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Improving the safety and quality of fermented shrimp paste (*terasi*) through supplementation of betel leaf (*Piper betle* L.) essential oil

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Abstract

Terasi is a traditional fermented shrimp paste that has been an integral part of Indonesian cuisine and food culture. Despite its broad use and complex distinctive flavor, shrimp paste could be harmful for human consumption, particularly when its quality has deteriorated. Indeed, many chemical reactions and microbial activities taking place during shrimp paste fermentation could lead to a decrease in quality and the formation of toxic compounds that are potentially injurious to human health. Thus, this study aimed to explore to improve the safety and quality of shrimp paste by incorporating betel leaf (*Piper betle* L.) essential oil, known to exert antioxidant and antimicrobial properties. The supplementation of betel leaf essential oil (1% and 5%) reduced lipid peroxidation as well as the formation of toxic compounds, including histamine and acrylamide in the shrimp paste fermented for 30 and 60 days. This phenomenon was associated with the inhibition of microbial growth in shrimp paste supplemented with betel leaf essential oil. The sensory analysis revealed that the supplementation of 1% betel leaf essential oil did not affect the consumer's preference for shrimp paste. Therefore, the present study highlights the potential of betel leaf essential oil as a food additive to improve the safety and quality of fermented shrimp paste.

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1. Introduction

Terasi is an Indonesian traditional fermented seafood paste made mainly from crushed and salted planktonic shrimp (*Acetes indicus*) locally known as *udang rebon* (1). Related products are also found in other Southeast Asian countries, such as *belacan* in Malaysia, *kapi* in Thailand and Cambodia, *bagoong alamang* in the Philippines, *ngapi* in Myanmar, and *mam tom* in Vietnam (2). In Indonesia, *terasi* is mainly produced in a traditional manner by local producers. Its production steps consist of salting shrimp, grinding to form a homogenous mixture, drying, and fermentation for several months (2). Besides shrimp, some traditional producers often incorporate fish and other seafood (squid, mussels, etc.) in the production of *terasi* (3). In traditional culinary practice, *terasi* is mainly used as flavor enhancer in different cuisines, such as soup, vegetable salad, fruit salad, and chili paste (4).

Shrimp paste possesses a complex flavor due to microbial activities and a series of chemical modifications taking place during the fermentation process (5). Some major bacterial genus present in *terasi* are *Tetragenococcus*, *Aloicoccus*, *Atopostipes*, *Alkalibacillus*, and *Alkalibacterium* (6). Despite developing the flavor of shrimp paste, these chemical reactions often lead to reduction in quality and safety of shrimp paste. Microorganisms in

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shrimp paste are responsible for lipid oxidation that can cause rancidity, an essential factor indicating the quality reduction of shrimp paste (7). Histamine, an allergenic compound that causes scromboid food poisoning in humans, results from protein degradation by microorganisms in shrimp paste (8). The non-enzymatic browning process in shrimp paste also leads to the formation of a myriad of compounds, one of which is acrylamide that is known to be probably carcinogenic to humans (9). Therefore, it appears primordial to find strategies to improve the safety and quality of shrimp paste by inhibiting lipid peroxidation as well as the formation of histamine and acrylamide in shrimp paste.

Betel (*Piper betle* L.) is a species of herbal plant in the pepper family Piperaceae, native to Southeast Asia (10). Betel leaf has a strong cultural significance in South and Southeast Asian countries since it is commonly used as a flavoring agent in the traditional betel nut (*Areca catechu*) chewing practice (10). Betel leaf essential oil contains a plethora of chemical compounds, with the major ones being eugenol, isoeugenol, germacene D, chavicol, hydroxychavicol, and caryophyllene (11). Betel leaf essential oil has been suggested to be incorporated in foods as a preservative and organoleptic enhancer (12). In addition, it also exerts antioxidant and antimicrobial properties, as well as providing multiple health benefits (13). In general, betel leaf essential oil is considered to be practically non-toxic and not an irritant (toxicity class IV) (14,15). The median lethal dose (LD50) of betel leaf methanol extract is higher than 5,000 mg/kg body weight when administered orally in both male and female mice (16).

