Utilization of Shrimp Waste *(Litopenaeus vannamei)* as Powdered Broth: Effects of Roasting Duration on Protein Content, Color Changes, and FTIR

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Abstract

Shrimp waste, such as shells and heads, can still be utilized to create powdered broth commonly used as a flavor enhancer in food. This research is intriguing because shrimp waste can substitute for MSG *(monosodium glutamate*). This study aims to investigate the impact of the roasting process duration on the protein content and physical properties of powdered broth derived from shrimp heads and shells. The primary treatment in this study involves varying roasting times, divided into four groups: K1 (30 min), K2 (40 min), K3 (50 min), and K4 (60 min). The protein content aligns with the quality requirements for flavor enhancers. Additionally, the L* color value ranges from 65-12-71.33, the a* value ranges from 2.23-5.61, and the b* value ranges from 22.71-25.32. Due to the prolonged drying process, the peaks of amide A, amide B, amide I, amide II, and amide III shift. The opportunity to utilize shrimp waste in the form of shells and heads for powdered broth is wide open for commercialization.

Keywords : FTIR, protein content, powdered broth, vaname shrimp

Introduction

Generally, Indonesian society consumes powdered broth from synthetic materials known as MSG *(monosodium glutamate)*. Powdered broth serves as a flavor enhancer in dishes. However, excessive consumption of synthetic powdered broth can negatively affect the body. Excessive and continuous consumption of monosodium glutamate can lead to stomach disorders, sleep disturbances, nausea, and allergic reactions, as well as trigger hypertension, asthma, cancer, diabetes, paralysis, decreased intelligence, and spermatogenesis disorders due to free radicals and oxidative stress in the body (Ningsih, 2018).

Shrimp shells and heads contain various beneficial nutrients for health. When dried and ground into powder, shrimp shells, and heads offer a variety of nutritional benefits. The proteins they contain are essential for muscle repair and growth. The fiber in this powder aids digestion and can help regulate blood sugar levels. Additionally, shrimp shell and head powder are rich in minerals such as calcium, crucial for bone health; potassium, which helps control blood pressure; selenium, with antioxidant properties that support the immune system; and zinc, essential for metabolism and immune function (Milati *et al*., 2022).

Shrimp shells and heads should be dried before being used as flavor-enhancer broth powder because drying has several essential benefits. Drying removes moisture from the shells and heads, preventing decay and ensuring the resulting powder is long-lasting. Drying also concentrates the nutrients in shrimp shells and heads, producing a powder that contains higher amounts of protein, fiber, minerals, vitamins, and antioxidant compounds (Bawinto, 2015). Once in powder form, shrimp shells and heads provide a rich umami flavor. Ribonucleotide compounds in shrimp shells enhance the savory taste in dishes. Therefore, drying is crucial to maximize the benefits of shrimp shells and heads in broth powder production.

Based on the explanations above, research on the utilization of shrimp shells and heads as flavor-enhancer broth powder in food is up-and-coming and worthy of investigation because it has the potential to benefit the broader community. This study aims to evaluate the influence of roasting duration on protein content, color changes, and molecular interactions in broth powder derived from vannamei shrimp heads and shells.

Materials and Methods

Material

The main raw materials in this study are waste from vannamei shrimp *(Litopenaeus vannamei)*, sourced from the heads and skins, obtained from vannamei shrimp ponds in Sawojajar Village, Brebes Regency. Additionally, other supplementary ingredients include garlic, shallots, pepper, ginger, and salt. Tapioca and flour are also used as fillers.

Broth Powder process

Making broth powder is based on research conducted by Sintya *et al*. (2023) with several modifications. The production of broth powder begins with a washing process lasting 1 hour. The shrimp shells, once washed, are then manually sorted to separate impurities from the shells. After sorting, the shrimp shells are blanched with seasoning added for 20 min. Subsequently, the shrimp shells are oven-dried for 30 minutes at 100°C. The shrimp shells undergo roasting with varying durations based on treatments (K1 = 30 min, K2 = 40 min, K3 = 50 min, and K4 = 60 min). A concentration of filler material at 4% (w/w) is also added. Afterward, the broth powder is blended for 3 min to ensure uniform size, followed by sieving through a 60 mesh sieve. The filler material is added to enhance the broth powder's texture, moisture, and texture distribution.

