Alternative Proteins as a Source of Bioactive Peptides: The Edible Snail and Generation of Hydrolysates Containing Peptides with Bioactive Potential for Use as Functional Foods

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Abstract: Members of the Phylum Mollusca include shellfish such as oysters and squid but also the edible garden snail known as Helix aspersa. This snail species is consumed as a delicacy in countries including France (where they are known as petit-gris), southern Spain (where they are known as Bobe), Nigeria, Greece, Portugal and Italy but is not a traditional food in many other countries. However, it is considered an excellent protein source with a balanced amino acid profile and an environmentally friendly, sustainable protein source. The aim of this work was to develop a different dietary form of snail protein by generating protein hydrolysate ingredients from the edible snail using enzyme technology. A second aim was to assess the bioactive peptide content and potential health benefits of these hydrolysates. H. aspersa hydrolysates were made using the enzyme Alcalase® and the nutritional profile of these hydrolysates was determined. In addition, the bioactive peptide content of developed hydrolysates was identified using mass spectrometry. The potential heart health benefits of developed snail hydrolysates were measured in vitro using the Angiotensin-I-converting Enzyme (ACE-1; EC 3.4.15.1) inhibition assay, and the ACE-1 inhibitory drug Captopril® was used as a positive control. The generated H. aspersa hydrolysates were found to inhibit ACE-1 by 95.60% (±0.011) when assayed at a concentration of 1 mg/mL (n = 9) compared to the positive control Captopril® which inhibited ACE-1 by 96.53% (±0.0156) when assayed at a concentration of 0.005 mg/mL (n = 3). A total of 113 unique peptide sequences were identified following MS analysis with peptides identified ranging from 628.35 Da (peptide GGGLVGGI—protein accession number sp|P54334|XKDO_BACSU) to 2343.14 Da (peptide GPAGVPGLPGAKGDHFPGSSGRRGD—protein accession number sp|Q7SIB2|CO4A1_BOVIN) in size using the BIOPEP-UWM database.

Keywords: edible garden snail; Helix aspersa; protein; hydrolysis; Alcalase®; heart health; angiotensin-converting enzyme; mass spectrometry; sustainable protein

1. Introduction

Snails from the genus Helix (H. aspersa, H. pomatia, H. lucorum or Turkish snail) are terrestrial gastropods commonly used as niche food sources in some European and African countries. In other countries such as China and Japan they are commonly consumed in the breeding and capture forms [1]. H. aspersa is known as H. aspersa maxima, which is a hermaphrodite snail grown in several temperate climatic regions.

Snail meat is low in cholesterol and fat and Bazán [2] previously reported that it contains 10 mg cholesterol/10 g of meat. There is an increasing demand for alternative protein sources and a requirement for cheaper and more sustainable proteins to feed the growing global population. Snail protein is considered nutritious with an excellent amino acid profile. Snails can be produced in humid countries and European climates generally suit production of H. aspersa. However, the consumption of snails is not preferred in several countries in Western Europe and the USA. Despite this, global consumption is
400,000–450,000 tons per year, of which only 15% comes from snail breeding units, while the remaining 85% comes from snails collected from nature [3].

Snails are considered a delicacy and snail meat is usually consumed as an appetizer and prepared in butter. The snail shell is discarded as waste. Several reports exist concerning the nutritional quality of snails [4–6]. In addition, there are some reports in the literature regarding the potential benefits of snail meat and eggs. Górka and colleagues [7] recently reported the nutrient and antioxidant properties of *H. aspersa maxima* eggs. Estimated global annual consumption of snail meat is predicted to increase five-fold in the next twenty years [7]. However, there are no studies on the development of snail protein hydrolysates to date as a method to make snail protein more stable and accessible to consumers. Due to the high nutritional value of snail meat products, spoilage sets in after one or two days after harvesting, and preservation methods are required. Hydrolysis can also improve and increase the health benefits of snail proteins.

