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Antioxidant and Antimicrobial Activities of Enzymatic Hydrolysates of Camel's Milk Whey Protein and Casein

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ABSTRACT

Healthy skimmed camel milk's, casein and whey proteins precipitated and freeze dried, and were treated with 1% of five proteolytic microbial renin, pepsin, trypsin, collagenase and microbial protease. Hydrolysates were analyzed for pH, degree hydrolysis and scavenging properties. The pH decreased, and the degree of protein hydrolysis increased reaching 16.22, 17.0, 22.12, 22.88, 15.85, 16.23, 15.68, 16.08, 23.75, 24.44 for casein and whey after 8 hours of By increasing the proteolysis The inhibition % of DPPH increased by increasing the proteolysis to 21.4, 22.9, 20.18, 25.2, 23.2 for casein hydrolysate and 34, 31, 30, 30, 28.5 for whey proteins hydrolystes after 8 hours at 37° C. Whey proteins resulted in higher scavenging properties than casein under the same conditions. Both casein and whey protein hydrolysates had a significant antibacterial properties, against undigested or digested protein. The highest antibacterial activity The undigested whey proteins and collagenase digested whey protein and microbial protease digested casein characterized with the highest antibacterial activities (18.0, 17.0 and 20.0 mm) for *Staphylococcus aureus, Streptococcus pyogenes*, and *Escherichia coli* respectively. All of the digested enzymes were of antifungal effect against *Aspergillus flavus* and *Aspergillus fumigates*, but of no effect against *Aspergillus niger*. Casein was found on antifungal against *Aspergillus flavus*. Only undigested casein, collagenase and trypsin digested casein were also found of anti-fungal activity against *Aspergillus niger*, which recorded 9.0, 8.0 and 8.0. respectively.

keywords: casein , whey protein, camel milk, antimicrobial , antioxidant.

INTRODUCTION

The one-humped camels (Camelus dromedarius) are well-known producers of milk which differs from bovine milk in the composition and structure of its protein content and are thus of different functional and medicinal properties. Casein fractions of camel milk are α-, β- and κ-CN constitutes about 65, 21 and 3.47%. respectively, of total caseins (Camel milk is similar to human milk as it contains a high amount of β-CN; this can reflect the high digestibility and low sensitivity in infants, since β-CN is more sensitive to gastrointestinal degradation than α-CN (El-Agamy *et al* 2009). from the estimated molecular mass CN-CN and α-CN in camel milk using SDS-PAGE technique are 28.6 kD and 35 kD respectively, and are higher than those in milk Cows. The molecular weight of α-lactalbumin from camel milk is 14.6 kDa, and it contains 123 amino acids which is similar to those in cow's milk, humans and goats. Peptides derived from milk proteins have been shown to perform various functions such as antioxidant activities, anticancer and antihypertensive (ACE) activities, opioid activities, mineral binding, growth stimulation and antimicrobial activities;Meisel 2005;).Consequently, casein may play important biological functions after decomposition with different proteases. The enzymatic hydrolysis of casein produce specific peptides exert bioactivety which reduce the risk of heart disease, diabetes and cancer (Beg *et al*. 1985, Mohammad 1993, Farah 1996, Clare and Swaisgood 2000, Rival *et al*. 2001, Kappeler *et al*. 2003; Aimutis 2004, Meisel, 2005 Chen *et al*. (1998 and Chen *et al*. (1998)

The antioxidant properties of the bio-active peptides are attributed to their composition, structure and hydrophobicity as well as position of amino acid residue, and the molecular weight. The bioactivity of peptides obtained from camel milk casein has not been extensively studied so far, so it needs a lot of research in the future. There is a high degree of protein degradation of camel casein from that in casein cows by treatment with pancreatic enzymes.

