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Isolation and identification of bacteria in the process of making dry and wet *gaplek*

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ABSTRACT

Gaplek is a staple food widely consumed in tropical regions of developing countries. As a source of carbohydrate food, cassava can be processed through industrial development with the dehydration process, with the product being Gaplek. Despite its importance, research on the microorganisms and bacteria involved in the gaplek production process remains limited. This study aims to analyze microorganisms' total plate and yeast mold counts and identify the bacteria involved in dry and wet gaplek production. The research involved enumeration, isolation, and identification stages. Enumeration assessed total plate and yeast mold counts on various media, followed by bacterial identification on PCA media. The results showed that dried gaplek (GD) had the highest plate count on PCA media, with the highest yeast mold count observed in the 0-hour soaked sample (GS0) on PDA media. Enumeration revealed total microorganism, lactic acid bacteria, and yeast mold counts in dried gaplek to be 4.5 x 10^8, 1.0 x 10^5, and 2.2 x 10^8 cfu/gram, respectively. In contrast, for wet gaplek, counts were 3.2 x 10^8, 2.5 x 10^8, and 7.1 x 10^8 cfu/gram, respectively. Bacterial identification involved Gram staining, catalase testing, and biochemical assays, revealing Staphylococcus, Serratia marcescens, and Leuconostoc mesenteroides as potential contributors to dried gaplek production. In contrast, Staphylococcus, Leuconostoc mesenteroides, and Pediococcus pentosaceus were implicated in wet gaplek production.

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INTRODUCTION

Cassava (*Manihot esculenta*) serves as a significant carbohydrate source and is widely consumed as a staple food, particularly in developing pantropical countries. Cassava can be processed into many food products, either intermediate or ready-to-eat, such as cassava, chips, tapioca, and gaplek (Aswita and Chayati 2021). However, cassava tubers have a limited shelf life, typically lasting only 1 to 2 days. To prolong its shelf life, cassava can be processed into gaplek. Gaplek is a traditional intermediate food product from Indonesia, traditionally produced by sun-drying cassava tuber as whole tuber, sliced, or in log shapes, after it has been peeled (Ikhram and Chotimah 2022). The drying process used to produce gaplek can extend its shelf life for 1 to 2 months.

Throughout the years, gaplek production has expanded in several regions of Java Island, such as Lampung, Gunung Kidul, Wonogiri, Trenggalek, and Ponorogo, where the local communities still keep gaplek as a food reserve (Widowati 2020). It was used for food security when rice was not affordable or unavailable. Nowadays, it has the potential to reduce Indonesian rice and wheat flour imports, supported by the relatively affordable price of cassava.

Based on the processing method, gaplek is divided into two types: dry gaplek and wet gaplek. The dry gaplek method involves drying cassava by sun-drying without soaking (Krisbianto et al. 2024), while the wet gaplek method differs in its production process compared to dry gaplek, particularly in the soaking process. Wet gaplek is made by washing and soaking the cassava for 1-2 days before undergoing the drying process (Hrp et al. 2022).

There are other processed food products made from cassava products that are similar to gaplek but produced through slightly different methods, including *tiwul* and *gatot*. *Tiwul* is a ready-to-eat dish made from gaplek flour, typically prepared by boiling or steaming it. (Manarul et al. 2019). On the other hand, gatot is a semi-processed food similar to *gatot*. Still, it involves an additional step where the cassava tubers are incubated for several days until they turn black before being dried. (Ambarsari et al. 2021). This study aims to compare the bacterial growth in dry and wet gaplek processing methods, excluding similar products such as tiwul and gatot.

The spontaneous fermentation process in gaplek involves indigenous microbes naturally present in cassava (Putri et al. 2021). According to previous research, the dominant bacteria in the fermentation process of gaplek using tannia cocoyam, not cassava tuber, were expected as lactic acid bacteria *Lactococcus lactis* and *Leuconostoc mesenteroides* as well as non-pathogenic *staphylococci* and yeast (Krisbianto et al. 2024). Spontaneous fermentation occurs during the drying process, leading to changes in color and texture, as well as mold growth during storage. Some previous studies have shown that lactic acid bacteria (LAB) present in fermented products can affect acidity levels (Siregar et al. 2020). However, no research has been conducted on the isolation and identification of bacteria in the production of both dry and wet gaplek.

