MOL2NET, International Conference Series on Multidisciplinary Sciences
MODEC-05: Nat. Prod. & Agro-Indust. Proc. in Amazon, UEA, Puyo, Ecuador, 2020

Valorization of shrimp *Metapenaeus monoceros* bio-waste as a source of bioactive protein hydrolysate

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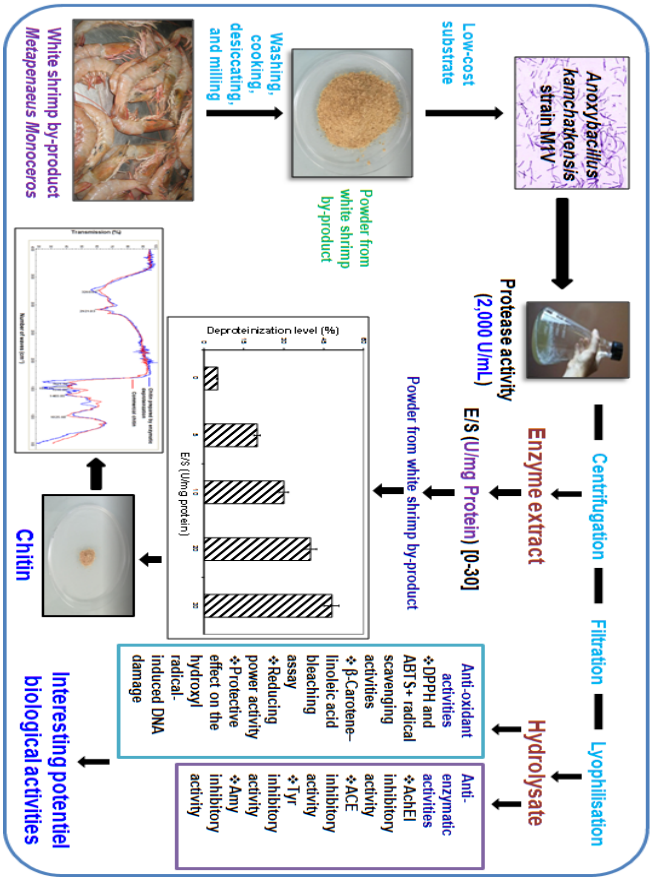
Graphical Abstract



Abstract.

Mediterranean sea is threatened by a large number of factors such as habitat alteration, urbanization, climate change, pollution and more specifically by the introduction of autochton and non-native species. Shrimps are caught all along the Tunisian coasts. They are found in the Gulf of Gabes area, wherein the largest concentrations of these species are located, especially the king shrimp *Penaeus kerathurus*. In addition, fishing with both trawlers and inshore fishing units have been recorded as relatively large quantities of another shrimp species, commonly known as white or speckled shrimp, *Metapenaeus monoceros*. The efficiency of the proteolytic strain *Anoxybacillus kamchatkensis* M1V in the fermentation of speckled shrimp by-product was investigated for the recovery of a deproteinized bioactive hydrolysate. The biological activities of the resulting hydrolysate were also examined by applying several antioxidant and enzyme inhibitory assays. The strain M1V was found to produce high level of protease activity (2,000 U/mL) when grown in media containing only shrimp powder at 25 g/L. The obtained hydrolysate showed a significant enzymatic inhibitory potential against acetylcholinesterase, tyrosinase, amylase, and angiotensin I convertase, and a strong antioxidant activity.

