

## *Staphylococcus aureus* contamination in traditional Balinese Indonesian stem food: *Tum bungkil*, *Tum pusuh* and *Tum biu batu*

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**ABSTRACT.** *Tum* is a traditional Balinese Indonesian dish made from various parts of the stone banana tree, including the stone banana root bulb (*bungkil*), stone banana flower (*pusuh*), and stone banana fruit (*biu batu*). However, when *tum* is made by hand without gloves, there is a risk of contamination with *Staphylococcus aureus*. Although the steaming process may reduce some bacteria, contamination can still occur. *S. aureus* can survive in ready-to-eat foods prepared under poor hygiene conditions. The toxins produced by *S. aureus* can lead to skin infections, respiratory problems, and sepsis. This study aims to investigate the presence of *S. aureus* in processed *tum*. Purposive sampling was conducted in two districts that sell *tum bungkil*, *pusuh*, and *biu batu*. Six samples were tested using mannitol salt agar (MSA) which is specific media from a dilution level of  $10^{-3}$ . Identification is conducted based on morphology, gram staining and total plate count (TPC) results. The results indicated that the highest level of contamination was found in sample 3 (S3) were  $1.7 \times 10^2$  CFU/g, which contained *tum pusuh*. This study implies that it is essential to improve hygiene practices during the preparation of *tum pusuh* to minimize the risk of contamination.

**Keywords:** food handlers; food poisoning; food safety; pathogen bacteria; stem foods

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## INTRODUCTION

*Tum* is a traditional Balinese Indonesian food preparation made from meat, fish, vegetables, fruit or a mixture of both (Dewantari *et al.*, 2022). Particularly in Bali, *tum* is a traditional dish categorized as a wet side dish, cooked using a spice called *base genep gede* and wrapped in banana leaves (Rahmadaeni *et al.*, 2019). The steaming process of *tum*, which is a menu at the *pewiwahan* or marriage ceremony in Bali, has a meaning for the bride and groom, which is that during married life in facing problems, they must support each other, not blame each other and introspect themselves (Widani *et al.*, 2021). One of the typical Balinese *tum* is processed *tum* that uses parts of the stone banana tree such as the root bulb or tuber, it is called *tum bungkil*, from banana flower commonly called *tum pusuh* and stone banana fruit called *tum biu batu* (Suja *et al.*, 2021). The existence of this processed *tum* is very popular among the Balinese people including in Klungkung and Gianyar districts (Sunada, 2019). The processed *tum bungkil*, *pusuh* and *biu batu* are not only delicious but also good for digestion due to the fiber content of the root bulb, banana flower and stone banana (Komalasari *et al.*, 2016). Healthy traditional food must be supported by the quality of hygiene and free from pathogenic bacteria contamination (Agustini *et al.*, 2020).

Pathogenic bacterial contamination in processed food can be affected by sanitary hygiene conditions during processing (Trisdayanti *et al.*, 2015). Some of them are personal hygiene of the handler when processing food, cleanliness of the equipment and materials used, water as a cleaning medium or mixture in processing (Hamaideh *et al.*, 2024). Furthermore, the condition and cleanliness of the environment where food is stored and sold can be a source of growth and contamination of pathogenic microorganisms (Bintsis, 2017). One bacterium that is known to cause poisoning and cause disease due to consuming contaminated food is *Staphylococcus aureus* (Kadariya *et al.*, 2014). The ability of *S. aureus* to adhere to food surfaces and form biofilms is a significant concern in the food industry, as it heightens the risk of microbial cross-contamination among food products. The

potential presence of other pathogenic microorganisms that may coexist with *S. aureus* in these biofilms further increases the likelihood of disease transmission. Biofilms are primarily composed of an extracellular matrix made of exopolysaccharides (EPS), which enable *S. aureus* to defend itself against environmental threats by restricting mobility and limiting the deeper penetration of biocides into the biofilm matrix (Léguillier *et al.*, 2024).

The purpose of this study was to determine the presence of *S. aureus* in traditional *tum* dishes made from stone banana trees. Through this research, it is hoped that it can add to the reference knowledge about the factors that affect the microbiological quality of processed *tum bungkil*/banana root bulb, *tum pusuh*/ banana flower and *tum batu biu*/stone banana fruit, especially the contamination of *S. aureus*.