Protein content

Protein content measurement employs the Kjeldahl method. The procedure commences by weighing 5 grams of finely ground samples. Subsequently, the sample is placed into a pre-prepared Kjeldahl flask. Next, the flask adds 7 grams of K_2SO_4 and 0.8 grams of $CuSO_4$ hitherto. The destruction process involves heating the sample in the Kjeldahl flask using an electric heater until its color changes to light green. Afterward, the Kjeldahl flask is allowed to cool for 20 min before adding 25 mL of distilled water. Following this, 50 mL of 40% NaOH solution and a few boiling stones are added to the flask, and distillation is conducted using a distillation apparatus. Finally, the distillate is titrated with a 0.1 N HCl standard solution until it turns pink. Thus, the Kjeldahl method offers a systematic approach for accurately measuring sample protein content (Hasdar*et al*., 2019).

Color measuremet

The measurement of film color characteristics in this study follows the methodology described by Saputra & Mursyid (2023). The Konica Minolta Chroma Meter CR-400/410 colorimeter, manufactured in Tokyo, Japan, was selected for its accuracy and reliability in measuring essential color parameters such as L*, a*, and b*. L* represents the overall brightness of the sample, while a* and b*, respectively, describe the intensity of red and yellow. In addition to these basic color parameters, the study also considered ΔE (color difference) as an objective measure to detect differences in color among samples. Furthermore, Whiteness Index (WI), Yellowness Index (YI), and Browning Index (BI) were used to evaluate additional aspects of color characteristics that may be significant in the context of film applications. Before the measurement, the colorimeter was calibrated using a white background standard to ensure the instrument's precision in providing accurate and reproducible data. Calibration procedures are crucial in minimizing measurement errors from environmental factors or instrument variations.

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR measurement method applied in this study is an adaptation of the approach developed by Pajooh *et al*. (2024), with specific modifications to enhance the accuracy and precision of results. Measurements were conducted using the Bruker INVENIO® spectrometer integrated with OPUS v8.5 software, a combination proven effective in spectral analysis across various fields of study. The spectrometer operated in transmission mode over a spectrum range from 4000 to 500 cm^{-1}, with a high resolution of 4 cm^{-1} to enable precise detection of subtle changes in molecular structure. Each spectrum was acquired 32 times to reduce variability and ensure data consistency. Subsequently, the data were normalized against a background spectrum taken at a constant temperature of 25 °C, a crucial step to eliminate disturbances from temperature or humidity fluctuations in the environment. The recorded spectra were converted into transmittance, providing the necessary data foundation for in-depth analysis of the material's structure under investigation. Data analysis processes were performed using OriginPro®2023 software, a leading complex scientific data analysis platform. OriginPro® not only facilitated in-depth analysis of FTIR spectra and enabled meticulous and systematic data processing. With this approach, the research aims to contribute to a deeper understanding of the investigated material's structure and composition while maintaining high accuracy and reproducibility standards in experimental results.

Research design

This study employed a Completely Randomized Design (CRD) with three replications (Hasdar *et al*., 2021). The drying time duration was a primary factor in this research. The study was divided into four treatments: K1 (30 min), K2 (40 min), K3 (50 min), and K4 (60 min).

Data analysis

Data analysis was conducted using ANOVA (Analysis of Variance). If the statistical test using ANOVA yielded significant results, a post hoc Duncan test was performed to determine which treatment groups showed the most critical outcomes.

Results and Discussion

Protein content

Protein is a highly essential nutrient for the body because it functions as fuel and as a building and regulatory substance (Pagarra *et al*., 2022). Table 1 shows the protein content of the broth powder with various roasting durations.