1. Introduction

Having a rich natural heritage, a high biodiversity and a relatively unspoiled coastal environment, the Mediterranean is endowed with a tremendous potential for the development of fishery sector. Particularly, occupying a focal position in the Mediterranean, Tunisia is largely open to the sea typically on its eastern and southern shores. Its coastline exceeds 1,300 km. Thus, the fishery and the aquaculture are considered highly significant in both socio-economic and food sectors. For particular interest, the global crustacean world production intended for human consumption keeps up a continuous growing scale. In turn, such growth has led to a much greater volume of by-produts and non-edible. It is reported that approximately 6-8 million tons of crustacean bio-waste is produced worldwide every year [1]. Shrimp has constituted a major part of crustacean consumption in recent years. In industries, shrimp is usually processed as meat, since remaining head, shell, and tail portions are considered as by-products. Discarding shrimp bio-waste, i.e. its inadequate disposal, poses a critical environmental and burdensome economic problem. However, during the industrial processing, 45 to 60% of total shrimp parts weight is regarded as a by-product, which varies among processing modes and species [2]. Modern scientific investigations have shown that shrimp by-product can be biotechnologically exploited for the production of useful marketable products with high benefit such as protein, chitin, carotenoid pigments, flavor compounds, lipids, and calcium carbonate, as well as chitin and chitosan [3, 4].

Fermentation, using specific microorganisms, which is a cost-effective alternative process and characterized by higher efficiency in bioconversion of fish bio-waste, can be applied for the production of a bioactive hydrolysate [5]. Moreover, proteolytic microorganisms have been reported to utilize shrimp by-products as the sole and unique source of carbon and nitrogen resulting in the production of metabolites which have varying levels of bioactivity [6-8].

The current work was designed to study the efficiency of the proteolytic *Anoxybacillus kamchatkensis* strain M1V in the fermentation of white shrimp shell by-product for bioactive hydrolysate recovery. The biological activities of obtained hydrolysate were investigated. These activities concerned the inhibitory potential of the tyrosinase (Tyr), the acetylcholinesterase (AchE), the amylase (Amy), and the angiotensin I convertase (ACE). Accordingly, this is the first research discussing the inhibitory potential of a hydrolysate recovered through the M1V strain fermented shrimp by-product.

2. Materials and Methods

2. 1. Biological material, microorganism, culture conditions, and protease production

Fresh shrimp by-product was obtained from a fish market in Sfax city (Tunisia). The material was fully washed with tap water and cooked at 90 °C for 20 min. Then, it was dried and milled with a Stainless Steel Cross Beater Mill Model Sk1 to obtain a fine homogeneous powder with particle sizes < 1.23 mm and stored at room temperature.

Anoxybacillus kamchatkensis strain M1V is a proteolytic bacteria newly isolated from Hammam Righa's geothermal waters in Algeria. It was identified on the basis of the *16S* rDNA gene [9]. For the production of protease, the M1V strain was cultured in a one-liter Erlenmeyer flask with a working volume of 50 mL, containing 25 g/L shrimp waste at pH 7.4 for 48 h at 45 °C in a rotary shaker (200 rpm). Before each activity assay, the biomass was taken away by centrifugation at 10,000×*g* for 30 min, and the supernatant was used for the evaluation of protease activity and chitin extraction. Furthermore, the cleared supernatant was filtered through a coarse cloth followed by the use of a sieve (22 mesh size). Subsequently, the resulting filtrate (hydrolysate) was concentrated in a rotary evaporator at 40 °C, freeze dried, and stored at -20 °C. Next, the composition of the lyophilized hydrolysate and its biological activities were evaluated.

2.2. Methods

2.2.1. Chemical analysis

The total nitrogen content of raw the material was determined using the Kjeldahl method as previously reported [10]. Protein concentration of freeze-dried hydrolysate was estimated with the

Bradford method, using bovin serum albumin as a reference [11]. The total sugar and lipid contents were determined using the Dubois and Flochl methods, respectively [12, 13].

2.2.2. Protease activity Assay

The peptidase activity was assayed using Kembhavi method [14]. One unit (U) of peptidase was defined as the amount of enzyme releasing 1 µg of tyrosine released under the assay conditions detailed.

2.2.3. Determination of biological activities

2.2.3.1. Acetylcholinesterase inhibitory activity (AchEI)

AChEI was assessed by a spectrophotometric method [15] with slight modifications [16]. The % of inhibition of AChE was determined by a comparison of reaction rates of tested samples relative to the control (10% dimethyl sulfoxide in Tris-HCl buffer) using the attached formula:

$$AchEI\% = [1 - (\delta A_S / \delta A_C)] \times 100 \quad \text{(Eq. 1)}$$

Where δA_S: Sample absorbance at zero time - Sample absorbance at the end of reaction, and δA_C: Control absorbance at zero time - Control absorbance at the end of reaction. Galanthamine was used as a positive control.

