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Antioxidant and Antimicrobial Activities of Enzymatic Hydrolysis Products from Sunflower Protein Isolate

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Abstract: The goal of the present work was to prepare bioactive hydrolysates and peptides from sunflower protein isolate (free of chlorogenic acid). To reach this goal the protein isolate was hydrolysed using several enzymes and enzyme mixtures, including: pepsin, trypsin, chymotrypsin and a mixture of the three enzymes (mixture I); also alcalase, flavourzyme and a mixture of the two enzymes (mixture II). These enzymes were used at 2% concentration and at pH and temperature reported by the manufacturer. During hydrolysis, at certain time intervals, 30, 60 and 120 min. aliquots were withdrawn from the reaction mixture to give peptide fractions and at the end of 3h give hydrolysate. The peptic fractions exhibited prooxidant activities at 30, 60 and 120 min. Other hydrolysis products revealed moderate antioxidant activity (AOA). Tryptic peptide at 60 min. hydrolysis showed the highest AOA (94.32%), followed by 61.21% for the hydrolysate at 180 min. and very low AOA values were exhibited by all chymotryptic peptides and hydrolysate. The peptides resulting from enzyme mixture I were prooxidative after 30 and 60 min. because they resulted from peptic hydrolysis then on adding the two other enzymes, the AOA was raised to 38.75 and 44.06% after 120 and 180 min., respectively. Hydrolysis using alcalase and flavourzyme gave peptides with moderate AOA ranging from 31.10-48.44% and from 10.5-43.19 %, respectively. Upon using the mixture II the AOA was improved to 53.05 and 79.37%, after 120 and 180 min., respectively. Some peptides and hydrolysates were chosen for testing their antimicrobial activity. All tested hydrolysis products showed different antimicrobial activity. Enzymatic hydrolysis of the protein isolate with enzyme mixture II (alcalase and flavourzyme) resulted in products that inhibited all the five microbial strains tested. Chymotryptic hydrolysate, mixture I Hydrolysate and a peptide fraction from flavourzyme inhibited four bacterial strains.

Key words: Sunflower protein isolate · Bioactive hydrolysates and peptides · Antioxidant activity · Prooxidant activity · Antimicrobial activity.

INTRODUCTION

Protein hydrolysis is the breakdown of protein into smaller peptides and free amino acids. Specialty enzymes has animal-based proteases like trypsin, plant based proteases like papain and a variety of microbial source proteases. Protein hydrolysates constitute an alternative to intact proteins and elemental formulas in the development of special formulations designed to provide nutritional support to patients with different needs. The production of extensive protein hydrolysates by sequential action of endopeptidases and exoproteases coupled with the development of posthydrolysis procedures is considered the most effective way to obtain protein hydrolysates with defined characteristics. These hydrolysates are suitable for dietary treatment of patients with phenylketonuria, food allergy and chronic liver failure [1], as diets for the elderly [2], in sports nutrition [3] and in weight-control diets [4]. Lately it has been reported that besides the dietary value of the protein hydrolysates, the hydrolysates and their peptides exhibit several biological activities. They act as antioxidants [5, 6, 7] have antimicrobial [8, 9] and are considered as immunomodulatory [10, 11], antihypertensive [12, 13], anticoagulant [14], anticarcinogenic agent [12, 15] and have several other physiological functions.