## MATERIALS AND METHODS

**Samples.** The research was conducted from August to October 2024. We collected the samples from 6 sellers of *tum bungkil*, *pusuh* and *biu batu* in 2 districts namely Klungkung and Gianyar using simple random sampling method (Erlita & Maria, 2019). The population of *tum* sellers made from banana trees in Klungkung and Gianyar consisted of six individuals. *Tum* samples were collected once, with the requirement freshly cooked in the morning. Four *tum* samples were taken from each seller, resulting in a total of 24 samples. The number of four samples was determined using the Solvin formula. These four *tum* samples from each seller were analyzed for the presence of *S. aureus*, representing the tums available from each seller. The samples were processed at the Medical Research laboratory of Universitas Warmadewa in Bali.

**Procedures.** All of *tum* samples consisting of 2 *tum bungkil*/root bulb samples, 2 *tum pusuh*/flower and 2 *tum biu batu*/stone banana fruit were diluted  $10^{-1}$  to  $10^{-5}$  in 0.9% NaCl. Each sample was taken 1 g and then put into 0.9% NaCl in a graduated test tube. At dilution  $10^{-3}$  as much as 100  $\mu$ l was poured on the surface of Mannitol Salt Agar (MSA) (Nurliana *et al.*, 2022), specific media for the growth of *S. aureus*, by the spread method. The media containing the sample was incubated for 24 hours at 37°C, then morphological observations, gram stain test and total plate count were made on the growing bacteria. The total plate number is calculated using the following formula (Kuncara *et al.*, 2021; Ramadani *et al.*, 2023):

$$\text{Bacterial number TPC (CFU/ml)} \approx \frac{\text{colony number} \times \text{dilution factor}}{\text{volume}}$$

**Data analysis.** The data were analyzed using descriptive analysis based on the data obtained in table (histogram) and potrait (Mailoa *et al.*, 2017; Mutiarasari *et al.*, 2020). Morphological analysis of *S. aureus* was determined based on macroscopic and microscopic observation of the colony, further on the ability to ferment mannitol (Jiwantoro & Putri, 2023; Znad & Zghair, 2023; Wan *et al.*, 2025.)

## RESULTS AND DISCUSSION

The six samples that have been planted on MSA media showed growth of *S. aureus* bacterial colonies after 24 hours of incubation at 37°C. The characteristics of *S. aureus* colonies on MSA media are distinguished by their golden yellow color (Table 1.), MSA media contains carbohydrates and high levels of salt. *S. aureus* has the ability to ferment mannitol, which is a carbohydrate, producing organic products such as lactic acid, formic acid, and acetic acid. This fermentation process allows *S. aureus* to thrive in high-salinity environments, resulting in a color change of the media from red to yellow (Yambise *et al.*, 2020). The analysis of colonies on MSA from sample S1 probability indicates *S. epidermidis*. Due to inability to ferment mannitol is evident from the media remaining red, confirming the presence of this bacterial species (Estrella *et al.*, 2015). Gram staining of both sample colonies shows a gram-positive result, with blue coloring and a cocci-shaped morphology (Afrin *et al.*, 2019). The growth was observed in samples S1 and S3, which were derived from stone banana

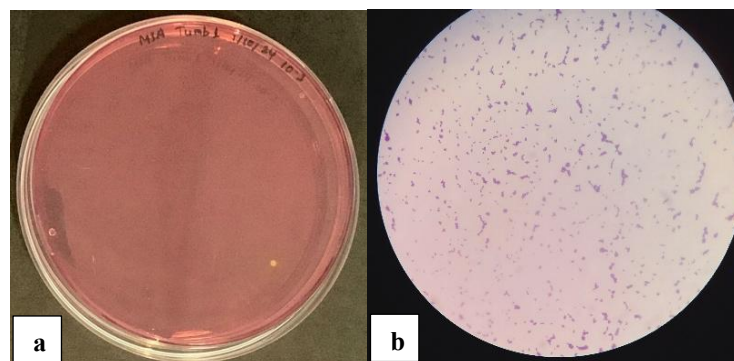
root bulb *tum* and stone banana flower *tum*, respectively. Notably, the highest level of *S. aureus* growth was found in the stone banana flower *tum*.



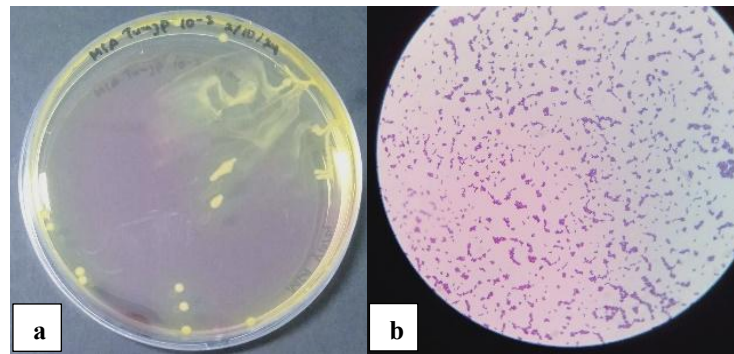
**Fig. 1.** *Tum pusuh* or flower stone banana *tum*