2.2.3.2. Angiotensine inhibitory activity (ACEI)

The ACEI of the hydrolysate was measured according to the method described by Cushman and Cheung [17] with slight modifications using captopril was as a positive control. The ACEI% was determined using the attached equation:

$$ACEI\% = 1 - \left[\frac{(A-B)}{C-D}\right] \times 100 \quad \text{(Eq. 2)}$$

Where A: absorbance of the sample, B: absorbance of the sample blank, C: absorbance of the control, and D: absorbance of the control blank.

2.2.3.3. Tyrosinase inhibitory activity (TyrI)

The TyrI assessment was evaluated using L-tyrosine as substrate and carried out in a 96-well micro plate following the protocol suggested by Rangkadilok [18] with slight modifications. Kojic acid was used as a positive control. The data were expressed as percent inhibition of Tyr, TyrI% following the formula:

$$TyrI\% = [1 - (\delta A\ sample\ /\ \delta A\ control)] \times 100 \quad \text{(Eq. 3)}$$

Where δA sample: sample absorbance at zero-time - sample absorbance at the end of the reaction, δA control: control absorbance at zero-time - control absorbance at the end of the reaction.

2.2.3.4. Amylase inhibitory activity (AmyI)

The AmyI assay was determined using a modified Sahnoun's method [19] with starch at concentration of 10 g/L as a substrate. Acarbose was used as reference substance and the activity percentage was calculated following this equation:

$$AmyI\% = \left[\frac{Absorbance\ control - Absorbance\ test}{Absorbance\ control}\right] \times 100 \quad \text{(Eq. 4)}$$

2.2.3.5. DPPH radical scavenging assay

This assay determines the scavenging of DPPH radicals according to the method of Kirby and Schmidt, with slight modifications [20]. The scavenging capacities of both sample and standard were determined spectrophotometrically by monitoring the absorbance decrease at 517 nm versus a blank. The results were expressed as the EC_{50} defined as the concentration (µg/mL) needed for the reduction of 50% of the initial DPPH radicals. The percentage of antiradical activity (ArA%) was calculated as follows:

$$ArA\% = [(Absorbance\ of\ control - Absorbance\ of\ test\ sample)/Absorbance\ of\ control] \times 100$$

(Eq. 5)

2.2.3.6. ABTS radical scavenging activity

The scavenging potential of the protein hydrolysate was also assessed against a cation radical using the ABTS free radical discoloration assay performed as previously described elsewhere [21] and modified by the authors [16].

The percentage of scavenging activity of $ABTS^{\cdot+}$, SA%, was expressed by the following formula:

$$SA\% = [(Absorbance\ of\ control - Absorbance\ of\ test\ sample)/Absorbance\ of\ control] \times 100 \quad \text{(Eq. 6)}$$

2.2.3.7. β-Carotene bleaching assay

The hydrolysate anti-oxidant activity was assessed using to the β-carotene bleaching method optimized by Pratt [22] using a serial dilution of hydrolysate and BHT from 125 to 1,000 µg/mL. The antioxidant activity in β-carotene bleaching model was expressed in percentage AAOx% based on the subsequent equation:

$$AAOx\% = [1 - (A0S - A120S)/(A0C - A120C)] \times 100 \quad \text{(Eq. 7)}$$

Where A^0_S and A^0_C are the absorbance of the sample and the control respectively, measured at zero time, and A^{120}_S and A^{120}_C are the absorbance of the sample and the control respectively, measured after 120 min.

2.2.3.8. Ferrous chelating activity

Ferrous chelating activity of the hydrolysate was predicted according to the method described elsewhere [23]. The absorbance of the Fe^{2+}-ferrozine complex was assessed at 562 nm. The chelating antioxidant activity for Fe^{2+}, chelating rate%, was calculated according to the subsequent formula:

$$Chelating\ rate\% = [(Acontrol - Asample)/Acontrol] \times 100 \quad \text{(Eq. 8)}$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the hydrolysate. EDTA was adopted as a reference. The control was conducted under the same experimental conditions, excluding that distilled water was used instead of the sample.

