Flavored powder from shrimp shells with bromelain enzymatic process and adding of flour and spices

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Abstract. Shrimp shells consisting of head and skin can be extracted to get a filtrate that is rich in protein and delicious taste. The shrimp shell powder is refluxed in 2 stages, the first without enzymes and followed by the reflux stage with enzymatic bromelain. The function of enzymatic extraction is to get a protein that has more shrimp flavor. The filtrate without and with the enzyme are made into filtrate then added with flour and spices to become shrimp flavoring powder. Products analyzed and tested are shrimp flavor and filtrate. Tests carried out were tests of protein content, water content, analysis with FTIR, GCMS, and TEM. The results of the filtrate contain 24.6% total protein in the condition of adding bromelain enzymes as much as 2% (b/b) at 55 °C. Analysis of functional groups flavoring consist of amines, carboxylic acids, alcohols and phenols, amides, and sulfates. The powder is evenly distributed (not agglomerated) between 1-8 nm with a dominant diameter of 6 nm of 26.1%.

1. Introduction
Indonesia is a country that has great potential in the fishing industry, one of which is shrimp farming. Indonesia is the largest shrimp exporter, especially the market in Japan, the United States, and Europe [1]. Most of the shrimp products are exported in frozen conditions that have undergone a process of separation from their shells, which are then frozen [2]. Indonesia has great potential in the fishing industry, one of which is shrimp farming.

Most of the shrimp products are exported in frozen conditions that have undergone a process of separation from their shells [2]. Shrimp for freezing usually involves the removal of the head and body shells. Shrimp processing produces several solid wastes. Based on data from Indonesian Statistics 2017, the total exports of shrimp that have been separated from their shells in 2016 were 13.68 kilotons, increasing in 2017 to 15.04 kilotons. A large amount of shrimp production will produce a lot of waste and also consider side production. The trash produced from frozen shrimp, canned shrimp, and processing of shrimp crackers ranges from 30-75% of the weight of shrimp [3]. Generally, tropical head shrimp is 34-45%, and the body shell is 10-15% [4]. The head and shell of the shrimp are usually processed as animal feed at low selling prices [5]. Though most of the shrimp waste produced by the shrimp processing business comes, protein (25% -40%) can be used as animal feed [6]. Delicate shrimp skin can be mixed with soybean meal, which serves as brackish fish feed [7].
Increasing the amount of shrimp waste is still a problem and needs to be sought for other uses. The addition of added value from shrimp shells can be a shrimp processing business, but it can also overcome the environmental pollution problems that are caused. Many shrimp shells are processed into chitin and chitosan, but this is unfortunate if the scent of shrimp is not taken first and lost during the process of making chitin and chitosan. Although the most specific shrimp aroma can be found in the shrimp shell [8], this shrimp shell has a potential value for technological innovation in food products as flavoring shrimp.

2. Material Procedures
There are several steps in making flavoring in the preparation of refining shrimp shell powder, extracting shrimp shell powder with water, analysis of shrimp shell filtrate, flavor powder making, and analysis of quality including protein content analysis using the Micro-Kjeldahl method, water content analysis, FTIR Shimadzu 8201 PC, GC-MS Clarus 680, and TEM J-1400 analysis.

2.1. Preparation of refining shrimp shell
Shrimp shells are sorted to get a good sample condition and not rotten. The shell is washed thoroughly. Furthermore, the sample consisting of the head and skin of the shrimp was dried in a hot air oven at 60°C for 24 hours. This dry sample is easily finely ground by blending three times for 2 minutes each time. The dried shrimp skin becomes shrimp flour prepared to be extracted.

2.2. Extracting shrimp shell powder
Shrimp flour is added with distilled water containing 0.5% NaCl solution with a ratio of 1:2 (w/v). The mixture is then boiled using reflux, which is set at 85°C for 30 minutes. The addition of NaCl to the mixture aims to achieve shrimp shell filtrate with the best taste of the first filtrate [20]. The filtrate is separated from the pulp. Then the first slurry from the mixture is used again because it still contains protein in it. The pulp is then refluxed into 1:2 (b/v) distilled water by adding some bromelain enzyme concentrations of 0%, 1%, 2%, and 3% (w/w)). The heated mixture uses reflux at 50°C, 55°C, and 60°C for 2 hours to obtain optimal conditions in making the second filtrate with the performance of the enzyme. Then the second filtrate is filtered to be separated from the pulp. The first and second filtrate is then combined with a ratio of 1:1 (v/v) to obtain a filtrate at a certain temperature and bromelain.

2.3. Making the flavored powder.
The shrimp shell filtrate is prepared to be a ready-made flavoring. The trick is to add the filtrate with 5% flour and tapioca flour with a ratio of 1:1 (b/b) as a binder for shrimp shell filtrate. Furthermore, the mixture was added by 1.8% (w/v) of garlic, onions 2.23% (w/v), turmeric 0.1% (w/v), ginger 0.3% (w/v) and 7.5% (w/v) of refined salt. Then the mixture is mixed evenly and dried into an oven at 105 °C until the weight is constant. After the mixture dries, it is carried out grinding and sieving to 200 mesh.