Antimicrobial, radical-scavenging and angiotensin 1converting enzyme inhibitory (ACE) activities of camel milk have camel casein hydrolysate by pepsin and pancreatin could have significant therapeutic attributes such as anti-cancer and anti-diabetic properties. Caseins and whey proteins considered a good source of bio-active peptides. The precursor protein sequence contains peptides in a latent state which can be released by enzymatic proteolysis Hernández-Ledesma *et al* (2007). Once bioactive peptides are released, they may act as regulatory compounds in the host organism with specific activities such as antihypertensive, antioxidant, antimicrobial or opioid. Search for milk-based bioactive peptides has been focused until now mainly on bovine and to smaller extent on ovine and caprine milk proteins. Therefore, this investigation was conducted to produce casein and whey protein hydrolysates from camel milk using specific proteolytic enzymes from different sources to estimate the antioxidant and antimicrobial properties to use in further investigation as a food additives or natural food preservations of the resultant hydrolysates (Chen *et al*. 1995, Korhonen *et al* 2001, Agrawal *et al*. 2003; Magjeed 2005, Hernández-Ledesma *et al* 2007., Li *et al*. 2008, Salami *et al*. 2008 and Jrad *et al*. 2014)

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MATERIALS AND METHODS

Microbial rennin (EC: 3.4.23.4), pepsin (EC 3.4.23.1), trypsin (EC: 3.4.21.4)), collagenase (EC:3.4.24.3) and microbial protease (EC: 3.4. 21-24) were obtained from Sigma–Aldrich Chemical Co., India (M P Biomedicals, India). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtain from Sigma–Aldrich Chemical Co. India. All solutions, prepared with double-distilled water and kept at 4°C before further use.

Antimicrobial activity of the protein hydrolysate of camel casein and whey protein was determined against three bacterial strains, namely *Staphylococcus aureus, E.coli and Staphylococcus aureus* from Animal & Environmental Hygiene, Fac. of Vet. Medicine, Assiut University, while *Aspergillus fumigatus, Aspergillus flavus*and *Aspergillus niger* were isolated from dairy products and identified at plant and microbiology department Fac. of Sci. Assiut university. The organisms were periodically subcultured and maintained in nutrient agar slant at 4°C.

Casein and whey protein powder preparation:

The one-humped healthy female Camels (Camelus dromedarius) located Marsa Matrouh farm, milk samples was obtained. The milk samples kept in closed ice box at 5^0 C., and transfered to the laboratory at the same day of milking. The milk samples were centrifugated at 3000 × g for 10 min at 4°C. The pH of the defatted milk was adjusted to 4.6 using 1.0 N HCl to precipitate the whole caseins. The obtained supernatant was adjusted to pH 7.0 with 1.0 N NaOH and re-centrifugated at 10,000 × g for 30 min at 4°C. The resultant supernatant after is used for precipitation of the whole whey proteins by salting out using ammonium sulphate and dialysis against distilled water to remove the rest of ammonium sulphate (750 g./1 L of liquied whey) .Both of the caseins and whey proteins concentrates were freeze-dried and stored in a desiccator until farther analyses.

Total nitrogen contents of the camel milk samples were estimated in triplicate using Kjeldahl procedure according to AOAC (1995). Total protein content was calculated as N × 6.38.

Enzymatic hydrolysis of CMCP and CMWP in phosphate buffer at 5% total solid and pH adjusted to (6.5 for microbial rennet ,2.5 for,pepsin and 7.4 for trypsin, collagenase and microbial protease) casein and whey protein separated from camel milk were dissolved. The CMCP and CMWP solutions heated in water path for 5 min to kill the microorganisms, which may produce proteolytic enzymes during the proteolysis process, to denature the indigenous enzymes of milk and denature the proteins, which increases its susceptibility to proteolytic enzymes. The enzyme/substrate ratio (E:S ratio) was kept constant (1:100) for all the enzymes. The hydrolysis was carried out by incubating the samples at 37 °C for in stirred water bathand samples were drawn when fresh and after, 2, 4, 6 and 8 hour of incubation. The hydrolyzed sample heated at 85 °C for 15 minin water bath, then cooled immediately, and centrifuged under cooling at 10,000 rpm for 25 min; and the supernatants were collected and stored at −20 °C until further analysis.

