (FAO/WHO, 1991).

Amino acid composition of freeze-dried casein hydrolysates (after 10 hrs hydrolysis) (Table 2) exhibited minor changes compared to unhydrolyzed casein, such as decrease in ratio of some amino acids like methionine, leucine, histidine, aspartic and glutamic acid and increase in ratio of arginine. But the overall amino acid composition of casein hydrolysates was similar to that of casein. Clemente (2000) explained that enzymatic hydrolysis is developed under mild conditions of pH and temperature, avoiding the extremes usually required for chemical and physical treatments and minimizing side reactions. Compared to essential amino acid levels in the reference protein (FAO/WHO/UNU, 1985) camel casein and its hydrolysates (Table 2) contain these essential amino acid in higher levels than that in the reference pattern. Sulphur amino acids are usually considered as limiting in milk (Sawaya et al., 1984). Hussein and Hajos (1993) prepared chymotryptic hydrolysate of buffalo milk proteins that was enriched in methionine by enzymatic peptide modification to improve the biological value.

Amino acid composition of Al-Ban protein isolate and hydrolysates was shown in Table (3). Al-Ban protein isolate contains low level of lysine, high levels of arginine and glutamic acid and fair amounts of most of the essential amino acids. The amino acid profile as shown in Table (3) is comparable to the amino acid composition of Al-Ban protein reported by Al-Housein and Abu-Tarboush (1997). The effect of enzyme hydrolysis on the composition of the amino acid of Al-Ban protein could be explained as follows; trypsin + chymotrypsin hydrolysis resulted in a slight increase in the ratios of most of the amino acids (Table 3), while pepsin followed by pancreatin hydrolysis resulted in slight increase in arginine and phenyl alanine and a slight decrease in most of the other amino acids as shown in Table (3).

Table 2: Effect of enzymatic treatment on amino acid profile of Camel casein and casein hydrolysates (g amino acid/100g) protein.

Amino acids	Casein	Casein hydrolysate (1)	Casein hydrolysate (2)	Reference protein FAO/WHO/UNU (1985)
Essential				
Lysine	*7.85^{a}±0.035	7.28^{c}±0.066	7.57^{b}±0.077	5.8
Threonine	5.07^{a}±0.043	4.87^{b}±0.043	4.96^{ab}±0.043	3.4
Valine	5.80^{a}±0.017	5.82^{a}±0.003	5.09^{b}±0.080	3.5
Methionine	3.39^{a}±0.011	2.97^{b}±0.056	2.73^{b}±0.180	
Methionine + Cystine				2.5
Isoleucine	4.95^{a}±0.005	5.00^{a}±0.011	4.71^{b}±0.029	2.8
Leucine	10.36^{a}±0.01	9.78^{b}±0.097	9.74^{b}±0.049	6.6
Phenylalanine (p)	4.98^{a}±0.011	4.86^{a}±0.023	4.65^{a}±0.168	
Tyrosine (T)	5.57^{a}±0.000	5.37^{b}±0.075	5.19^{c}±0.046	
P+T				6.3
Histidine	3.15^{a}±0.011	2.78^{b}±0.032	2.61^{c}±0.036	1.9
Non Essential				
Arginine	5.63^{b}±0.003	6.66^{a}±0.077	6.59^{a}±0.031	
Aspartic acid	7.10^{a}±0.008	6.95^{b}±0.043	5.55^{c}±0.037	
Glutamic acid	23.28^{a}±0.21	21.49^{b}±0.233	21.79^{b}±0.052	
Glycine	1.21^{b}±0.003	1.43^{a}±0.008	1.10^{c}±0.010	
Alanine	2.72^{a}±0.020	2.64^{b}±0.026	2.15^{c}±0.008	
Serine	4.86^{a}±0.105	4.74^{a}±0.051	4.04^{b}±0.066	

*Each value is the mean ± SE of three determinations. Means with different letters in each row are significantly different ($P<0.05$).
Casein hydrolysate (1) = prepared by pepsin/pancreatin hydrolysis for 10 hrs.
Casein hydrolysate (2) = prepared by trypsin/chymotrypsin hydrolysis for 10 hrs.