Hydrolysates consist of short-chains of amino acids known as bioactive peptides. Bioactive peptides are encrypted within the sequence of a parent protein and may be released by hydrolysis using enzymes or acids or by fermentation with lactic acid bacteria (LAB) [8]. The majority of the biologically active peptides identified to date can be found in the BIOPEP-UWM database [9] and include antimicrobial, dipeptidyl peptidase-IV (DPP-IV; EC 3.4.14.5), angiotensin-I-converting enzyme (ACE-I; EC 3.4.15.1) and prolyl endopeptidase (PEP; EC 3.4.21.26)-inhibitory peptides, as well as antioxidant peptides) [10]. Recently, protein hydrolysates were developed from the spotted Babylon snail *Babylonia areolata* using pepsin and pancreatin and the antioxidant activity of the resultant hydrolysate was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity assays as well as a cellular antioxidant assay [11]. Two antioxidant peptides with the amino acid sequences HTYHEVTKH and WPVLAYHFT were identified using MS and WPVLAYHFT had higher radical scavenging activities likely due to the hydrophobic nature of the amino acids within its structure.

The aim of this work was to develop snail protein hydrolysates using the common garden snail and the proteolytic enzyme Alcalase® and to determine yields, nutritional profiles and assess the potential health benefits of this new form of snail protein.

2. Materials and Methods

2.1. Chemical and Substrate Materials

*Helix aspersa maxima* by-product snails were supplied by Mr Stephen Ryan, Tuam, Co. Galway (Ireland) who farms these snails. By-product snails are those considered to be under-sized and not suitable for market. By-product *Helix aspersa maxima* were carefully washed using running water prior to placement on ice and subsequent freezing at −80 °C. Alcalase® 2.4 L FG was kindly supplied by Novozymes (Bagsvaerd, Denmark). The ACE-I inhibition assay kit was kindly supplied by NBS Biologicals Ltd. (Cambridgeshire, England, UK). The positive control Captopril® was purchased from Sigma-Aldrich (Sigma-Aldrich, Dublin, Ireland). All other chemicals used were of analytical grade.

2.2. Generation of Snail Protein Hydrolysates

Generation of snail protein hydrolysates was performed according to the method of Hamid et al. [12] with some modifications. Briefly, 44 g of cleaned, whole by-product *H. aspersa maxima* snail was ground using a Pestle and Mortar and to this 130 mL of distilled deionised water was added. Native enzymes present in the mixture were heat-deactivated by heating the mixture to 85 °C for 15 min in a water bath followed by cooling to room temperature. Following this, the pH was adjusted to pH 8 using 1 M NaOH. The mixture was placed in a shaking incubator at 45 °C and 1.76 mL of Alcalase® enzyme (Sigma Aldrich, Dublin, Ireland) was added to the mixture in an enzyme to substrate ratio of 4%. The temperature was maintained at 45 °C, with shaking at 150 rpm for 180 min. The pH was kept constant at the optimum for Alcalase® using 0.1 M NaOH. After this time, the
hydrolysates were heat-deactivated by heating to 90 °C for 15 min. The mixtures were cooled and subsequently centrifuged at 5000 rpm, 4 °C for 20 min after which time the supernatant was recovered, frozen at −80 °C and subsequently freeze-dried along with the shell fraction. The degree of hydrolysis (DH) was calculated using the pHstat technique after 180 min according to the Adler-Nissen method [13].

2.3. Freeze-Drying

The supernatant containing peptides was frozen, freeze-dried using an industrial-scale FD 80 model freeze-drier (Cuddon Engineering, Christchurch, New Zealand) and stored at −20 °C until further use. The yield of hydrolysates was calculated based on the percentage of supernatant recovered on a dry weight basis.

2.4. Proximate Analysis

The yield of stabilised hydrolysate products was calculated after freeze-drying and weighing the samples, and is expressed as a percentage of the product in total mass of initial snail hydrolysate.

2.4.1. Protein

The total protein content of the samples was determined using the Dumas combustion method using a LECO FP328 Protein analyser (LECO Corp., Saint Joseph, MI, USA), according to the Association of Official Analytical Chemists (AOAC) method 992.15 [14]. The conversion factor of 6.25 was used to convert total nitrogen to protein.

2.4.2. Ash

The ash content was determined gravimetrically, as previously described by Kolar [15].

2.4.3. Lipid Content

The total fat content was determined gravimetrically using an Ankom XT15 Extractor (Ankom Technology, Macedon, NY, USA) for lipid extraction, after previous acid hydrolysis using Ankom HCl Hydrolysis System according to manufacturers’ operating manual and as previously described [16].

2.4.4. Water Activity

The water activity ($a_w$) of all samples was measured using an AquaLab Lite meter (Decagon Devices Inc., Munich, Germany). Approximately 0.25 g of finely powdered sample was placed in the water activity metre and $a_w$ and the temperature was recorded.