Research on microorganisms in dry and wet gaplek is still limited, so the types of bacteria involved in the fermentation process are not yet well understood. Therefore, this study aims to isolate and identify the bacteria involved in producing both dry and wet gaplek. The methods include analyzing the total plate count (TPC), lactic acid bacteria count (LABC), and total yeast and mold count (TYMC) in both dry and wet gaplek, as well as identifying the types of bacteria that play a role in the production process.

METHOD

Materials and Instruments

The main material used in this study was cassava tubers bought from local vendors at Benowo Market, Surabaya. The materials used for sample analysis include plate count agar (PCA, Merck, Merck KGaA, Germany), potato dextrose agar (PDA, Himedia, HiMedia Laboratories, India), Man Rogosa Sharpe Agar (MRS-A, Merck, Merck KGaA, Germany), nutrient broth (NB, Merck, Merck KGaA, Germany), nutrient agar (NA, Merck, Merck KGaA, Germany), Bromocresol blue, arabinose (Merck, Merck KGaA, Germany), lactose (Merck, Merck KGaA, Germany), mannose (Himedia, HiMedia Laboratories, India), sucrose (Himedia, HiMedia Laboratories, India), dextrose (Himedia, HiMedia Laboratories, India), dulcitol (Himedia, HiMedia Laboratories, India), maltose (Himedia, HiMedia Laboratories, India), rhamnose (Himedia,

HiMedia Laboratories, India), and salicin (Himedia, HiMedia Laboratories, India).

The instruments used in this study include an autoclave (HICLAVE HVE-50, Hirayama, Indonesia), a colony counter (Colony Star, Funke Gerber, Germany), a trinocular microscope, an incubator (Memmert IN 55, Germany), a laminar airflow cabinet (General Model B-CV-04, CHCbiolus, Korea), a vortex mixer (Thermo Scientific, USA), a water bath (PolyScience General Purpose, USA), various glassware, and disposable Petri dishes for microbiological analysis.

Research Design

The research design used in this study was a Pre-Experimental Design with an exploratory approach. The experiment consisted of several stages, including enumeration, isolation, and identification of microorganisms, which were conducted at three sampling points. The first point was gaplek after drying at 40°C (GD), the second point was gaplek with 0 days of soaking (GS0), and the third point was gaplek after 48 hours of soaking (GS48).

Production of Gaplek

Cassava was washed and peeled. Next, it was sliced thinly to a thickness of about 2.5 mm. The slices were then dried using a dehydrator for three days at 40°C until they reached a dryness level where they were crisp but could still be broken with some effort. The dry gaplek sample was then sampled.

The next phase was to soak the dried cassava in water for 0, 24, and 48 hours, with the water being changed every 24 hours, for the wet gaplek method. The cassava soaked for 48 hours was subsequently dried using a dehydrator at 50°C. The wet fermentation process occurred in containers covered with cloth to protect them from light and was carried out at ambient room temperature (Krisbianto et al. 2024).

Serial Dilution

The dilution technique used followed the standards outlined in SNI 2897:2008. A total of 25 grams of the sample was dissolved in 225 mL of 0.1% sterile BPW and homogenized using a vortex mixer. Serial dilutions were performed by transferring 1 mL of the initial sample into 9 mL of 0.1% BPW in the next test tube. This process was continued until the desired dilutions of 10^{-4} to 10^{-8} for PCA tests, 10^{-2} to 10^{-6} for MRS-A tests, and 10^{-3} to 10^{-7} for PDA tests were achieved (Krisbianto et al. 2024).

Total Plate Count (TPC) Analysis

The TPC analysis followed the SNI 2332.7:2015 standard. The PCA test was performed using the pour plate technique. Diluted samples (10^{-4} to 10^{-8}) were pipetted with 1 mL into petri dishes, after which 20 mL of PCA was poured into each Petri dish containing the sample and mixed. The Petri dishes were then incubated for 24-48 hours at 37°C. After incubation, observations were made, and the bacterial colony was counted (Krisbianto et al. 2024).

Lactic Acid Bacteria (LAB) Analysis

The LAB analysis followed the SNI 2332.7:2015 standard. The MRS-A test was performed using the pour plate technique. Diluted samples (10^{-4} to 10^{-6}) were pipetted with 1 mL into petri dishes, after which 20 mL of PCA was poured into each petri dish containing the sample and mixed. The petri dishes were then incubated for 24-48 hours at 37°C. After incubation, observations were made, and the bacterial colonies were counted. (Krisbianto et al. 2024).