Table 3: Amino acid profile of Al-Ban Protein Isolate and Al-Ban Hydrolysates (g amino acid/100g protein)

Amino acids	Al-Ban Isolate	Al-Ban hydrolysate (1)	Al-Ban hydrolysate (2)	Reference protein FAO/WHO/UNU (1985)
Essential				
Lysine	*1.07^{b}±0.077	0.91^{b}±0.008	1.30^{a}±0.051	5.8
Threonine	3.53^{b}±0.017	3.36^{c}±0.005	3.89^{a}±0.005	3.4
Valine	3.95^{c}±0.014	4.07^{b}±0.005	4.24^{a}±0.003	3.5
Methionine	1.86^{a}±0.043	1.72^{b}±0.008	1.92^{a}±0.003	
Methionine + Cystine				2.5
Isoleucine	2.71^{b}±0.006	2.66^{c}±0.008	2.89^{a}±0.000	2.8
Leucine	6.71^{b}±0.011	6.55^{c}±0.017	7.07^{a}±0.003	6.6
Phenylalanine (p)	5.76^{b}±0.017	5.88^{c}±0.005	6.00^{a}±0.008	
Tyrosine (T)	2.46^{b}±0.029	1.82^{c}±0.000	2.69^{a}±0.012	
P+T				6.3
Histidine	3.24^{a}±0.051	3.03^{b}±0.011	3.28^{a}±0.014	1.9
Non Essential				
Arginine	16.04^{b}±0.072	16.47^{b}±0.043	17.76^{a}±0.242	
Aspartic acid	6.52^{b}±0.147	4.50^{c}±0.003	7.27^{a}±0.011	
Glutamic acid	19.91^{a}±0.031	18.96^{c}±0.014	19.04^{b}±0.003	
Glycine	5.99^{a}±0.001	3.73^{b}±0.173	6.04^{a}±0.008	
Alanine	5.03^{b}±0.046	3.26^{c}±0.011	5.28^{a}±0.003	
Serine	2.89^{b}±0.066	2.09^{c}±0.000	3.39^{a}±0.034	

*Each value is the mean ± SE of three determinations. Means with different letters in each row are significantly different ($P<0.05$).

Al-Ban hydrolysate (1) = prepared by pepsin/pancreatin hydrolysis for 10 hrs.

Al-Ban hydrolysate (2) = prepared by trypsin/chymotrypsin hydrolysis for 10 hrs

The decrease in amino acid content after hydrolysis could be due to the enzymatic activity and/or conformational aspects that limit the enzymatic action during the hydrolysis process (Clemente et al., 1999). The increase in hydrophobic amino acids such as isoleucine, leucine and lysine (as shown in Table 3) is an advantage due to effects that these amino acids have on physical and functional properties of food proteins (Periago et al., 1998). However, the overall amino acid profile of Al-Ban hydrolysates was similar to that of the starting material, and the levels of essential amino acids of Al-Ban protein and hydrolysates met the amino acid requirements in comparison with reported reference pattern (FAO/WHO/UNU, 1985), except for lysine which is the first limiting amino acid in Al-Ban protein (Al-Housein and Abu-Tarboush, 1997).

Amino acid composition of karkade protein isolate as shown in Table (4) is comparable to that reported by Abu-Tarboush et al. (1997) for karkade protein isolate except for a decrease in lysine and glutamic acid and an increase in leucine contents in this study. Treatment of karkade protein by pepsin followed by pancreatin resulted in a significant reduction ($P \leq 0.05$) in the contents of some amino acids (as shown in Table 4). These losses could be due to the extensive enzyme activity or conformational changes as stated before, for karkade hydrolysate produced by trypsin + chymotrypsin hydrolysis some amino acid ratios increased such as threonine, glutamic acid, alanine and some amino acids encountered a slight decrease as in lysine, tyrosine, arginine and glycine, but the general amino acid profile of this hydrolysate was similar to the starting material. The levels of histidine, phenylalanine + tyrosine and leucine in karkade, isolate and hydrolysates (Table 4) met the requirements for these amino acids in the reference protein (FAO/WHO/UNU, 1985), while level of lysine, threonine, isoleucine and methionine (alone) did not meet the recommended levels, although Young and Pellett (1994) proposed tentative pattern for adult requirements that assigned 3.0, 1.5, 2.3 gaa/100 protein for lysine, threonine and isoleucine, respectively. In this case karkade protein and hydrolysates satisfied the requirements for these amino acids in the adults as proposed by Young and Pellett (1994).