The pH of hydrolysate samples was measured using combined glass electrode of Mettler Toledo pH meter (Model FiveEasyTM plus FEP 20, Switzerland). The pH of each sample was measured just before heating to inactivate the residual enzyme.

Degree of proteolysis (DH) of casein and whey protein was estimated by detecting of solubilized protein in 10% (w/v) trichloroacetic acid (TCA), compared to the total protein content of the sample according to Hoyle and Merritt 1994 and Devendra Kumar1 *et al* 2016). The DH was calculated according to the equation:

DH (%) = [Solubilised protein content in 10% TCA (mg)/ Total protein content (mg)] x 100.

The ability to scavenge DPPH radical by added antioxidants in samples was estimated according to the method of Brand-Williams *et al*. (1995) and modified byDevendra Kumar *et al* (2016).

Scavenging activity (% inhibition) = 100-[(At20/At0) x 100].

Where: asabsorbency in time t=20 min (t20) and time t= 0 min (t0).

Antibacterial activity was conducted for five different enzymes for both casein and whey protein after proteolysis for 6 hours at 37C° at the suitable pH by agar disc diffusion method adopted by Mounyr *et al* (2016) against four bacteria and four molds *Staphylococcus aureus* (MTCC 3160) and*Pseudomonas aeruginosa* (MTCC 424).

Statistical analysis was conducted in triplicate; data were expressed as means with standard deviation.

RESULTS AND DISCUSSION

Results presented in Table (1) show slight decrease in pH of both CC and CWP hydrolysates with the advancement of hydrolysis. it was also observed that the deceasing rate depends on the protein substrate and the intial pH. Comparing to pepsin, the rate of pH decrease was lower for micrbial protease. Rate of pH decrease was higher in case of whey protein hydrolysate. The decreasing was also higher in the first 4 hours, and then tends to be persistence to some extent.

Table 1. pH measurements of casein and whey protein at different enzymatic hydrolysis.

Time / hours.	pH measurements									
	MR		PEP		TRY		COLL		MP	
	CCH	CWPH	CCH	CWPH	CCH	CWPH	CCH	CWPH	CCH	CWPH
0	6.5± 0.24	6.5± 0.25	2.5± 0.24	2.5± 0.25	7.4±0.24	7.4±0.25	7.4±0.25	7.4± 0.25	7.4±0.24	7.4± 0.25
2	6.4± 0.04	6.3±0.14	2.3± 0.20	2.3± 0.02	7.39± 0.11	7.35±0.23	7.34±0.04	7.32± 0.00	7.31±0.11	7.30± 0.22
4	6.39± 0.01	6.35±0.00	2.1± 0.10	2.2± 0.08	7.38± 0.08	7.32±0.01	7.24±0.44	7.20± 0.43	7.28±0.14	7.26± 0.04
6	6.35± 0.02	6.2±0.14	2.00± 0.24	2.00± 0.03	7.37± 0.09	7.30±0.02	7.23±0.07	7.19± 0.00	7.22±0.11	7.18± 0.12
8	6.35± 0.00	6.30± 0.04	2.00± 0.10	1.99± 0.17	7.37± 0.22	7.30± 0.04	7.23±0.00	7.19± 0.12	7.21±0.08	7.15± 0.06

MR: microbial rennet
PEP: Pepsin TRY: Trypsin COLL: Collagenase
MP: Microbial protease CH : casein hydrolysate. WPH: whey protein hydrolysate

Change in pH during hydrolysis may not only affect the enzyme structure, but also the occurrence of the changes in the structure or properties of the substrate ,which took place in the enzyme-substrate binding and thereby hydrolysis. To avoid sharp change and rapid decline of pH , phosphate buffers of specific pH for each enzymes were used. This decrease might be attributed to release of protons into hydrolysis medium, which results in the reduction in the pH as reported by Ovissipour *et al*, 2013, Daroit *et al* 2012, and Kumar *et al* 2016)