Table 1. Powdered Broth Protein Content Results

Component	Powdered Broth Protein Content Results			
	K1	K2	K3	K4
Protein (%)	35.78 ± 2.14^{a}	32.84 ± 1.97^{b}	$35.11 \pm 3,37^{a}$	36.55 ± 3.12^{c}

Note: ^{a,b,c,d} use as letter notations indicate no significant difference. Duncan's test level is at 5%

Based on the data presented in Table 1, there is a significant difference ($P<0.05$) in the protein content of shrimp waste-based broth powder with varying roasting times. The analysis shows that broth powder K4 roasted for 60 min had the highest protein content, reaching 36.55%. This treatment is significantly higher than other broth treatments roasted for 30, 40, and 50 min, with protein contents of 33.21%, 34.12%, and 35.05%, respectively.

Additionally, both treatments, K2 roasted for 40 min, exhibited the lowest moisture content, at 32.84%, compared to other treatments (for instance, K1= 33.72%, K3= 34.18%, K4= 35.29%). However, it should be noted that treatments K2 and K3 showed fluctuations in

the protein content produced. These fluctuations are suspected to be due to variations in the drying process of the raw materials, which can affect the structure of the protein double helix, leading to instability. Differences in moisture content among treatments may also influence the structural conditions of the proteins.

This study also reveals that longer roasting times increase protein content. This process may facilitate protein denaturation or interaction with other components in the broth powder, thereby enhancing the nutritional value and final product taste. The reduction in moisture content in the broth powder also plays a crucial role in improving its protein structure quality (Maryam and Andi, 2023). These findings underscore the importance of precise processing time management and conditions as critical factors in optimizing protein content and other nutritional properties in shrimp waste-based broth powder for potential applications in food science.

Color

Color quality has three attributes: L*, a*, and b*. The value of L* indicates the brightness of the sample (chromatic color, 0 indicates black, and 100 indicates white). The value of a* reflects the chromatic color from red to green (a*= 0 to 100 for red, a*= 0 to -80 for green), while the value of b* reflects the chromatic color from blue to yellow (b*= 0 to 70 for yellow, b*= 0 to -70 for blue) (Safithri*et al*, 2019). The analysis of broth powder color with variations in roasting time can be seen in Table 2.

Table 2. Results of Powder Broth Analysis

Component	Color of Broth Powder			
	K1	K2	K3	K4
L*	1.33 ± 3.240^{a}	64.93 ± 16.334^{b}	65.23 ± 4.057^{c}	65.12 ± 1.088^{d}
a*	.35 ± 0.427^{a}	5.61 ± 7.337^{b}	4.14 ± 0.394^{c}	5.05 ± 0.716^{b}
b*	5.32 ± 1.438^{a}	23.35 ± 1.428^{ab}	24.76 ± 0.782^{ab}	22.71 ± 3.844^{c}
ΔE	1.25 ± 3.291^{a}	36.20 ± 0.148^{b}	35.85 ± 2.933^{c}	34.15 ± 1.006^{d}
WI	1.66 ± 3.317^{a}	62.57 ± 2.546^{b}	61.93 ± 2.769^{a}	63.59 ± 3.198^{c}
YI	0.91 ± 4.985^{a}	57.78 ± 30.837^{b}	54.29 ± 1.893^{c}	46.94 ± 1.265^{d}
BI	5.01 ± 5.636^{a}	63.18 ± 54.032^{b}	50.91 ± 2.836^{c}	44.28 ± 1.302^{d}

Note: ^{a,b,c,d} use as letter notations indicate no significant difference. Duncan's test level is at 5%