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In the course of preparing hydrolysates for human consumption it is preferable to use enzymatic hydrolysis rather than acid or alkali hydrolysis, because the conditions of enzymatic hydrolysis are much milder and no amino acids are destroyed. Acid and alkali hydrolysis can destroy L-form amino acids and produce form amino acids and can form toxic substances such as lysino-alanine [16]. Hydrolysis is a chemical reaction in which water is used to break the bonds of certain substances. In biotechnology and living organisms, these substances are often polymers. In a hydrolysis reaction involving an ester link, such as that found between two amino acids in a protein, the products that result include one that receives the hydroxyl (OH) group from the water molecule and another that becomes a carboxylic acid with the addition of the remaining proton (H^+). The biochemical reactions that breakdown polymers such as proteins (peptide bonds between amino acids) use the enzymes called proteinases. These are divided into several classes according to their specificity: a) Broad specificity e.g. papain, bromelain and ficin enzymes. b) Moderate, structural or group specificity e.g. pepsin, trypsin, chymotrypsin, amino peptidase and carboxypeptidase enzymes. In this type of specificity the enzyme is specific to not only the type of bond but also to the structure surrounding it. Proteinases are also divided into: endoproteinase which are protelytic peptidases that break peptide bonds of nonterminal amino acids (i.e. within the molecule) and exopeptidases, which break peptide bonds from their end-pieces. For this reason, endopeptidases cannot breakdown peptides into monomers, while exopeptidases can breakdown proteins into monomers.

The hydrolysis products exhibit antioxidant activity [5-7]. Yet the mechanism of inhibiting lipid peroxidation by hydrolysis products is not very clear. However previous research on di-or tri-peptides in oil or metal catalyzed liposomal suspensions seemed to indicate that it was attributed to chelation to prooxidative metal ions and termination of the free radical chain reactions by the presence of antioxidative peptides either through the specific amino acid residue side-chain groups or through the specific peptide structure [17-20]. The hydrolysis products are also reported to have antimicrobial activity [8, 21]. Antimicrobial peptides encompass a wide variety of structural motifs. Many peptides have α-helical structures. The majority of these peptides are cationic and amphipathic but there are also hydrophobic α-helical peptides which possess antimicrobial activity. In addition some β-sheet peptides have antimicrobial activity and even antimicrobial α-helical peptides which have been modified to possess a β-structure retain part of their antimicrobial activity. There are also antimicrobial peptides which are rich in certain specific amino acid such as tryptophan and histidine. In addition antimicrobial peptides exist with thio-ether rings which are lipopeptides or which have macrocyclic Cys knots. In spite of the structure diversity a common feature of the cationic antimicrobial peptides is that they all have an amphipathic structure which allows them to bind to the membrane interface. Indeed most antimicrobial peptides interact with membranes and may be cytotoxic as a result of the disturbance of the inner or outer bacterial membrane. Alternatively, a necessary but not a sufficient property of these peptides may be able to pass through the membrane to reach a target inside the cell. The interaction of these peptides with biological membranes is not just a function of the peptide but is also modulated by the lipid components of the membrane. It is not likely that this diverse group of peptides has a single mechanism of action, but interaction of the peptides with membranes is an important requirement for most, if not all, antimicrobial peptides [8].

The aim of the present study is to prepare sunflower protein hydrolysis products from protein isolate-free of chlorogenic acid (CGA) and to examine their biological activities. Several enzymes and their mixtures will be utilized for the process of hydrolysis. Peptides resulting from partial hydrolysis at different time intervals will be collected as well as the final hydrolysates and tested for their antioxidant and antimicrobial activity.

MATERIALS AND METHODS

Materials: Sunflower protein isolate was prepared from sunflower seed meal from which chlorogenic acid has been removed as reported by Taha *et al.* [22]. The protein isolate (PI) was prepared by alkali solubilization of the protein then isoelectric precipitation [23]. The precipitated isolate was washed with acetone and then ethyl ether, then air-dried.

Enzymes: Pepsin (P), trypsin (T), chymotrypsin (C), alcalase (A) and flavourzyme (F) are obtained from Sigma-Aldrich.

Microorganisms: All the used microorganisms were (*E. coli* o157:H7 ATCC 51659, *Staphylococcus aureus* ATCC 13565, *Bacillus cereus* EMCC 1080, *Listeria mono-cytogenes* EMCC 1875 and *Salmonella typhimurium* ATCC25566) were obtained from the Microbiological Resources Centre (Cairo MIRCEN) Faculty of Agriculture, Ain Shams University.