Functional properties

Solubility:

Figure (4) demonstrates the pH-solubility curves of untreated and enzyme-hydorlyzed camel casein. Casein 1 and 2 referred to the hydrolysate produced by the action of pepsin followed by pancreatin and trypsin + chymotrypsin, respectively. The pH of minimum solubility for camel casein was at pH 4 (Figure 4), the solubility was less than 40% at pH 2 while the solubility was greater than 80% at and above pH6. It could be said that the solubility of untreated camel casein was minimum at the acidic range and maximum at the alkaline range. Solubility as stated by Murphy and Fox (1991) is a primary functional requirement of dairy proteins. They observed that the pH of minimum solubility for sodium caseinate and casein enriched fractions was at pH 3.5-4.5, and the solubility was greater than 90% above pH 5.5 for all samples.

Table 4: Amino acid profile of Karkade Protein Isolate and Karkade Hydrolysates (g amino acid/100g protein)

Amino acids	Karkade Isolate	Karkade hydrolysate (1)	Karkade hydrolysate (2)	Reference protein FAO/WHO/UNU (1985)
Essential				
Lysine	*4.16^{a}±0.003	3.98^{b}±0.020	3.93^{b}±0.049	5.8
Threonine	3.05^{a}±0.049	2.84^{b}±0.027	3.16^{a}±0.083	3.4
Valine	3.55^{a}±0.017	3.00^{b}±0.014	3.53^{a}±0.071	3.5
Methionine	1.54^{a}±0.006	1.10^{c}±0.057	1.37^{b}±0.014	
Methionine + Cystine				2.5
Isoleucine	2.69^{a}±0.008	2.43^{a}±0.125	2.66^{a}0.054	2.8
Leucine	7.23^{a}±0.037	7.28^{a}±0.031	7.36^{a}±0.150	6.6
Phenylalanine (p)	5.20^{a}±0.017	4.84^{b}±0.031	5.17^{a}±0.107	
Tyrosine (T)	3.31^{a}±0.038	2.91^{b}±0.014	2.92^{b}±0.104	
P+T				6.3
Histidine	2.66^{a}±0.003	2.37^{c}±0.020	2.57^{b}±0.024	1.9
Non Essential				
Arginine	12.61^{b}±0.167	13.53^{a}±0.122	10.30^{c}±0.072	
Aspartic acid	10.00^{a}±0.083	7.21^{b}±0.056	10.08^{a}±0.219	
Glutamic acid	19.36^{a}±0.063	19.18^{a}±0.101	19.44^{a}±0.118	
Glycine	3.91^{a}±0.020	3.87^{a}±0.020	3.79^{a}±0.072	
Alanine	4.67^{b}±0.011	3.30^{c}±0.014	4.84^{a}±0.060	
Serine	4.61^{a}±0.127	3.43^{b}±0.011	4.65^{a}±0.150	

*Each value is the mean ± SE of three determinations. Means with different letters in each row are significantly different (P<0.05).

Al-Ban hydrolysate (1) = prepared by pepsin/pancreatin hydrolysis for 10 hrs.

Al-Ban hydrolysate (2) = prepared by trypsin/chymotrypsin hydrolysis for 10 hrs.