2.2.3.9. Reducing power activity

The reducing power of hydrolysate was measured according to the method of Oyaizu, with a slight modification [24]. The BHT was the reference antioxidant in this assessment.

2.2.3.10. Protective effect against hydroxyl radical-induced DNA deterioration

DNA nicking assay was performed using pUT57 plasmid. The investigation was tested as previously developed [25] with few improvements.

3. Results and Discussion

3.1. Production of protease in economical medium

During industrial enzymes production, mostly 30 to 40% of the production cost is substantiated by the price of the growth substrate [6]. To overcome such inconvenience, researchers have, therefore, become increasingly interested in the exploitation and valorization of fish by-products for producing higher added value products [26]. Recently, the valorization of crustacean bio-wastes is becoming an increasingly “hot” topic [27-29]. The composition of fresh powder from speckled shrimp by-product showed that it has a high protein content making up to 38% ± 0.6 of dry matter with 4% ± 0.01 of sugar and a very low amount of fats (1.8% ± 0.04). So far, recycling protein or other functional ingredients from shrimp processing by-products has attracted an increasing attention [30, 31]. Various processing methods; including enzymatic hydrolysis and fermentation have been applied. Particularly, processes for producing microbial proteases using inexpensive media have been investigated. Interestingly, fish by-products including guts, viscera, bones, chitinous material, skin, fish head, blood, gonads, and waste water were prepared and tested as growth substrates for microbial enzymes production such as proteolytic, chitinolytic, lipolytic, and ligninolytic enzymes. The *Anoxybacillus kamchatkensis* strain M1V was found to produce a high level of proteolytic activity (2,000 U/mL) using 25 g/L of shrimp powder.

3.2. Shrimp bio-waste hydrolysate: Composition, and biological activities

3.2.1. Shrimp by-product hydrolysate composition

The protease production by strain M1V was under-taken in the optimal medium. The extent of protein hydrolysis during fermentation by *Anoxybacillus kamchatkensis* strain M1V was measured by

assessing the proteolytic activity and hydrolysate composition. The hydrolysate has a maximum amount of protein (42 ± 1.02%), which coincides with the optimum of proteolytic production (2,000 U/mL) after 48 h of culture. The sugar level in the hydrolysate was about 2.5 ± 0.12% of sugar and a very low amount of fats (2 ± 0.06%). The obtained hydrolysate was noticeable due to its high protein content. Several studies have apprised that the controlled enzymatic digestion is the broadly applied method for the production of protein hydrolysates [5]. Within the time course of hydrolysis, a large variety of peptides and free amino acids are generated depending on the protease specificity. Definitely, the level, the changes in size, and the composition of peptides and free amino acids modulate the biological activities. Here, a few investigations have been conducted on the generation of biologically active proteins using microbial fermentation. In fact, the high protease activity (2,000 U/mL) reflects the generation of small sized bioactive peptides in the hydrolysate mixture. Indeed, the hydrolysate was assessed for its antioxidant and enzyme inhibitory capacities using various tests.

