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Research Article

Antimicrobial Effect of Wasabia japonica or Wasabi on Raw Salmon in Served Sashimi at Japanese Restaurants in Medan

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Abstract:
Sashimi, a delicacy made of thinly cut fresh raw ingredients, is one of Japan's most popular foods. As a raw ready-to-eat dish, sashimi has the potential to cause foodborne illnesses due to pathogenic bacteria present in it. Wasabi (*Wasabia japonica*), a type of condiment served alongside sashimi, is believed to be an antimicrobial agent against bacteria in sashimi. This study was conducted to find out whether the wasabi served at all Japanese restaurants in Medan has antimicrobial effects and to identify the bacteria found in sashimi as well. The study was conducted through an experiment with a pretest-posttest one-group design using total plate count tests to determine the effects of wasabi's antimicrobial activity. Bacterial identification in sashimi was carried out by using gram staining and biochemical reaction test consisting of IMViC test, motility test, urease test, triple sugar iron test, and sugar fermentation test. Based on data analysis using Wilcoxon signed ranks test, this study showed that wasabi had antimicrobial effects (Z = -2,803; p = 0,005). Enterobacteriaceae group and *Bacillus sp*. were commonly found in our study. Therefore, it was concluded that wasabi had antimicrobial effects by its reduction effect on the number of pathogenic bacteria.

Keywords: isothiocyanate, salmon, sashimi, wasabi

Introduction

Sashimi is a Japanese dish consisting of fillets of marine fish, molluscs, crustaceans, fish roe or other seafood to be eaten in its raw state.[1] Raw dishes such as sashimi are considered to be high risked for health.[2] There are reports of various cases or outbreaks of foodborne illness associated with sashimi and sushi consumption. In Hong Kong, between 1997-1999, there were 45 outbreaks of food poisoning due to sashimi and sushi consumption, of which 30 outbreaks (66.7%) were due to sashimi consumption.[1] In 2004 in Queensland, Australia, there were reported that outbreaks of food poisoning that attacked 12 people with causes of outbreaks associated with sushi consumption.[3] Pathogenic bacteria including *Salmonella* spp., *Staphylococcus aureus, Vibrio parahaemolyticus, Vibrio cholerae, Listeria monocytogenes, Bacillus cereus* can be found in ingredients used in producing of sushi and sashimi, such as fish, seafood products, rice, and vegetables.[2] In Indonesia, a study which examined sashimi served at various Japanese restaurants in Medan found positive results on the presence of *Staphylococcus aureus*.[4] Another study using the same samples showed positive results on *Escherichia coli*.[5]
In its consumption, sashimi is usually served alongside wasabi (Japanese spicy radish). Wasabi (*Wasabia japonica*) is a native Japanese plant of the Brassicaceae family (also called Cruciferae) which also includes cabbage, cauliflower, broccoli, radish, and horseradish.This plant adds a unique flavor, heat and greenish color to foods, making it a valuable plant in Japanese culinary. As a condiment, wasabi is served as a paste of grated stems (rhizomes). Sometimes grated wasabi is mixed with other ingredients such as soy sauce and vinegar to be served as a dip, in accordance with consumer's choice.[6] Because of low production rate due to difficult cultivation, the wasabi served as paste at Japanese restaurants often do not contain wasabi, but rather horseradish that taste similar to wasabi.[7]
Both wasabi and horseradish (*Armoracio rusticana*) – which are still in the same family as wasabi– have pungent flavors and aromas, which are caused by a volatile chemical compound called isothiocyanate (ITC). ITC is a degradation product of glucosinolate, a chemical found in all plants belonging to the Cruciferae family. Glucosinolate is stored in cell vacuoles and reacts with myrosinase (thioglucosidase) located in the cell wall or cytoplasm during tissue damage. Glucosinolate is then hydrolyzed to a number of products, with ITC as the most quantitatively predominant compound. In wasabi and spicy radishes, the main type of ITC is allyl isothiocyanate (AITC).[8] ITC shows biocidal properties against various pathogenic bacteria such as *Vibrio parahaemolyticus,*[8] *Campylobacter jejuni,*[9] *E. coli* O157: H7, *Staphylococcus aureus,*[10] *Salmonella* spp.[11] and *Helicobacter pylori.*[12]

Methods

This study is an experimental research using pretest-posttest design through laboratory tests. The research was conducted between September and November 2017 at Department of Microbiology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. The data collection in our study was performed in two stages: first, total plate count test was conducted to figure out the number of bacterial colonies in samples before and after wasabi administration; second, bacterial identification tests was carried out to determine the type of bacteria species found in sashimi. The entire data retrieval process was carried out in a biosafety cabinet. Samples were acquired from ten Japanese restaurants serving sashimi in Medan.

The total plate count test began by emulsifying the salmon using a blender and diluting it using pepton water five times (dilution 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}). This was done twice per sample: without the addition of wasabi in the pretest group and with the addition of wasabi in the posttest group. The amount of wasabi used was about the size of a grain of rice which, when measured using an electronic scale, showed numbers between 20-25 mg. It is based on the behavior of Japanese people who add the amount of wasabi to a grain of rice.[13] Afterward, it was incubated upside down for 24 hours in the incubator at 37°C. The colonies grown on agar were subsequently calculated manually and reported based on SNI 2897: 2008 on microbial contamination examination in meat, eggs, and milk and their processed products.

The bacterial identification tests conducted were gram staining and biochemical reaction tests consisting of IMViC test (indole, Methyl red, Voges-Proskauer, and citrate test), motility test, urease test, triple sugar iron test, and sugar fermentation test. Subsequently, the results of biochemical reaction tests were used to identify the species of each microbe according to Manual of Clinical Microbiology 9th edition by Murray et al.

