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Extraction And Characterization Of Collagen And Development Of Value - Added Food Product By Blending With Fresh Cheese

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ABSTRACT

Despite its reputation as a useless byproduct, fish viscera has been shown to be rich in useful proteins, peptides, and amino acids. In this research, we explore the potential of Labeorohita fish visceral hydrolysate in the food, pharmaceutical, and other product manufacturing sectors. Enzymatic hydrolysis with alcalase and protease enzymes was used to produce the hydrolysate. Protein and antioxidant activity were both elevated in the resultant hydrolysate. The hydrolysate was used into food products as a functional component to improve their nutritional profile. It was also discovered that the hydrolysate possessed antibacterial qualities, meaning it might be used as a natural preservative in foods. Hydrolysate was evaluated for its potential as a wound healer and anticoagulant by the pharmaceutical sector. The findings suggested that the hydrolysate might be utilised as a natural anticoagulant and also had a substantial impact on wound healing. Hydrolysate of Labeorohita fish viscera has promising uses in the food, pharmaceutical, and other industries due to its high concentration of bioactive chemicals.

Keywords: *Fish viscera, hydrolysate, Labeorohita, protease enzymes, bioactive compounds.*

1. INTRODUCTION

Type I collagen(CGT1) is a fibrillous protein and has well organized structure because of its both amino acid arrangement and flexible nature. It is the most omnious protein type even among the other types of collagen. Collagen (CG) consists 29 sub types and the most abundant macromolecule which present in almost all types of tissues like skin, connective tissues, bones, tendons and also in extra cellular matrix. CG has a share of 25-35% of whole protien content present in animal (Gauza-Włodarczyk et al., 2017)

CGT1 is highly used in commercial industries like food and cosmetic industries(Wu et al., 2017) because of its superior characteristics like its structural stability which is solely due to its polypeptide intervened structure which greatly contributes to the stability of protien.

C Gcan be extracted from higher order animals like horses, cows and pigs(Ogawa et al., 2004). Due to the antipathetic reactions and possibility to spread of contagious diseases(Gauza et al., 2017) and also due to the religious beliefs this method of extraction is being avoided. This created an interest in identifying a alternative sources like fishes to extract the collagen or a similar protein with CG characteristics. The processed copious fish waste that dumped at landfills may cause pollution and several allergies and also gives an un-pleasant odor (Rasool et al.,2014). To overcome this problem, we can use the fish waste for the production of CG similar protein present in the fishes (Bhagwat and Dandge, 2016).

Catla

Catla (Scientific name: Catlacatla) is a freshwater fish species native to South Asia. It is a popular and economically significant fish found in rivers, lakes, and reservoirs throughout the region. Catla is known for its distinctive appearance, characterized by a large, deeply forked tail and a slightly flattened body. It has a silvery coloration with a white belly and scales that are often larger compared to other freshwater fish species. Catla is highly valued for its delicious taste and is considered a delicacy in many cuisines. It is a herbivorous fish, primarily feeding on phytoplankton and vegetation. Due to

its size and impressive fighting ability, catla is also sought after by recreational anglers. In aquaculture, catla is an important species due to its fast growth rate and adaptability to various environmental conditions. Its cultivation contributes significantly to the freshwater fish farming industry in South Asia.

2. LITERATURE REVIEW

During animal cell culture, cells attain confluence which hinders their total harvesting. They need dissociation from substratum which is achieved by treatment with enzyme trypsin (Freshney, 2006)[14]. However, Huang et al., (2010) have reported trypsinization as down-regulator of growth and metabolic proteins and up-regulator of apoptotic proteins[15]. Commercial bovine trypsin is costly enzyme and its proteolytic action affects cell physiology and viability. An alternative source of trypsin and examination of its impact over cell lines is of prime importance. In the present study, the efficacy of isolated trypsin was investigated on cancerous cell line in terms of cell viability. Among the studied applications, lowering of the cost of cell/tissue dissociation in animal cell culture is a challenge and has not been worked out. Research in maximizing the use of fish waste, especially in biotechnology is still needed. The goal of the current study was to determine the most affordable methods for extracting trypsin from the visceral waste of the common freshwater carps, Catla and Labeorohita[16].

Due to the annual increase in fish output, a significant amount of fish waste is produced[17]. A World Bank analysis projects that by 2030, there will be an additional 5 lac metric tons of fish available worldwide. Only 4 lac metric tons of this will be used; the rest will be squandered. By-catch discards and processing trash are included in this garbage. While processing waste comprises head, frames, fins, tails, viscera, and skin, by-catch includes non-targeted creatures such young fishes, marine benthic animals, etc. In India alone, there are more than 2 million tons of fish processing wastes produced, of which 300,000 tons are viscera, according to Kumaran et al. (2013)[18].

This material is being dumped into the ocean in a highly unscientific manner (PNPPRC, 2010; AMEC, 2011). Decomposition of waste in a body of water produces harmful byproducts. The result is eutrophication, which is caused by an excess of nitrogen, phosphate, and ammonia and a drop in dissolved oxygen and pH. In turn, this encourages plankton biomass (U.S. Environment Protection Agency Report, 2010). The reduced oxygen content in water leads to release of stinking gases such as hydrogen sulfide and ammonia. Additionally, improper methods of handling, storing, and disposing of this waste result in noxious odors like indole, sketol, cadavarine, and putricine as well as environmental and health issues[19]. According to Darwina et al., (2012), fish flesh promotes the growth of heterotrophic bacteria. Other cascade effects of eutrophication include changes in nutrient flux and energy, change in structure of planktonic community; fish stock; mineral cycling and deterioration of ground water quality. Some countries like Iceland, Norway and New Zealand follow no- discard policy according to which “everything that is caught must be landed” (Bellido, et al., 2011). Landings and bycatch are now subject to requirements under the European Commission's "zero waste" program. These laws ban returning landings and by catch to the water and place an emphasis on using fish waste in a planned way[20].

Conventional uses of fish waste include recovering marketable by-products such as animal feed, manure and generating renewable energy in the form of biogas (Nnali and Oke, 2013). In 2001, around 30 million metric tons of fish waste products, including trimmings and other small bony fish, were used in aqua feeds. However, temperature above 105 ºC has been found to affect digestibility of visceral waste to be used as fish feed (Esteban et al., 2007)[21]. Also, prior knowledge of visceral waste is required to use it for composting as significant percentage of heavy metals (Cd, Pb and Cu) have been reported in fish meal derived from fish visceral waste. Thus, utilization of fish visceral waste requires extra investment and power consumption. Also, these methods turn waste into low value product. Valorization of fish visceral waste has been proved better over traditional waste management methods (composting, incineration and land-filling) by Ferraro et al., (2010). It can reduce hectares of occupied land and pressure on aquaculture resources[22].