Experimental: Protein hydrolysates were prepared by dissolving sunflower isolate II at a meal: water ratio of 1: 10 (w/v) by continuous stirring, while adjusting the temperature then the pH to the optimum temperature and pH of the enzyme. The enzyme was then added (2% enzyme concentration). During hydrolysis both the temperature and pH were kept constant. Twenty ml aliquots were withdrawn from the hydrolysate solution, at intervals, after 30, 60, 120 min. These aliquots will give the peptide fractions (PF) and hydrolysis continued to 180 min. to give hydrolysate (H). The enzyme was deactivated directly after withdrawal of the aliquot and then the solutions were used for the determination of their antioxidant and antimicrobial activities. In the hydrolysis experiment carried out by enzyme mixture I (P and T and C, at 1:1:1 ratio), the condition of hydrolysis was first adjusted to pH 2.0 and 37°C for 1h., two aliquots taken at 30 min. and 60 min., then pH shifted to 7.8-8.0 and temperature adjusted to 25-30°C. Two other aliquots were withdrawn after 120 and 180 min. When using the enzyme mixture II (A and F) the pH was adjusted to 8.0 for (A) and temperature to 50°C, aliquots taken after 30 min. and 60 min., then pH changed to 7 for (F) and aliquots taken after 120 and 180 min. With each hydrolysis a control experiment was carried under same conditions but without the addition of the enzyme.

Methods of Analysis: Protein, residual oil, ash, crude fiber and moisture were determined according to A.O.A.C. [24]. Antioxidant activity was determined by the β-carotene/linoleate method according to Al-Shaikhan *et al.* [25]. Antimicrobial activity the survivor experiments were carried out on Trypticase soy agar supplemented with yeast extract (TSAYE: 17g tryptone, 3 g soy peptone, 2.5 g glucose, 5 g NaCl, 2.5 g K_2HPO_4, 5g yeast extract and 15 g agar in litre of distilled water, pH 7). Number of viable cells was determined with plate count method after incubation at 37°C for 24-48h. Antibacterial assay was carried out using the disc diffusion method as described by Kotzekido *et al.* [26].

RESULTS AND DISCUSSION

The goal in this work was to add value to the meal remaining after the extraction of CGA. The nutritional properties of protein isolates are well documented. But if we prepare an isolate with the aim of subjecting it to enzymatic hydrolysis in order to obtain protein hydrolysis products having biological activities, then we have added value to the protein isolate. Protein isolate had to be prepared from meal free of chlorogenic acid, because chlorogenic acid was proved to possess antioxidant (AOA) and antimicrobial (AMA) activities [22], which might give us false results if present with the hydrolysis products. Table 2 show the chemical composition of the protein isolate and the meal from which it was prepared. It is clear from the values in Table 2 that the isolate is a better starting material for hydrolysis because of its high protein content (95.1%) and little crude fibre (0.7%) and little carbohydrate content (2.5%). The carbohydrates and fibre can interfere during the process of hydrolysis.

Table 1: Optimum conditions of enzymes

Enzyme	pH	Temperature °C
Pepsin	2.0	37
Trypsin	8.0	30
Chymotrypsin	7.8	25
Mixture I	2.0 then 7.8-8.0	25-30
Alcalase	8.0-10	45-50
Flavourzyme	7.0	50
Mixture II	8.0 then 7.0	50

Table 2: Chemical composition of protein isolate and the meal from which it was prepared (dry weight basis)

Composition (%)	Meal	Protein isolate
Protein	53.8	95.1
Lipids	0.1	0.1
Ash	9.0	1.6
Fibre	8.4	0.7
Nitrogen free extract	28.7	2.5

Table 3: The antioxidant activity % of peptide fractions and protein hydrolysates prepared from sunflower protein isolate using pepsin, trypsin and chymotrypsin enzymes and their mixture