Yeast and Mold Count (YMC)

The YMC test followed the SNI 2332.7:2015. Diluted samples (10^{-3} to 10^{-7}) were pipetted with 1 mL into petri dishes. Then, 20 mL of PDA was poured into each Petri dish containing the sample and mixed. The prepared petri dishes were incubated for 24-48 hours at 37°C. After incubation, observations were made, and the number of colonies was counted (Krisbianto et al. 2024).

Microorganism Isolation

Microorganism isolation in this study used Nutrient Agar. The inoculating loop was first sterilized by heating before picking colonies from the PCA medium. After cooling, the loop, which had picked the colonies, was gently streaked onto the NA medium using the quadrant streaking technique. The petri dishes were then incubated for 24-48 hours at 37°C (Krisbianto et al. 2024).

Slant Agar Preservation

Slant agar was used to cultivate microorganisms and as a stock culture in biochemical testing. To prepare a slant agar, Nutrient Agar (NA) was used. 5 mL of NA was

poured into a test tube and allowed to solidify while the tube was positioned at an angle, creating a slanted surface for cultivation. (Prihanto et al. 2018).

Gram Staining

The Gram Staining procedure has followed the guidelines outlined in SNI 2332.9:2011. Generally, Gram staining is a biochemical technique employed to differentiate between Gram-positive and Gram-negative bacteria through a specific staining sequence. Initially, the sample was transferred from the slanted agar medium to a glass slide and fixed. Subsequently, 2-3 drops of crystal violet solution were applied, left for 1 minute, and then rinsed with distilled water. Similarly, 2-3 drops of iodine solution were applied and rinsed after 1 minute. Next, 95% alcohol was applied and rinsed off after 5-10 seconds. Finally, 2-3 drops of safranin solution were applied, left for 1 minute, and then rinsed with distilled water. Following the staining process, a cover glass was placed over the sample on the glass slide, allowing for observation under a microscope (Krisbianto et al. 2024).

Catalase Test

This method was based on SNI 2332.9:2011. This test was conducted by placing a few drops of 3% H_2O_2 solution into a glass slide and then using an inoculating loop to transfer bacteria to the slide. The reaction was then observed. If bubbles form, it indicates a positive reaction, while the absence of bubbles indicates a negative reaction (Krisbianto et al. 2024).

Motility Test

The motility test is a biochemical test used to observe bacterial movement within a medium. For this test, Sulfide Indole Motility (SIM) medium was used, which was prepared at one-tenth of the standard concentration to create a semi-solid inoculation medium. The test was performed by transferring a bacterial culture with a sterilized inoculating loop and inoculating it vertically into the semi-solid SIM medium. The cultures are then incubated for 24 hours at 37°C to observe bacterial growth. A positive result is indicated by the spreading of bacterial colonies and the medium becoming turbid (Krisbianto et al. 2024)

Starch Hydrolysis

The starch hydrolysis test was performed by preparing starch agar medium and pouring it into sterile Petri dishes. Once the medium had solidified, each isolate was inoculated onto the starch agar and incubated at 27°C for 24 hours. A positive result was indicated when iodine or Lugol's solution was added around the colonies, revealing a clear zone. This clear zone indicates that the bacteria hydrolyzed the starch (Lal and Cheeptham 2012).

Triple Sugar Iron Agar (TSIA) Test

This testing method was based on SNI 2897:2008. The TSIA test was performed using a TSIA medium in a slanted position within a test tube. The procedure involves stabbing the medium with a sterile inoculating loop to a depth of about one-third of the tube's bottom, then withdrawing the loop and streaking the surface of the medium in a zig-zag pattern. The tube was then incubated for 24 hours at 37°C. The results of the TSIA test were observed based on the color changes in the butt (the bottom part of the medium) and the slant (the surface of the medium). If the microorganism can only ferment glucose, the butt of the medium will turn yellow (acidic), while the slant will remain red (basic). If the microorganism can ferment both lactose and sucrose, both the butt and the slant will turn yellow (acidic) (Krisbianto et al. 2024).