Trypsin from fish visceral waste has been extracted by researchers using extraction buffer or liquid nitrogen. This crude extract is then clarified using salting-in, salting-out precipitation method. Trypsin is then purified by chromatographic technique. Different precipitants such as solvent and salt have been analysed. The present investigation proposed precipitation by solvent and salt both in terms of protein content and weight. In order to make process more economic, a simple low-cost method has been followed using DEAE- cellulose chromatography, which would otherwise limit the commercialization of fish processing waste as an enzyme source. Scientists have suggested great potential in fish processing waste for recovery of trypsin. Recovery of around 1g enzyme from 1Kg viscera has been reported by Esposito et al., (2010)[23]. Despite the fact that trypsin has been isolated from a number of marine species, little is known about the digestive proteases found in tropical freshwater fish, particularly carps.

The study of enzyme properties is imperative for successful application in respective industry. Enzyme activity is affected by pH and temperature. Trypsin with lower temperature optima is desirable in food processing industry which involves digestion of protein at low temperature, such as chill- proofing in beer[24]. Also monitoring enzyme activity at different temperatures enables the enzyme to carry out controlled proteolysis as in manufacture of bakery items. On the other hand, trypsin with higher temperature optima is utilized in detergent formulation. Fish trypsin has been found active over broad range of temperature 30º C to 80º C. Also, trypsin being an alkaline protease exhibits activity at higher pH. In the present

study, activity of trypsin was monitored over a pH range of 4-10 and temperature range of 20°C - 70°C. Fish trypsin generally exhibits molecular weight within a range 23-28kDa upon performing SDS-PAGE. Hence, the technique of SDS-PAGE to determine molecular weight of purified trypsin was carried out. Characterization of fish trypsin has been performed in terms of pH, temperature, and molecular weight[25].

Kinetic parameters determine enzyme activity. These should be determined so as to extrapolate enzyme activity as occurring in vivo. The enzyme isolated from different visceral sources and their physiological efficiency over commercial trypsin can be evaluated. Fish trypsin resembles mammalian trypsin in terms of enzyme activity. In some cases, fish trypsin shows better activity than mammalian counterpart[26]. The study of kinetic parameters proves fish trypsin better over commercial trypsin such as higher turnover number and physiological efficiency. The present work focused on studying Km, Vmax and kcat values of the isolated trypsin. These relate the efficiency of fish trypsin over mammalian trypsin and make it suitable for commercial applications. On constructing Lineweaver-Burk plot, the values of Km and Vmax can be obtained from which, turnover number of the enzyme and efficiency can be calculated[27].

The similarity of fish trypsin with mammalian trypsin can also be determined through N-terminal sequencing. But this method requires labeling of the enzyme which is laborious. LC-MS is a high throughput proteomic technique based on Mass Spectrometry that generates MS spectra of peptides[28]. The MS data is then compared with standard known sequence using suitable search engine with which it shows closest homology. This is a 'bottom-up' approach, which identifies chromatogram and generates a sequence coverage map[29]. The present investigation focused on obtaining a sequence coverage map to compare fish trypsin with commercial trypsin so as to determine its applicability in animal cell culture[30].

3. MATERIALS AND METHODS

Pre-treatment and extraction of collagen

P. P was freshly collected, cleaned, dissected to separate the skin and pretreated with sodium hydroxide and butanol. After the pretreatment, the extraction of CG was done which was explained clearly in the below Figure (4.1) (Nazeer et al., 2014).

Characterization of collagen

Characterization of CG was done by using UV-Vis and FTIR spectroscopy. UV-Vis spectrophotometer (UV-1800, Schimadzu Corporation, Japan) was used to perform the UV-Visible spectroscopy from range of 200-420 nm on the freeze dried CG which was solubilized in 0.5 M acetic acid. During the same time the spectrum of the collagen was noted using FTIR spectroscopy (Perkin Elmer)from the range of 500-4000cm^{-1} with a 2cm^{-1} resolution.

SDS – PAGE analysis

By comparing the samples to a standard CGT1 and high molecular weight marker (), the molecular weights of acid soluble collagen (ASC) and pepsin soluble collagen (pp PSC) were determined, using the standard methodology (Laemmli, 1970).

The gel consists of two gels : stack in gels and resolving gels. For SDS-PAGE the following reagents were used:

(a) 0.5M,pH6.8Tris-Hclbufferfor(4x)stackingeland1.5M,pH8.8Tris-

HCl buffer for(4x)resolve in gel

(b) Acrylamide-bisacrylamide:29%(w/v)acrylamideand1%(w/v)N,N'- methylenebisacrylamide were prepared in distilled water.

(c) Ammonium persulfate: freshly prepared 10%(w/v)aqueous solution

(d) SDS:10%(w/v)aqueous solution.

(e) TEMED solution

(f) Sample buffer

(g) Resolve ingel(8%)

(h) Stack in gel(5%)

(i) Staining solution (2x)

(j) Destaining solution

Two spacers were used to give some space to the gel in between the two glass plates and the space between plates is sealed to prevent the leak of gel with the agarose solution. After that, these glass plates were fixed and erected on the gel casting stand. Above the Resolving gel, a layer of isobuitanol was poured to prevent the diffusion of oxygen. After some time the resolving gel is polymerized and the isobutanol was drained washed with deionized water to remove it pour the stacking gel

on the resolving gel. After the inducing of gel the wells were formed by inserting a comb in the stacking gel. The gel cast was transferred into the electrophoresis chamber and both reservoirs (lower and upper) of the chamber were filled with electrode buffer.

Before the induction of samples into wells they were boiled for 1 minute along with the gel loading buffer. Then the lid of electrophoresis chamber is closed and turned on for the separation with a Maximum of 100 V voltage. The samples laded in the wells were allowed to run until the dye reaches the bottom of the resolving gel. After completion of run, the molecular weight of the sample is determined by removing the gel from the glass plates. Then the gel was stained for 30 min to allow the sample to absorb the stain. Now this stained gel is destained using a destainede solution for a minimum time of 16 hrs and this solution was changed at regular intervals to remove the excess starin from gel. Then, after destaining the gel has bands which indicates the presence of sample. This band fed gel was compared with the bands in the ladder and the molecular weights of the sample was founded.

Amino acid composition

At 40 degrees Celsius and using 338 and 262 nanometer detection (Yan et al., 2008), high performance liquid chromatography using a C18 column (Agilent 1100 coupled to Zorbax 80 A) was used.