3.2.2. In-vitro biological activities

3.2.2.1. Enzymatic inhibitory potential

The obtained hydrolysate was assessed for its enzymatic inhibitory power against 4 enzymes: AChE, ACE, Tyr, and Amy and compared to their respective standards: galanthamine, captopril, Kojic acid, and acarbose. The results were presented in graphics displaying the relationsip between the different used concentrations of the hydrolysate and their corresponding percentages of inhibitory activities. Enzymatic inhibition has become an interesting therapeutic strategy, since many enzymes are crucial factors in several pathological processes. Therefore, the enzymatic inhibitory power of the shrimp *Metapenaeus monoceros* shell hydrolysate was investigated. Alzheimer's Disease (AD) is the most common neurodegenerative disorder, with unclear pathogenesis to date. The "Cholinergic hypothesis" is considered as one of the most accepted theories. The inhibition of AChE preserves the levels of acetylcholine and ameliorates the cholinergic function and therefore has become the standard approach in the symptomatic treatment of AD [32]. Our results show the AChEI reached 100% at 620.23 ± 0.8 µg/mL shrimp shell hydrolysate with an IC_{50} of 268.01 ± 2.15 µg/mL. However, with such a value, the hydrolysate appears to be so far weaker than the AChE inhibitor galanthamine (IC_{50} = 0.118 ± 0.037 µg/mL). The AchEI potential by fish bio-waste hydrolysate is being reported for the first time. At this stage, further studies are required to evaluate the origin of the AChEI, which could be explained by the presence of positively charged amino acids. It has been found that positively charged amino acids could form a stable complex with the peripheral anionic site of the AChE protein, which would hinder the substances enter into active sites of AchE [33]. ACE plays a fondamental role in the modulation of blood pressure via renin-angiotensin and the kinin-kallikrein systems. Different studies have highlighted the remarkable ACEI of protein hydrolysates. ACE inhibitors such as enalapril, captopril, alacepril, or lisinopril are often used to clinically treat hypertension, myocardial infraction, and cardio-related diseases. In the present investigation, a stronger ACEI was recorded for shrimp shell hydrolysate compared to captopril activity. The corresponding IC_{50} were 71.52 ± 1.48 and 85.33 ± 1.26 µg/mL for the hydrolysate and captopril, respectively. The IC_{50} value is nearly similar to that of smooth hound viscera hydrolysate obtained by treatment with Puraf protease (75 µg/mL) [34]. The IC_{50} value of the hydrolysate is lower than those of liquid state lentils fermented by *Lactobacillus plantarum* (0.20 mg/mL) and squid gelatine hydrolysates obtained with different proteases (0.34 and 1.6 mg/mL) [35, 36]. ACEI was earlier demonstrated for sardinelle protein hydrolysates from fermentations using *Bacillus subtilis* strain A26 (SPH-A26) and *Bacillus amyloliquefaciens* strain An6 (SPH-An6) reaching 75.7% ± 0.66 and 79.5% ± 0.33 at 0.6 mg/mL, respectively [37]. Both hydrolysates showed IC_{50} values reaching 0.21 mg/mL for SPH-An6 and 0.26 mg/mL for SPH-A26. Moreover, no ACEI was detected with undigested sardinelle proteins. Therefore, the ACEI is closely related to the sardinelle proteins and could be released through the action of proteolytic enzymes secreted by proteolytic bacteria during fermentation. The inhibition mechanism may be due to the degradation of the initial peptide and the elaboration of newly generated peptide fragments [38]. Several studies suggested the presence of hydrophobic (branched side or aromatic chains) amino acid residues at the three C-terminal positions and the presence of lysin and arginin residues at the C-terminal are supposed to promote the ACEI of protein hydrolysates [37, 39].

Tyrosinase is mainly involved in the melanin biosynthesis. In mammals, this enzyme is involved in the Parkinson's and other neuro-degenerative illnesses. Besides being used for the treatment of some dermatological disorders, associated with melanin hyperpigmentation of humans as well as animals, tyrosinase inhibitors have offered a great potential with respect to pharmaceutical and cosmetic industries owing to their skin-whitening effect and depigmentation after sunburn. Shrimp by-product hydrolysate showed an important Tyrosinase inhibitory effect even though the IC_{50} was higher than the value determined for Kojic acid; 107.67 ± 2.33 and 4.05 ± 0.25 µg/mL, respectively. The collagen hydrolysate fraction obtained from the frozen squid *Todarodes pacificus*, by Alcalase 2.4L inhibited Tyr by 39.65% at 1 mg/mL [40]. Once again, this study gives the first report on the TyrI potential of shrimp shell hydrolysate. Thus, the use of protein hydrolysates as Tyr inhibitors is gaining much more concern in the cosmetic industry thanks to its skin whitening and preventive effects. Several potent TyrI have been used, such as Kojic acid, hydroquinones, and arbutin. However, Kojic acid and arbutin hardly showed inhibitory activity against pigmentation in intact melanocytes or in a clinical trials, and hydroquinones are considered to be cytotoxic to melanocytes and potentially mutagenic to mammalian cells. Therefore, the screening of new tyrosinase inhibitors remains a necessity [41]. These authors have demonstrated that protein hydrolysates containing low molecular weight peptides showed the greatest tyrosinase inhibitory effect. Furthermore, these authors have also linked this activity with the presence of hydrophobic and aliphatic amino acid residues. In protein hydrolysate, the TyrI has been essentially assigned to cyclic peptides as Pro-Val-Pro-Tyr [42].

