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Studies on Mass and Nutrients Balance During Enzymatic Hydrolysis of Cuttlefish Skin Waste

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Abstract

Processed cuttlefish is one of the major seafood export commodities of India. Approximately 35% of raw material is converted into by-products during processing of cuttlefish. The cuttlefish skin waste (CSW) accounted to roughly 9% of the raw material and 28% of the by-products generated. In the present investigation, five different proteases namely alcalase, protease from *Streptomyces griesus*, protamex, papain and bromelain were screened for hydrolysis efficiency on CSW and found to produce soluble content of 80.65, 79.85, 73.47, 66.41 and 67.71%, respectively. Though the bacterial proteases were relatively more efficient in terms of soluble production based on the cost factor, papain was used for studying the mass and nutrients balance during the process of production than the plant proteases of cuttlefish skin protein hydrolysates. Papain enzyme was useful in converting the CSW in to two protein rich products namely water soluble protein hydrolysates and insoluble protein hydrolysates with 82.86 and 71.25% crude protein, respectively. The data presented would be useful for setting up industries for protein hydrolysates production from cuttlefish skin waste, which is abundantly available in India.

Keywords: Cuttlefish, enymatic hydrolysis, cuttlefish skin waste, papain, mass balance

Introduction

Processed frozen cuttlefish is one of the seafood export commodities. A quantity of 70906 tonnes of frozen cuttlefish has been exported during the period of 2019-2020 from India (MPEDA, 2021). Cuttlefish processing generate waste which includes skin, visceral mass, cuttlebone and ink. Approximately, 35% of raw material is converted into by-products during processing of cuttlefish. The cuttlefish skin waste (CSW) accounted to roughly 9% of the raw material and 28% of the by-products generated (Unpublished data from authors). CSW is relatively easier to handle and less complex as a raw material. The skin waste includes ventral skin and dorsal skin. Cuttlefish skin waste has been studied for production of sulphated polysaccharides (Jridi et al., 2019), collagen (Jridi et al., 2015), gelatin (Hoque et al., 2010) and gelatin hydrolysates (Jridi et al., 2014). Fish protein hydrolysates (FPH) have been researched extensively in the last decade due to its various bio-functional properties and nutraceutical potential (Elavarasan et al., 2021). There is a growing demand for FPH from feed industry especially as a protein ingredient in aquaculture feed formulations (Tejpal et al., 2019). Commercial production of fish protein hydrolysates, on the other hand, is still in its infancy. Mass balance is one of the essential information that should be made available for successful entrepreneurship development. In the present investigation we have profiled the mass balance and nutrients flow during production of cuttlefish skin waste protein hydrolysates. From the academic point, the present study would help to understand the effect of papain on hydrolysis of cuttlefish skin waste which is available in abundance and can be better utilized for producing value added products like protein hydrolysates. Reports on hydrolysis of cuttlefish skin waste with reference to mass balance is rarely found.

Materials and Methods

Cuttlefish skin waste were collected from the local seafood processing plants (Torry Harris Seafoods Pvt Ltd, Aroor, Alappuzha, India) and brought to the laboratory in iced condition. Enyzmes, protease from *Streptomyces griseus*, protamex from *Bacillus*

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amyloliquifaciens, bromelain and alcalase from *Bacillus licheniformis* were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, Missouri, United States) and papain was purchased from HiMedia, (HiMedia, Mumbai, India). Other chemicals used in analysis were of analytical grade or reagent grade.

Cuttlefish skin waste (CSW) was rinsed briefly with chilled potable water and cut into small pieces manually. The cut pieces of CSW were further broken into small particles by chopping for 30 min in a bowl chopper to obtain a fine paste. The CSW paste was mixed with distilled water at 1:2 ratio and homogenized using a household blender. Cuttlefish skin waste homogenate was incubated in a water bath to attain the optimum temperature (Table 1) needed for the enzyme used. After temperature pre-equilibration, the hydrolysis was initiated by adding the enzyme as given in Table 1 and incubated for 60 min. Hydrolysis was terminated by keeping in boiling water bath for 15 min. The hydrolysed slurry was subjected to centrifugation at 5000 x g for 30 min (Thermo Fisher Scientific, Heraeus Fresco 17, Germany). The supernatant and the residues representing soluble and insoluble, respectively were collected and subjected to freeze drying. The amount of soluble formed indirectly indicates the efficiency of hydrolysis and is calculated using the following equation on dry basis (db).

$$\text{Recovery of solubles or insolubles (\%; db)} = \frac{\text{Weight of soluble or insolubles (g; db)}}{\text{Weight of CSW (g; db)}}$$

Simultaneously, two controls were prepared without adding enzyme (Control-1 [C1] -Heat inactivation and incubation; Control-2 [C2]- Incubation and heat inactivation without adding enzyme). Control CSW homogenate was prepared as explained for samples. Control-1 was prepared to understand the effect of incubation and heat inactivation. Control-2 was prepared to understand the effect of endogenous enzymes and heat inactivation. For further study, papain enzyme was used for hydrolysis.