Data presented in Table (2) illustrate the content of soluble peptide released from crude protein during the proteolysis using different five proteolytic enzymes. DH (%) increased with the increase in hydrolysis time, however, after 6h of hydrolysis, the DH% increased slowly and after 8h of hydrolysis it became static. This might be due to the decreased availability of cleavable peptide bonds within the substrate as well as the changing of surrounding medium. Adler-Nissen (1986) attributed the reduction in hydrolysis rate due to the competition between unhydrolysed protein and the peptides being constantly formed during hydrolysis. The reduction of hydrolysis rate in latter hours might also be due to decrease in pH of the medium, which might cause denaturation of protein structure of the enzyme or the disturbances of the ionic character of the substrate, would in turn affect enzyme-substrate binding. The microbial protease treated casein and whey protein showed higher DH% for all time intervals as compared to other enzymes, followed by pepsin with mean values of 23.75 ± 0.05, 24.44 ±0.07, 22.12 ± 0.09, 22.88 ±0.00 for casein hydrolysate and whey protein hydrolysate respectively.on the other hand the lowest HD% was obtained by using microbial rennet which recorded 16.25 ±2.04 and 17.00 ± 1.34 of casein and whey protein hydrolysate after 8 hours of hydrolysis.The highest levels of DH% obtained with both microbial protease and pepsin suggested that this enzyme has more affinity for the substrate and thus more efficient than the others enzymes for the production of protein hydrolysates of camel milk peroteins. Similar results were also reported by Graszkiewicz *et al* 2010, Lira *et al* 2010, and Kumar *et al* 2016) .From these data it was noticed that the DH% after 8hours of hydrolysis did not increase significantly. This might be attributed to enzyme specificity which could not further hydrolyse the remaining bonds within the generated peptides.

Table 2. Degree of hydrolysis (mean±SE) of CMCP and CMWP:

Time / hours.	Degree of hydrolysis									
	MR		PEP		TRY		COLL		MP	
	CH	WPH	CH	WPH	CH	WPH	CH	WPH	CH	WPH
0.00	0.45±0.01	0.21± 0.04	0.45±0.01	0.21±0.04	0.45±0.01	0.21±0.04	0.45±0.01	0.21±0.04	0.45±0.01	0.21±0.04
2.00	8.22±0.27	9.14± 0.28	11.8± 0.03	12.04±0.88	7.87± 0.07	7.56±0.03	6.48±0.01	6.75±0.07	12.4± 0.05	12.51±0.08
4.00	13.99±0.04	14.35±0.04	18.62± 0.04	19.21±0.04	13.24± 0.04	13.88±0.04	11.22±0.04	12.11±0.04	19.87± 0.04	20.11±0.04
6.00	15.55±1.02	16.21± 0.90	21.11± 0.03	22.03±0.33	14.99± 0.12	15.32±1.01	14.78±1.08	15.23±0.90	23.02± 0.07	23.57±0.88
8.00	16.25±2.04	17.00± 1.34	22.12± 0.09	22.88±0.00	15.85± 1.04	16.23±2.00	15.68±1.14	16.08±0.09	23.75± 0.05	24.44±0.07

MR: microbial rennet PEP: Pepsin TRY: Trypsin
COLL: Collagenase MP:Microbial protease CH : Camel casein hydrolysate WPH: Camel whey protein hydrolysate

Data presented in Figs. (1 and 2) by using different proteolytic enzymes in hydrolysis lyophilized camel whey protein isolate and camel casein, to evaluate the antioxidant properties through using DPPH scavenger. It could observed that there was a significant increase in DPPH activity with the progress in hydrolysis time, and a positive relationship between hydrolysis time and DPPH activity could be noticed. Both of camel casein and camel whey prtein hydrolysates produced by all of the examined 5 enzymes resulted in an increase in the DPPH-scavenging activity upto 6h of hydrolysis period.