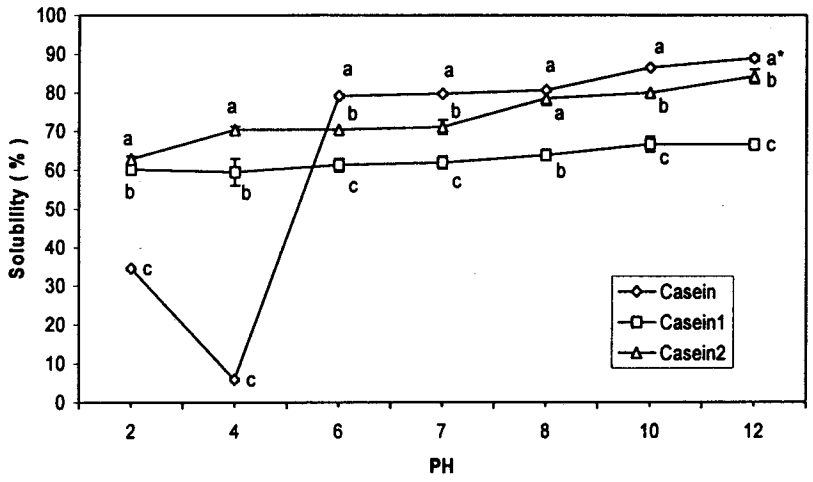

Fig.4. Protein solubility curves of camel casein, casein hydrolysate(1) treated by pepsin followed by pancreatin and casein hydrolysate(2) treated by trypsin + chymotrypsin.
* Unlike letters differ significantly (P<0.05)

Hydrolysis of camel casein with pepsin followed by pancreatin resulted in pH-solubility profile with almost similar solubility values at the pH range 2-12 (Figure 4). Compared to solubility curve of untreated casein, pepsin / pancreatin hydrolyzed casein, solubility was increased at pH 2 and pH 4, and decreased at pH 6 to pH 12. The solubility of casein hydrolysate produced by trypsin + chymotrypsin showed also an increase at the acidic range compared to unhydrolyzed camel casein. Improvement in protein solubility due to hydrolysis by different proteases has been reported (DeLaBarca et al., 2000; Mahmoud, 1994; Chan and Ma, 1999; Ortiz and Wanger, 2002; Periago et al., 1998; and Clemente et al., 1999). The enhanced solubility of the hydrolysates is due to their smaller molecular size and the newly exposed ionizable amino and carboxyl groups that increase the hydrolysates hydrophilicity (Mahmoud, 1994). Although some studies reported increase in solubility of hydrolysates over a wide pH range (Periago et al., 1998 and Clemente et al., 1999), in this study (Figure 4) the solubility of casein hydrolysates was only enhanced at the acidic range, while hydrolysates solubilities, showed a decrease compared to the intact casein at pH values more than 6. The soluble fractions of casein hydrolysates mainly composed of small free peptides, these peptides as described by Ortiz and Wanger (2002) would not be affected by pH gradient.

The solubility profiles of Al-Ban protein isolate and hydrolysates were shown in Figure (5). Compared to karkade protein isolate and casein in this study and to other plant sources (Al-Kahtani and Abu-Arab, 1993), Al-Ban protein isolate was less soluble particularly at the alkaline range. Hydrolysis of Al-Ban isolate by trypsin + chymotrypsin and pepsin followed by pancreatin (Figure 5) accompanied by improvement in solubility over a wide pH range except at the extremes (pH 2 and pH 12). This is due to the smaller molecular size of the peptides formed and the exposure of end groups as stated before. Solubility profile of Al-Ban hydrolysate produced by pepsin / pancreatin seemed to be pH-independent, according to degree of hydrolysis of this hydrolysate (29.9%) more free small peptides were expected to be formed. Mild hydrolysis of Al-Ban isolate by trypsin + chymotrypsin (DH = 7%) produced a hydrolysate with improved solubility compared to the intact protein over a wide pH range, and this hydrolysate is more soluble than pepsin / pancreatin hydrolysate at the alkaline pH range.

Karkade protein isolate (Figure 6) showed high solubility values particularly at pH 10 (89%) and pH 12 (88%) and minimum solubility at pH 4 (16.4%). These results were comparable to that of Abu-Tarboush et al. (1997). They stated that the good solubility of karkade protein isolate might contribute benificial functional properties. Improvemnt of solubility of karkade protein hydrolysates at acidic pH range compared to protein isolate (Figure 6) was also evidenced for camel casein hydrolysates and Al-Ban hydrolysates in this study. Hydrolysates increased solubility at the isoelectric point is often utilized in the supplementation of fruit drinks (having an acidic pH) with additional nitrogen to enhance their nutritional quality (Mahmoud, 1994).

Clarity:

Clarity profiles, expressed as absorbance values, of the intact proteins and their hydrolysates are shown in Figures 7, 8 and 9. Clarity or turbidity of a protein solution (turbidity) is a functional property that is related to solubility, viscosity and other physiochemical properties depending on its molecular size (Mahmoud, 1994). Casein hydrolysates (Figure 7) showed increased clarity values compared to the starting material, in this case the effect of pH was little for the two hydrolysates, while the affect of molecular size of the hydrolysates was very remarkable on clarity values. Casein hydrolysates can be considered clear at all the tested pHs.

