Characterization of Microparticles Extracted from Wasabi (*Wasabia japonica*) as a Natural Food Preservative for Fresh Chicken Meat

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Submitted: October 12, 2023; Revised: May 24, 2024, July 8, 2024, July 29, 2024, September 30, 2024;
Accepted: October 2, 2024; Published: May 28, 2025

ABSTRACT

Chicken meat is a food ingredient vulnerable to contamination, requiring effective treatment to extend its shelf life and maintain quality. Wasabi (*Wasabia japonica*), a typical Japanese plant, contains an allyl isothiocyanate compound with antibacterial properties, making it a potential natural preservative. Therefore, this study aimed to develop microparticles of wasabi extract for application as a preservative for fresh chicken meat. In the process, microparticles were produced using the dry spray method, with maltodextrin as a coating material. The formulations tested were 0% (F0), 1% (F1), 2% (F2), and 3% wasabi extracts (F3), combined with 100 grams of maltodextrin and 1000 mL of distilled water. The produced microparticles were subjected to characterization to determine particle size, solubility test, particle morphology, crystal structure, and functional group identification. Subsequently, inhibition zone tests and total plate count were conducted to assess the effectiveness of the particles as a natural food preservative. The results showed that the F3 treatment had the widest inhibition zone (7.1 mm). Immersing chicken meat in the extract solution reduced the bacterial colony count from 16.8x106 CFU/gram to 12.5x106 CFU/gram over 6 days of storage. Therefore, F3 was the best formulation based on microbiological test results. This signified the potential of wasabi extract microparticles as a natural food preservative.

Keywords: Allyl isothiocyanate; chicken meat; microparticles; natural food preservative; wasabi extract

INTRODUCTION

Chicken meat is a widely consumed protein source in Indonesia, valued for its affordability, palatability, and nutritional content. However, it is highly susceptible to contamination by pathogenic bacteria when left unrefrigerated and exposed to air. Lack of food hygiene has led to a rise in cases of poisoning, such as the incident reported in Lembang (Pradana, 2023). In addition to bacterial contamination, fat oxidation can also lead to a decrease in the quality of chicken meat, such as color, taste, and texture (Zhang et al., 2016).

To extend the shelf life and maintain quality, chicken meat is generally stored by freezing, cooling, thermal processes, or the addition of preservatives such as salt, sugar, synthetic compounds, or natural agents. According to Indonesian Food and Drug Authority (BPOM) Regulation number 11 of 2019 concerning food additives, the types of preservatives permitted in food products are sorbic acid, benzoic acid, ethyl-parahydroxybenzoate, methyl para-hydroxybenzoate, sulfite, nisin, nitrite, nitrate, propionic acid, and lysozyme hydrochloride. Long-term accumulation of synthetic preservatives can have adverse effects on

DOI: http://doi.org/10.22146/agritech.89696
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

health, as it increases the risk of cancer (Lu et al., 2016). Therefore, the use of natural preservatives (natural food preservatives) is recommended due to greater environmental friendliness and minimal health risks. Studies on plants with natural preservative properties have gained traction as an effort to reduce the use of synthetic additives.

Plant is the prime source of natural preservatives, particularly spices that are rich in phenolic compounds and have antimicrobial activity (Zhang et al., 2016). Wasabi (*Wasabia japonica*), a widely known complementary seasoning, consists of the main root, leaf stalks, and leaves, all of which have a distinctive aroma and spicy taste when eaten. The spiciness is caused by the ally isothiocyanate, a compound with antibacterial and antimicrobial properties. Study by Lu et al. (2016) showed that wasabi extract at low concentrations inhibited bacterial growth. It had bactericidal effects against *E. coli* and *S. aureus* bacteria at high concentration, reflecting the potential as a natural food preservative.

Allyl isothiocyanate is a volatile compound that easily evaporates, making it unstable at high temperatures. A protection process is needed for the wasabi stem extract to prevent damage to the compound. Syamsinar et al. (2018) stated that microencapsulation offers a viable solution by coating the material with a microscopic layer, creating capsules with diameters ranging from 1 to 800 µm. It is effectively conducted through spray drying which include freezing the extract, thereby minimizing exposure to high temperatures. Therefore, this study aimed to determine the best formulation of wasabi extract microparticles as a natural food preservative in fresh chicken meat. The result is expected to be a reference for the development of natural preservatives derived from wasabi stems in the future.