Acid from Glucose Test

This method was based on SNI 2897:2008. In this test, Nutrient Broth (NB) medium was used, with 1% sugar (arabinose, lactose, mannose, sucrose, mannitol, dextrose, dulcitol, maltose, rhamnose, salicin) added to the sterilized NB medium. Before sterilization, the medium was supplemented with bromocresol blue reagent, causing the medium to turn green. After sterilization, colonies were taken using an inoculating loop and introduced into the NB medium in test tubes. The test tubes were then incubated for 24 hours at 37°C. The color change was observed in the medium. If the medium turned yellow, it indicated that the bacterial colonies produced acid (Krisbianto et al. 2024).

Indole Test

A Sulfide Indole Motility (SIM) medium was used in this test. The indole test was performed concurrently with the motility test. After the bacterial isolate had grown in the SIM medium, Kovac's reagent was added. A positive result for the indole test was indicated by the formation of a red ring at the surface of the

medium after incubation for 24 hours at 37°C (Cappuccino and Welsh 2019).

RESULT AND DISCUSSION

Microbial Counts

The microbial count data for Total Plate Count (TPC), Lactic Acid Bacteria Count (LABC), and Total Yeast and Mold Count (TYMC) were presented in Table 1.

Table 1 Number of TPC, LABC, and TMYC

Sample	TPC (CFU/g)	LABC (CFU/g)	TMYC (CFU/g)
GD	$4{,}5 \times 10^8$	$1{,}0 \times 10^5$	$2{,}2 \times 10^8$
GS0	$4{,}6 \times 10^7$	$3{,}8 \times 10^4$	$9{,}6 \times 10^8$
GS48	$3{,}2 \times 10^8$	$2{,}5 \times 10^8$	$7{,}1 \times 10^8$

GD: Dried Gaplek; GS0: Gaplek soaked for 0 hour; GS48: Gaplek soaked for 48 hours

Sample GD, which underwent drying at 40°C, showed that lactic acid bacteria, molds, and yeasts were capable of growing at this temperature. This was why the Total Plate Count (TPC), Lactic Acid Bacteria Count (LABC), and Total Yeast and Mold Count (TYMC) values for sample GD were relatively high (Krisbianto et al. 2024). Based on the data in Table 1, the Total Plate Count (TPC) for sample GS0 was 1 log cycle lower than that for sample GD. This decrease was likely due to the bacteria having entered the lag phase at this stage. During the lag phase, bacteria were adjusting to the new environment, and cell division was minimal. The addition of water during soaking in GD created pressure that caused bacterial cells to spread apart, thereby reducing bacterial density and decreasing nutrient concentration as the cells became more dispersed. This contributes to the observed lower TPC in GS0 (Achinas et al. 2019). However, at this stage, some bacteria still did not have a strong attachment to the gaplek. The 'attachment' referred to the condition where water was tightly bound to the components of the material, with a water activity (aw) level below 1. This lower water activity meant that the bacteria had less available moisture to thrive and establish themselves on the gaplek (Hayati 2017). Microorganisms in food are influenced by physical characteristics, nutritional content, extrinsic and intrinsic factors, and their interactions (Sulastri et al. 2022). The difference in bacterial types present in gaplek had a significant impact. Obligate aerobic bacteria were more likely to be found in dried gaplek, while facultative aerobic and microaerophilic bacteria were more commonly found in soaked gaplek. The low TPC value in GS0 was due to environmental changes that led to bacterial death (Purnowo et al. 2023). Ecological changes could lead to alterations in physiological characteristics, potentially affecting the growth and survival of microorganisms. In this study, such environmental changes occurred when dried gaplek was soaked in water.

In sample GS48, the TPC value was higher compared to GS0. This increase could have been due to the fermentation process, which increased the number of active bacteria, resulting in the breakdown of substrates (Kinteki et al. 2019). Additionally, bacterial growth during fermentation was supported by the environmental temperature. Gaplek was processed at room temperature, around 28-30°C, which was an optimal temperature for bacterial growth (Sipayung et al. 2023).

The TPC value for GS48 was lower compared to GD. This was because the initial bacterial count before fermentation consisted of microbes from sample GS0. Additionally, every 24 hours, the gaplek was washed with a change of soaking water, which affected the number of microbes adhering to the surface of the food material (Saidi and Wulandari 2019). Furthermore, GD and GS48 had different environmental conditions, so GD might have contained obligate aerobic microbes, while GS48 harbored facultative aerobic and microaerophilic microbes.