Clarity values of Al-Ban protein isolate (Figure 8) were low over the pH range selected. This may be due the insoluble nature of the protein at the isolectric region. Clarity of Al-Ban hydrolysate produced by trypsin + chymotrypsin was lower because it contained less hydrolyzed poplypeptides (DH = 7.0%) when compared to the hydrolysate, produced by pepsin / pancreatin (DH = 29%). Both hydrolysates of Al-Ban protein were less clear than casein hydrolysates, this may due to the less hydrolyzed peptides present and the insoluble nature of the starting material.

The effects of pH and molecular size on the clarity value of karkade hydrolysates (Figure 9) were observable. Compared to clarity of unhydrolyzed karkade protein isolate.

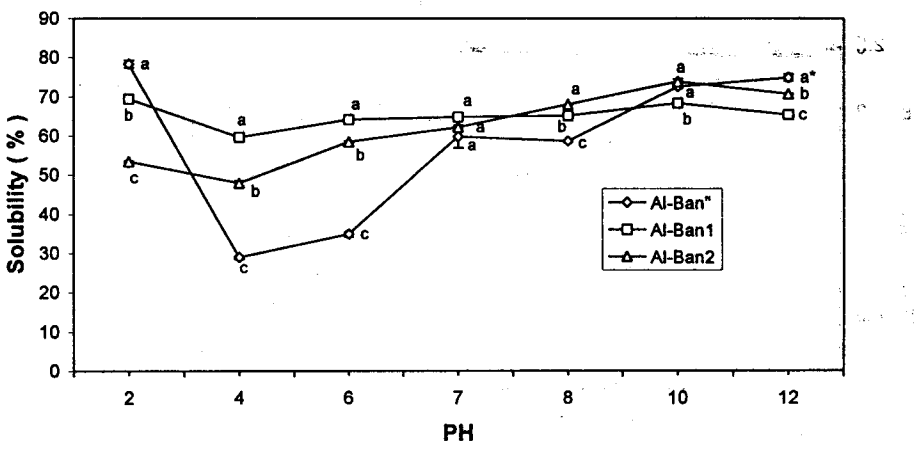

Fig.5. Protein solubility curves of Al-Ban protein Isolat, Al-Ban protein hydrolysate (1) treated by pepsin followed by pancreatin and Al-Ban protein hydrolysate (2) treated by trypsin + chymotrypsin .

*Unlike letters differ significantly (P<0.05)

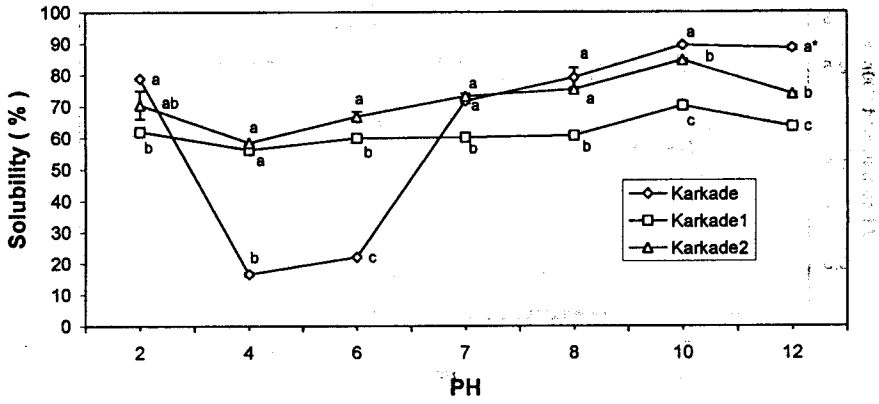

Fig.6. Protein solubility curves of Karkade protein Isolate , Karkade protein hydrolysate (1) treated by pepsin followed by pancreatin and Karkade protein hydrolysate(2) treated by trypsin + chymotrypsin.