3. Protein Content Analysis
There are three steps in the Micro-Kjeldahl, namely digestion, distillation, and titration. Where in the digestion step, the sample that contains protein break into an amino acid. The nitrogen reacts with H2SO4 into ammonium ion (NH4+). Furthermore, in the distillation step, ammonium ion (NH4+) react with sodium hydroxide (NaOH) into ammonia (NH3). The ammonia (NH3) condensed during the distillation process then trapped by boric acid (H3BO3), and methyl red indicator solution becomes ammonium ion (NH4+). The concentration of trapped NH4+ titrated using HCl 0.01 N. The protein content calculated using the following equation (1).
Where, \( N \) is HCl normality; \( f_k \) is a conversion factor (6.25), and \( f_p \) is dilution factor (0.5).

Based on Figure 1, along with the increased use of the bromelain enzyme, it appears that the protein content increases. At an increase in temperature of 50, 55 and 60°C, and the reflux temperature of 55°C also showed the highest increase and protein content. At 50°C the reflux temperature, the protein content increases to 27.8% at 2% (w/w) the addition of the bromelain enzyme, then constant at 3% (w/w) the addition of the bromelain enzyme. Optimal performance of the bromelain enzyme occurs at a temperature of 50-60°C depending on the activity and type. According, the optimum crude bromelain temperature is 50 °C, while pure bromelain is 60°C. It can be analyzed that the purity of enzymes can affect activity. The enzymatic process used in this study works optimally at 55°C, which means that the enzyme condition is not pure enough. When the temperature increases, more molecules have kinetic energy to undergo the reaction. When the temperature rises above the optimal temperature, performance increases above the biochemical threshold that disrupts peptide and disulfide bonds and thus deactivates the enzyme [11]. Besides, the production of the enzyme is also influenced by the concentration of the enzyme. If the enzyme concentration is low, the ability of enzyme activity can be inhibited. While at a high level, this catalyst activity will be deflected due to a reduced substrate during the enzymatic process [12].

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\text{% protein content} = \frac{(V_1-V_2) x N x 0.014 x f_k x f_p}{W} x 100\% \tag{1}
\]

Figure 1. the effect of temperature and addition of bromelin to protein content

4. FTIR Analysis
Comparison of the infrared (IR) spectrum of flavoring products, as shown in Figure 2. Identification of functional groups from flavoring products that have become powder products is the vibration stretching of the second amine group (N-H) at 3421 cm⁻¹, which has vigorous intensity. The absorbance at 2930 cm⁻¹ shows the stretching vibration of the carboxyl acid (O-H) group. This strong intensity and stretches of vibration shown as the aldehyde (C-H) group. The group of carboxyl acid derivatives (C = O) was identified as amide compounds obtained at 1694 cm⁻¹ and 1653 cm⁻¹. The absorption band of about 1416 cm⁻¹ represents a group of sulfates (S = O) with strong intensity and an aromatic skeleton that joins aldehyde (C-H) in a deformed and stretched plane. Absorption of about 1156 cm⁻¹ was identified as stretching the ether (C-O-C) group, carboxylic acid group (C-O), lactone (cyclic ester) (C = O) stretching group. Absorption of about 1022 cm⁻¹ was identified as a carboxylic acid group (C-O), vibration aldehyde (C-H), and stretching of the aliphatic amine group (C-N).
Amines (N-H) has only one absorption near 3420 cm⁻¹ and compounds derived from protein in shrimp that show the rate of degradation of protein and non-protein nitrogen compounds that are identified as the specific aroma of shrimp [13]. Absorption of the carboxylic acid group (OH) 2930 cm⁻¹ derived from shrimp fatty acid compound [14]. While the asymmetric stretching aldehyde group (CH) comes from the ginger [16] which can be caused by oxidation of lipids [17]. Amides of carboxylic acid derivatives group (C = O) on the absorption of 1694 cm⁻¹ and 1653.52 cm⁻¹. The sulfate group (S = O) adsorption in 1416 cm⁻¹ derived from onion and garlic in the form of allicin-containing flavoring flavor [22], [23]. Absorption at 1156 cm⁻¹ is identified as being ether (C-O-C) derived from turmeric [24], and Ginger [16], [15], the group lactone (cyclic ester) (C = O) comes from the ginger [16], and stretching aliphatic amine group (CN) obtained from shrimp.

Figure 2. FTIR Spectra of flavored powder from Vannamei shrimp shell waste

5. GC-MS Analysis

Figure 3. shown GC-MS spectra of the filtrate without and with enzym. The filtrate without the addition of bromelain has a high retention time of each peak identified, as shown in Table 1. Most of the compounds in the first filtrate are volatile compounds due to the addition of NaCl from the primary reflux. It builds a hydration shell to bind water molecules. Finally, the release of taste compounds and increased mobility is caused by a decrease in water molecules to dissolve aroma compounds [13].