Denaturation temperature

Samples of collagen were ready to be put into Ostwald's viscometer when they were dissolved in 0.1 M acetic acid at 10oC. Both samples were subjected to a gradual temperature increase from 10 to 40 degrees Celsius by submerging the viscometer in a water bath. This estimate of fractional viscosity was calculated using the following formula:

Calculating the fractional viscosity is as simple as dividing the maximum viscosity by the minimum.

4..2.6 Influenceof pH and salt on solubility

The pH of a solution containing 0.5 M acetic acid was varied from 2 to 12 and used to dissolve CG samples with a concentration of 4mg/ml. It was centrifuged at 8000 g for 15 minutes at 6 degrees Celsius. The protein concentration was calculated using the Kjeldahl method (AOAC, 1991) on the obtained supernatant. Samples were prepared and collected in fresh test tubes containing an equivalent volume of NaCl (at concentrations 0-5%).After centrifuging the contents of the test tube at 8000 g for 15 minutes at 6 oC, we gave it a thorough mix. Standard methods (AOAC, 1991) were used to determine the protein concentration in the collected supernatant.

Zeta potential

Two samples i.e were dissolved in double distilled water and assessed for their isoelectric point (pI) of collagen and pepsin was determined using a Zeta potential analyzer by adjusting the pH between 2 to 12. (SZ-100, HORIBA scientific, Japan), after theywere preserved for 2days at 6 ºC in the0.05 M acetic acid (0.4 mg/mL).

Collagen supplement edinfresh cheese

Two separate batches of the Standardized buffalo milk with 6% fat was prepared. One batch of milk is heated to 80 ºC for 10 min, then3% collagen was added and cooled to 70 ºC. Ater that, both samples were coagulated using citric acid (2%). The batches were constantly mixed until clear whey is formed. The whey was separated carefully from the batches and the residual solid fraction was immersed into cold water. Then it is pressed for 20 min to remove the water content by applying physical pressure. Soon after that, the solid residue was pressed against a wooden plank to remove the water content in the solid residue (Kumar et al., 2008) (Figure 2). The physicochemical characteristics and sensory analysis of the newly made cheese have been analysed with Association of Analytical Chemists standard procedures (Felicio et al., 2016). Five expert tasters evaluated the items' sensory qualities on a 9-point hedonic scale (9 being the judges' highest rating of enjoyment and 1 their lowest) (Felicio et al., 2016).

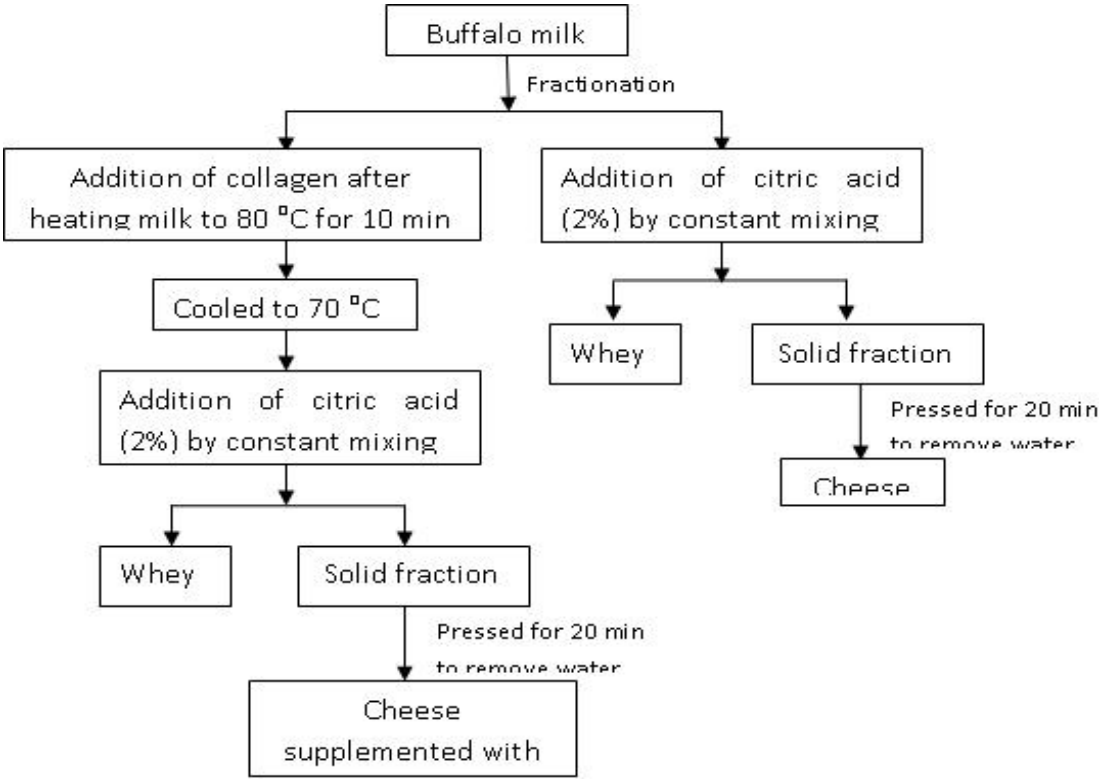

Figure 1 : Preparation of collagen supplemented cheese

4. RESULTS AND DISCUSSION

Pretreatment and extraction of collagen

Fish visceral is extracted. After extraction process, the content of collagen and pepsin were observed to be 19.6% and 23.8% respectively (wet weight basis). The collagen yield in the was found to be higher than that of observed in black drum (Ogawa et al., 2004) and threadfin bream (Nalinanon et al., 2011). In general, the factors such as age, species, body part, habit and habitat of the fish determine the percentage of the collagen in the organism(Rigby, 1968). Other factors such as experimental conditions and preparative methods also plays a predominant role in determining the yield pepsin treatment lead to the high yield of collagen than the acid treatment.

The explainable answer for this is the high density cross linking in the tissue due to the interaction between aldehydes and lysine/ hydroxylysine and alsothe complexity of the molecules (Chen et al., 2016a; 2016b).

The molecular integrity of the collagen is highly effected by the amino acids present in the telopeptide helical region of tropocollagen . To break these bonds solubilizing the protein requires Optimum concentration of acetic acid/enzyme and time. Various proteolytic enzymes such as collagenases, trypsin and pepsin can be employed to cleave these bonds in the helical region to improve yield (Suphatharaprateep et al., 2011). Considering cost and efficiency in releasing the carboxyl groups and hydrophilic amide in CG; pepsin was considered for the extraction process.