For mass and nutrient balance study, 200 g of CSW was hydrolysed as three independent batches and representative samples maintained independently (200 g × 3 batches x 6 sets=18 independent samples) were drawn at appropriate stage of operation (Set-1: raw material, Set-2:homogenate, Set-3:homogenate-supernatant, Set-3:homogenate-solid, Set-4:reaction slurry, Set-5:slurry supernatant, Set-5:slurry solid, Set-6:soluble hydrolysate spray dried and Set-6: insoluble hydrolysate oven dried) recorded the weight and analysed for moisture, solid, protein, fat and ash content according to the methods described in AOAC (2019). The schematic flow of sample preparations is represented in Fig. 2. Weight of the sample was used to analyse the mass flow. The proximate composition data was used to understand the nutrient flow during the hydrolysis process.

Descriptive statistical analysis was carried out for finding the mean and standard deviation using Excel programme of MS office.

Results and Discussion

Efficiency of five different enzymes in hydrolysing the CSW is presented in Fig.1. Alcalase, enzyme from *B. licheniformis* and protease from *S. griesus* produced 80% of solids as solubles, whereas protamex produced 73.46% solubles. Plant proteases bromelain and papain produced 66.41 and 67.70% solubles. Results indicate that among the enzymes studied, bacterial proteases are more efficient in hydrolysing the cuttlefish skin compared to plant proteases. Cuttlefish skin waste incubated without enzymes and subjected to heat inactivation (C2) produced 36.41% solubles which is comparatively

Table 1. Conditions employed for hydrolysis of cuttlefish skin waste by different protease

Enzyme	E/S (on protein basis)	Temperature (°C)	pH	Time (min)
Protease from *S. griesus*	0.5%	40	6.5±0.2	60
Protamex	0.5%	50	6.5±0.2	60
Papain	0.5%	60	6.5±0.2	60
Bromelain	0.5%	50	6.5±0.2	60
Alcalase	0.5%	50	8.5±0.2	60

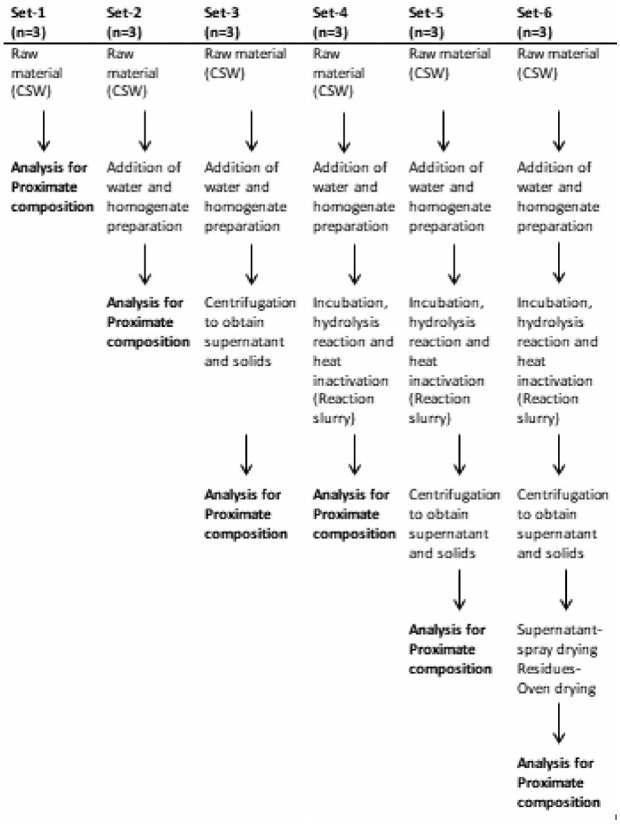

Fig. 1. Screening of enzymes for hydrolysis efficiency against cuttlefish skin waste