*Unlike letters differ significantly(P<0.05)

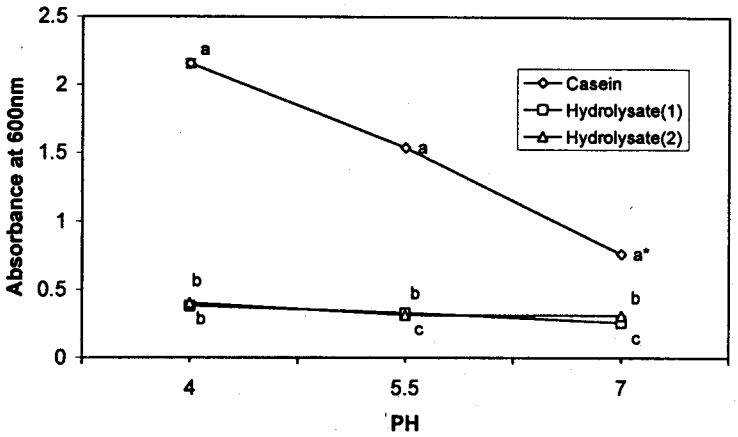

Fig.7. Clarity of Camel casein ,casein hydrolysate(1) treated by pepsin followed by pancreatin and casein hydrolysate(2) treated by trypsin + chymotrypsin.

* Unlike letters differ significantly (P<0.05)

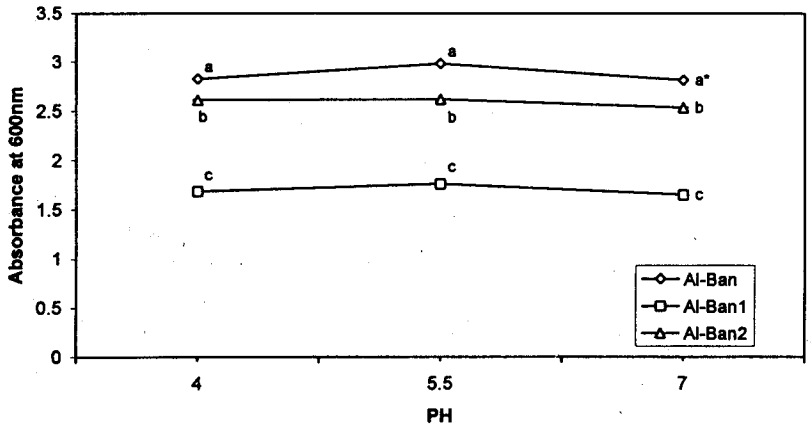

Fig.8. Clarity of Al-Ban protein Isolate , Al-Ban protein hydrolysate(1) treated by pepsin followed by pancreatin and Al-Ban hydrolysate(2) treated by trypsin + chymotrypsin.

* Unlike letters differ significantly (P<0.05)

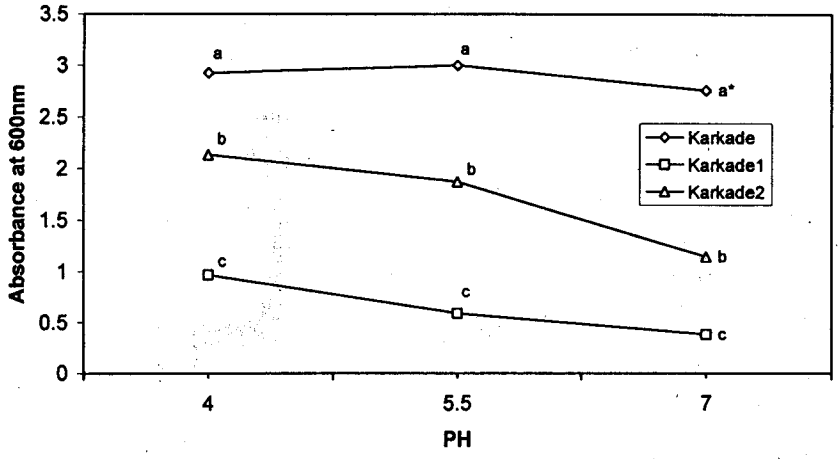

Fig.9. Clarity of Karkade protein Isolate , Karkade protein hydrolysate (1) treated by pepsin followed by pancreatin and Karkade hydrolysate (2) treated by trypsin + chymotrypsin.