Finally, the shrimp hydrolysate also displayed a promising activity against Amy, its IC_{50} (133.24 µg/mL ± 0.67) was remarkably lower than that obtained with acarbose (570.16 µg/mL ± 1.82). The protein hydrolysate from shrimp shell wastes, *Fenneropenaeus chinensis*, exhibit an AmyI of 43% at 5.4% (w/w) of commercial protease, Neutrase [43]. This activity could be speculated by the presence of peptides containing cationic and branched chain residues, such as Phe, Tyr, Trp, and Lys [44]. Previous work suggested that Amy inhibitors are a class of compounds that facilitate and assist in the control of diabetes by diminishing the absorption of glucose [44].

3.2.2.2. Antioxidant activities

Hydrolysate antioxidant potential was emphasized through several tests: DPPH and ABTS radicals scavenging, ferrous ion chelating capacity, β-carotene-linoleic acid bleaching system, and ferric reducing antioxidant power (FRAP). BHT was used as reference, except the ferrous ion chelating capacity where EDTA was used.

The antiradical power (DPPH and ABTS) of the hydrolysate was significant, especially against the ABTS cation radical where the hydrolysate was as effective as BHT at 100 µg/mL which caused the neutralization of 95% of the radicals. However, at lower concentrations, BHT showed a stronger potential in inhibiting ABTS radicals which is better explained by a lower EC_{50} of 5.13 against 46.88 µg/mL for the hydrolysate. For the scavenging ability against DPPH radical, the hydrolysate showed only an EC_{50} value of 423.07 µg/mL against 238.52 for the BHT. These findings are in line with previous studies which reported that the difference between the two tests could be elucidated by the property of the two radicals. While $ABTS^{\cdot+}$ radicals dissolve in aqueous media, the DPPH radicals is better solubilized in alcoholic media. This fact may affect the interaction between antioxidant peptides and the radicals. Therefore, the observed antioxidant capacity could be assigned to the presence of certain amino-acids residues, such as Met, His, Trp, Phe, Tyr, Leu, Pro, and Gly [45].

In the same context, the production of antioxidant activities from shrimp, *Penaeus mondon* by-product fermented by proteolytic strain CFR2182 has also been reported. Thus, it was found that protein hydrolysate showed EC_{50} values of 1.26 and 0.2 mg/mL respectively for the DPPH and ABTS radicals [46].

The hydrolysate inhibitory power in the β-carotene-linoleic acid bleaching system was moderate, likewise its ion chelating capacity against BHT and EDTA.

For the β-carotene bleaching, the hydrolysate showed an inhibition of 62.8% at 1,000 µg/mL with an EC_{50} value of 410.21 µg/mL against 215.40 µg/mL for the BHT. Whereas, the protein hydrolysate obtained with fermentation of the same species *Metapenaeus monoceros* by *Pseudomonas aeruginosa* strain A2 exhibited an EC_{50} of 3,000 µg/mL. In addition, an independent report revealed that at 6

mg/mL, the protein hydrolystates SPHA1, SPHEE, and SPHA2, obtained by several proteolytic enzymes caused an inhibition of β-carotene bleaching by 50, 70, and 75%, respectively [47]. This activity could be attributed to the presence of hydrophobic amino acids in the peptide chains present in the hydolysate [48]. Indeed, hydrophobic amino acids could have higher efficiency than hydrophilic amino acids in terms of preventing oxidation in oil and water emulsion system. The displayed activity could be assigned to the presence of Gly and His rich peptidic structures [49].