Results and Discussion

In Table 1, the results showed that the number of colonies in the posttest group treated with wasabi was less than the untreated pretest group. This evident in all samples and our findings can also be seen in Table 2 which showed lower mean, median and standard deviation values in the posttest group. Before we analyse the data, firstly, normality test had to be carried out, the test was aimed to determine whether the data is normally distributed or not. The normality test was performed by using Shapiro-Wilk and the result of the normality test showed that p-value in pretest group was > 0.05 while in posttest group p-value was <0,05. These findings showed that the data in the posttest group was not normally distributed, therefore, the hypothesis test that we utilized was a non-parametric test, Wilcoxon signed ranks test which is an alternative for the parametric dependent T-test. After data analysis, we obtained the results that the value of Z = -2.803 and p-value = 0.005. The p-value of < 0.05 on this test indicated that the difference between the number of bacteria in the pretest and posttest group was significant.

Table 1. Total Plate Count (TPC) test

Restaurant	Numbers of Bacteria (CFU/ml)	
	Pretest	*Posttest*
A	78×10^4	96×10^3
B	10×10^5	52×10^4
C	55×10^4	74×10^3
D	41×10^3	12×10^3
E	11×10^3	54×10^3
F	46×10^4	85×10^3
G	41×10^4	11×10^4
H	48×10^4	90×10^3
I	93×10^3	18×10^3
J	45×10^4	15×10^4

Table 2. Total Plate Count (TPC) Statistic

Statistics	Pretest	Posttest
n	10	10
Mean	437.750,00	120.000,00
Median	455.000,00	87.500,00
Standard deviation	303.765,557	144.615,774

The results of the total plate count test were consistent with previous studies on antimicrobial effects of isothiocyanate (ITC) contained in wasabi and horseradish against various types of bacteria. The antimicrobial effects of wasabi which was obtained from all of the Japanese restaurants in Medan can be explained by the content of the ITC in it, in addition it also gave strong flavor and aroma when consumed. The wasabi served was assumed to contain little or no wasabi, but contained grated horseradish root which had an ITC content and a taste similar to wasabi. This assumption is based on the high price and the scarcity of wasabi in circulation,[7] whereas the author observed the wasabi pastes served at Japanese restaurants in Medan were available in large quantities and free of charge when servings of sashimi were purchased.

Meanwhile, We definitely identified ten species of bacteria in sashimi with *Enterobacter* sp. as the most common species followed by *Klebsiella sp*, *Bacillus subtilis*, and *Yersinia sp*. Most of bacteria found belong to the Enterobacteriaceae family, except *Bacillus subtilis* which was a member of the Bacillaceae family. Bacteria belonging to the Enterobacteriaceae family were rod-shaped gram-negative bacteria, facultative anaerobes and sugar fermenting. Many of these bacteria were part of the normal human and animal flora; others were found in soil and water. All of the Enterobacteriaceae bacteria found in this study were opportunistic pathogens that can cause various infections in humans, ranging from diarrhea, urinary tract infections to eye infections, except for *Yersinia kristensenii*, which is just potentially infectious in mice.[14] Enterobacteriaceae can be used as an overall hygienic indicator of food, but not as a fecal indicator.[15]

Bacillus subtilis found with Enterobacteriaceae was a gram-positive rod-shaped bacteria capable of producing resistant endospores in terms of exposure to air. Most species of the *Bacillus* genus were common contaminants in the environment, present in water and all soil types, and have little or no pathogenic traits and were rarely associated with diseases, except for *B. anthracis* and *B. cereus*.[14] However, the discovery of bacteria in sashimi does not directly make it unsafe for consumption. According to the Health Protection Agency,[15] there are certain numbers of bacteria based on their groups which determine food safety. With regard to this, total plate count result of < 10^6 CFU/gr in dishes serving raw ingredients indicates food safety.

Table 3. Bacteria Identification

Bacterium	Frequency	Percentage (%)
Bacillus subtilis	4	14.3
Citrobacter sp.	5	17.7
Edwardsiella sp.	1	3.6
Enterobacter sp.	6	21.4
Hafnia sp.	1	3.6
Klebsiella sp.	4	14.3
Kluyvera sp.	1	3.6
Providencia sp.	1	3.6
*Serratia*sp.	1	3.6
Yersinia sp.	4	14.3
Total	**28**	**100**

The discovery of various types of bacteria in sashimi in this study is consistent with previous studies conducted. The bacteria found in sashimi reflect the microbiological status of the instrument used in its production process.[2] The discovery of Enterobacteriaceae in this study is consistent with the discovery of bacteria belonging to the same group by Muscolino in Italy. The discovery of *Bacillus subtilis* in this study was suspected to be a result of cross-contamination during the research.

Conclusion and Suggestion

Wasabi's antimicrobial effect was evident in our study. Therefore, Wasabi may be one of the food ingredients that can directly reduce the number of pathogenic bacteria. Meanwhile, its administration alongside Sashimi, a raw food, has been a distinct advantage among human populations since its effect on bacterial proliferation. In addition, our study also revealed the high prevalent Enterobacteriaceae group among Sashimi served in several Japanese restaurants in Medan still in a safe level.

In addition, our findings are expected to encourage consumers to consume Wasabi alongside Sashimi which also may reduce food-borne transmission by its reduction effect against pathogenic bacteria. Therefore, it is also suggested that proper managements of the restaurants must continue its regulations concerning the storage and presentation of foods in Indonesia.

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