METHOD

Material

The materials utilized in this study include matured wasabi (*Wasabia japonica*) obtained from a farm in Yogyakarta, Indonesia and 96% ethanol (IPA5) from CV. Makmur Sejati, maltodextrin DE 18 (food grade), distilled water (WaterOne), and fresh chicken breast meat from local market in Pasuruan, East Java. Other materials were *E. coli* bacterial culture from the Biomedical Laboratory of the University of Muhammadiyah Malang, PCA media (Oxoid), MHA media (Oxoid), and blank disk (Oxoid).

The tools used were glassware, stirring rods, petri dishes, spray dryers, rotary vacuum evaporators (EYELA SB-1000, Japan), scanning electron microscopy/ SEM (FEI Inspect-S50, America), particle size analyzer/ PSA (Type 10/90 CILAS, France), and X-Ray Diffraction/ XRD (PANalytical X'pert PRO, Netherlands). Fourier Transform-Infra Red/FTIR (Shimadzu IR Prestige 21, Japan), ose rods, autoclaves, micropipettes, Erlenmeyer flasks (Pyrex, America), incubators, and calipers were also utilized.

Wasabi Stem Extraction

A total of 500 g of wasabi stem was washed thoroughly, cut into small pieces, dried in an oven at 40 ° C for 3 hours, and ground to powder form. The simplicia was then extracted using the maceration method with 96% ethanol as the solvent at a ratio of 1:5. The extraction was conducted for 3x24 hours at room temperature. The macerate obtained was concentrated with a rotary evaporator (EYELA SB-1000) to obtain wasabi stem extract.

Microparticle Formulation of Wasabi Stem Extract

Microparticle formulation of wasabi stem extract was prepared using spray drying microencapsulation method with maltodextrin as a coating material. The formulation variations are presented in Table 1.

Microencapsulation Technique of Wasabi Stem Extract

Microencapsulation of wasabi stem extract was conducted using the spray drying method. The extract mixed with maltodextrin and distilled water according to the specified formulation was stirred until dissolved. Subsequently, the 3 formulations were dried using a spray dryer.

Table 1. Microparticle formulation of wasabi stem extract

No.	Formulation	Wasabi stem extract (%)	Maltodextrin (%)	Distilled water (mL)
1	F0 (control)	0	10	1000
2	F1 (1% wasabi extract)	1	10	1000
3	F2 (2% wasabi extract)	2	10	1000
4	F3 (3% wasabi extract)	3	10	1000

Microparticle Analysis

The microparticles produced from each treatment were analyzed physicochemically. This included a solubility test, as well as identification of particle size, surface morphology, crystal diffraction patterns, and functional groups using a PSA (particle size analyzer), scanning electron microscopy (SEM), an X-ray diffraction (XRD) tool, and a Fourier transform infrared (FTIR), respectively.

Effectiveness Test of Microparticles as Antibacterial

The effectiveness of microparticles as antibacterial was tested against *E. coli* bacterial colonies. The cultures were grown on media containing wasabi stem extract and the inhibition zones were measured using the disc diffusion method.

Microparticle Application on Fresh Chicken Meat

The best microencapsulation formulation was applied to fresh chicken meat to test the shelf life. A total of 25 g of the meat was soaked in 10% wasabi stem extract microparticle solution for 30 minutes and then stored in a refrigerator at 4 °C for 6 days. The total number of bacterial colonies was observed on days 0, 2, 4, and 6 using the Total Plate Count (TPC) method.

Data Analysis

Inhibition zone diameter was analyzed using the One-Way ANOVA method and Minitab 19 software to determine the effect of extract concentration on each treatment. Furthermore, this research was repeated 5 times from 4 treatments to obtain 20 samples. In the case of a significant difference (p <0.05), further examination was conducted using the Dunnet Test (α = 0.05). TPC and organoleptic test data were analyzed using linear regression and the Friedmann test, respectively.

Table 2. PSA analysis results

No.	Formulation	Microparticle Size (µm)
1	F0 (control)	158.37
2	F1 (1% wasabi extract)	142.99
3	F2 (2% wasabi extract)	145.87
4	F3 (3% wasabi extract)	169.40

RESULT AND DISCUSSION

Microparticle Characterization

Identification of microparticle size

Particle size distribution analysis was performed using PSA, and the results are presented in Table 2.