* Unlike letters differ significantly ($P<0.05$)

Karkade hydrolysate produced by pepsin followed by pancreatin was more clear than karkade hydrolysate produced by trypsin + chymotrypsin, this may due to the presence of more hydrolyzed peptides in pepsin followed by pancreatin hydrolysate (DH = 42%). A good correlation between clarity or turbidity and solubility was obtained in this study, for casein and its hydrolysate, r= -0.986, for Al-Ban and hydrolysates r= -0.882 and for karkade and karkade hydrolysates the correlation coefficient (r) between clarity and solubility was −0.777. Casein hydrolysates and karkade hydrolysates produced by pepsin / pancreatin action, because of their solubility and clarity at low pH, could be used as stated by DeLaBarca et al. (2000) in sparkling or carbonated fortified beverages.

Water absorption capacity

Figure (10) shows water absorption capacities of camel casein, Al-Ban isolate and karkade isolate and their hydrolysates. Camel casein showed low water absorption capacity (0.4 ml/g). No data was available from the literature on water absorption of camel casein. Water absorption capacity of Al-Ban protein isolate in this study (2.0 ml/g) was comparable to that of Al-Kahtani and Abu-Arab (1993). Karkade protein isolate had water absorption capacity equal to 2.17 ml/g protein, Abu-Tarboush, et al. (1997) reported 2.47 ml/g as water absorption of karkade isolate. The degree of water retention is considered to be useful as an indication of performance in several food formulations (Circle and Smith, 1972).

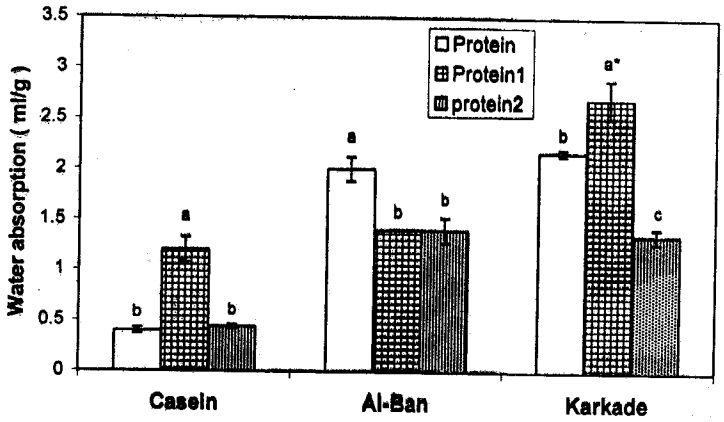

Fig.10.Water absorption of Camel casein,Al-Ban isolate,Karkade isolate and the proteins hydrolysates(protein1 treated by pepsin followed by pancreatin; protein2 treated by trypsin+chymotrypsin)
*Unlike letters differ significantly($P<0.05$)

Casein and karkade hydrolysates produced by pepsin followed by pancreatin hydrolysis (Figure 10) showed a significant increase ($P\geq0.05$) in water holding capacity compared to unhydrolyzed proteins. Water absorption capacity of proteins may be affected by conformation and environmental factors (Kinsella, 1976). Kinsella (1976) explained that conformational changes in the protein molecules may expose previously enclosed amino acid side chains, thereby making them available to ineract with water. Since proteolytic modification encounter protein conformational changes, the improvement in water absorption capacity of casein and karkade hydrolysates may be due to improvement in water uptake by hydrolysates since this is related to the liberation of amino and carboxyl groups (Chan and Ma, 1999). The lower water absorption capacities of Al-Ban hydrolysates (Figure 10) compared to the starting material may be due the low true protein content of the hydrolysates, since water absorption capacity depends on the protein content (Periaga et al., 1998).

Oil absorption capacity:

Oil absorption capacities of camel casein, Al-Ban isolate, karkade isolate and their protein hydrolysates, were shown in Figure (11). Oil absorption is mainly attributed to the physical entrapment of oil, it is an important functional property in food products because it improves mouth feel and flavour retention (Kinsella, 1976). Camel casein had remarkable high oil absorption capacity (5.76 ml/g) as shown in Figure (11). Casein hydrosates showed lower oil absorption capacity compared to unhydrolyzed casein. Lorenzen (2000) reported that oil binding capacity of sodium caseinate decreased slightly by enzymatic treatment, this may be due to the reduction of the number of nonpolar side chains on proteins which, as stated by Kinsella (1976), bind hydrocarbon chains on the fatty acids.