As previously mentioned, the preparation of *tum* involves mixing spices and packaging the mixture in banana leaves using hands, which raises the possibility of contamination with *S. aureus* from the handler's hands. This contamination can be prevented if the handler uses personal protective equipment, such as gloves. The presence of *S. aureus* in the processed *tum* may occur if the handler did not wash their hands thoroughly, did not wear gloves, or if they were suffering from skin conditions caused by *S. aureus* (Ho *et al.*, 2015). The *S. aureus* is a bacterium commonly found on the surface of human skin as a commensal organism. However, if it enters the bloodstream through an injury, it can lead to disease. The hygiene level of those handling processed food, like Tum, is crucial in preventing contamination by *S. aureus*. While these bacteria typically do not cause disease when present on the skin, if ingested or allowed to enter the body through pores or blood vessels, it can cause symptoms such as nausea, vomiting, abdominal pain, and diarrhea (Tong *et al.*, 2015).

*Staphylococcus aureus* can spread to other organs and cause skin infections, including boils and impetigo. Therefore, maintaining cleanliness is essential for anyone handling food. It is recommended that handlers thoroughly wash their bodies and hands with soap, wear clean clothes, and use personal protective equipment, especially on the hands, during food processing (Hasbi *et al.*, 2024). Kadariya *et al.* (2014) explained that *S. aureus* can proliferate during food preparation and processing. Studies by Habib *et al.* (2024) found that raw vegetable salads sold in the United Arab Emirates tested positive for *S. aureus* due to hand contamination. This is concerning because *S. aureus* can grow across a broad range of temperatures and acidity levels, specifically between 7°C and 48°C (with an optimum temperature of 30°- 37°C) and a pH range of 4.2 - 9.3 (with an optimum pH of 7 - 7.5).



**Fig. 2.** Morphology (a) and Gram stain (b) of *Staphylococcus aureus* contamination in *tum bungkil* sample bp1 (S1). Dilution:  $10^{-3}$ ; number of colonies:  $1 \times 10^2$  CFU/g



**Fig. 3.** Morphology (a) and Gram stain (b) of *Staphylococcus aureus* contamination in *tum pusuh* sample jp3 (S3). Dilution:  $10^{-3}$ ; number of colonies:  $1.7 \times 10^2$  CFU/g

To prevent growth during processing, steaming the food at temperatures above  $50^{\circ}\text{C}$  for at least 60 minutes is recommended. For large-scale or industrial steaming, two methods can be employed: saturated steam (SS) at  $100^{\circ}\text{C}$  and superheated steam (SHS) at  $150^{\circ}\text{C}$ , both with a maximum exposure time of 20 seconds. The equipment used for steaming should be made of stainless steel (STS), high-density polyethylene (HDPE), or polypropylene (PP) to ensure stability during heating. Utilizing the steaming methods described can effectively reduce the growth of *S. aureus* (Kim *et al.*, 2019; Lehotová *et al.*, 2021). Processed *tum* is typically steamed in a stainless steel pot, and using banana leaves as a wrapper has antimicrobial benefits. The flavonoids, phenolics, and tannins found in banana leaves, along with the tight pore structure on their surface, help protect the processed *tum* from bacterial contamination by preventing exposure to airborne bacteria during sale at room temperature ( $27^{\circ}\text{C}$ ). Additionally, wrapping in banana leaves helps preserve the aroma of the *tum*, making it fragrant and fresh (Sari *et al.*, 2019; Nafiusokhib *et al.*, 2022; Rinaldi *et al.*, 2023). The only source of *S. aureus* contamination in *tum* samples S1 and S3 was attributed to the sanitary hygiene practices of the handlers.