Based on the ANOVA analysis results in Table 2 for the color parameters L*, a*, b*, ΔE, WI, YI, and BI, it can be observed that there are no significant differences (P>0.05) among different treatments. The L* values ranging from 65.12 to 71.33 indicate that shrimp waste-based powder broth with varying roasting times exhibited significant brightness, predominantly white. A significant decrease in brightness was evident in the powdered product with increased temperature and roasting time. This phenomenon is attributed to the oxidation of phenolic compounds in the raw materials, leading to the formation of darker compounds through the Maillard reaction. This process affects color parameters and influences sensory characteristics and overall product quality. These findings align with existing knowledge regarding the influence of temperature and time on chemical reactions in food processing, where more intense heating conditions can alter product compound profiles and physical characteristics. The practical implications of this discovery underscore the importance of temperature and time control during production processes to maintain desired product quality, optimize production efficiency, and enhance consumer satisfaction.

The* value in the shrimp waste powder broth test showed a positive result, indicating that the broth tends to have a red hue. However, the* values were relatively the same, possibly due to suboptimal processing. Previous research by Murali *et al*. (2021) suggested that

increased redness could occur due to the release of astaxanthin during protein breakdown binding carotenoids in the roasting process. Additionally, the b* value is used in the color system to measure yellow intensity. Test results indicated that shrimp waste powder broth had a positive b* value, indicating a tendency towards yellow coloration. Graphs from treatment K3 showed unstable results, with a water activity 24.76 after roasting for 50 min, higher than K2's 23.35 after 40 min. Furthermore, the b* value (yellowness) in the shrimp waste powder with varying roasting times showed a significant decrease with increasing roasting time, from 25.32 to 22.71. This suggests that roasting time significantly affects the yellow intensity of shrimp waste powder broth.

The yellowness value in K1 is influenced by the amount of carotenoid pigments (yellow color). These pigments are mainly found in marine animals and provide bright red or pink hues; however, with prolonged roasting time, browning reactions occur, fading the carotenoid color (Pramudya *et al.*, 2022).

The ΔE value is used to determine how far the color difference is between the treatment groups (Mursyid *et al*., 2022). The unstable K1 value is caused by a less-than-optimal processing process, such as an unstable roasting process, which affects the analyzed powdered broth. The K2, K3, and K4 values decreased so that the ΔE value differed slightly from the roasting time. This is in line with the powdered broth's brightness level (L*), which decreases with increasing roasting time. Changes in the color of the powdered broth are caused by the heating given. The longer the drying time, the more significant the difference in the resulting ΔE value.

The Whiteness Index (WI) is a parameter used to measure a food material's degree of whiteness or white color. A higher WI value indicates that the material has a whiter color. The graph from treatment K3 shows unstable results, where the water activity of K3 reached 61.93 after a roasting time of 50 min, indicating a lower WI. Conversely, the WI values in treatments K1, K2, and K4 of shrimp waste powder with varying roasting times significantly increased with longer roasting times, rising from 61.66 to 63.59. This demonstrates that the duration of the roasting process positively influences the degree of whiteness of the shrimp waste powder. Factors affecting the whiteness of food materials include heme proteins, high-fat content, and drying temperature (Muslimin *et al*., 2022). This study underscores the importance of controlling these variables during processing to achieve the desired level of whiteness in the final product.

The effect of drying time on the Yellowness Index (YI) is evident from the variation in drying durations that influence Maillard and caramelization reactions. In K1 (30 min), the shorter drying time means these reactions have not fully developed, resulting in a lower YI than other treatments but still higher than K4. In K2 (40 min), Maillard and caramelization reactions develop further, increasing YI due to more brown and yellow pigment formation. The reactions peak in K3 (50 min), so the YI increase is not as significant as in K2. However, in K4 (60 min), the reactions decline, or the pigments degrade, causing YI to drop drastically. Pigment degradation in K4 occurs because prolonged drying can damage the chemical structure of the pigments, transforming them into colorless or darker compounds. Additionally, volatile compounds that provide color may evaporate, reducing the intensity of the yellow color. In conclusion, the differences in YI are due to the dynamics of chemical reactions during drying, with the lowest YI in K4 resulting from pigment degradation and the volatility of colored compounds (Yuliawaty *et al.*, 2015).