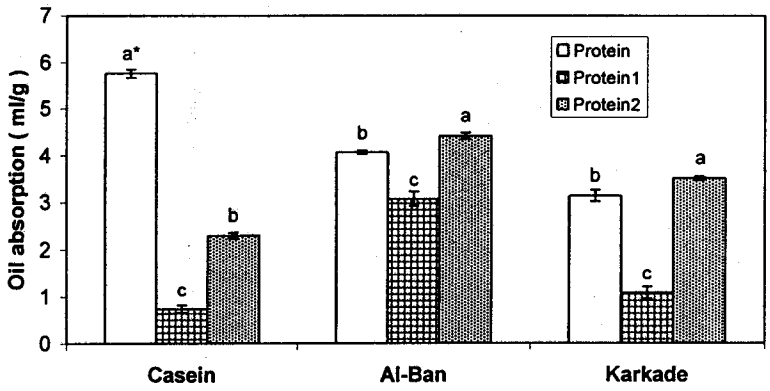

Fig.11. Oil absorption of Camel casein,Al-Ban isolate,Karkade isolate and the proteins hydrolysates (protein1 treated by pepsin followed by pancreatin; protein 2 treated by trypsin + chymotrypsin) .

*Unlike letters differ significantly ($P<0.05$)

Oil absorption capacity of Al-Ban protein isolate in this study was found to be 4.07 ml/g protein. Al-Kahtani and Abu-Arab (1993) reported a lower value for Al-Ban isolate oil absorption which was 2.4 ml/g. Oil absorption capacity of karkade protein isolate (Figure 11) was slightly higher than that reported by Abu-Tarboush et al. (1997) which was 2.77 ml/g. Al-Ban and karkade hydrolysates, produced by trypsin + chymotrypsin hydrolysis (Figure 11), showed significantly ($P\geq0.05$) higher oil absorption capacities compared to the unhydrolyzed proteins. The increase in oil absorption capacity may be attributed to the increase in the amino acids during enzymatic treatment, since lipid binding depends on the surface available of hydrophobic amino acids (Periago et al., 1998).

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خصائص البروتينات المحللة إنزيمياً المحضرة من كازين الإبل ومعزولات بروتين بذرة ألبان (*Moringa peregrina*) وبذرة الكركدي (*Hibiscuc sabderiffa*)

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ص.ب ٢٤٦٠- الرياض ١١٤٥١- المملكة العربية السعودية

الملخص: أجري التحليل الإنزيمي لكازين الإبل ومعزولات بروتين بذرة ألبان والكركدي كما تم تقدير الخصائص الوظيفية لهذه المحللات. درجة التحلل لكل العينات باستخدام إنزيمات البيسين المتبوعة بالبنكرياتين كانت أعلى من درجة التحلل باستخدام التربسين والكيموتربسين. قدرت نسب الأحماض الأمينية في البروتينات التي تم دراستها ومحللاتها الإنزيمية أيضاً. التركيب العام للأحماض الأمينية في محللات البروتينات يشابه ذلك الموجود في البروتينات الأصلية عدا بعض التغيرات الطفيفة. مستويات الأحماض الأمينية الأساسية في البروتينات الأصلية ومحللاتها البروتينية أستوفى بمتطلبات هذه الأحماض مقارنة بالبروتين المرجعي عدا اللايسين في بروتين ألبان واللايسين والثيرونين والأيزوليوسين في بروتين الكركدي. قابلية الذوبان للمحللات الثلاث ارتفعت مقارنة بالبروتينات غير المحللة. كازين الإبل وبروتين الكركدي المحللة إنزيمياً أظهرت انخفاضاً في قيم العكارة في حين أن بروتين ألبان المحلل إنزيمياً كانت له قيم عكارة عالية. الكازين وبروتين الكركدي المحللة بإنزيمات البيسين المتبوع بالبنكرياتين لها قيم قدرة على الاحتفاظ بالماء مرتفعة معنوياً مقارنة ببروتيناتها غير المحللة. وفي المقابل فإن بروتينات ألبان والكركدي المحللة بخليط إنزيمات التربسين والكيموتربسين لها قيم قدرة على الاحتفاظ بالزيت مرتفعة معنوياً مقارنة ببروتيناتها غير المحللة إنزيمياً.