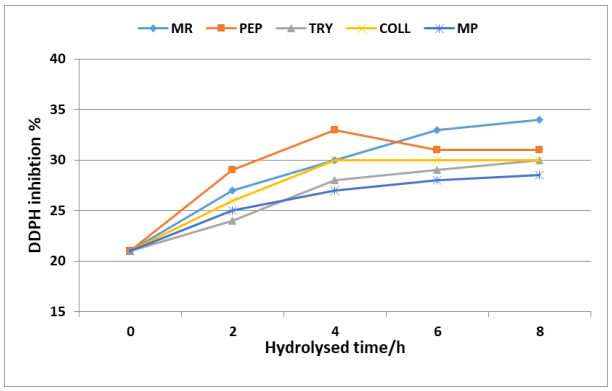


Fig.1. DPPH-scavenging activity of camel whey proteinat different enzymatic hydrolysis.

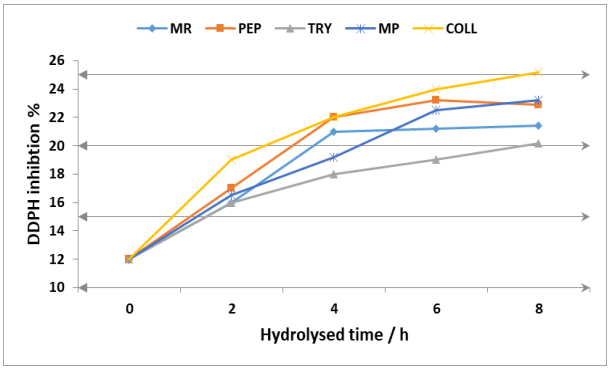


Fig. 2. DPPH-scavenging activity of camel casein hydrolysate at different enzymatic hydrolysis.

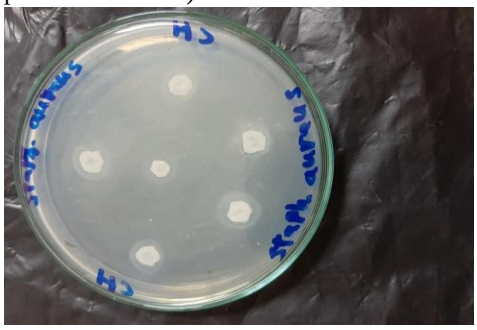
As compared to other 4 enzymes, the pepsin produced hydrolysates which had higher antioxidant activity at 4h of hydrolysis and it remained higher up to 8th h of hydyolysis, except for the microbial rennet which exhibited higher value of DPPH activity after 6 hours of hydrolysis time. However, the camel casein hydrolysates produced by all enzymes showed slight or no increase in DPPH-scavenging activity. While in case of camel whey protein as shown in Fig (2) the collagenase had the higher antioxidant properties (DPPH scavenging activety %) flowed by pepsin enzyme, and the trypsin enzyme was the lowest antioxidant properties.

All enzymes used in this study showed no antioxidant properties after 7 hours of hydrolysis time. From the DPPH-scavenging activity, it could be hypothesised that both hydrolysed camel casein and camel whey protein contain some electron donating substances that could interact with free radicals, making them more stable molecules and stopping the radical chain reaction. The increase in DPPH radical scavenging activity of camel milk protein hydrolysates was in agreement with results obtained by Mao *et al* 2011, Thiansilakul *et al* 2007 and Khantaphanta *et al* 2011).

Antimicrobial activity:

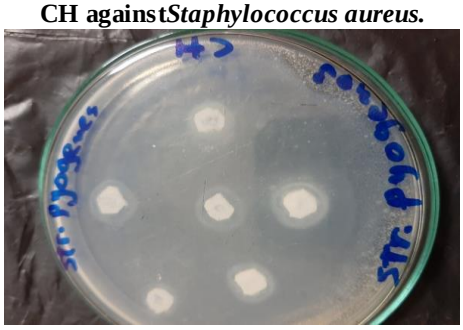
Five different proteolytic enzymes used to produce casein and whey protein hydrolysates which examined for antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*.