The particle size ranged from 142.99 µm - 169.40 µm and was categorized as microparticles. Lengyel et al. (2019) stated that the size of microparticles ranged between 1 and 1000 µm. Treatment F1, F2, and F3 had sizes of 142.99 µm, 145.87 µm, and 169.40 µm, respectively. As a result, the variation in particle size is directly proportional to the concentration of the extract.

Microparticle solubility test

The solubility of the microparticles in various solvents was determined using the appropriate test. In this study, the solvents used were distilled water, 96% ethanol, and n-hexane. The results showed that the wasabi extract microparticles were only soluble in the distilled water solvent. Meanwhile, in an n-hexane solvent, the particles remained in the form of granules. In 96% ethanol solvent, there was no dissolution, and clumps were formed. The solubility times of the microparticles in the respective solvents are presented in Table 4. Meanwhile, the results of solubility test are presented in Table 3.

The control treatment, F0, had the fastest dissolution time compared to all treatments. For microparticles, the time was directly proportional to the extract concentration. This relationship is associated with particle size, as smaller particles tend to dissolve more easily due to greater surface area, which facilitates absorption. In this study, the F1 treatment had the

Table 3. Results of the microparticle solubility test

No.	Parameter	Solubility		
		Distilled water	Ethanol 96%	n-hexane
1	Solubility	Dissolve	Insoluble (granules)	Insoluble (lumps)
2	Color	Yellow	Colorless	Colorless
3	Clarity	Turbid	Clear	Clear

Table 4. Microparticle solubility level

No.	Sample	Solubility	Dissolution time
1	F0	98.6 %	90 second
2	F1	85.8 %	119 second
3	F2	85.4%	124 second
4	F3	83.2%	138 second

smallest particle size (142.99) and correspondingly shorter dissolution times.

This result is in line with the study by Nugrahani et al. (2021) where the solubility of bean extract powder was influenced by the small size of the powder grains and the greater number of granules. Small particle size produces a large surface area, enhancing the interaction between the solvent and the solute. Additionally, Higher solubility correlates with better particle quality, as it ensures faster dispersion and dissolution during processing (Ummah et al., 2021).

Identification of microparticle morphology

Morphological analysis of wasabi extract microparticles with a magnification of 1000x using an SEM is shown in Figure 1.

Based on the results of SEM analysis, the morphology of wasabi extract microparticles showed irregular shapes and uneven size distribution. Treatments F1 and F2 had smaller sizes compared to the control (F0). This is due to the addition of wasabi extract to F1 and F2. Treatment F3 had a larger particle size than F1 and F2 but was smaller than the control (F0).