2.5. ACE-I Inhibition Assay for Potential to Reduce Blood Pressure

The ACE-I inhibition assay was carried out according to the manufacturer’s instructions and as described previously [16]. In brief, 20 µL of each sample aqueous solution at a concentration of 1 mg/mL was added to 20 µL substrate and 20 µL enzyme working solution in triplicate. Captopril was used as a positive control. Samples were incubated at 37 °C for 1 h. A 200 µL aliquot of indicator working solution was then added to each well, and subsequent incubation at room temperature was carried out for 10 min. Absorbance at 450 nm was read using a FLUOstarOmega microplate reader (BMG LABTECH GmbH, Offenburg, Germany). The percentage of inhibition was calculated using the following equation:

\[
\% \text{ ACE-I inhibition} = 100\% \text{ Initial activity} - \text{Inhibitor} \times \frac{100}{100\% \text{ Initial activity}}
\]  

(1)

2.6. LC-MS/MS Analysis

Peptides were analysed in a mass spectrometer nanoESI qQTOF (6600 plus TripleTOF, AB SCIEX, Framingham, MA, U.S.A.) using liquid chromatography and tandem mass spectrometry (LC–MS/MS). A total of 1 µL of snail hydrolysate was loaded onto a trap
column (3 µ C18-CL 120 Å, 350 µm × 0.5 mm; Eksigent) and desalted with 0.1% TFA (trifluoroacetic acid) at 5 µL/min during 5 min. The peptides were then loaded onto an analytical column (3 µ C18-CL 120 Å, 0.075 × 150 mm; Eksigent) equilibrated in 5% acetonitrile 0.1% FA (formic acid). Elution was carried out with a linear gradient from 7 to 45% B in A for 20 min, where solvent A was 0.1% FA and solvent B was ACN (acetonitrile) with 0.1% FA) at a flow rate of 300 nL/min. The sample was ionised in an electrospray source Optiflow < 1 µL Nano applying 3.0 kV to the spray emitter at 200 ºC. Analysis was carried out in a data-dependent mode. Survey MS1 scans were acquired from 350 to 1400 m/z for 250 ms. The quadrupole resolution was set to ‘LOW’ for MS2 experiments, which were acquired from 100 to 1500 m/z for 25 ms in ‘high sensitivity’ mode. The following switch criteria were used: charge: 1+ to 4+; minimum intensity; 100 counts per second (cps). Up to 50 ions were selected for fragmentation after each survey scan. Dynamic exclusion was set to 15 s. The system sensitivity was controlled by analysing 500 ng of K562 protein extract digest (SCIEX); in these conditions, 2260 proteins were identified (FDR < 1%) in a 45 min gradient.

2.7. Data Analysis of MS/MS Results

Protein Pilot v 5.0. (SCIEX) default parameters were used to generate peak list directly from 6600 plus TripleTOF wiff files. The Paragon algorithm [17] of ProteinPilot v 5.0 was used to search different databases with the following parameters: Uniprot Mollusca database (201,001, 340,255 proteins) and none enzyme. Peptides were identified with a confidence of ≥90%.

2.8. In Silico Identification of Novel Peptides

Peptides identified in Table 1 were compared to 4132 bioactive peptides previously reported in the literature [18] and those found in BIOPEP-UWM http://www.uwm.edu.pl/biochemia/index.php/en/biopep (accessed on the 21 December 2020) [19].

Table 1. Identification of snail hydrolysate sequences of peptides by mass spectrometry in tandem using the Uniprot Mollusca database (201,001, 340,255 proteins).