*Staphylococcus aureus* can survive in both acidic and alkaline environments. The results of research by (Lee *et al.*, 2014) and (Liao *et al.*, 2023) on the ability of *S. aureus* to survive in acidic environments ranging from pH 3-4.5, due to the expression of *clpB*, *zwf*, *nuoF*, and *gnd* genes that regulate modification and repair of protein synthesis and cause an increase in the internal pH of bacterial cells when in an acidic environment. Different parts of the banana, including the stem, root bulb, flower, and both unripe and ripe fruit, have acidity (pH) values of 6, 3, 5, and 4-6, respectively. The acidity of unripe bananas is approximately 5, as the oxalic acid has not yet converted into malic acid. Including pH of *bungkil* and *pusuh* stone banana *tum*. These pH levels still allow for the growth and survival of *S. aureus*. Food preparations made from vegetables or plants typically have an alkaline pH (Swe, 2012; Kookal & Thimmaiah, 2018; Mubarak *et al.*, 2021; Zaini & Kormin, 2022; Rahmawati *et al.*, 2023; Triardianto & Bintoro, 2024). Steaming vegetables for more than 15 minutes at a minimum temperature of  $50^{\circ}\text{C}$  only slightly reduces their acidity (pH). For example, *tabah* bamboo leaves steamed at temperatures ranging from  $50^{\circ}\text{C}$  -  $70^{\circ}\text{C}$  for 10 - 20 minutes have a pH ranging from 6.32 - 6.59 (Purnama *et al.*, 2020). Chicken nuggets with a pH of 6.0, when mixed with broccoli and steamed for 45 minutes at  $100^{\circ}\text{C}$ , had their pH increase between 6.02 and 6.33, as the pH of broccoli ranges from 6.30 - 6.85 (Kariang *et al.*, 2023). Similarly, with *tum bungkil* and *pusuh*, *S. aureus* may survive if it is not steamed for a sufficient amount of time. Steaming at temperatures above  $50^{\circ}\text{C}$  for at least 60 minutes is necessary to effectively kill *S. aureus*, even though the end product may not be very acidic. *S. aureus* is a bacterium that can adapt well to high salinity environments (greater than 15% NaCl), which means that processed *tum* that contains salt can create a favorable environment for the growth of *S. aureus* (Kim *et al.*, 2019; Purnama *et al.*, 2020).

The transmission of *S. aureus* to humans through food is particularly higher in hand-prepared, ready-to-eat foods such as *bakpia*, *gado-gado*, *sushi*, *salad*, and *lawar* (Lestari *et al.*, 2018;

Rahmautami *et al.*, 2022; Yennie *et al.*, 2022; Agitian *et al.*, 2023; Savini *et al.*, 2023). Ready-to-eat foods containing meat ingredients have a higher contamination rate of 8.87% compared to those made from vegetables, which have a contamination rate of only about 0.56%. The highest level of contamination in meat products, both raw and cooked, reaches 59.51% (Léguillier *et al.*, 2024). Although the levels of *S. aureus* contamination in processed *tum bungkil* and *pusuh*, which are classified as wet snacks, the total plate count (TPC) value of *tum bungkil* is within the range that still permitted in the SNI 7388:2009 standar, but the *tum pusuh* is above the maximum limit of  $< 1 \times 10^2$  (BPOM, 2012), it is advisable to steam the *tum* again at temperatures above 50°C for a minimum of 60 minutes before consumption. This precaution is to prevent the risk of staphyloenterotoxigenesis, staphyloenterotoxemia, and leukocidin poisoning (Cieza *et al.*, 2024). It's important to consider storage temperatures, as not all sellers are aware of the guidelines. The maximum safe storage temperature to prevent bacterial growth is  $\leq 10^\circ\text{C}$ , as *S. aureus* can begin to grow by day three under these conditions. However, if the food must be sold in an environment with a temperature around 25°C, it is recommended to consume it within a maximum of 2.5 hours (Kothe *et al.*, 2021). The identification of *S. aureus* can be effectively conducted using the PCR method, which provides more accurate results (Zwe *et al.*, 2025). Several studies have indicated that the presence of *S. aureus* in ready to eat foods is not always linked to the hygiene levels of food handlers or the cleanliness of the processing environment. This is evident as healthy food handlers can still carry *S. aureus*, which can be detected through nasal swabs (Shafizi *et al.*, 2016). Additionally, establishments that appear clean may still harbor *S. aureus*. To better understand the sources of *S. aureus* in ready to eat foods, further research is necessary. This should include regular screenings for *S. aureus* among food handlers and in ready to eat foods processing and sales areas. Implementing strategies to reduce and eliminate the transmission of *S. aureus* from handlers to food and the environment is crucial. This can include identifying appropriate personal protective equipment and effective cleaning agents capable of killing *S. aureus* in ready to eat foods processing area and selling environments.

## CONCLUSION

The presence of *S. aureus* in *tum pusuh* ready-to-eat food raises significant concerns about food safety. This issue primarily stems from a lack of proper hygiene practices among food processors. Additionally, the resilient nature of *S. aureus* allows it to survive inadequate processing conditions, which may include insufficient temperature control and improper steaming times. Furthermore, the ambient temperature at which *tum pusuh* is displayed and sold can further exacerbate the risk of contamination. To ensure public health, it is crucial that stringent hygiene standards and cooking practices are implemented consistently in the preparation and handling of this food.

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