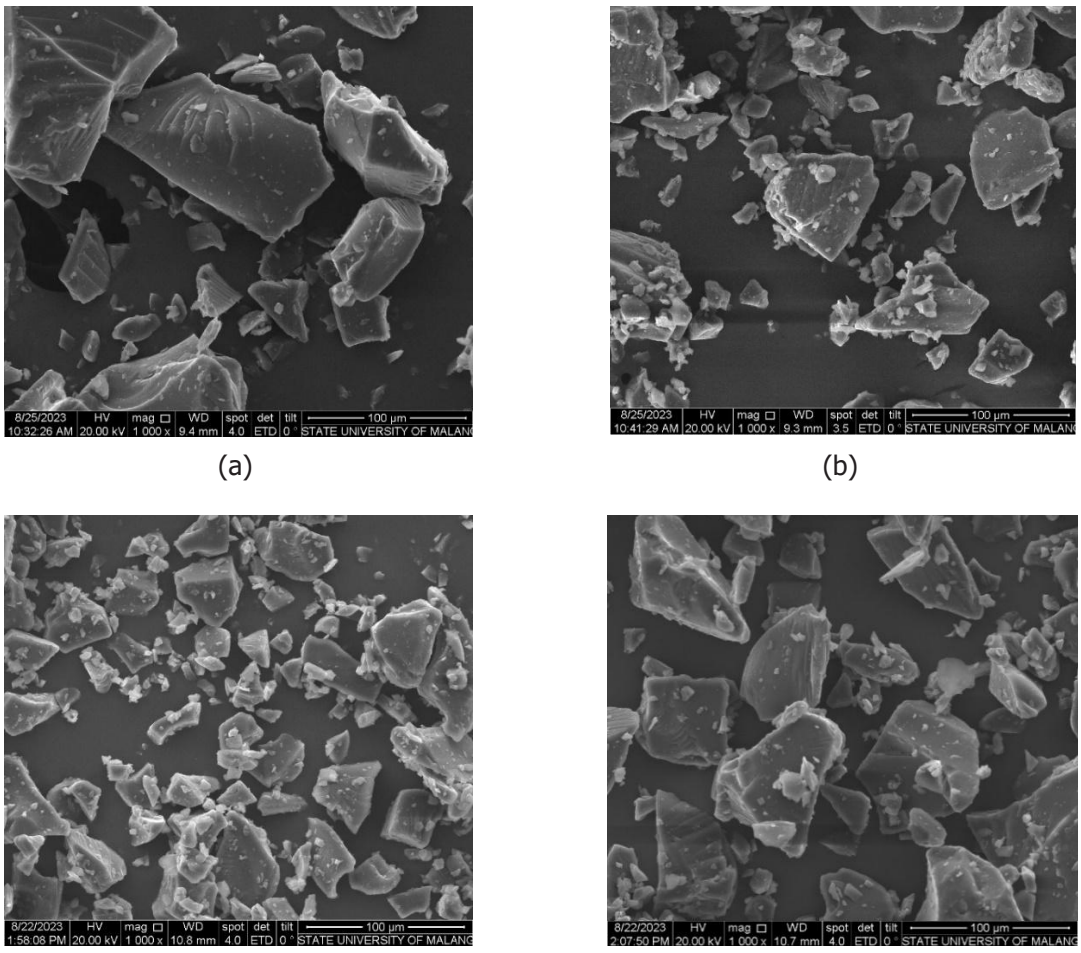

Figure 1. SEM Analysis Results (a) F0 (wasabi stem extract 0%, maltodextrin 10%, and 1000 mL of distilled water), (b) F1 (wasabi stem extract 1%, maltodextrin 10% and 1000 mL of distilled water), (c) F2 (wasabi stem extract 2%, maltodextrin 10% and 1000 mL of distilled water), and (d) F3 (wasabi stem extract 3%, maltodextrin 10% and 1000 mL of distilled water)