| Protein Accession Number | Peptide Sequences Identified a | Theoretical Molecular Weight (MW) b |
|--------------------------|-------------------------------|-----------------------------------|
| sp|P00780|SUBC_BACL1 | AAGNOSGSGNTNIGYPAKY | 1928.89 |
| sp|P00780|SUBC_BACL1 | AQTVPYPILIK | 1298.76 |
| sp|P00780|SUBC_BACL1 | AQTVPYPILIKADKQVAQQ | 2039.14 |
| sp|P00780|SUBC_BACL1 | ASHPDLNVVGG | 1135.56 |
| sp|P00780|SUBC_BACL1 | ASHPDLNVVGGAS | 1222.59 |
| sp|P00780|SUBC_BACL1 | AVDSSNRSASFS | 1253.56 |
| sp|P00780|SUBC_BACL1 | AYNTDNGHHTHA | 1413.59 |
| sp|P00780|SUBC_BACL1 | DNTTGVLGVAPVSLY | 1591.81 |
| sp|P00780|SUBC_BACL1 | DTGIQASHPDNLVVGGA | 1649.80 |
| sp|P00780|SUBC_BACL1 | GAVDSSNRSASFS | 1310.59 |
| sp|P00780|SUBC_BACL1 | GIPLIKADKQVAQQ | 1379.81 |
| sp|P00780|SUBC_BACL1 | ILSKHPNLSAS | 1165.65 |
| sp|P00780|SUBC_BACL1 | NIGNGSNGSGVGGSCJE | 1499.67 |
| sp|P00780|SUBC_BACL1 | NSSGSGSYSGVSGGWEATTN | 2072.93 |
| sp|P00780|SUBC_BACL1 | NTGDNGHHTHA | 1179.49 |
| sp|P00780|SUBC_BACL1 | SLGGASGSTMKNQ | 1209.57 |
| sp|P00780|SUBC_BACL1 | STYPTNTYATL | 1230.58 |
| sp|P00780|SUBC_BACL1 | STYPTNTYATLN | 1344.62 |
| sp|P00780|SUBC_BACL1 | VDSSNRSASFS | 1182.53 |
| sp|O16808|ACT_MAYDE | AGDDAPRAVFPS | 1201.57 |
| sp|O16808|ACT_MAYDE | GQKDSVYVDADQKSRKILTL | 2164.11 |
| sp|O16808|ACT_MAYDE | KSYELPDQQVITIG | 1518.79 |
| sp|O16808|ACT_MAYDE | KSYELPDQQVITIGNE | 1761.88 |
Table 1. Cont.