Data presented in Figs. (3) and Table (3) show that both casein hydrolysate and whey protein hydrolyste were of positive effect of antibacterial activity with the three examined bacterial strains.

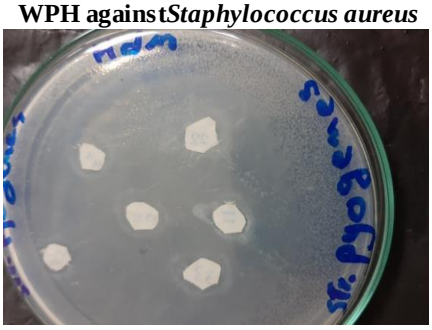


CH against*Staphylococcus aureus.*

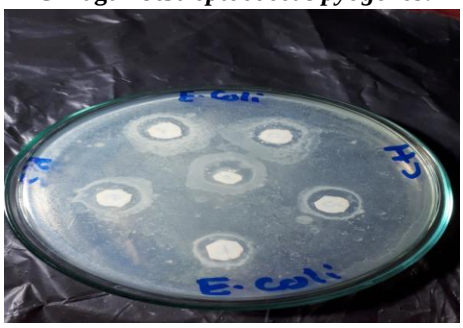
WPH against*Staphylococcus aureus*



CH against*Streptococcus pyogenes*.



WPHagainst*Streptococcus pyogenes*



CH against*Escherichia coli*.

WPHagainst*Escherichia coli*

Fig. 3. Antibacterial activity of camel casein and whey protein hydrolysate at different enzymatic hydrolysis.

Table 3 . Antibacterial Activity of five different enzymes digested protein hydrolysates of CMWP and CMCP.

	Bacterial inhibition zone in mm.											
	Control		**MR**		**PEP**		**TRY**		**COLL**		**MP**	
	CH	**WPH**	**CH**	**WPH**	**CH**	**WPH**	**CH**	**WPH**	**CH**	**WPH**	**CH**	**WPH**
Staphylococcus aureus	10.0	18.0	9.0	10.0	11.0	10.0	8.0	9.0	10.0	10.0	11.0	12.0
Streptococcus pyogenes	11.0	11.0	11.0	9.0	13.0	11.0	12.0	10.0	12.0	17.0	10.0	9.0
Escherichia coli	20.0	13.0	13.0	11.0	15.0	10.0	13.0	13.0	13.0	12.0	20.0	13.0
	Fungal inhibition zone in mm.											
	Control		MR		PEP		TRY		COLL		MP	
	CH	WPH	CH	WPH	CH	WPH	CH	WPH	CH	WPH	CH	WPH
Aspergillus niger	9.0	NE	CH	WPH	NE	NE	CH	WPH	8.0	NE	NE	NE
Aspergillus flavus	0.0	19.0	NE	NE	NE	10.0	8.0	NE	NE	11.0	NE	12.0
Aspergillus fumigatus	10.0	14.0	NE	11.0	9.0	12.0	NE	8.0	7.0	18.0	8.0	11.0

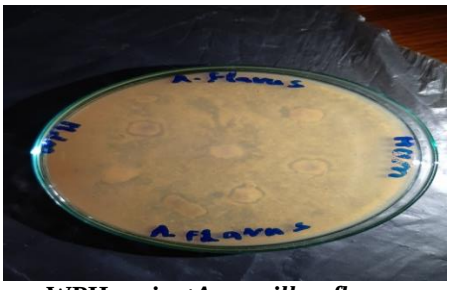
MR: microbial rennet PEP: Pepsin TRY: Trypsin COLL: Collagenase
MP:Microbial protease CH: Camel casein hydrolysate . WPH:Camel whey protein hydrolysate NE: No effects