The present study aimed to explore the possibility of improving the safety and quality of shrimp paste by incorporating betel leaf essential oil. Plausibly, the supplementation of betel leaf could lead to reduction of lipid peroxidation as well as the formation of histamine and acrylamide in shrimp paste. Betel leaf essential oil was added into dried shrimp paste under two different concentrations (1% and 5%) prior to fermentation for 60 days. The formation of lipid peroxidation byproducts, histamine, and acrylamide was recorded at the beginning of fermentation (day 0), day 30, and day 60. In addition, the amount of microorganisms and lactic acid bacteria was also analyzed to assess the influence of betel leaf essential oil on microbial growth in shrimp paste. Finally, a sensory analysis was conducted to analyze whether the supplementation of betel leaf essential oil in shrimp paste would affect consumer's acceptance.

2. Materials and Methods

2.1. Preparation of Shrimp Paste

The shrimp paste in the present study was traditionally prepared as described by a local producer as previously published (17). Briefly, raw planktonic shrimp acquired from a local fish market was blanched in boiling water for 5 min, drained, and mixed with solar salt (15% 15 g salt for 100 g shrimp). The salted shrimp was left in an enclosed jar for 48 h at room temperature (25^{o}C) prior to grinding with a blender. The paste was then formed into flattened balls (diameter 8-10 cm) manually and dried using an oven (50^{o}C, 4 h) to reduce the moisture content. After cooling, food-grade pure betel leaf essential oil (Brand Serambi Botani, IPB University, Bogor, West Java, Indonesia) was added to the dried shrimp paste under two concentrations: 1% (v/w) and 5% (v/w) by dropping using a pipette. The shrimp paste was then covered in banana leaves and left fermented at room temperature (25^{o}C) for 60 days. Prior to utilization, the banana leaves were cleaned using flowing tap water to remove dirt and wiped using a clean cloth.

2.2. Chemical Analysis

The chemical analysis was done as previously described (17). The thiobarbituric acid reactive substances (TBARS) assay expressing the lipid peroxidation byproducts was examined using a commercial TBARS assay kit (QuantiChrom, BioAssay Systems, CA, USA) according to the manufacturer's instructions followed by UV-Vis spectrophotometry (Spectroquant Prove 100, EMD Millipore, MA, USA) at 532 nm. The results were expressed as mg malondialdehyde (MDA)/kg dry sample. The histamine analysis was performed using a commercial histamine assay kit (Megazyme, Dublin, Ireland) according to the manufacturer's instructions followed by UV-Vis spectrophotometry (Spectroquant Prove 100, EMD Millipore, MA, USA) at 492 nm. The results were expressed as mg histamine/kg dry sample. The acrylamide analysis in the *terasi* samples was analyzed using a commercial Acrylamide-ES ELISA kit (Life Technologies, Delhi, India) according to the manufacturer's instructions. Following incubation with acrylamide-linked antibody solution and substrate (color) solution, the absorbance was read using a microplate ELISA photometer (Infinite 200 PRO, Tecan, Männedord, Switzerland) at 450 nm. The results were expressed as mg acrylamide/kg dry sample.

2.3. Microbial Load Analysis

The shrimp paste samples were diluted in peptone water containing 0.85% NaCl in serial tenfold steps prior to application onto agar by the spread plate technique and followed by incubation. The total viable count was analyzed using a standard plate count agar (pH 7.5) according to Bacteriological Analytical Manual (BAM) as previously described (18). The lactic acid bacteria count was performed using De Man-Rogosa-Sharpe (MRS) agar (19). All the samples were then incubated for 5 days at 35°C.