higher than the soluble obtained for C1 samples which are heat inactivated and incubated. This indicates that the endogenous enzymes present in the CSW exhibit a weaker hydrolysis activity. Though CSW expected to be rich in collagen protein, conversion into gelatin by direct boiling for 15 min is possible only to a very lesser extent. Earlier studies have reported use of proteases for isolation of gelatin from CSW and production of gelatin hydrolysates and collagen hydrolysates. Microbial enzymes, particularly alcalase found to be useful in CSW gelatin hydrolysates with improved antioxidant activities (Balti et al., 2011; Jridi et al., 2014). Similarly, collagen hydrolysates prepared using collagenase reported to have antioxidant properties. The study conducted on mass balance during papain mediated hydrolysis without water addition using fish frame waste has revealed the production of 90 kg soluble protein and 40 kg insoluble protein from 130 kg of whole protein (Himonides et al., 2011). In the present investigation, whole CSW was subjected to hydrolysis by proteases to evaluate their hydrolysis efficiency.

Information on mass flow in a CSW hydrolysis process is very much essential for working out the vessel capacity, energy requirement man power requirement. In the present study, data was generated for 200 g of sample from three independent batch of processing. In protein hydrolysis, the stages of processing can be broadly grouped into three viz., raw material handling and preparation, protein hydrolysis and separation of soluble and concentration and drying. The addition of water which facilitates the hydrolysis process increases the bulkiness of material handling (300%). From 200 g of wet material, 18.57 g was recovered as hydrolysates (9.2%) and 9.69 g (4.84%) was recovered as insoluble hydrolysates. The supernatant after hydrolysis reaction contained 3.4% solid and without hydrolysis (Homogenate-supernatant) this was nearly 1.22% (Table 2).

Information on moisture content on various stages of enzymatic hydrolysis of protein is essential for handling the raw material, designing the process equipment, energy requirements, product stability, etc. The moisture content of CSW was 87.12%. At the end of the process, the supernatant and residue were dehydrated via spray drying and oven drying, respectively. Both the drying process is energy intensive and the former one is cost intensive too. From the hydrolysed supernatant, 99.80% of water content was removed by spray drying process, resulted in the hydrolysates powder with 7.0% residual moisture. Similarly, from insoluble residues 98.39% of water content was removed to obtain the dry solids with the residual moisture of 7.0%. The moisture content in the end product i.e protein hydrolysates affects its shelf life, browning reaction and fat oxidation.

Information on the protein balance during the processing is essential as it is related to process

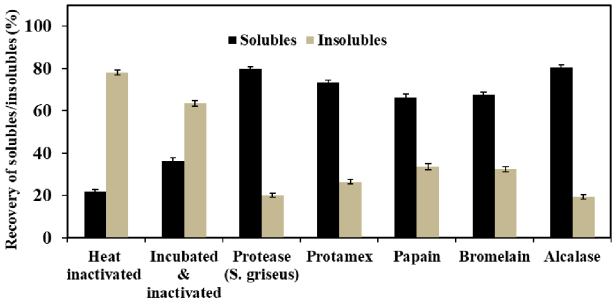

Fig. 2. Schematic representation of sample preparation for mass balance and nutrient flow study

Table 2. Mass balance and nutrients flow during hydrolysis of cuttlefish skin (200g wet material) waste using papain

Stage of hydrolysis	Weight (g)	Moisture (g)	Dry solids (g)	Protein (g)	Fat (g)	Ash (g)
Raw material (CSW)	200.00±0.00	174.25±0.34	25.75±0.34	20.77±0.53	0.72±0.15	1.15±0.27
Homogenate	594.67±2.08	570.32±1.70	24.34±1.17	19.15±0.98	0.99±0.24	1.13±0.13
Homogenate-Supernatant	472.00±1.00	466.24±0.82	5.76±0.18	1.92±0.77	0.37±0.06	0.83±0.12
Homogenate solid	121.67±1.53	100.75±1.43	20.92±0.31	17.22±0.55	0.62±0.12	0.30±0.05
Reaction slurry	562.00±7.94	536.41±7.70	25.59±0.36	21.08±0.23	0.60±0.06	2.08±0.17
Slurry-Supernatant	512.67±4.51	495.35±3.66	17.32±0.85	14.63±0.23	-Not Analysed-	1.76±0.21
Slurry solid	51.33±5.69	42.28±4.31	9.05±1.39	6.45±1.05	2.37±0.46	0.31±0.04
Hydrolysate-soluble**	18.57±0.17	1.30±0.01	17.27±0.16	14.31±0.13	0.54±0.02	1.35±0.01
Hydrolysate- insoluble*	9.69±1.07	0.68±0.07	9.01±1.00	6.42±0.78	2.36±0.35	0.27±0.03