Spectral analysis of collagen and ppPSC

It is evident from the present study that both the collagen and pepsin samples show maximum absorbance at 235 nm eventhouh all proteins show maximum absorbance at 280 nm. The collagen is exceptional to this, because of its amino acid composition and arrangement it shows maximum absorbance from 210-240 nm (Kittiphattanabawon et al., 2010; Yan et al., 2008).due to lack of tryptophan. In this case, because of influential tryptophanim participation this shows maximum

The pp ASC & pp PSC solubilised collagen extracted was 19.6 and 23.8% on wet weight basis. It is high when compared with other marine and fresh water fishes like black drum (ogawa et al.,2004)*Catla Catla, cirrhinusmrigala* (Shahid Mahaboob et al.,2015), *Siganusfuscens, kyphosusbigibbus, myliobatistobejei, Dasyatisakajei & D.laevigala (*Food chemistry, volume 108 , issue 1, May 2008), *Evenchelysmarcruva*(I bae et al., 2008).Whereas threadfin bream fish yield was high at 12 hrs extraction and low when compared at 24 hrs. By considering fish availability, and extraction procedure. Industrial cost, availability of the material the fish was not selected for further study.absorbance at 235 nm (Duan et al., 2009).

The illustrations of the FTIR spectral analysis of collagen extracted from skin of *P. pardalis* which is shown in (Figure 4). At 3321 cm^{-1}a peak is observed which indicates the N-H stretching of the secondary structure of collagen (Yan et al., 2008).

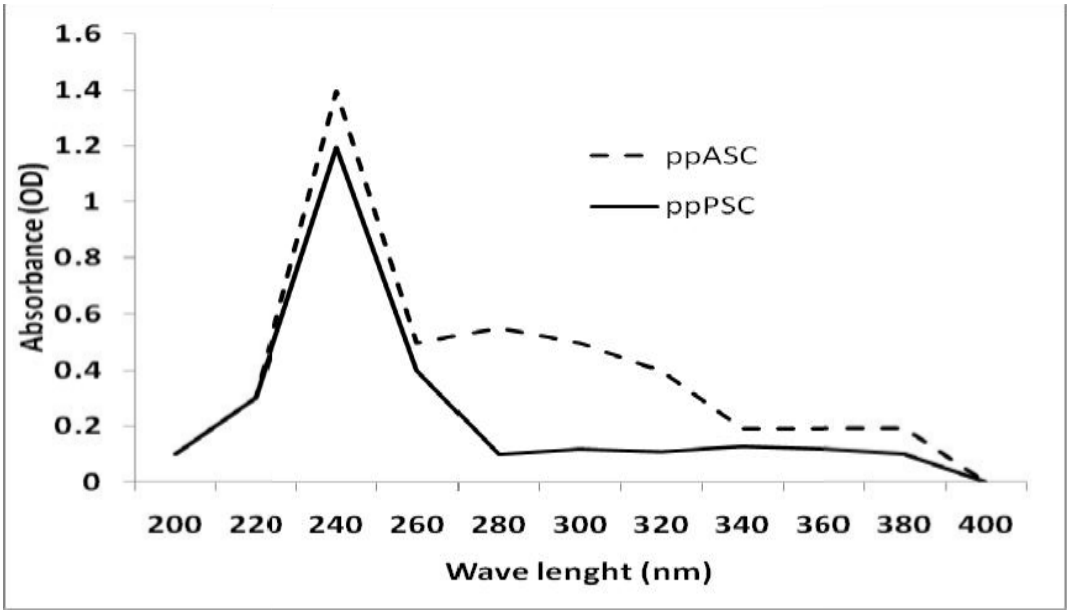

Figure 2 : UV-Vis spectral analysis of collagen and pp PSC

In both collagen and pepsin the amide peaks are shown. At which the amide I peak is observed in the range of1615-1680 cm^{-1} and the amide IIpeak is observed at a lower frequency in the range of1530-1600 cm^{-1}.This may be because of the hydrogen bonds that maintains the triple helical structure of collagen. By this spectral analysis and the literature we can confirmthattheextractedprotienisCGT1.

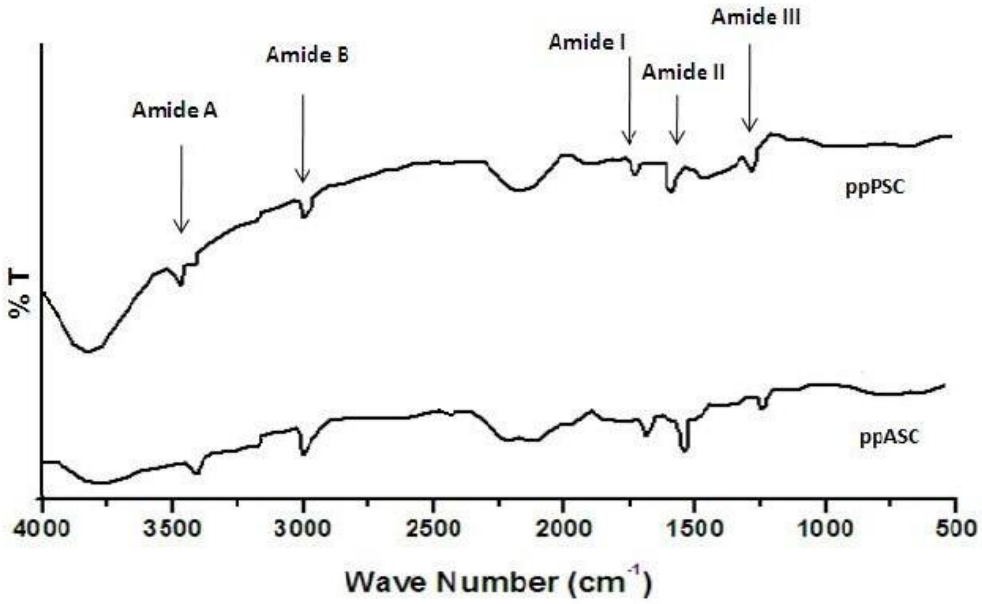

Figure 3: FTIR spectral analysis of collagen and ppPSC

Table1: Standard chart for FTIR analysis

REGION	RANGE (cm^{-1})	ASSIGNMENTS	REFERENCES
Amide A	3400-3440	NH stretch, couple with Hydrogen bond	Sai and Babu (2001)
Amide B	-3080	CH_2 asymmetrical stretch	Abe and Karim (1972)
Amide I	1600-1660	C = O stretch/hydrogen bond coupled with COO	Jackson *et al.* (1995)
Amide II	-1550	NH bend coupled with CN stretch	Jackson *et al.* (1995)
Amide III	1220-1320	NH bend coupled with CN stretch	Jackson *et al.* (1995)

Molecular weight determination

Electrophoresis of collagen isolated from P. pardalis skin revealed three distinct bands: one with a molecular weight of 205 kDa (b chain), and the other two with a molecular weight of 116 kDa (a1 and a2). For this experiment, we employed four lanes: lane 1 contained the high molecular weight protein marker; lane 2 had the reference bovine derived collagen; lanes 3 and 4 contained the collagen and pepsin; and lanes 5 and 6 contained the control solution. (Noitup et al., 2008) Band locations in lanes 3 and 4 are very similar to those in lane 2.