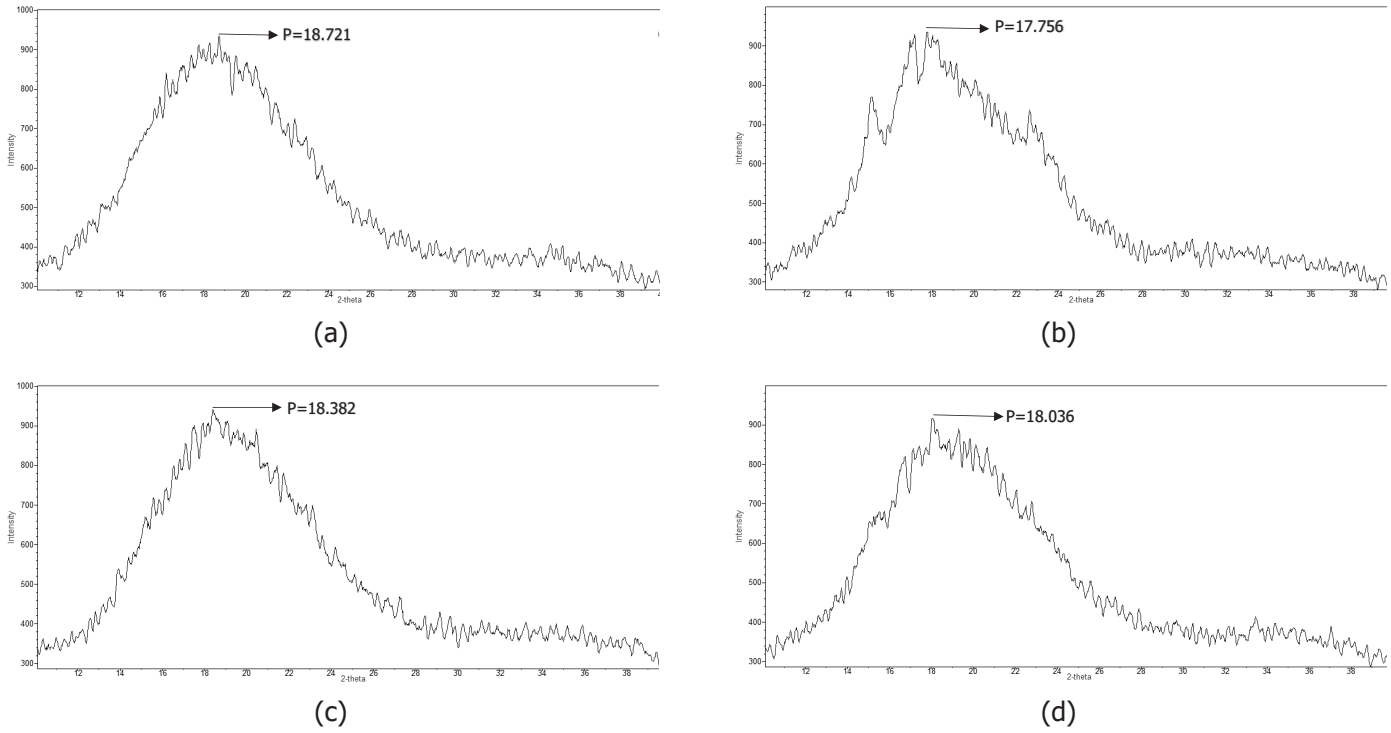

Figure 2. XRD analysis results (a) F0 (wasabi stem extract 0%, maltodextrin 10%, and 1000 mL of distilled water), (b) F1 (wasabi stem extract 1%, maltodextrin 10% and 1000 mL of distilled water), (c) F2 (wasabi stem extract 2%, maltodextrin 10% and 1000 mL of distilled water), and (d) F3 (wasabi stem extract 3%, maltodextrin 10% and 1000 mL of distilled water)

The addition of wasabi extract affects the morphology of microparticles. The more extract is added, the larger the particle size is, but it remained smaller when compared to the control. The irregular shape of the microparticles is attributed to the use of ethanol solvents during the extraction process. Aryanti et al. (2015) stated that the morphology of glucomannan flour extracted with ethanol tended to have a needle-like or fiber shape with an uneven distribution. The particle distribution appeared more even in treatments F1 and F2. It was important to acknowledge that homogeneous particle distribution can increase the stability of microparticles.

XRD analysis result

XRD analysis was conducted to identify the presence of crystal structure in the microparticle samples of wasabi extract, and the results are presented in Figure 2.

The XRD analysis results showed similar diffraction patterns in the 4 samples of wasabi extract microparticles. The samples featured a diffraction pattern of peaks that were broad and amorphous in structure. The peaks in each treatment ranged from an angle of 2Θ 17°-18°. The higher the degree of crystallinity, the more crystals were formed (Maghfiroh & Utomo, 2023). Sharp and narrow peaks with high intensity signified a crystalline structure in the particles (Widodo et al., 2021). Conversely, a broad peak presented an amorphous particle structure. The crystallinity of a particle can be affected by temperature in direct proportionality (Mutia et al., 2023).

The results of the XRD analysis showed small-sized particles with an amorphous structure. The sparse peaks in the F1 treatment signified that the particles were small. The resulting peaks were increasingly close together in F2 and F3. This shows that differences in wasabi extract concentration affect the density of the diffraction pattern, thereby influencing particle size.

FTIR analysis results

FTIR analysis was conducted to identify functional groups in the samples. The characteristic peaks appeared in the spectrum of the interpreted microparticles, and the graph is presented in Figure 3.

The FTIR spectrum for allyl isothiocyanate was at a wavelength of 500-4000 cm-1. The results of spectrophotometric absorption (FTIR) showed that the functional groups in wasabi extract microparticles were alkenes (strong), aromatic rings (strong),