Based on the Lactic Acid Bacteria (LAB) count data, sample GS0 showed a reduction of 1 log cycle compared to GD. This decrease may have been attributed to environmental factors. Sample GS0 was derived from GD with added water, which changed the environment from aerobic to anaerobic conditions. Although LAB could survive and grow in limited oxygen environments, the shift from an oxygen-rich to an oxygen-poor environment may have affected their growth and abundance (Krisbianto et al. 2024).

The LAB count in GS48 was higher compared to GS0, as the presence of water played a crucial role in increasing the bacterial population (Ferdian et al. 2019). Additionally, the nutrients in the gaplek sample, such as sugars, could also

support bacterial growth (Altatri et al. 2021). During soaking, cassava undergoes fermentation, where LAB converts starch into simple sugars like glucose and maltose through the process of hydrolysis (Faozi et al. 2023). Additionally, the temperature required for cassava fermentation was around 27-35°C, with the optimal temperature being 33°C (Yerizam et al. 2020). The LAB count tended to be lower than the Total Plate Count (TPC) in each treatment. This indicated that the number of LAB was less compared to non-LAB bacteria, as the medium used for TPC, Plate Count Agar (PCA), was non-selective and supported the growth of various bacterial types.

Based on the Total Yeast and Mold Count (TYMC) data, the highest count was found in the GS0 sample. This is due to the soaking water's temperature and pH, which supported the growth of molds and yeasts, particularly at a temperature of 30°C (Wu and Wong 2022). Additionally, molds had an optimal acidity level at a pH range of 3.5 to 8, while yeasts thrived best at a pH between 3.5 and 4.5 for growth (Racchi et al. 2020). The TYMC count in GS48 was lower than in GS0, which was suspected to be due to bacteriocins. LAB produces Bacteriocins and inhibits the growth of other microorganisms, one of which is diacetyl (Gaffar and Suryani 2023).

Morphology of Microorganisms

Based on Table 2, 10 types of microorganisms were found on PCA media from the three different treatments. The variation in bacterial types in each treatment was due to the differences in growth media conditions. In the treatment of dried gaplek, four types of bacteria were identified with the codes GD101, GD201, GD301, and GD401. Meanwhile, in the 0-hour soaking treatment, bacteria with the codes GS011, GS021, and GS031 were also successfully identified. On the other hand, in the gaplek soaked for 48 hours, three types of bacteria with the codes GS481, GS482, and GS483 were also found. Bacteria with codes GD401, GS031, and GS481 dominate each treatment and share common characteristics: small size, smooth texture, regular edges, convex elevation, white color, and surface growth on the media. Based on this morphology, they were likely to be lactic acid bacteria (LAB) (Putri and Kusdiyantini 2018).

Gram Staining and Catalase Test on Bacteria from PCA Media

Gram staining and catalase tests for bacteria on PCA media were useful in helping to identify the bacteria found. The results of Gram staining and catalase tests are presented in Table 3. There were three categories of bacteria: Gram-positive cocci (GD101, GD401, GS011, GS031, GS481, and GS483), Gram-negative cocci (GD201, GS021, and GS482), and Gram-negative bacilli (GD301). Gram-negative bacteria were often found in the air and on laboratory floor surfaces, which may have been due to airborne contamination from room users (Despita et al. 2021). Gram-positive cocci with a negative catalase test indicated the genus *Streptococcus*. In contrast, Gram-positive cocci with a positive catalase test belonged to the genus *Diplococcus*. Additionally, Gram-positive bacteria with a positive catalase test were classified under the genus *Staphylococcus*.

Table 3 Gram Staining Results of Colonies on PCA Media

Sample	Colony Code	Gram Reaction	Cell Shape	Catalase
GD	**GD101**	+	*coccus*	+
	GD201	-	*coccus*	+
	GD301	-	*bacillus*	+
	GD401	+	*coccus*	-
GS0	**GS011**	+	*coccus*	+
	GS021	-	*coccus*	+
	GS031	+	*coccus*	-
GS48	**GS481**	+	*coccus*	-
	GS482	-	*coccus*	+
	GS483	+	*coccus*	-

(+): Positive (-): Negative
GD: Dried Gaplek; GS0: Gaplek soaked for 0 hour; GS48: Gaplek soaked for 48 hours

Gram-negative bacteria with a positive catalase test fall into the genus *Bacillus*. Samples GD401, GS031, GS481, and GS483 were categorized as LAB because these bacteria were Gram-positive and exhibited a negative catalase test, which aligned with the characteristics of LAB (Khushboo et al. 2023) (Wang et al. 2021). Bacterial colonies that showed a Gram-negative staining result were not selected for further biochemical testing due to the absence of LAB characteristics in those bacteria.