Enzyme	Time of hydrolysis (min)	Protein Fraction (PF)	Antioxidant Activity (%)
Pepsin (P)	30	PFP-1	-51.41
	60	PFP-2	-42.24
	120	PFP-3	-36.12
	180	HP-4	48.61
Trypsin (T)	30	PFT-1	14.01
	60	PFT-2	94.32
	120	PFT-3	57.19
	180	HT-4	61.21
Chymotrypsin (C)	30	PFC-1	12.50
	60	PFC-2	15.40
	120	PFC-3	17.45
	180	HC-4	19.94
Mixture I (P and T and C)	30	PFMI-1	-137.12
	60	PFMI-2	-78.44
	120	PFMI-3	38.75
	180	HMI-4	44.06

Antioxidant Activity of Peptide Fractions and Hydrolysates: Results in Table 3 and Fig.1 revealed that all examined Peptide fraction (PF) exhibited antioxidant activity except for the PF resulting from hydrolysis of

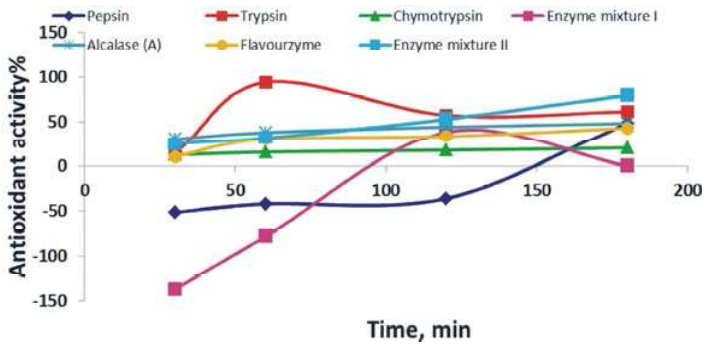

Fig. 1: Antioxidant activity of different sunflower protein peptides and hydrolysates.
Enzyme mixture I = mixture of P and T and C
Enzyme mixture II = mixture of A and F

protein isolate using enzyme Pepsin. PFP-1, PFP-2 and PFP-3 exhibited prooxidant activity. This might be due to the type of amino acids present in the peptides and their sequence. Also sunflower protein contains substantial amounts of cysteine which is reported to be prooxidative by Marcuse [27]. Shahidi and Zhong [12] reported that protein digests have varied antioxidant activities depending on the peptide structure i.e., size of the peptides and their amino acid sequences, which are influenced by the source of protein and conditions of the hydrolysis process involved. Penta-Ramos and Xiong [28] obtained soy peptides from native or heated soy protein by different enzymes such as pepsin, papain, chymotrypsin, alcalase, protamex and flavourzyme. They found that this resulted in peptides with different degrees of hydrolysis, ranging from 1.7 to 20.6% and antioxidant activity ranging from 28 to 65% measured as inhibition against formation of thiobarbituric acid reactive substances in a liposome-oxidizing system. Marcuse [27], studying the effect of some amino acids on the oxidation of linoleic acid and its methyl ester, reported that the phenomenon of substances being pro-oxidative as well as antioxidative, depending on the circumstances is well known. He also found that with increasing concentration the antioxidative effect may pass a maximum and may at further increasing concentrations be inverted into a prooxidative effect. Hayes *et al.* [29] reported that the amino acids may be antioxidative less than one set of conditions, but prooxidative or borderline under another set of conditions. Low pH values favored the tendency towards prooxidative inversion. It is worthy to mention that pepsin enzyme works at pH 2.0. After 3h hydrolysis of sunflower protein isolate with enzyme (P), the hydrolysate (HP) showed moderate antioxidant activity 48.6%.