The difference in the Browning Index (BI) in this study is attributed to the duration of drying using roasting methods, which affects the Maillard reaction and caramelization in broth powder. Shorter drying times (30 min, K1) result in a lower BI due to suboptimal reaction conditions. The optimal drying time for brown compound formation is 40 min (K2), which shows the highest BI. Longer drying times (50 and 60 min, K3 and K4) tend to decrease BI

due to the potential degradation of brown compounds formed from the Maillard reaction and caramelization caused by excessive heat (Irfan A. & Lestari N., 2022).

Functional group analysis

Shrimp powder broth can be analyzed using Fourier Transform Infrared Spectroscopy (FTIR), which is highly useful for identifying molecular structures in compounds. FTIR technique utilizes the Fourier Transform concept to observe functional groups in shrimp waste powder broth. Analysis results reveal distinctive absorption peaks in the amide region, such as amide A, amide B, amide I, amide II, and amide III, as seen in Table 3. The FTIR spectrum of shrimp waste powder broth shows the absorption bands of each amide in the infrared radiation (IR) range: amide A at 3300 - 3500 cm-1, amide B at 2925 - 2935 cm-1, amide I at 1600 - 1700 cm-1, amide II at 1480 - 1575 cm-1, and amide III at 1229 - 1301 cm-1. This technique provides a clear understanding of the chemical structure of shrimp waste powder broth, which is crucial for applications in the food or pharmaceutical industries to ensure product quality (Safithri *et al*., 2019).

Table 3. Wavenumber Powdered Broth

Type of compound	Wavenumber (cm^{-1})				
	Shrimp shells	K1	K2	K3	K4
Amida A	3338	3417	3327	3308	3366
Amida B	2935	2927	2925	2925	2922
Amida I	1636	1635	1637	1641	1641
Amida II	1556	1566	1562	1555	1552
Amida III	1232	1259	1234	1236	1235

This study evaluated the influence of prolonged drying time using the roasting method on shrimp shells using FTIR analysis. FTIR (Fourier Transform Infrared) is a spectroscopic technique employed to examine chemical bonds in samples based on the infrared absorption by molecules in various compounds. FTIR analysis indicated changes in characteristic peaks such as Amide A (3308 - 3417 cm-1), typically associated with NH vibration bands often involved in hydrogen bonding. Shifts in wavenumber values indicated alterations in hydrogen bonding strength and hydration level of shrimp shell during drying, with decreasing wavenumber values from K1 to K4 potentially indicating reduced formation of hydrogen bonds or loss of free water in the shrimp shell. Amide B (2922 - 2935 cm-1) relates to CH vibration bands reflecting the presence of CH groups in organic compounds. The relatively stable wavenumber values suggested that carbon-hydrogen bonds in this compound did not undergo significant changes during drying (Guerrero-Rodríguez et al., 2021).

Amide I (1636 - 1641 cm-1) is frequently associated with C=O vibration bands, indicating the state of carbonyl (C=O) bonds in compounds. Minor shifts in wavenumber values may indicate changes in hydrogen bonding or molecular conformation due to drying processes. Amide II (1552 - 1566 cm-1) is associated with N-H vibration bands and is also involved in hydrogen bonding. Changes in wavenumber values reflected alterations in hydrogen bond interactions within the molecular structure of shrimp shells during drying. Amide III (1232 - 1259 cm-1) is related to C-N vibration bands, reflecting bonds between carbon and nitrogen atoms in molecules. Changes in wavenumber values may indicate changes

in the chemical state of C-N groups, which could be related to changes in protein or molecular structure within the shrimp shell (Rahayu et al., 2022).