| Protein Accession Number | Peptide Sequences Identified \(^a\) | Theoretical Molecular Weight (MW) \(^b\) |
|--------------------------|-------------------------------------|--------------------------------------|
| sp| P80057|GSEP_BACLD | SEPGQAGSPGPPGPPGAIGPSGP | 1937.91 |
| sp| P80057|GSEP_BACLD | WQHQSPGPAISE | 1223.59 |
| sp| P02481|C03A1_HUMAN | GECPQAGSGPPGPPGAIGPSGP | 1937.91 |
| sp| Q8R867|D1N1_CALS4 | GECQGGRGPP | 1139.52 |
| sp| Q86XJ0|CQI5_HUMAN | GEAPQGPGTTGAP | 1037.48 |
| sp| B4UJ6|Y4073_ANASK | EGAVLAGAGLGSER | 1526.81 |
| sp| O4S218|ADAS_CAEEL | LDPANIFASNLIDI | 1565.84 |
| sp| Q03A38|ATPB_LACP3 | GDDPGGEAFGP | 1130.49 |
| sp| A5GE21|LPXA_PSYYW | IGNPVILGGNAG | 1097.58 |
| sp| O60784|TOM1_HUMAN | SAEPPGPSPGPA | 1119.52 |
| sp| P45866|CU79A_LOMCI | LGGGLGGLGL | 812.48 |
| sp| P02385|CP17_SOLUT | VIDDKDFIPF | 1209.56 |
| sp| Q8X9A1|GPA1_LACSS | MPTINAJNVL | 1247.66 |
| sp| Q5RF96|SPCS1_PONAB | SGAVAIAFPFLEGPPA | 1452.76 |
| sp| P54341|XKDO_BACSL | GGLVGLGL | 626.35 |
| sp| Q7F531|CLCC_DICDI | LIGGLGL | 641.41 |
| sp| Q3UE01|PITM3_MOUSE | AGPSGDPSCSSR | 1142.90 |
| sp| Q8IQC1|M02B_DROME | LLGGILG | 641.41 |
| sp| Q8X501|MRAY_LACSS | IGGGLGL | 641.41 |
| sp| A4R21|NST1_MAGO7 | NQHYPAPGPAPNAP | 1490.72 |
| sp| L0E307|PHQO_PENFE | YLKPVPIVPGLP | 1319.79 |
| sp| Q80X17|VOME_MOUSE | GGLGGLGGL | 755.45 |
| sp| Q8BM72|HSP13_MOUSE | YTGVAGQIDGGGSG | 1526.77 |
| sp| C1A5U3|DAF_GEMAT | FVKGMTSGGDF | 1233.53 |
| sp| P80057|GSEP_BACLD | GYPDQTAQQWQHSGPAISE | 2299.09 |
| sp| Q42550|CO1A2_LITCT | AGLNGLGPGPAP | 1067.52 |
| sp| P00780|SUBC_BACLI | SHPDLNPGGAG | 1064.53 |
| sp| P00780|SUBC_BACLI | AAGNSGSGNTGNYPA | 1637.73 |
| sp| Q5FRT2|PRO_GLUOX | LIDAAIPAL | 966.58 |
| sp| P00780|SUBC_BACLI | SKHPNLSAS | 939.48 |
| sp| P00780|SUBC_BACLI | APVRPGAGVY | 630.31 |
| sp| Q9MB80|PME34_ARATH | MPVQSOQADIV | 1341.67 |
| sp| Q9Z0J0|AROA_CORGL | ATAGAAIGLAVDG | 1127.62 |
| sp| A1X831|TI214_RANMC | LIVLPSLI | 866.58 |
| sp| D4G4P1|KGSIH_HALVD | GATLVAGGGVPF | 1026.53 |
| sp| Q8XX26|C51_CRYNH | FGLWVLNLWI | 1147.61 |
| sp| Q6CV31|AGLS8_ARATH | AGAGAGAAPL | 754.40 |
| sp| P27483|GRP1_ARATH | GAGGGLGSGGSGG | 2447.13 |
| sp| P27393|CO4A2_ASCSU | GDDGLPGAPGRPG | 1146.54 |
| sp| P00780|SUBC_BACLI | NSSSGSSGYSIVS | 1200.53 |
| sp| B2A865|ACCCARDNAUTT | EGGSCGALTV | 1030.53 |
| sp| Q8WX71|MUC16_HUMAN | PSLSLFSATTSP | 1182.65 |
| sp| Q9H7P9|PHK12_HUMAN | RGCGGGGTP | 769.39 |
| sp| Q1HV77|EBNA1_EBVA | GAGGAGAGGGAGGAGGAGGAGG | 1555.67 |
| sp| Q8X176|VOME_MOUSE | GEEGGGGLGGLL | 1055.56 |
| sp| Q7W0A1|MUTL_BORPE | AGVPDQAAPDTAYAGEPA | 1628.73 |
| sp| Q8IA41|GLT11_DROME | EPIILLNQ | 939.50 |
| sp| Q8K418|BPIA1_RAT | NGLVNGGLG | 798.46 |
| sp| B8TX31|MCH_METNO | VAEAGVPL | 825.46 |
| sp| Q9LE10|EN01_HEVBR | SIEDFQDQ | 1064.43 |
| sp| P86950|SLP2_PINMA | GIGGGGIGGIGG | 1023.57 |
| sp| Q5U9X3|GP6C6A_DNAR | IGGGLPFI | 828.51 |
| sp| Q9N9O8|TOP3A_DROME | GGGGGPGPAGG | 879.38 |
| sp| Q1H808|ACT_MAYDE | RVAPVEHPVLL | 1258.70 |
| sp| P34804|COL40_CAEEL | SEPGPAGPAGDGAGPGAGPGA | 2114.91 |
| sp| Q6KN51|HEM3_BOVIN | WSLNGAEIMQ | 1136.48 |
| sp| Q9EF24|FOX1_ARATH | PAGTTPKTLGRP | 1305.78 |
| sp| P12575|FUS_SENDF | IVVMVVI | 884.58 |
| sp| B0TMM7|ADEC_SHEH | LDALAPI | 824.50 |
| sp| P00780|SUBC_BACLI | SHPDLNNGGAG | 1151.56 |
| sp| P49571|P2C6_ARATH | AGPFPFRE | 790.41 |
| sp| Q7TZN1|PKNE_MYCO | TEALPIE | 868.45 |
Table 1. Cont.