These results clearly identify type I collagen as the isolated protein (Figure 5). Similar to what was published by Kumar and Rani (2017) and Zhang et al. (2016), we discovered that the conventional type I collagen had a higher intensity of a-bands than the extracted collagen and pp PSC.

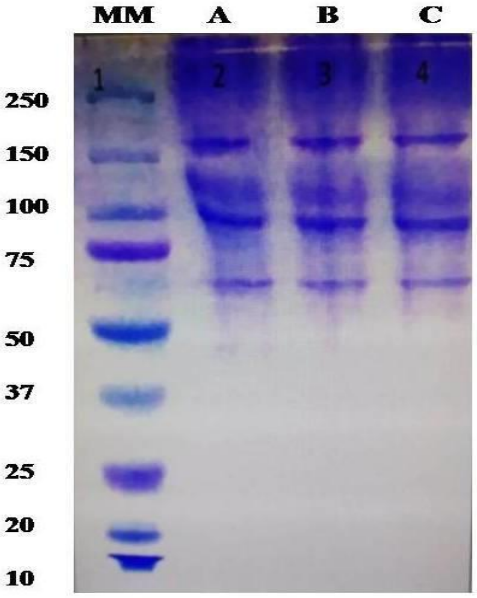

Figure 4: SDSPAGE analysisofCollagen(Lane1-Protein marker;Lane2– standard type I collagen; Lane 3 – collagen; Lane 4 – ppPSC)

(Aoand Li,2012)

The glycine percentages in collagen and pepsin were 28.3% and 27.9%, respectively, using the conventional HPLC method, which yielded a profile of 16 amino acids. Collagen percentages from mammals (30%) and a variety of marine and fresh water fishes (80%) were found to have similar amino acid compositions in the present investigation.

One sixth of collagen's amino acid makeup is made up of the recurring Gly-x-y sequence, with x and y frequently switching between proline and hydroxyproline. The proline and hydroxylproline content of P. pardalis collagen extract for ASC was

14.6% and 16.3%, respectively. While PSC's are 14.3 and 16.8 percent, respectively. The proline and hydroxyproline are essential for collagen's structural integrity. Collagen obtained in the present study is employed as a replacement for commercial collagen since its amino acid content is identical to that of collagen obtained from other mammalian and aquatic animals.

Cysteine is absent from collagen and pepsin, while tyrosine, methionine, and histidine are present in relatively modest amounts (1.2, 0.4, 0.8, and 1.4, respectively). Protease activity may be responsible for the modest differences in collagen and pepsin amino acid percentages (Matmaroh et al., 2011).

Denaturation temperature(Td)

By subjecting the samples of collagen and pp PSC to different temperature and measuring their viscosities using a viscometer the denaturation temperature can be determined(Table 2).

In this study the solubility of the protein tends to decrease with the rise in temperature from 10 °C to 40 °C (Figure 6).

It was shown that both collagen and pepsin get denatured at 25ºC which was lower than that of porcine collagen (37 ºC). On the other hand, the denaturation temperature of the fresh water fishes is in the range of 19.9 - 26.9ºC respectively, which is in the close range with the collagen and pp PSC extracted in the present study

(Kimuraetal.,1988).

Table 2 : Denaturation temperature of collagen and pp PSC

°C	collagen	ppPSC
10	1.20±0.03	1.50±0.04
15	1.10±0.01	1.20±0.03
20	0.90±0.06	1.00±0.07
25	0.20±0.9	0.20±0.02
30	0.11±0.03	0.15±0.07
35	0.09±0.04	0.10±0.01
40	0.05±0.01	0.08±0.03

Results are represented as mean ± SD (n=3)

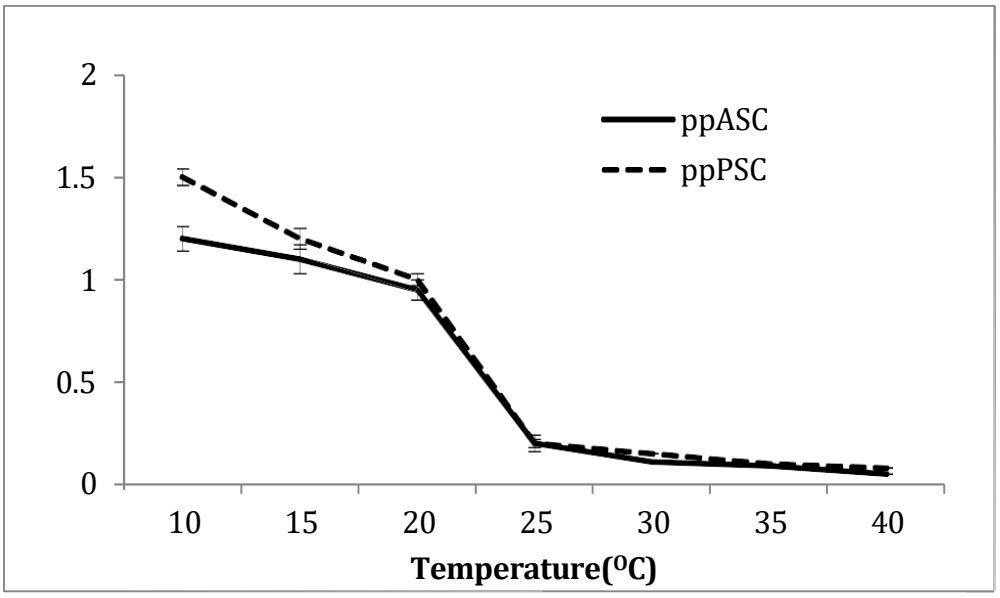

Figure 5: Denaturation temperature of acid and pepsin soluble collagen

Table 3: Influence of P^H on solubility of collagen and ppPSC

pH	collagen	pp PSC
0	79.6±0.9	82.3±1.3
2	91.6±0.7	94.5±1.03
4	86.2±1.1	83.6±2.1
6	19.9±1.6	24.5±1.07
8	19.1±1.0	21.3±1.05
10	18.6±1.5	20.0±0.9
12	19.1±1.2	19.9±0.7