The undigested whey protein resulted in a halo of 18mm against *S.aureus*, followed by the collagenase digested whey protein hydrolysate with a halo of 17.0mm against *Str. pyogenes*. While in case of casein hydrolystes it

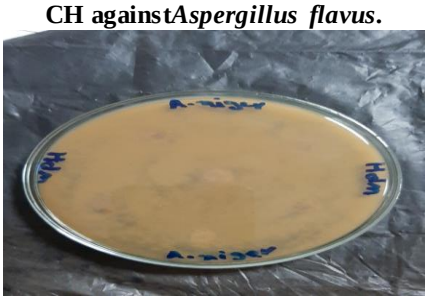
was observed that, the undigested camel casein and microbial protease digested casein were of higher antibacterial effects with a halo of 20.0 mm.) against *Escherichia coli* flowed by pepsin digested casein with a halo of (15.0 mm). Fom these results it could be clouded that, both undigested casein and undigested whey protein had the highest antibacterial effects than the enzymatic digested proteins. A slight decreasing in the antibacterial activity of digest proteins may be attributed to the degradation of native milk protein polypeptide structure which had a high antibacterial activity. These findings were in accordance with Memarpoor-Yazdi *et al*. 2012 and Najafian and Babji, 2012 who stated that, the amino acid sequence is closely related to the antimicrobial activity against both Gram-positive and Gram-negative microorganisms.

Regarding to the antifungal activity of both casein and whey protein hydrolystes Table (3) and Fig (4) showed that both of casein hydrolysate and undigested casein had no effect against *Aspergillus flavus*, while the whey protein whether digested or hydrolysates had a significant antifungal activity properties.The undigested whey protein had the highest antifungal activity with measured halo (19.0 mm) followed by digested microbial protease whey protein with halo of 12.0 mm.From Table (3) it could also be noticed significant antifungal effects of both casein and whey protein against *Aspergillus fumigates*, collagenase digested casein, which recorded the largest halo diameter of 18.0 mm, while the smallest halo diameter of 7.0 mm. was recorded for microbial rennin digested whey protein. Lastly, the antifungal properties of both casein and whey protein hydrolyastes against *Aspergillus niger* was noticed as a positive effects only in case of undigested casein , collagenase digested casein and trypsin digested casein with halo diameter of 9.0, 8.0 and 8.0 mm respectively. The protein hydrolysates which cotain cationic acids amino acids in their composition will show higher antimicrobial activity)Najafian and Babji (2012).

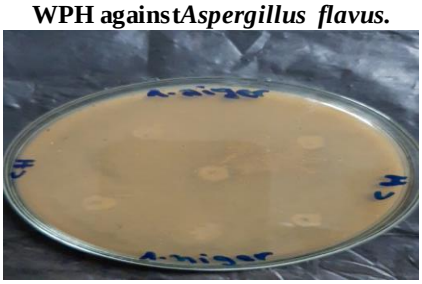
CH against*Aspergillus flavus*.



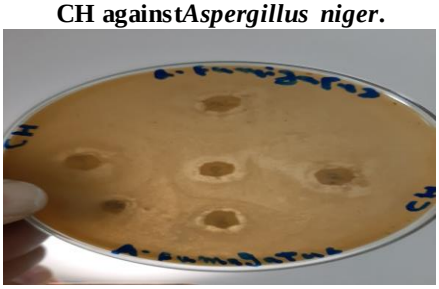
WPH against*Aspergillus flavus*.



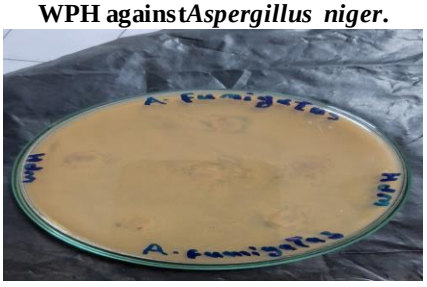
CH against*Aspergillus niger*.