| Protein Accession Number | Peptide Sequences Identified a | Theoretical Molecular Weight (MW) b |
|--------------------------|-------------------------------|-------------------------------------|
| sp|Q6AZY7|SCAR3_HUMAN | GDPGSLGPLGPQ 1094.52 |
| sp|P171401|CO4A2_CAEEL | QDGLGPLCNHG 1152.58 |
| sp|A5GQG6|LEUC_ACICJ | LGMPDKLKPGE 1297.67 |
| sp|Q7SIB21|CO4A1_BOVIN | GPAGVPGAGKDHGPPGSGPGRGD 2343.14 |
| sp|Q6MP8|KCTD7_HUMAN | FGDVLNF 810.39 |
| sp|Q8TZZ21|MPTA_PYRFU | EDALEMDI 1047.48 |
| sp|Q7Z5A41|PR542_HUMAN | AFGPEAEPLL 904.47 |
| sp|O48531|DEXHD_ARATH | VQVGAIVNGE 984.52 |
| sp|Q9QY61|MYO9B_MOUSE | DAGLSPGQGSDK 1217.55 |
| sp|Q4K758|SYM_PSEF5 | ITQYFDPE 1011.45 |
| sp|Q9Y4K41|M4K5_HUMAN | SSDPNFMLQ 1038.43 |
| sp|Q8WH61|Y206_ENCCU | DVPVEEMAVG 1044.48 |
| sp|Q8EX71|DNAJ1_STRAW | GAGGGFGGGI 748.35 |
| sp|B2GDS9|DNLJ_LACF3 | AGDIIPE 713.36 |
| sp|Q9Z8N11|PHSG_CHLPN | AIEDIALI 856.49 |
| sp|Q9FCC11|BIOD_STRTO | GAPLLGAVPGAGS 1136.62 |
| sp|Q6CRZ31|GPH_PECAS | IGGDDDIVK 914.51 |
| sp|P0C61|GRSA_BREBRE | GGEGLARDGYK 1192.60 |
| sp|Q218401|VSM5_TRYBR | GEDQETFHSRFDQ 1781.73 |
| sp|Q9CT761|METK_THEVO | DTSGVGFAP 996.46 |
| sp|Q2KJ581|R3GEF_BOVIN | SVGPCKSHERSLGLPGE 1751.86 |
| sp|Q1Y051|RIMO_TRIEI | GTPAYNLPN 945.46 |
| sp|Q4KSPF1|PROA_PSEF5 | NEVDSSSVMVNASTRF 1741.79 |
| sp|Q1D5V41|TTK_RHOCB | FVMEFGASAPA 1239.56 |
| sp|Q9KPH11|Y2383_VIBCH | AAQALGGMGL 1014.55 |
| sp|Q8SY411|BCAS3_DROME | GLGVQVWAIPANGEAVE 1708.88 |
| sp|Q9DR481|MKNR2_SERQU | GGGAGGGAGGAGGGCP 1239.56 |
| sp|O489281|C77A3_SOYBN | TALAFFSLIF 1298.73 |

a The sequence of the peptide identified by the search; b The theoretical precursor molecular weight for peptide sequence, including modifications.

3. Results

3.1. Yields and Proximate Analysis of Snail Protein Hydrolysates

The yield of snail protein hydrolysate recovered following treatment with the proteolytic enzyme Alcalase® was 3.98 g (±0.275) from 44 g of ground snail (9.05% dry weight). The yield of clean shell recovered following hydrolysis was 7.98 g (±0.68) (18.13% dry weight). The degree of hydrolysis was calculated. The protein content of recovered snail protein hydrolysate was 65.49% (±0.90) when measured using the LECO method with a nitrogen conversion factor of 6.25. The lipid content of the developed hydrolysate was 0.51% (±0.31) and the remainder was ash. The a_w value of the freeze-dried snail protein Alcalase® hydrolysate was 0.49 (±0.90). All analyses were carried out in triplicate (n = 3). The degree of hydrolysis was calculated as 10.8% (±0.78) using the pH stat technique [13], where B (mL) is the volume of NaOH consumed, NB is the normality of the NaOH used, 1/α is the average degree of dissociation of the α-amino groups related with the pK of the amino groups at particular pH and temperatures, MP (g) is the amount of protein in the reaction mixture, and htot (meq/g) is the sum of the millimoles of individual amino acids per gram of protein associated with the source of protein used in the experiment. Values for htot and 1/α were obtained from the study conducted by Adler-Nissen [13].