2.4. Sensory Analysis

The hedonic rating analysis was performed using 187 untrained Indonesian panelists who were all household women aged 23-47 years old claiming to like shrimp paste and consume it on regular basis. The analysis was done at three traditional markets in Jakarta, Indonesia on April 29-30, 2023. The protocol for the sensory analysis has been reviewed and approved by the Ethics Committee at Bina Nusantara University (Jakarta, Indonesia). Briefly, each panelist was asked to rate four attributes (color, texture, aroma, and overall characteristics) of shrimp paste samples based on their personal preference using the Likert scale of 1-9 (1 for strongly dislike and 9 for strongly like). Each session was held through a series of one-on-one interview between a panelist and an interviewer.

2.5. Statistical analysis

All data ($n \geq 3$) were reported as mean±SD and analyzed using one- or two-way Anova followed by Tukey's HSD post hoc test in case of significant difference ($p < 0.05$). The software Systat 10 for Windows was used to perform statistical data analysis.

3. Results and Discussion

3.1. Effects of Betel Leaf Essential Oil Supplementation on Lipid Peroxidation and the Formation of Toxic Compounds in Shrimp Paste

Figure 1A-C demonstrates the formation of lipid peroxidation byproducts, histamine, and acrylamide during the fermentation of shrimp paste. In general, the formation of such compounds was shown to decrease with the supplementation of betel leaf essential oil in the shrimp paste. Higher essential oil concentration in shrimp paste (5%) led to a stronger reduction of lipid peroxidation byproducts, histamine, and acrylamide compared to lower concentration (1%). As demonstrated by two-way Anova statistical analysis, fermentation time, essential oil concentration, as well as their interaction significantly influenced the formation of lipid peroxidation byproducts, histamine, and acrylamide in shrimp paste ($p<0.05$).