WPH against*Aspergillus niger*.



CH against*Aspergillus fumigatus*



WPH against*Aspergillus fumigatus*

Fig. 4. Antifungal activity of camel casein and whey protein hydrolysate at different enzymatic hydrolysis

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الخواص المضادة للأكسدة والمضادة للميكروبات لبروتينات شرش وكازين لبن الجمال المحللة إنزيمياً

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في هذة الدراسة تم نزع الدهن من لبن النوق المأخوذ من انثي سليمة وتم فصل الكازين وكذلك بروتينات الشرش تجفيدهم وبعد ذلك تم معاملة كل من الكازين وبروتينات الشرش بخمس انزيمات محللة للبرويتن أنزيم الرينين الميكروبي و البيبسين و التربسين و الكولاجينيز و البروتيز الميكروبي بنسبة ١% وتم دراسة تلك الإنزيمات علي كل من درجة الحموضة وكذلك درجة تحلل البروتين وأخيرا خواص البرويتن المضادة للأكسدة وأوضحت النتائج أن باضافة الانزيمات المحللة للبروتين لكل من الكازين وبروتينات الشرش الي إنخفاض درجة الحموضة انخفاضات بسيطة ...كما ادي معاملة البروتينات بانزيمات محللة للبروتين الي زيادة درجة تحلل البروتنيات والتي بلغت ١٦,٢٢, ١٧,٠, ٢٢,١٢, ٢٢,٨٨, ١٥,٨٥, ١٦,٢٣, ١٥,٦٨, ١٦,٠٨, ٢٣,٧٥, ٢٤,٤٤ لكل من الكازين وبروتينات الشرش بعد ٨ ساعات من التحلل البروتيني علي التوالي. كما اوضحت النتائج خصائص كل من الكازين وبروتينات الشرش زادت بزيادة درجة التحللالبروتيني والتي بلغت درجة تثبيط مادة DPPHبلغت ٢١,٤, ٢٢,٩, ٢٠,١٨, ٢٥,٢, ٢٣,٢ للكازين المتحل بينما بلغت ٣٤, ٣١, ٣٠, ٣٠, ٢٨,٥ لبرويتنات الشرش المتحللة بعد ٨ ساعات علي درجة ٣٧ م كما لوحظ أن برتينات الشرش لها خصائص مضادة للاكسدة أعلي من الكازين المعامل بنفس الظروف . كما قامت الدراسة بدراسة الاثر المضاد لكل من البكريا والفطريات ودلت النتائج علي ان جميع البكتيريا المستخدمة تاثُرت تاثراً ملحوظا سواء بإستخدام الكازين الغير معامل بالإنزيمات أو بروتينات الشرش الغير معاملة بالانزيمات , وكان أعلي نشاط مضاد للبكتيريا لبروتينات الشرش الغير متحل ' وبروتينات الشرش المعاملة بانزيم الكولاجينيز , والكازين المعامل بانزيم البروتيز الميكروبي (١٨,٠, ١٧,٠ و ٢٠,٠ مم) لكل من *Staphylococcus aureus* و *Streptococcus pyogenes* و *Escherichia coli* علي التوالي . كما أوضحت النتائج أن كل من ....بروتينات الشرش المعاملة بالانزيمات كانت لها تاثيرا مضادا للفطرياتفي مواجهة كل من*Aspergillus flavus* و *Aspergillus fumigates* بينما لم يكن لها أي اثر يذكر علي فطر *Aspergillus niger* كما اوضحت النتائج ان كازين لبن الجمال كان لة اثرا مضادا لفطر *Aspergillus fumigates* ولم يسجل اي أثر مضاد في حالة فطر *Aspergillus flavus* وعلي العكس من ذلك أظهر كل من الكازين الغير معامل و الكازين المعامل بانزيم البيبسين و التربسين خواصاً مضادة لفطر *Aspergillus niger* والتي بلغت ٩,٠, ٨,٠, ٨,٠ مم علي التوالي.