Obviously, at 1,000 µg/mL, the ferrous chelating power was 44.95% with an EC_{50} of 1120.04 µg/mL against, 80.10 µg/mL for the EDTA. The ferrous chelating activity in the protein hydrolysate could be related to the Gly and His-rich peptides [37]. The screening of all fermented protein hydrolysates from *Metapenaeus monoceros* described in the literature, only the one obtained with *Bacillus licheniformis* strain RP1 was reported to exhibit 98% of ferrous chelating activity at 5 mg/mL [50]. Morover, our results indicated that the ferrous chelating activity of the recovered hydrolysate is comprised with those obtained with fermented sardinelle protein hydrolysates, SPH-A26 (20%) and SPHAn6 (60%), at 1,000 µg/mL [37].

Regarding the FRAP assay, shrimp hydrolysate displayed a remarkable potential nearly similar to that of BHT as indicated by the absorbance at 700 nm. At 1,000 µg/mL, the sample displayed a significant FRAP, rached 1.55 of absorbance against 1.634 for the BHT. In the same context, the fermented protein hydrolysate from *Metapenaeus monoceros* obtained by *Bacillus licheniformis* strain A1 exhibited an absorbance of 1.55 at 1,500 µg/mL [50]. Each fermented fish meat protein hydrolysates prepared from sardinelle, zebra blenny, goby, and ray showed lower reducing power activities than BHA as positive control at the same concentrations [51]. The observed FRAP activity could be ascribed to the presence of peptides capable of donating electron to free radicals, resulting in the prevention or retardation of the oxidation propagation.

The protective effect againt the hydroxyl radical-induced DNA damage in supercoiled pUT57 plasmidic system was also evaluated. In the initial condition (1), the plasmid migration led to the appearence of three bands: the lower one presents the supercoiled DNA form followed by the nicked open circular form, and the upper one is the linear form. When the DNA sample was treated with Fenton's reagent, the supercoiled form was totally converted to the other two forms. When shrimp hydrolysate was added at 100 or 1,000 µg/mL, the DNA denaturation was successfully precluded. These findings are in agreement with previous investigations, which prove that protein hydrolysates from marine sources exhibited a strong protection against hydroxyl radicals inducing DNA damage. Likewise, the protein hydrolysate from sardinelle, zebra blenny, goby, and ray displayed a potentiel hydroxyl radical scavenging activity and inhibited the conversion of supercoiled pCRII^{TM}-TOPO$^{®}$ plasmid DNA to the open circular form [51]. Excessive free radicals production in living cells have been shown to be responsable for mutations and carcinogenesis by inducing base modification and DNA strand breakage [52, 53]. For example, hydroxyl radicals, having the highest reduction potential, can react with lipids, proteins, and DNA, especially thiamine and guanosine. The protective effect of protein hydrolysates against DNA damage has been previously discussed. It has been reported that gelatin hydrolysates were effective in preventing DNA damage caused by Fenton reaction and peroxyl radicals [54]. Essentially, numerous factors could affect the antioxydant capacity of hydrolysate samples, such as the degree of hydrolysis, amino acids content, substrate affinity, and especially polarities, peptide sizes, and structures.

4. Conclusions

For a better management and valorization of fish by-products, the integrated process developed in this study allowed to obtain proteolytic enzyme by *Anoxybacillus kamchatkensis* strain M1V while using an alternate cheap substrate from white shrimp bio-waste. Lyophilized hydrolysate from fermented shrimp, *Metapenaeus monoceros*, by-product exhibited both antioxidant activities and enzymes inhibitory potential. Supplementary and comprehensively studies will be requisite to isolate and identify the specific compounds responsible for the biological activities, notably for tyrosinase and acetylcholinesterase inhibition, and to evaluate *in-vivo* activities of the hydrolysate fom *Metapenaeus monoceros*.

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