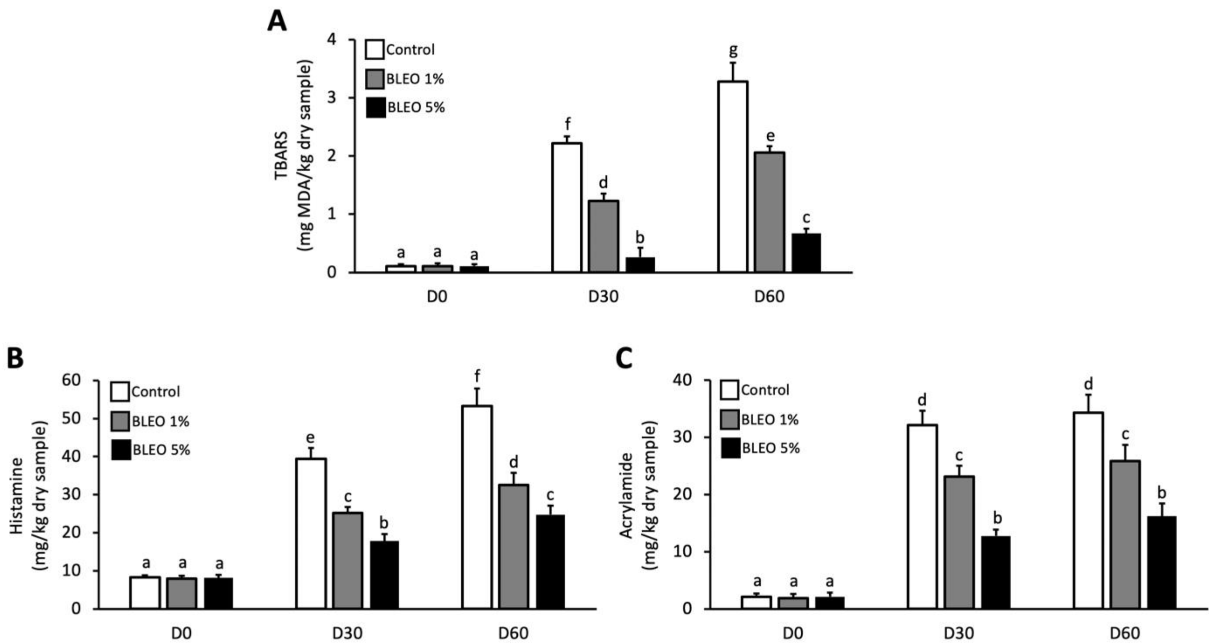

Figure 1. Chemical characteristics of shrimp paste supplemented or not with betel leaf essential oil (BLEO) at the beginning of fermentation (D0), day 30 (D30), and day 60 (D60): A) concentration of thiobarbituric acid reactive substances (TBARS), B) concentration of histamine, and C) concentration of acrylamide. Data (n=3) were expressed as mean±SD. Different letters indicate significant difference ($p<0.05$) following two-way Anova and Tukey's HSD post hoc test. MDA: malondialdehyde.

The thiobarbituric acid reactive substances (TBARS) reflect the byproducts resulting from lipid peroxidation in food matrix (20). Lipid peroxidation is a series of chain reactions of lipid degradation, particularly unsaturated fatty acids involving oxygen and the formation of free radicals (20). The major end products of lipid peroxidation are reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) (20). In foods rich in lipid, lipid peroxidation is the main phenomenon associated with rancidity and quality reduction Oxidative processes in meat and meat products: quality (21). In shrimp paste, the accumulation of lipid peroxidation byproducts results in the formation of off-flavor that could render the product inedible. In Figure 1A, the supplementation of betel leaf essential oil (1%

and 5%) significantly reduced the TBARS level in shrimp paste on day 30 and day 60. The reduction appeared to be stronger when higher concentration of essential oil was applied. This phenomenon could be related to the antioxidative properties of betel leaf essential oil (22) that would potentially inhibit oxygen-mediated lipid peroxidation in shrimp paste. Therefore, the use of betel leaf essential oil in shrimp paste can be considered to maintain the quality of shrimp paste, particularly with regard to its flavor, by inhibiting lipid peroxidation.

Microbial activities occurring during the fermentation of shrimp paste could lead to the formation of toxic components. Biogenic amines are formed through microbial degradation of amino acids (23). Histamine is a common biogenic amine derived from histidine in seafood products and exposure to an excessive amount of oral histamine may lead to scombroid food poisoning resembling an allergic reactions in humans (20). The U.S. Food and Drugs Administration (FDA) considers fish and fishery products containing 35 ppm or more histamine to be adulterated and those containing 200 ppm or more histamine to be injurious to humans (24). According to Figure 1B, the supplementation of betel leaf essential oil significantly reduced the formation of histamine in shrimp paste. On day 60, the concentration of histamine in the final product could be suppressed from 53.23 ppm to 32.55 ppm and 24.75 ppm when betel oil essential oil (1% and 5% respectively) was incorporated in the paste. Compounds with antioxidant activities have been reported to inhibit the activity of histidine decarboxylase, the enzyme involved in the microbial production of histamine (25).

Acrylamide is a toxic compound classified as probably carcinogenic (Group 2A) according to the World Health Organization (WHO) (26). It results from heat-catalyzed non-enzymatic browning (Maillard) reaction between reducing sugars and asparagine (27). The optimal temperature to achieve the Maillard reaction is 140-165°C (28). The Maillard reaction in shrimp paste happens particularly during the drying process since heat is applied to facilitate water evaporation (17). Such a reaction also contributes to the flavor development of shrimp paste (29). The tolerable daily intake of acrylamide is set at 2.6 μg/kg body weight (30). Figure 1C shows a reduction of acrylamide in the shrimp paste supplemented with betel leaf essential oil compared to the control shrimp paste. On day 60, the concentration of acrylamide in shrimp paste decreased from 34.43 ppm to 25.86 ppm and 16.25 ppm with the presence of betel oil essential oil (1% and 5% respectively). Previously, natural antioxidants extracted from green tea and bamboo leaves were reported to inhibit the formation of acrylamide in an equimolar asparagine-glucose mode system at 180°C and pH 6.80 (31). The presence of antioxidants could disrupt the Maillard reaction that is based on redox reactions.