Results are represented as mean ± SD (n=3)

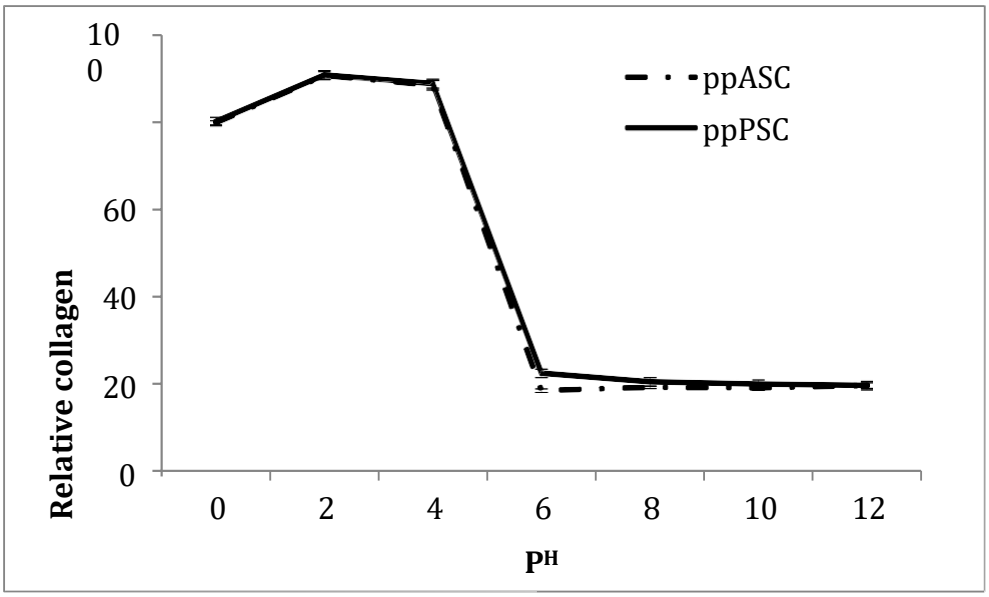

Figure 6: Influence of pH on solubility of acid and pepsin soluble collagen

Solubility of collagen

Both collagen and ppPSC were extracted into 0.5 M Macetic acid at a pH of 2.5.Table3 displays the results of research into how changes in pH and NaCl concentration affect the solubility of proteins. Figure 7 shows that the solubility of collagen and ppPSC is greatest between pH 1 and 4, and falls after pH 4 with the lowest solubility at pH 6. Due to the isoelectric point (pI), which causes a net neutralisation of charge and precipitation. These two proteins are the least stable because their isoelectric point is at a pH of 6 (Jongjareonrak et al., 2005).

Salt concentration

Collagen and pepsin solubility in 0.5 M acetic acid after addition of 0–6% NaCl was evaluated (Table 4). The concentration of NaCl present might be anywhere from 0% to 6%. Proteins maintain a constant solubility of 0-3% (w/v). Then, when the amount of NaCl in the solution increases, the solubility drops (Figure8).Changes in their content, structure, and characteristics may account for the enhanced solubility of PSC in comparison to ASC (Chen et al., 2016a; 2016b). The solubility of collagen is drastically reduced above a NaCl concentration of 6%, possibly as a result of the increased ionic strength of NaCl in water.

Zeta potential

Table (5) displays the results of an analysis of the zeta potential response of collagen and pepsin to changes in pH. According to the results of the current investigation, the pH of collagen and pepsin are 6.39 and 6.41, respectively (Figure 8), indicating that their isolectric points are in the pH range of 6-7. In the pH range of 6-7, where the +ve charges become equal to the -ve

charge golden carp, collagen has a net zero charge (Ali et al., 2018). The availability of charged amino acids like Lys, Asp, and Glu in the pepsin solubilized or the deletion of numerous amino acids from the non-helical area in the acid solubilized causes pepsin to have a higher isoelectric point than collagen (Benjakul et al., 2010).

Table4: Effect of salt on solubility of collagen and ppPSC

NaCl	collagen	ppPSC
0	96.0±2.7	97.1±2.1
1	89.1±1.4	95.6±1.5
2	84.2±1.2	91.3±1.3
3	81.5±1.1	86.9±0.8
4	48.2±0.9	43.1±1.1
5	42.3±1.5	41.6±1.7
6	41.6±1.7	40.9±1.6

Results are represented as mean ± SD (n=3)

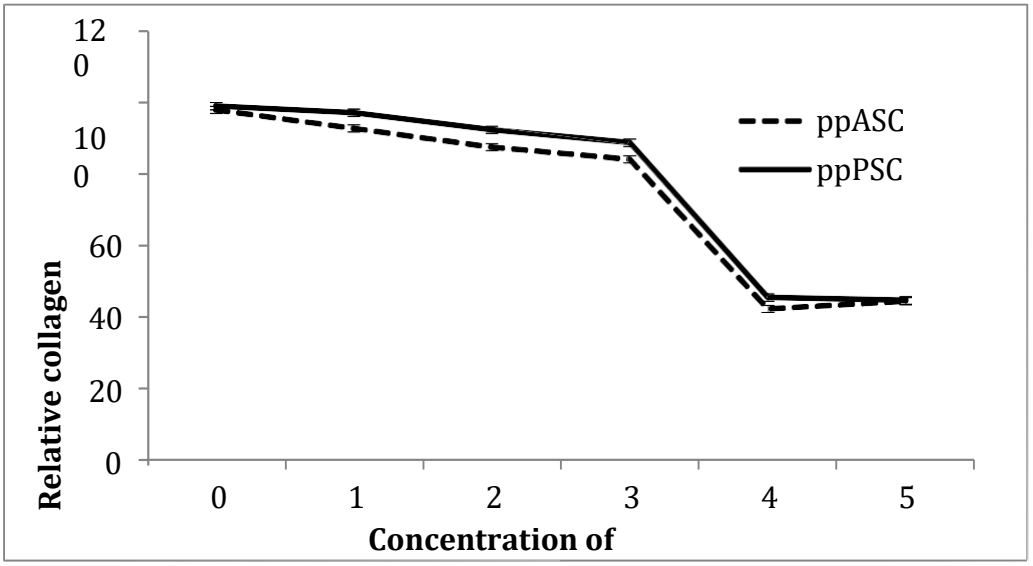

Figure7: Effect of pH on solubility of acid and pepsin soluble collagen

Table5: Zeta potential of collagen and ppPSC at different pH

	pH	collagen	ppPSC
2	27.3±1.9	29.8	±1.9
4	8.5±1.2	10.2	±1.2
6	0.39±0.03	0.41	±0.7
8	-2.1±0.1	-1.9	±0.3
10	-4.6±0.5	-3.5	±0.9
12	-6.1±0.7	-5.8	±0.8