3.2. Angiotensin Converting Enzyme I Inhibition Assay

The snail Alcalase® hydrolysates showed appreciable ACE-1 inhibition compared to the positive control Captopril©. The percentage ACE-1 inhibition was 95.60 ± 0.011% as compared to 96.53 ± 0.0156% inhibition of ACE-1 by the positive control (Figure 1) when assayed at a concentration of 1 mg/mL as it is shown in Figure 1. Further, the IC50 value was calculated as 0.2944 mg/mL, which is comparable to other reported values for marine mollusca hydrolysates (1.50–2.54 mg/mL) [15].
3.3. Identification of Proteins and Peptides in Snail Alcalase® Hydrolysates

A total of 113 peptides were identified in the snail Alcalase® hydrolysates. Several (nineteen) of the identified peptides originated from the Alcalase® enzyme, while others (five) were cleaved from the protein actin (Accession number sp|O16808|ACT_MAYDE). Peptides and their parent proteins are shown in Table 1. Several of the peptides shown in Table 1 contain the previously identified di-peptide sequences YG, YA, VY, FG/GF, DF, SF and VW identified previously from Helix aspersa as antihypertensive by Drevet [18]. DF is found in four of the peptides and SF in five of the peptides identified in Table 1. These peptides were previously reported to reduce systolic blood pressure in vivo in spontaneously hypertensive rats by 20 mmHg after a single oral administration at doses of 400 and 800 mg kg−1 [18].

4. Discussion

The yield of protein recovered following the hydrolysis of H. aspersa was 9.05% but the protein content of the hydrolysate was significant—65.49% (±0.90). The protein content of the recovered hydrolysate compares favorably with those of marine and animal origin. Previously, Drevet [20] identified seven di-peptides from a hydrolysate of H. aspersa. This work identified 113 peptides from an H. aspersa hydrolysate generated using the proteolytic enzyme Alcalase®. Several of the peptides identified in the hydrolysate contained the di-peptide sequences identified previously within their amino acid sequences and collectively the hydrolysate inhibited the enzyme ACE-1 by 95.60 ± 0.011%. However, the peptides identified were not chemically synthesised and assessed for their ability to inhibit ACE-1 individually. Thus, each peptide is not specifically an ACE-1 inhibitory peptide. However, some of the identified peptides contained shorter sequences with confirmed ACE-1 inhibitory bioactivity. The fact that some regularities were observed previously between the presence of the specific amino acid(s) and the function of a whole peptide sequence has prompted some scientists to find the foundations for the fragmentomic idea of research [21–23]. According to the fragmentomic idea, shorter peptide fragments with known bioactivity encrypted in a larger peptide of interest may affect the presence of ACE-1 inhibitory dipeptides in the parent sequence (understood as the identified longer peptides in this study) could decide the ACE-1 bioactivity of the whole fragment.

None of the peptides identified in this study are reported in the peptide database BIOPEP-UWM http://www.uwm.edu.pl/biochemia/index.php/en/biopep (accessed on the 22 of December 2020) [19]. This database contains over 4132 bioactive peptides and, as it is a comprehensive source of bioactive peptides, no other database was used in this
study to assess the novelty of the identified peptides. The usability of in silico methods and databases such as BIOPEP-UWM in supporting the analysis of bioactive peptides derived from food sources was demonstrated previously by authors including Udenigwe [24], Tahir [25] and Minkiewicz and colleagues [19]. In this study, we used it to determine if the identified peptide sequences were novel, but further work to assess the bitterness and further bioactivities of the peptides could be determined using an in silico approach.

Treatment of the garden snail with the enzyme Alcalase® serves as a cheap and effective method of producing protein hydrolysates with ACE-1 inhibitory activities. The ACE-1 inhibitory activity obtained for this hydrolysate is superior to those reported previously in other studies where members of the Mollusca family—green-lipped mussel Perna canaliculus—were hydrolysed with Alcalase® and assessed for ACE-1 inhibitory activities [26]. Jayaprakash and Perera [26] reported ACE-1 inhibition values of ~5% in this study. The hydrolysis of H. aspersa generates bioactive ACE-1 inhibitory peptides with potential for the maintenance of normotensive blood pressure in consumers. It also supplies protein in a form that is more convenient than consuming snails in their traditional form.

5. Conclusions

In this study, a different dietary form of snail protein by generating peptides from Alcalase enzyme hydrolysis has been described. The obtained peptides have been characterized by mass spectrometry and some of them are potential bioactive peptides that could exert health benefits. In fact, the potential heart health benefits of developed snail hydrolysates were measured in vitro using the ACE-1 inhibitory assay obtaining a significant ACE1 inhibitory activity. Thus, snail Alcalase hydrolysates results an interesting and novel source of bioactive peptides that could exert a protective effect in cardiovascular diseases as well as a sustainable source of high quality protein.

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