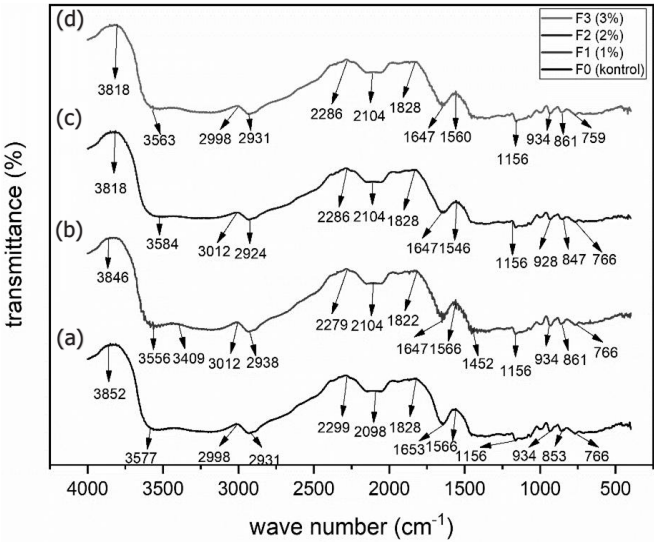

Figure 3. Graph of FTIR analysis results (a) F0 (wasabi stem extract 0%, maltodextrin 10%, and 1000 mL of distilled water), (b) F1 (wasabi stem extract 1%, maltodextrin 10% and 1000 mL of distilled water), (c) F2 (wasabi stem extract 2%, maltodextrin 10% and 1000 mL of distilled water), and (d) F3 (wasabi stem extract 3%, maltodextrin 10% and 1000 mL of distilled water)

alkynes (variable), amines/amides (strong), and phenols (variable).

The presence of allyl isothiocyanate compounds in wasabi extract was identified through the functional groups that appear in the FTIR spectrum. Allyl isothiocyanate is a chemical compound included in the isothiocyanate group and it consists of an allyl chain (C3H5) bound to nitrogen (N) and sulfur (S). A significant compound in this functional group is sinigrin, a glucosinolate discovered in plants of the Brassicaceae family. Isothiocyanates are degradation products of glucosinolates, which are secondary metabolites derived from amino acids, glucose, nitrogen, and sulfur. When plant tissue is damaged, glucosinolates in cell vacuoles interact with the myrosine enzyme located in the cell wall, leading to hydrolysis and the formation of various products, including isothiocyanate (Saada & Othman, 2019).

The presence of allyl double bonds with nitrogen (C=N) at a wavelength of 2210-2280 signified that wasabi extract microparticles contain allyl isothiocyanate compounds with strong intensity. Based on the results of FTIR analysis, the 4 samples with different concentration treatments showed no significant changes in the graph structure.

Table 5. Functional groups of microparticles

No.	Wave number (cm-1)	treatment				Functional groups
		F0	F1	F2	F3	
1	675-995	766 853 934	766 861 934	766 847 920	759 861 934	C-H (alkene)
2	690-900	766 853	766 861	766 847	759 861	C-H (aromatic ring)
3	1144-1185	1156	1156	1156	1156	C-OH (aliphatic alcohol/carbohydrate)
4	1500-1600	1566	1566 1586	1546	1560	C=C (aromatic ring)
5	1610-1680	1653	1647	1647	1647	C=C (alkene)
6	1790-2000	1828	1822	1828	1828	Si-O
7	2100-2260	-	2104	2104	2104	C=H alkyne
8	2800-3010	2931 2998	2938	2924	2931 2998	C-H Aliphatic
9	2210-2280	2299	2279	2286	2286	C=N (nitrile)
10	3300-3500	3577	3409 3556	3583	3563	N-H (amine/amide)
11	3200-3600	3577	3409 3556	3583	3563	O-H (hydrogen alcohol/phenol)

Table 6. Inhibition zone test results

Treatment	Inhibition zone diameter (mm)
F0 (Control)	5.7 ±0.3
F1	6.3 ±0.3
F2	6.7 ±0.5*
F3	7.1 ±0.3*

Note: (*) significantly different at the 5% level

Inhibition Zone Test

The difference in extract concentration affects the diameter of the inhibition zone in a direct proportionality. At an extract concentration of 1% (F1), 2% (F2), and 3% (F3), the area of the inhibition zone formed was 5.7 (±0.3) mm, 6.7 (±0.5) mm, and 7.1 (±0.3) mm. This confirms that the higher the concentration of wasabi extract, the area of the inhibition zone formed also increased. The results are in line with the study by (Asmawati, 2016), where wasabi stem extract at concentrations of 2.5% and 3% effectively inhibited the growth of *Propionibacterium acnes* bacteria that caused acne.