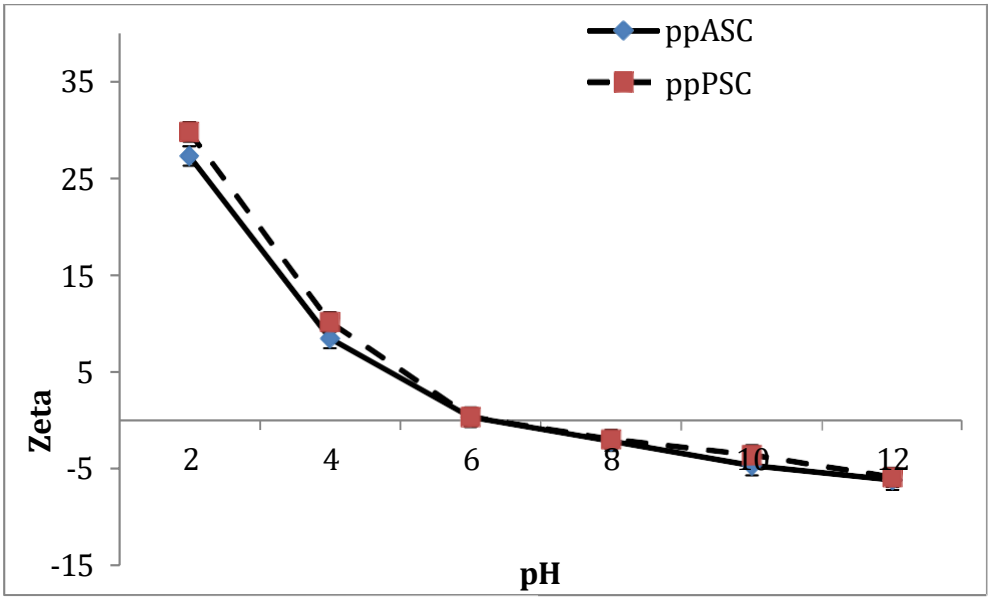

Figure 8: Zeta potential of acid and pepsin soluble collagen at different pH

Collagen supplement edin fresh cheese

Collagen is one of the fundamental creature proteins that gives tissues their flexibility and strength. As we become more established, our bodies become less ready to deliver collagen, and the collagen we as of now have weakens. Tissues become more slender and less strong accordingly. Collagen supplements, as nutrients, milk powder, pills, and moisturizers, are generally accessible available to assist with peopling manage these issues. Considering the meaning of collagen and the extensive variety of wellness situated items, there is a thought was imagined as a method for integrating collagen into cheddar (Figure 4.9).

As a result of its high protein content, this food is a decent wellspring of sustenance for the customer. Proximate examination (Table 6) was applied to both the collagen-upgraded cheddar and the standard cheddar (control), the making of which is portrayed exhaustively in Figure 2.

Figure 9: Fresh cheese blended with pepsin soluble collagen

The collagen-enhanced cheese (test) had higher moisture and protein contents than the usual cheese (control) in a close-range study (Table 6). Also, the test product's organoleptic evaluation showed that adding collagen with cheese did not affect the product's flavour, aroma, or overall acceptability (Hashim et al., 2015; Seda and Sibel, 2015). However, there was a noticeable shift in the hardness and adhesiveness of the samples tested.

Table 6 : Physico-chemical analysis of collagen supplemented cheese

Properties	Test	Control
Moisture(%)	48.44±0.53	45.80±0.39
Protein(%)	22.56±0.64	19.20±0.84
Ash (%)	3.12±0.24	3.74±0.1
Lactose (%) Fat (%)	2.46±0.04	2.82±0.06
Sensoryscore	23.84±0.56	26.25±0.24
	7.2±0.4	8.1±0.2

Test: Collagen supplemented cheese

Control: normal cheese Results are represented as mean±SD (n=3)

REFERENCES

[1] Abdelhedi O, Nasri R, Mora L, Jridi M, Toldrá F, Nasri M (2018) In silico analysis and molecular docking study of angiotensin I-converting enzyme inhibitory peptides from smooth-hound viscera protein hydrolysates fractionated by ultrafiltration. Food Chem 239:453–463. https://doi.org/10.1016/j.foodchem.2017.06.112

[2] Abdul-Hamid A, Bakar J, Bee GH (2002) Nutritional quality of spray dried protein hydrolysate from Black Tilapia (Oreochromis mossambicus). Food Chem 78(1):69–74. https://doi.org/10.1016/S0308-8146(01)00380-6

[3] Abejón R, Abejón A, Belleville MP, Sánchez-Marcano J, Garea A, Irabien Á (2016) Multiobjective Optimization of Membrane Networks for Fractionation of Protein Hydrolysate from Fish By-Products. In: Kravanja Z, Bogataj M (eds) Computer Aided Chemical Engineering. Elsevier, Amsterdam

[4] Abejón R, Belleville MP, Sanchez-Marcano J, Garea A, Irabien A (2018) Optimal design of industrial scale continuous process for fractionation by membrane technologies of protein hydrolysate derived from fish wastes. Sep Purif Technol 197:137–146. https://doi.org/10.1016/j.seppur.2017.12.057

[5] Aliani M, Eskin MNA (2017) Bitterness: perception, chemistry and food processing. Wiley, New Jersey

[6] Al-Mansour HE, Al-Busairi BH, Baker CGJ (2011) Energy consumption of a pilot-scale spray dryer. Dry Technol 29(16):1901–1910. https://doi.org/10.1080/07373937.2011.595563

[7] Alvares TS, Conte-Junior CA, Pierucci AP, de Oliveira GV, Cordeiro EM (2018) Acute effect of fish protein hydrolysate supplementation on vascular function in healthy individuals. Journal of Functional Foods 46:250–255. https://doi.org/10.1016/j.jff.2018.04.066

[8] ASHRAE (1999) ASHRAE handbook. American Society of Heating, Refrigerating and Air Conditioning Engineers, Atlanta

[9] Aubes-Dufau I, Capdevielle J, Seris JL, Combes D (1995) Bitter peptide from hemoglobin hydrolysate: isolation and characterization. FEBS Lett 364(2):115–119