Using enzyme trypsin (T) for hydrolyzing sunflower protein isolate, the highest antioxidant activity (AOA) was achieved after 60 min. of hydrolysis where the PFT-2 exhibited 94.32% AOA. The PFT-1, PFT-3 and HT exhibited 14.01, 57.19 and 61.21% AOA. Amarowicz and Shahidi [30] separated four peptide fractions from protein hydrolysates of Capelin. They found one fraction to possess notable AOA, another two with low AOA and the fourth with prooxidant activity. Hydrolysis of sunflower isolate with enzyme chymotrypsin (C) resulted in peptide fractions (PFC-1, PFC-2, PFC-3) and hydrolysate (HC) with very low AOA, 12.5, 15.4, 17.45 and 19.94 %, respectively. Leon Espinosa *et al.* [31] hydrolysing lentil protein with trypsin, chymotrypsin and pancreatin, found that maximum hydrolysis with trypsin was after 90 min., while that of chymotrypsin was after 60 min.The use of DPPH method showed that the highest antioxidant capacity was obtained in the hydrolysates with trypsin and pancreatin at 30 and 60 min., the obtained scavenging activity for pancreatin, trypsin and chymotrypsin hydrolysates were 87.44, 79.0 and 40 %, respectively.

Comparing the three enzymes it is clear that each enzyme behaves differently towards the same substrate. It was reported that the properties of the hydrolysates are dependent on the type of enzyme used, the degree of hydrolysis, the environmental conditions and the substrate pretreatment [32]. Using a mixture of the three enzymes was not very effective. The first hour of hydrolysis yielded prooxidative peptides because the enzyme used was pepsin. After the addition of the trypsin and chymotrypsin the resulting peptides revealed moderate AOA, PFM-I and HM-I had 38.75 and 44.01% antioxidant activity.

Table 4: The antioxidant activity % of peptide fractions and protein hydrolysates prepared from sunflower protein isolate using alcalase and flavourzyme enzymes and their mixture

Enzyme	Time of hydrolysis (min)	Protein Fraction (PF)	Antioxidant Activity (%)
Alcalase (A)	30	PFA-1	31.10
	60	PFA-2	38.40
	120	PFA-3	44.42
	180	HA-4	48.44
Flavourzyme (F)	30	PFF-1	10.5
	60	PFF-2	30.38
	120	PFF-3	34.17
	180	HF-4	43.18
Mixture II (A and F)	30	PFMII-1	24.92
	60	PFMII-2	32.16
	120	PFMII-3	53.05
	180	HMII-4	79.37

Results in Table 4 and Fig. 1, using enzymes, alcalase (A), flavourzine (F) and a mixture II (A and F) show that (A) enzyme alone and (F) enzyme alone resulted in peptides and hydrolysates with low to moderate AOA. The PFA-2, PFA-3, HA-4, PFF-1, PFF-2, PFF-3 and HF-4 exhibited 31.10, 38.40, 44.42, 48.44, 10.5, 30.38, 34.17 and 43.18 % AOA, respectively. Alcalase is an endopeptidase that breaks peptide bonds of nonterminal amino acids (i.e. within the molecule) and flavourzyme is an exopeptidase, which break peptide bonds from their end-pieces. For this reason, endopeptidases cannot break down peptides into monomers, while exopeptidases can breakdown peptides into monomers. Thus by using a mixture of the two enzymes first (A) enzyme, which results in a hydrolysate that is considered a predigestion causing an increase in the number of N-terminal sites for the exoprotease to follow by (F) enzyme a more advanced hydro-lysis should take place. Our results indicated that the PFMII-1 and PFMII-2 resulting from first hydrolysis with (A) possessed more or less the same AOA as peptides PFA-1and PFA-2 (Table 4). Adding (F) after that resulted in PFMII-3 and HMII-4 with much higher values 53.05 and 79.37 % AOA compared to PFF-3 and HF-4 with 34.17 and 43.18% AOA, respectively. Beva Kelfala *et al*. [33] working with Freeze dried protein powders of fresh minced meat (FMM) and hot water dip (HWD) from tilapia (*Oreochromis niloticus*) were hydrolysed by Alcalase 2.4 L (Alc), Flavourzyme (Flav) and Neutrase (Neut) and investigated for antioxidant activity and their functional properties.