The statistical analysis result using the One-Way ANOVA method showed a significant difference ($\alpha<0.05$) in the diameter area of the inhibition zone due to variation treatments. Further tests using Dunnet were conducted to determine the significance of the differences in each treatment against the control group at a level of 5% ($\alpha = 0.05$). The result showed that treatment F1 had no significant effect on the control group, while F2 and F3 showed significant effects. This signified that the extract concentration had a significant influence on the diameter area of the inhibition zone.

The effectiveness of allyl isothiocyanate as an antibacterial agents is also influenced by the wasabi extract concentration in a direct proportionality. The potential of the wasabi plant as an antibacterial is due to the content of secondary metabolite compounds. Among the characteristics of allyl isothiocyanate, include sharp odor and a burning sensation in the nose. This follows the study by Lu et al. (2016), where the compound inhibits and kills bacterial growth at low and high concentrations, respectively. The antibacterial properties mechanism of allyl isothiocyanate is the cause of damage to cell membranes, enhancing permeability, which interferes with cell metabolite activity and increases the 3-galactosidase compound (Kala et al., 2018).

Total Plate Count

The TPC test was conducted to determine the number of bacterial colonies (CFU/g) contained in the sample. Quantitative microbiological analysis of food ingredients was performed to assess the quality of the food ingredients (Yunita et al., 2015). This test aimed to compare the number of bacterial colonies in samples with the treatment of immersion in aquades and wasabi microparticle solution during the storage period of 0, 2, 4, and 6 days. The comparison of the total microbial colonies during the storage period is presented in Figure 4.

Based on the graph, soaking chicken meat with wasabi extract microparticles can reduce the number of bacterial colonies during storage. Meanwhile, the use of distilled water tends to increase the colonies along with the storage length. This was attributed to the secondary metabolite compound content in wasabi microparticles, which can inhibit bacterial growth. The presence of phytochemical compounds in plant extracts such as isothiocyanates, has been shown to inhibit microbial growth in meat (Maghfiroh & Daimatul, 2023).

The number of colonies formed in all chicken meat samples was quite high when compared to the standard of $1x10^6$ CFU/g set by BPOM. A fairly low extract concentration was considered less effective in reducing the number of bacterial colonies. Therefore, it is necessary to increase the concentration to meet the set standards.

The amount of wasabi extract used can influence the effectiveness of the microparticles as a natural food preservative. Zhang et al. (2016) stated that allyl isothiocyanate at low and high concentrations was bacteriostatic and bactericidal, respectively. This statement explains that the high number of bacterial colonies formed can be caused by the low concentration of extract added to the microparticle formulation. Additionally, the high number of microbial colonies can also be caused by the pH of the chicken meat. High pH values increased the growth of microorganisms in the meat (Cahyanti et al., 2020).