Biochemical Testing

Biochemical tests were methods used to identify bacterial cultures and determine isolation results based on their physiological characteristics. These tests include SIM, indole, starch hydrolysis, motility, TSIA, catalase, and carbohydrate fermentation tests. In this study, only Gram-positive bacteria underwent biochemical testing, with the exception of bacteria coded GD301.

Based on the data in Table 4, bacteria with codes GD101 and GS011 were identified as *Staphylococcus sp. Staphylococcus* species had enzyme activity that played a role in reducing cyanide during cassava fermentation. The presence of these bacteria may have been due to airborne contamination during the purchase of materials or while the research was being conducted (Balogun et al. 2021). Sample GD301 was suspected to be *Serratia marcescens*. This identification was based on the morphology of the colony, which was red in color. *Serratia marcescens* was known for its ability to produce the red pigment prodigiosin, which imparted a red color to its colonies. This bacterium could grow at temperatures between 5-40°C and at pH levels ranging from 5-9, and it has been found in cassava undergoing fermentation (Haddix and Shanks 2018).

Samples GD401, GS031, and GS481 were suspected to be *Leuconostoc mesenteroides*, a Gram-positive lactic acid bacterium typically found in fermented dairy products such as cheese and yogurt, as well as in other fermented products. Other LAB commonly found in fermented cassava products include *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus brevis*, and *Leuconostoc mesenteroides* (Fauziyah et al. 2023). Sample GS483 was identified as *Pediococcus pentosaceus*. *Pediococcus pentosaceus* was a Gram-positive LAB and belonged to the homofermentative group, impacting fermented products' flavor and aroma (Jiang et al. 2021).

Morphology of LAB on MRS-A Media

LAB morphology in dried and soaked gaplek was observed to confirm the presence of LAB during the production process of both types. According to the data in Table 5, seven types of microorganisms successfully developed on MRS-A medium across the three different treatments. In the dried gaplek treatment, two bacterial types were identified with codes GDA and GDB. For the 0-hour soaking treatment, bacteria with codes GS0A and GS0B were found. In the 48-hour soaking treatment, bacteria with codes GS48A, GS48B, and GS48C were identified.

Bacteria with codes GDA, GS0A, and GS48B had small, flat, spindle-shaped colonies with smooth edges, a smooth texture, and a white color growing in the center of the medium. In contrast, bacteria with codes GDB, GS0B, GS48A, and GS48C had small to medium-sized colonies with smooth edges, a smooth texture, a white color, and growth on the surface of the medium.

Gram Staining and Catalase Test on MRS-A Media

Gram staining and catalase testing on LAB were conducted to confirm the presence of LAB in the production process of dry and soaked gaplek. Based on Table 5, the Gram staining results for all colonies showed that they all belonged to a single group of bacteria, characterized as cocci, gram-positive, and catalase-negative. This suggested that these colonies might belong to the genus *Streptococcus*, which was typically spherical or oval. From the Gram staining and catalase test data, it was inferred that all isolates growing on MRS-A medium fall into the LAB category. This was indicated by their Gram-positive nature and negative catalase test results (Khushboo et al. 2023).

CONCLUSION

Based on the research findings, the total number of microorganisms, lactic acid bacteria, and yeast and mold counts in dry gaplek were 4.5 x 10^8, 1.0 x 10^5, 2.2 x 10^8cfu/gram, respectively,. In comparison,in soaked gaplek they were 3.2 x 10^8, 2.5 x 10^8, 7.1 x 10^8 cfu/gram, respectively. Additionally, the bacteria involved in the

production of dry gaplek were suspected to be *Staphylococcus*, *Serratia marcescens*, and *Leuconostoc mesenteroides*. In soaked gaplek, the bacteria likely involved were *Staphylococcus*, *Leuconostoc mesenteroides*, and *Pediococcus pentosaceus*.