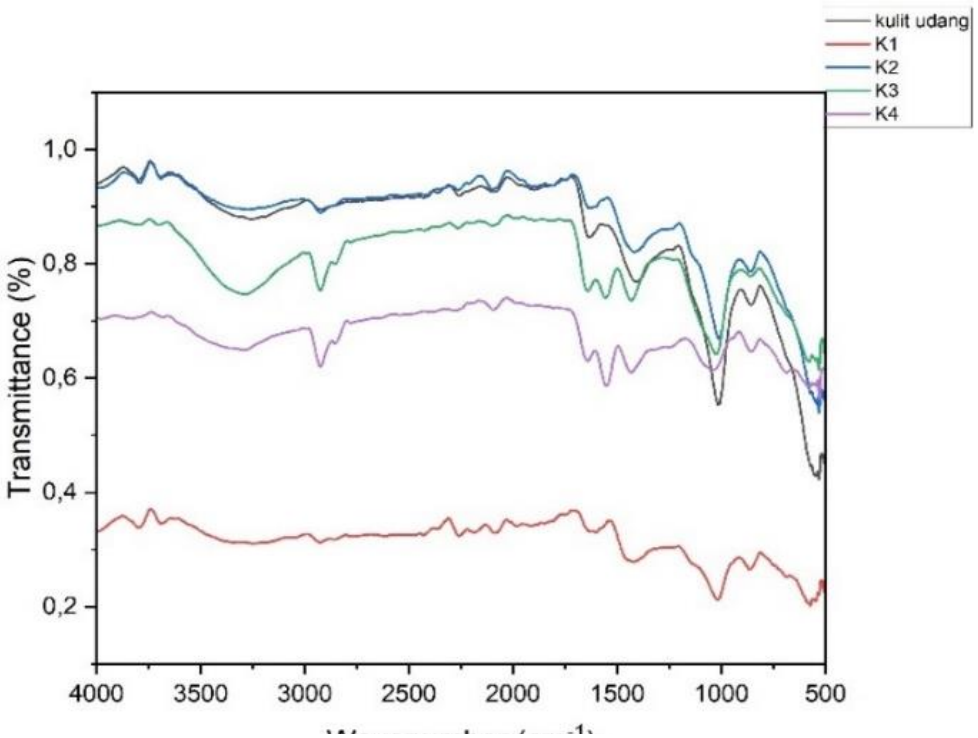

Figure 1. Graph of Functional Group Analysis of Broth Powder

During the drying process using the roasting method, several chemical reactions occur, including protein denaturation. Heating during roasting can cause protein denaturation in shrimp shells, altering the three-dimensional structure of proteins that affect intramolecular and intermolecular bonds, such as hydrogen bonds. Applied heat can also influence the strength of hydrogen bonds in compounds like amides A and II, as reflected in FTIR band intensity or position changes. Drying also removes water from the shrimp shell, affecting molecular hydration degree and protein structure stability, as observed in changes in FTIR band intensity or position related to hydrogen bonds (Liu *et al*., 2021). FTIR analysis of shrimp shells processed by roasting drying method revealed changes in chemical structure, mainly associated with hydrogen bonds and hydration conditions. These changes are crucial to understand in the context of further applications of shrimp shells, such as in food or pharmaceutical industries, to ensure the quality and stability of the final products.

Conclusion

This study provides valuable data for further research on shrimp waste-based broth powder. It reveals that the broth treated with K2, roasted for 40 min, exhibited the lowest moisture content at 32.84%, compared to K1= 33.72%, K3= 34.18%, and K4= 35.29%. However, treatments K2 and K3 showed fluctuating protein content, likely due to variations in the drying process affecting protein structure, which could impact protein structural conditions. The L* values ranged from 65.12 to 71.33, indicating significant brightness in shrimp waste-based powder broth with different roasting times, predominantly white; higher roasting temperatures decreased brightness due to phenolic compound oxidation, resulting in darker compounds via the Maillard reaction. FTIR analysis showed that roasting induced chemical

reactions, including protein denaturation in shrimp shells, altering protein structure, and affecting hydrogen bonds.

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