The FMM and HWD hydrolysed by Alc, exhibiting superior antioxidant activity, had estimated degrees of hydrolysis (DH) of 23.40% and 25.43%, respectively. The maximum values of the 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), 3-(2-pyridyl) 5, 6-bis (4-phenyl-sulphonic acid)-1, 2, 4-triazine (ferrozine), radical scavenging activities and metal chelating properties were 86.67%, 91.27% and 82.57% and 84.67%, 92.60 % and 78.00 % for FMM and HWD, respectively, with a significant difference ($P < 0.05$) between the samples. Ine *et al*. [34] prepared a protein hydrolysate from chia seed protein using the enzymes alcalase and flavourzyme sequentially. They reported the antioxidant activity of the Chia protein hydrolyzate, quantified and calculated as TEAC values (mm/mg), decreased as DH increased. The highest TEAC value was for the hydrolyzeate produced at 90 min (7.31 mm/mg protein), followed by those produced at 120 min. (4.66 mm/mg protein) and 150 min. (4.49 mm/mg protein); the latter two did not differ ($P<0.05$). Increased antioxidant activity in hydrolyzed proteins has also been reported for dairy, soy, potato, gelatin and egg yolk among other proteins. The mechanism of inhibiting lipid peroxidation by hydrolysis products is not very clear. However previous research on di-or tri-peptides in oil or metal catalyzed lipo-somal suspensions seemed to indicate that it was attributed to chelation to prooxidative metal ions and termination of the free radical chain reactions by the presence of antioxidative peptides either through the specific amino acid residue side-chain groups or through the specific peptide structure [17-20]. The present results of this study revealed that the enzymatic hydrolysis of sunflower protein isolate II should be carried out using trypsin enzyme at 2% enzyme concentration and hydrolysis time 1h or using alcalase and flavourzyme sequentially at 2 % enzyme concentration and hydrolysis time 3h and that this is recommended for the preparation of antioxidative peptides.

Antimicrobial Activity of Some Peptide Fractions and Hydrolysates: Antimicrobial agents from natural sources are nowadays being searched for, after many microbes acquired resistance against synthetic antibiotics. For this reason some of the peptide fractions and hydrolysates (exhibiting moderate good AOA) were chosen to be tested as antimicrobial agents. The power of inhibition of the chosen hydrolysis products was tested against five food pathogenic bacteria including: E. O157• (*E. coli* o157:H7 ATCC 51659), St•(*Staphylococcus aureus* ATCC 13565), B.c• (*Bacillus cereus* EMCC 1080), Lis• (*Listeria monocytogenes* EMCC 1875) and sa• (*Salmonella typhimurium* ATCC25566), using the disc diffusion

Table 5: Antimicrobial activity of different hydrolysates and peptides against some food pathogenic microorganisms

	Strains/ inhibition zone diameter (mm)				
Samples	B.c•	sa•	St•	E. O157•	Lis•
HP-4	ND	ND	ND	ND	ND
PFT-2	ND	ND	ND	ND	ND
HT-4	13	ND	ND	ND	ND
PFC-2	11	ND	ND	ND	9.6
HC-4	13	11	ND	9	12
HMI-4	11	ND	11	8	11
PFF-3	10	ND	ND	ND	ND
HF-4	ND	ND	ND	ND	10
PFA-3	11	ND	12	9	11
HA-4	10	ND	ND	8	ND
PFMII-3	13	9.7	13	9	19
HMII-4	16	9	10	9	19

E. O157• (*E.coli* o157:H7 ATCC 51659), St (*Staphylococcus aureus* ATCC 13565), B.c• (*Bacillus cereus* EMCC 1080), Lis• (*Listeria monocytogenes* EMCC 1875) and sa• (*Salmonella typhimurium* ATCC25566).