*Oven dried powder; **Spray dried powder

Note: Six sets in three independent batches were used to generate the data presented.

efficiency, nutritional quality of the product, product price fixation, deciding the marketable form of product. Cuttlefish skin waste contained 10.38% protein (10.38 g 100 g^{-1} CSW; wet basis). This indicates that the cuttlefish skin waste is a suitable raw material for developing the products with high protein. In this study, almost 69% of protein i.e 7.16 g from 100 g CSW was recovered either as liquid hydrolysates or spray dried hydrolysates. Remaining 31% protein forms the insoluble protein hydrolysates portion. The insoluble hydrolysed residues contain 71.25% crude protein. The soluble hydrolysates contain 82.86% protein. Mass balance for flow of protein has been reported for fish frames in a pilot scale system (Himonides et al., 2011). Both the products obtained in the present study are of high protein products, thus could find their applications as protein ingredients in food and feed formulations. Soluble fish protein hydrolysates offer a huge array of functional and bioactive properties. Hence, find its uses in functional food products formulations. The insoluble hydrolysates could be the ideal replacement for fish meal in feed formulation with added bio-functional properties as being a hydrolysed peptide fraction. Insoluble hydrolysate fraction would not impart water instability issues in feed formulation.

Information on fat balance during processing is mainly useful to understand the lipid stability and also for declaring the nutritional quality. In the present study, we have profiled the flow of fat at various stages of enzymatic hydrolysis of CSW. The crude fat content of CSW was 0.36% (2.8% on dry basis) which implies that the CSW not likely to impose any lipid oxidation related hurdles during the enzymatic hydrolysis process. However, the hydrolysed insoluble residues (insoluble hydrolysates) contained 24.35% ether extractable content. It should be mentioned that the smaller peptides could have been leached with solvent during fat extraction. Though the petroleum ether do not solvate the pure amino acids or peptides, the presence of lipid compounds in solvent during extraction process suspected to enhance the leaching of hydrophobic peptides and other compounds like pigments (cuttlefish skin contain pigments) so as to show more quantity of ether extractables. On the other side, the soluble hydrolysates contained 2.90% crude fat. The soluble hydrolysates fraction was likely to have abundance of smaller peptides and more of hydrophilic in nature. The insoluble hydrolysate was likely to have highly aggregated and hydrophobic protein fragments thus could have got extracted with solvent during extraction. This aspect of the study needs further investigation to clarify the assumption put forward. During centrifugation of fish protein hydrolysis, different fractions like sludge (bottom layer), middle aqueous layer and top oil layer are obtained. There are lipid and protein layer between aqueous and sludge fraction, aqueous-oil layer and oil layer on the top (Cui, 1996; Kristinsson & Rasco, 2000).

Information on minerals balance during the processing is mainly useful for declaring the nutritional quality. Cuttlefish skin waste contained 0.56% ash on wet basis (4.46% on dry basis) and get enriched

in the soluble hydrolysates fraction as result of hydrolysis (7.81% on dry basis). High ash content in the soluble hydrolysate fraction could have been due to extraction of, bound and water-soluble minerals with the water-soluble peptide fraction. Peptides also exhibits mineral binding properties. The values obtained for cuttlefish skin waste, soluble and insoluble protein hydrolysates are within the values reported for fish protein hydrolysate (0.45% to 27%) (Chalamaiah et al., 2012). There are no reports available on mineral profile during cuttlefish skin protein hydrolysis.

In the present study, a detailed investigation on material flow and major nutrients flow during various stages of enzymatic hydrolysis of cuttlefish skin waste was conducted and relevant information were generated. Present study is the first report on mass and nutrient balance aspects of CSW during papain hydrolysis. This information would be useful for setting up the industry for protein hydrolysates manufacturing from cuttlefish skin waste which is abundantly available in India. The protein recovery in the form of soluble protein hydrolysate from CSW was 69% and the rest forms insoluble protein hydrolysates. Papain enzyme is useful in converting the CSW waste in to two protein rich products namely water soluble protein hydrolysates and insoluble protein hydrolysates with the value of 82.86 and 71.25% crude protein, respectively.

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