3.2. Effects of Betel Leaf Essential Oil Supplementation on Microbial Development in Shrimp Paste

A plethora of microorganisms and their metabolites contribute to the complex flavor of shrimp paste. Figure 2A-B represents the microbiological profile of shrimp paste during fermentation. The supplementation of betel leaf essential oil was shown to inhibit the growth of microorganisms, including lactic acid bacteria. As demonstrated by two-way Anova statistical analysis, fermentation time, essential oil concentration, as well as their interaction significantly influenced the total microorganisms, including the lactic acid bacteria in shrimp paste ($p<0.05$).

On day 60, the supplementation of 1% and 5% betel leaf essential oil could reduce approximately by 1.5 and 2.9 log cycles of total microorganisms (Figure 2A). In accordance with this finding, the amount of lactic acid bacteria in shrimp paste was also reduced approximately by 0.7 and 1.2 log cycles upon the supplementation of 1% and 5% betel leaf essential oil respectively on day 60 (Figure 2B). Such a phenomenon could be due to the presence of antimicrobial compounds in betel leaf essential oil. Previously, betel leaf essential oil was reported to exert antimicrobial properties at a very low concentration (200 ppm) against bacteria and fungi, such as *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Vibrio*, *Candida*, *Aspergillus*, and *Penicillium* (32). The inhibition of microbial growth related to the use of betel leaf essential oil could be associated with the lower formation of lipid peroxidation byproducts, histamine, and acrylamide in the shrimp paste as previously shown in Figure 1A-C.

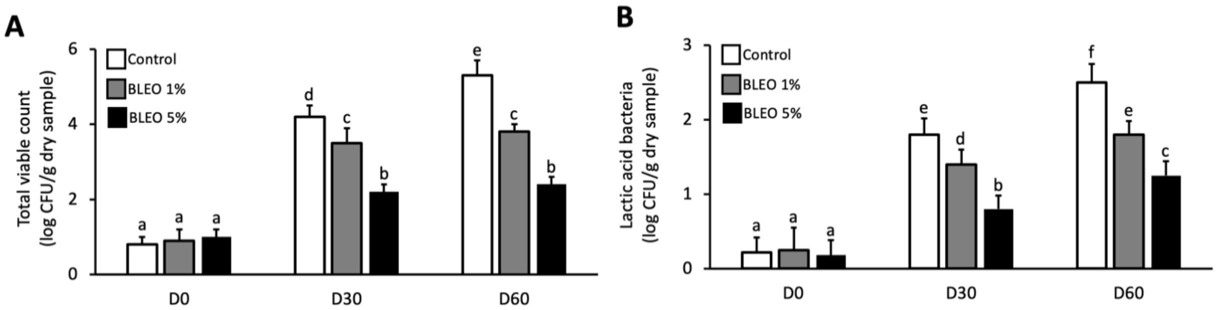

Figure 2. Microbial load in shrimp paste supplemented or not with betel leaf essential oil (BLEO) at the beginning of fermentation (D0), day 30 (D30), and day 60 (D60): A) total viable count and B) lactic acid bacteria count. Data (n=3) were expressed as mean±SD. Different letters indicate significant difference ($p<0.05$) following two-way Anova and Tukey's HSD post hoc test. CFU: colony forming unit.

3.3. Effects of Betel Leaf Essential Oil Supplementation on the Sensory Acceptance of Shrimp Paste

In the present study, the sensory testing was performed on shrimp paste samples that had been fermented for 60 days using 187 household women in traditional markets as panellists. The results are presented in Figure 3. The supplementation of 1% and 5% betel leaf essential oil did not alter the acceptance towards the color and texture of shrimp paste. In terms of aroma, the acceptance significantly dropped in the shrimp paste supplemented with 5% betel leaf essential oil but not in the shrimp paste with 1% betel leaf essential oil. Based on the interview with the panellists, it was revealed that the supplementation of 5% betel leaf essential oil resulted in a strong recognizable aroma of betel leaf in shrimp paste. Most panellists described the aroma of betel leaf essential oil as unpleasant, medicine-like, and not fit with shrimp paste. The decrease in sensory acceptance towards aroma in the shrimp paste supplemented with 5% betel leaf essential oil dragged down its acceptance towards overall characteristics. However, the sensory acceptance towards aroma and overall characteristics of the shrimp paste with 1% betel leaf essential oil did not differ from the control (unsupplemented) shrimp paste.