Besides, NaCl can reduce the ability of proteins to bind volatile compounds by modifying the surface polarity of proteins [14]. Esters and carboxylic acids are derived from the oxidation of lipids produced by unsaturated fatty acids. The unsaturated fatty acids (Octadecanoic acids) called oleic acids and saturated fatty acids (Glycidol stearate). Sulfur compounds can be derived from disulfide derivatives (Decyl sulfide) [15].

In the second filtrate, as shown in Table 2, the majority compounds caused by the enzymatic reaction. Bromelain enzyme included in the classification of a hydrolase. Cysteine proteinases can break peptide bonds and separate proteins into amino acids [16]. One of which amino acid that caused by bromelain enzyme is Trimethylamine. Trimethylamine (TMA) is a tertiary amine which is gaseous in average temperature that an indicator of decreasing fish (shrimp) quality and has the characteristic smell of rotting fish [17]. It is appropriate with that the fresh fish contained lower TMA value, good flavor, and highly acceptable. The carbonyl compounds (ketone and aldehyde) are the result of the protein oxidation involved in cross-linking of damaged proteins via Schiff base formation. Schiff bases are produced as a result of reactions between lipid oxidation products (aldehydes) and amino groups from the side chain of proteins [18]. The sulfide compound of the second filtrate can be caused by the
heating above the optimum temperature of the enzyme that makes the peptide and disulfide bonds are disrupted.

Figure 3. GC-MS Spectra of (a) Filtrate without Bromelain enzyme addition and (b) Filtrate with Bromelain enzyme addition.

Table 1. GC-MS spectra from the Filtrate without and with bromelain enzyme

| Retention Time | Area (%) | Compound of filtrate without enzyme | Compound of filtrate with enzyme |
|----------------|----------|-----------------------------------|---------------------------------|
| 14.14          | 7.29     | Octadecanoic acid                 |                                 |
| 14.45          | 5.98     | (2,14-Dioxocycloctadecyl) Acetic acid methyl ester |                                 |
| 15.25          | 10.69    | Glycidol stearate                  |                                 |
| 16.34          | 7.77     | Decyl sulfide                      |                                 |
| 17.08          | 4.84     | 7a-Isopropenyl-4,5-dimethyl-octahydroindene-4-carboxylic acid |                                 |
| 17.93          | 5.89     | Acetic acid, 13-hydroxy-4, 4, 6a, 6b, 8a, 11, tamethyl doco-sahydropicen-3-yl-ester |                                 |
| 22.30          | 6.15     | Silane, dimethyl(dodec-2-enyloxy) heptyloxy- |                                 |
| 25.39          | 2.41     | E-10, 13, 13-Trimethyl-11 Tetradecen-1-ol acetate |                                 |
| 27.98          | 26.52    | Butyl 9-octadecenoate or 9-18:1 |                                 |
| 29.39          | 7.94     | 18-Pentatriacontaneone             |                                 |
| 36.62          | 24.75    | 17-Pentatriacontene                |                                 |
| 36.99          | 48.92    | Octadecanoic acid                  |                                 |

6. TEM Analysis
The characterization of flavoring powder particles from white shrimp shell filtrate using TEM on a 20 nm particle size scale can be shown in Figure 4. The flavored shrimp shell distributed nanoparticles of almost uniform size. The shape of the granules close to the shape of the sphere with dispersion does not occur as agglomeration [19]. Flavor particle size can be seen in Figure 5, with a size distribution between 1 nm to 8 nm. The particle size with the size dominated at 6 nm is 26.1% of the total number of particles measured. The particle size of flavoring powder ranges from 53.78 to 71.68 nm [20]. The smaller the particle size signifies an increase in the quality of flavoring powder. Small particle size can increase the solubility of flavoring powder when applied to food ingredients [21].
Figure 4. Flavored powder particle using (a) TEM microscopy (b) Percentage of particle size

7. Conclusions
The quality of flavored powder from Vannamei (Litopenaeus vannamei) shrimp shell waste assisted by bromelain enzymes is the highest protein contents of flavored powder at 2% (w/w) of bromelain enzyme addition and reflux temperature 55°C with the most elevated protein 24.6%. The functional group of flavored powder using FTIR analysis consists of amines (N-H), carboxyl acid (O-H), aldehyde (C-H), carboxyl acid derivatives (C=O), sulfate (S=O), aldehyde (C-H), ether (C-O-C), lactone (a cyclic ester) (C=O), aliphatic amine (C-N), and vinyl (-CH=CH2) group. The specific compounds analysis using GC-MC shows that there is a protein present in the filtrate ie, dimethylamine that is caused by the enzymatic reaction by bromelain enzyme in shrimp shell pulp. Flavored particles are dispersed evenly, and no agglomeration occurs. The dominant particle size is 6 nm with a distribution between 1-8 nm.

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