HP-4 = pepsin hydrolysate (3h)
PFT-2 = trypsin peptide fraction (1h)
HT-4 = trypsin hydrolysate (3h)
HC-4 = chymotrypsin hyrolysate (3h)
PFF-3 = flavourzyme peptide fraction (2h)
PFA-3 = alcalase peptide fraction (2h)
PFMII-3 = mixture II peptide fraction (2h)
HMII-4 =mixture II hydrolysate (3h)
PFC-2 = chymotrypsin peptide fraction (1h)
HMI-4 =enzyme mixture I hydrolysate (3h)
HF-4 = flavourzyme hydrolysate (3h)
HA-4 = alcalase hydrolysate (3h)

method. The twelve peptides and hydrolysates showed various degrees of inhibition against five bacteria strains using the disc diffusion method as presented in Table 5. Samples with an enhanced inhibitory effect in decreasing order were: samples PFMII-3 and HMII-4 which inhibited all strains; samples HC-4, HMI-4 and PFA-3 (inhibition of four strains), samples PFC-2 and HA-4 (inhibition of two strains), samples HT-4, PFF-3 and HF-4 which inhibited one strain. *Staphylococcus aureus* strain was the most susceptible microorganisms inhibited by 9 samples, followed by *Listeria monocytogenes* which was inhibited by 7 samples then *Bacillus cereus* by 6 samples. Samples PFMII-3 and HMII-4 were most effective on *Listeria monocytogenes* (inhibition zone diameter 19 mm). HMII-4 was also the most effective on *Bacillus cereus* (inhibition zone diameter 16 mm). Biziulevicius *et al*. [35], in a study entitled "Food-protein enzymatic hydrolysates possess both antimicrobial and immune stimulatory activities: a cause and effect theory of bio-functionality", they examined the antimicrobial activity of twenty food protein hydrolysates which were hydrolysed with four gastrointestinal proteinases. All the food protein hydrolysates acted antimicrobially *in vitro* towards all twenty four microbial strains tested. Casein hydrolysates were the most active stimulants, with α-lactalbumin, β-lactoglobulin, serum albumin and ovalbumin hydrolysates following in decreasing order of activity. Similarly if the influence of the proteolytic enzyme used is evaluated, the sequence is as follows: tryptic hydrolysates > peptic hydrolyzate > chymotryptic hydrolysates > pancreatic hydrolysates. The autolysis activation induction level in various microorganisms was also very different. Thus the susceptibility of microbial strains to the food protein hydrolysates used indicates that the nature of the food protein and the proteolytic enzyme as well as the nature of the microorganism itself are all important. Nevertheless, the ability to stimulate the microbial autolytic system is likely to be the common denominator in protein hydrolysates. Despite their vast diversity, most antimicrobial peptides (AMPs) work directly against microbes through a mechanism involving membrane disruption and pore formation, allowing reflux of essential ions and nutrients. The molecular mechanism and pathway of membrane permeation may vary for different peptides depending on a number of parameters, such as the amino acid sequence, membrane lipid composition and peptide concentration [36]. Although the mechanisms by which peptides associate with and permeabilize microbial cell membranes are not entirely clear, AMPs are proposed to bind to the cytoplasmic membrane, creating micelle-like aggregates, leading to a disruptive effect. However, a mounting body of evidence indicates the presence of additional or complementary mechanisms such as intracellular targeting of cytoplasmic components crucial to proper cellular physiology [9, 37]. Thus, the initial interaction between the peptides and the microbial cell membrane would allow them to penetrate into the cell to bind intracellular molecules, resulting in the inhibition of cell wall biosynthesis and DNA, RNA and protein synthesis.

CONCLUSION

Sunflower protein hydrolysates are promising antioxidant and antimicrobial agents, especially those hydrolysed with a mixture of enzymes (alcalase and flavourzyme).

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