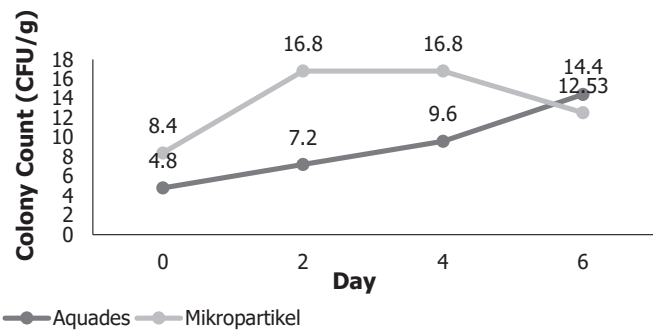

Figure 4. Graph of Total Plate Count test results

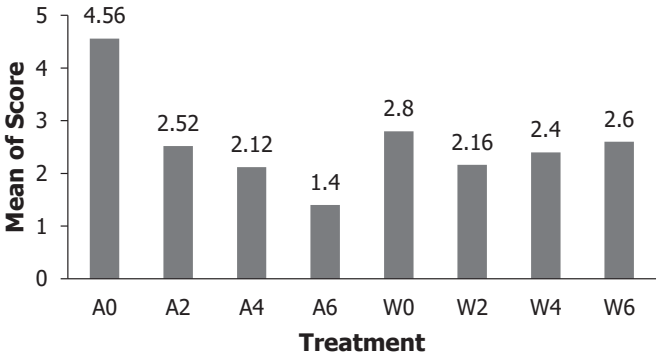

Figure 5. Bar chart of average color preference levels

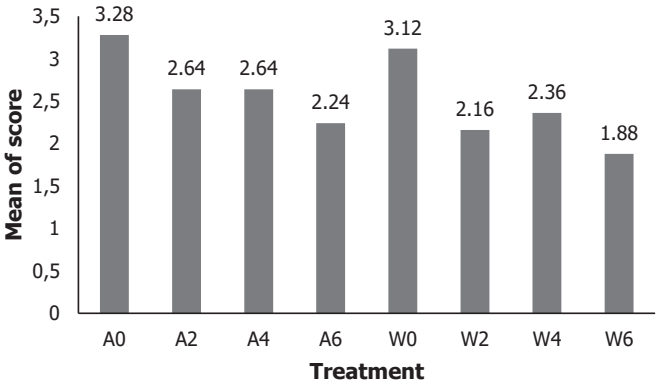

Figure 6. Bar chart of mean aroma preference levels

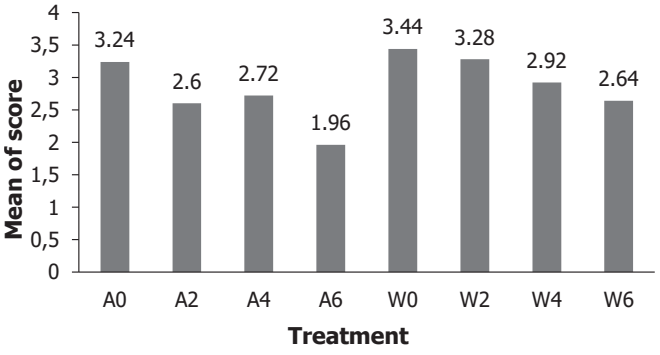

Figure 7. Bar chart of mean texture preference ratings

Organoleptic Test

Color

Color is an important parameter that determines the acceptability of the food by consumers. Based on the statistical analysis result, the soaking treatment has a significant effect (p<0.05) on the color of fresh chicken meat. The level of panelist's preference for the color of the chicken meat is presented in Figure 5.

The panelist's preference levels for the chicken meat color ranged from 1.4 (very dislike) to 4.5 (like). The color soaked in distilled water was preferred by the panelists compared to the color soaked in wasabi microparticle solution. This is because soaking chicken meat in a microparticle solution causes the meat's color to tend to be pale. Color changes in meat are closely related to muscle pigments, namely myoglobin and hemoglobin. The decrease in red color intensity is thought to be due to fat oxidation, which produces free radicals and affects the meat color (Zhang et al., 2016).

Aroma

Aroma is an important parameter in assessing the quality of fresh chicken meat. Based on the statistical test result, the treatment of immersion had a significant effect (p<0.05) on the fresh aroma of the meat, and the level of panelist preference for this parameter is presented in Figure 6.

The level of panelist's preference for the chicken meat aroma ranged from 1.8 (very dislike) to 3.2 (neutral). Panelists tended to like the aroma on day 0, both with distilled water and microparticle solution immersions. The storage period and fishy aroma of the meat are directly proportional. Changes in aroma can be caused by bacterial activity that breaks down proteins and lipids, causing an unpleasant smell (Floriantini et al., 2021).

Texture

The statistical analysis result showed that the soaking treatment had a significant effect (p<0.05) on the texture. The preference level for chicken meat ranged from 1.96 (very dislike) to 3.4 (like). Panelists tended to prefer the texture of the meat soaked in a wasabi microparticle solution compared to the distilled water. Soaking in microparticle solution can increase the strength of the cell wall. During storage, the texture of chicken meat changes due to protein denaturation. Several factors that can affect the texture include the age of livestock, variety, pH, type of feed, and storage time (Millan & Sirante, 2020).

CONCLUSION

In conclusion, the microparticles of wasabi stem extract inhibited the growth of *E. Coli* bacteria and reduced the number of bacterial colonies in fresh chicken meat during 6 days of storage. This proved its effectiveness as a natural food preservative with size characteristics of 142-169 µm and a solubility level of 83-85%.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Education, Culture, Research, and Technology for funding this study through the PKM-RE grant (SK No 2383/E2/DT.01.00/2023).

CONFLICT OF INTEREST

The author declared that there is no conflict of interest between the authors or with other parties.

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