4. Conclusions

To conclude, this present study revealed the potential of betel leaf essential oil as a food additive in fermented shrimp paste to improve its safety and quality. Considering all the findings, it is therefore suggested to apply 1% betel leaf essential oil during the preparation of salted shrimp to produce shrimp paste with lower concentration of lipid peroxidation byproducts and toxic compounds such as histamine and acrylamide. The mechanism by which betel leaf essential oil reduces the formation of toxic compounds includes, but not limited to the inhibition of microbial growth due to the antimicrobial properties of betel leaf essential oil. Indeed, the supplementation of 1% betel leaf essential oil resulted in a significant decrease of lipid peroxidation by products (37% reduction), histamine (39% reduction), and acrylamide (25% reduction). The supplementation of 1% betel leaf essential oil was chosen over the one with 5% betel leaf essential oil since the latter resulted in a lower consumer's acceptance. Previously, the supplementation of 5% and 10% torch ginger (*Etlingera elatior*) flowers was reported to improve the safety and quality of *terasi* in a similar direction (33).

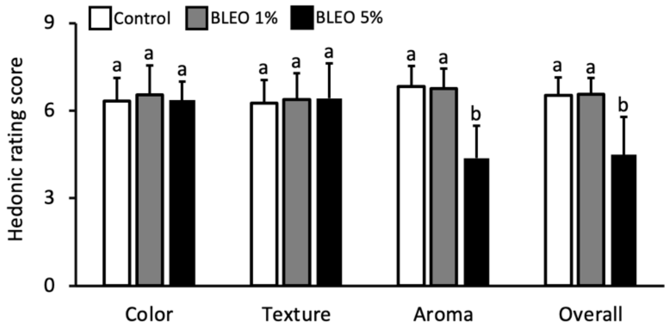

Figure 3. Consumer's acceptance of shrimp pastes supplemented or not with betel leaf essential oil (BLEO) following a 60-day fermentation. Data (n=187) were expressed as mean±SD. Different letters indicate significant difference ($p<0.05$) following one-way Anova and Tukey's HSD post hoc test.

We thus recommend incorporating lipid peroxidation byproducts, histamine, and acrylamide in the Indonesian national standards for fermented shrimp paste or *terasi* (SNI 2716:2016) (34) with regard to ensuring the quality and safety of commercial *terasi.* Currently, the national standards for shrimp paste encompass organoleptic characteristics (normal color, texture, and taste), water content (maximum 40%), mineral content without salt (maximum 20%), crude fiber content (maximum 8.5%), heavy metals (undetected presence of Cu, Hg, Pb, and As), coloring agent (permitted use by the National Health Department), coliform bacteria (undetected), molds (undetected), and foreign substances (undetected) (34). In addition, it is worth highlighting that *terasi* is generally fermented for 30-90 days according to the traditional process. However, a recent study recommended limiting the fermentation of shrimp paste to 21 days maximum in order to produce *terasi* with reduced health risks (6). Longer fermentation of *terasi* (28 days) was associated with the accumulation of toxic compounds and putrefactive products, as well as the alteration of bacterial community (6). Therefore, a shorter shrimp paste fermentation time should be taken into account in the future studies.

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Author Contributions

R.S. conceived and designed the experiments; R.S. and R.S.S. performed the experiments; R.S. and R.S.S. analyzed the data; R.S. wrote the paper."

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Institutional Review Board Statement

The use of humans in the sensory analysis has been reviewed and approved by the Ethics Committee of Bina Nusantara University, Jakarta, Indonesia.

Data Availability Statement

The data are available upon request.

Conflicts of Interest

The authors declared no conflict of interest.

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