[10] Baker CGJ, McKenzie KA (2005) Energy consumption of industrial spray dryers. Dryi Technol 23(1–2):365–386. https://doi.org/10.1081/DRT-200047665

[11] Bikov VP, Ionas GP, Golovkova GN, Didenko AP, Akulin VN, Perova LI, Odincov AB, Konstantinova LL, Dvinin UF, Christoferzen GS, Borisova LP (1998) Book of chemical content and technological properties of seawater and oceanic fishes. VNIRO Press, Moscow

[12] Blenford DE (1994) Protein hyddrolysates: functionalities and uses in nutritional products. Int Food Ingr 3:45–49

[13] Bourseau P, Vandanjon L, Jaouen P, Chaplain-Derouiniot M, Massé A, Guérard F, Chabeaud A, Fouchereau-Pérond M, Le Gal Y, Ravallec-Plé L, Bergé J-P, Picot L, Piot J-M, Batista I, Thorkelsson G, Delannoy C, Jakobsen G, Johansson I (2009) Fractionation of fish protein hydrolysates by ultrafiltration and nanofiltration: impact on peptidic populations. Desalination 244(1):303–320. https://doi.org/10.1016/j.desal.2008.05.026

[14] Chae HJ, In MJ, Kim MH (1998) Process development for the enzymatic hydrolysis of food protein: effects of pre-treatment and post-treatments on degree of hydrolysis and other product characteristics. BiotechnolBioproc E 3(1):35–39. https://doi.org/10.1007/BF02932481

[15] Chakraborty PK, Madhavan P (1977) A pilot scale set up for the manufacture of fish hydrolysate. Fish Technol

14(2):159

[16] Chalamaiah M, Dinesh Kumar B, Hemalatha R, Jyothirmayi T (2012) Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: A review. Food Chem 135(4):3020–3038. https://doi.org/10.1016/j.foodchem.2012.06.100

[17] Cui H (1996) The influence of degree of hydrolysis on the structural and functional properties of fish protein peptic hydrolysates. Masters thesis. University of Washington, Seattle

[18] Damodaran S, Parkin KL, Fennema OR (2008) Fennema's Food Chemistry. CRC Press, Boca Raton

[19] Danilov OI, Leontchik BI (1986) Energy Economic in Thermal Drying. Energoatomizdat, Moscow

[20] Di Pasquale MG (2008) Amino acids and proteins for the athlete: the anabolic edge. CRC Press, Boca Raton

[21] Elavarasan K, Shamasundar BA, Badii F, Howell N (2016) Angiotensin I-converting enzyme (ACE) inhibitory activity and structural properties of oven- and freeze-dried protein hydrolysate from fresh water fish (Cirrhinusmrigala). Food Chem 206:210–216. https://doi.org/10.1016/j.foodchem.2016.03.047

[22] European Comission (2012) https://ec.europa.eu/fisheries/cfp/aquaculture/species_en. Accessed 26 Sept 2018

[23] Gonzalez-Tello P, Camacho F, Jurado E, Paez MP, Guadix EM (1994) Enzymatic hydrolysis of whey proteins: i. Kinetic models. BiotechnolBioeng 44(4):523–528. https://doi.org/10.1002/bit.260440415

[24] Halim NRA, Azlan A, Yusof HM, Sarbon NM (2018) Antioxidant and anticancer activities of enzymatic eel (monopterussp) protein hydrolysate as influenced by different molecular weight. Biocatal Agric Biotechnol 16:10–16. https://doi.org/10.1016/j.bcab.2018.06.006

[25] He S, Franco C, Zhang W (2013) Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Res Int 50(1):289–297. https://doi.org/10.1016/j.foodres.2012.10.031

[26] Himonides AT, Taylor AKD, Morris AJ (2011) Enzymatic Hydrolysis of Fish Frames Using Pilot Plant Scale Systems. Food Nutr Sci 2:586–593

[27] Hoeling A, Volkov V (2015) Proteins from by-products - innovative components in sustainable industrial production. Proceedings of KGTU 38:83–92

[28] Huang S, Vignolles ML, Chen XD, Le LY, Jan G, Schuck P, Jeantet R (2017) Spray drying of probiotics and other food-grade bacteria: a review. Trends Food Sci Tech 63:1–17. https://doi.org/10.1016/j.tifs.2017.02.007

[29] Jaswal AS (1990) Amino Acid Hydrolysate from Crab Processing Waste. J Food Sci 55(2):379–380. https://doi.org/10.1111/j.1365-2621.1990.tb06768.x

[30] Jenkelunas PJ, Li-Chan ECY (2018) Production and assessment of Pacific hake (Merluccius productus) hydrolysates as cryoprotectants for frozen fish mince. Food Chem 239:535–543. https://doi.org/10.1016/j.foodchem.2017.06.148

[31] John TG (1993) Non-bitter protein hydrolyzates. US Patent 5266685, USA

[32] Juming THF, Shen GQ (2003) Drum Drying. In: Encyclopedia of agricultural, food, and biological engineering. Marcel Dekker, Inc., New York, pp 211–214. https://doi.org/10.1081/e-eafe 120007091

[33] Kinsella JE, Melachouris N (1976) Functional properties of proteins in foods: a survey. Crit Rev Food Sci 7(3):219–280. https://doi.org/10.1080/10408397609527208

[34] Kristinsson HG (1998) Reaction kinetics, biochemical and functional properties of salmon muscle proteins hydrolyzed by different alkaline proteases. Masters thesis. University of Washington, Seattle

[35] Kristinsson HG, Rasco BA (2000) Fish Protein Hydrolysates: production, Biochemical, and Functional Properties. Critical Rev Food Sci 40(1):43–81. https://doi.org/10.1080/10408690091189266

[36] Kuehler CA, Stine CM (1974) Effect of enzymatic hydrolysis on some functional properties of whey protein. J Food Sci 39(2):379–382. https://doi.org/10.1111/j.1365-2621.1974.tb02899.x

[37] Lahl WJ, Braun SD (1994) Enzymatic production of protein hydrolysates for food use. Food Sci 48:68–71

[38] Linder M, Fanni J, Parmentier M, Sergent M, Phan-Tan-Luu R (1995) Protein recovery from veal bones by enzymatic hydrolysis. J Food Sci 60(5):949–952. https://doi.org/10.1111/j.1365-2621.1995.tb06268.x

[39] Loffler A (1986) Proteolytic enzymes: sources and applications. Food Technol-Chicago 40(1):63

[40] Loosen P, Bressollier P, Julien R, Pejoan CH, Verneuil BG (1994) Method for preparing an enzymatic hydrolysate. United States Patent 5356637