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Table 2 Colony Morphology on PCA Media

Colony Code	Colony Count (CFU/gram)	Size	Shape	Margin	Elevation	Color	Transparency	Texture	Growth Location
GD101	$1,7 \times 10^8$	Medium	Circular	Smooth	Convex	Milky White	Opaque	Smooth	Surface
GD201	$4,0 \times 10^7$	Small	*Punctiform*	Smooth	Flat	Grayish White	Semi-Transparent	Smooth	Center
GD301	$0,3 \times 10^5$	Medium	Circular	Smooth	Convex	Red	Opaque	Smooth	Surface
GD401	$4,5 \times 10^8$	Small	Circular	Smooth	Convex	White	Opaque	Smooth	Surface
GS011	$8,5 \times 10^6$	Medium	Circular	Smooth	Convex	Milky White	Opaque	Smooth	Surface
GS021	$4,0 \times 10^6$	Small	*Punctiform*	Smooth	Flat	Grayish White	Semi-Transparent	Smooth	Center
GS031	$3,3 \times 10^7$	Small	Circular	Smooth	Convex	White	Opaque	Smooth	Surface
GS481	$2,4 \times 10^8$	Small	Circular	Smooth	Convex	White	Opaque	Smooth	Surface
GS482	$3,7 \times 10^7$	Small	*Punctiform*	Smooth	Flat	Grayish White	Semi-Transparent	Smooth	Center
GS483	$3,3 \times 10^7$	Medium	Circular	Smooth	Convex	Milky White	Opaque	Smooth	Surface

Table 4 Biochemical Test Results

Sample	**GD**			**GS0**		**GS48**	
Colony Code	**GD101**	**GD301**	**GD401**	**GS011**	**GS031**	**GS481**	**GS483**
Catalase	+	+	-	+	-	-	-
Gram Staining							
Reaction	+	-	+	+	+	+	+
Morphology	*Staphylococcus*	*Bacillus*	*Strepto-coccus*	*Staphylococcus*	*Strepto-coccus*	*Strepto-coccus*	*Strepto-coccus*
TSIA							
Gas	-	-	-	-	-	-	-
Fermentative	R/R	R/R	Y/Y	R/R	Y/Y	Y/Y	Y/Y

Sample	**GD**			**GS0**		**GS48**	
Colony Code	**GD101**	**GD301**	**GD401**	**GS011**	**GS031**	**GS481**	**GS483**
H2S	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-
Motility	+	+	-	+	-	-	-
Starch Hydrolysis	-	-	+	-	+	+	-
Acid From Sugars							
Arabinose	+	+	+	+	+	+	+
Dextrose	+	+	+	+	+	+	+
Dulcitol	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
Presumed	*Staphylococcus sp*	*Serratia marcescens*	*Leuconostoc mesenteroides*	*Staphylococcus sp*	*Leuconostoc mesenteroides*	*Leuconostoc mesenteroides*	*Pediococcus pentosaceus*

+: Positive R/R: Red/Red
-: Negative Y/Y: Yellow/Yellow
GD: Dried Gaplek; GS0: Gaplek soaked for 0 hour; GS48: Gaplek soaked for 48 hours

Table 5 Morphological Data Results of LAB Found in MRS-A Media

Colony Code	Colony Count (CFU/gram)	Size	Shape	Margin	Elevation	Color	Transparency	Texture	Growth Location
GDA	$8{,}5 \times 10^4$	Small	*Spindle*	Smooth	Convex	White	Opaque	Smooth	Center
GDB	$1{,}2 \times 10^4$	Small	Circular	Smooth	Convex	White	Opaque	Smooth	Surface
GS0A	$3{,}6 \times 10^4$	Small	*Spindle*	Smooth	Convex	White	Opaque	Smooth	Center
GS0B	$1{,}9 \times 10^3$	Small	Circular	Smooth	Convex	White	Opaque	Smooth	Surface
GS48A	$2{,}0 \times 10^8$	Small	Circular	Smooth	Convex	White	Opaque	Smooth	Surface
GS48B	$8{,}6 \times 10^6$	Small	*Spindle*	Smooth	Convex	White	Opaque	Smooth	Center
GS48C	$2{,}2 \times 10^7$	Medium	Circular	Smooth	Convex	White	Opaque	Smooth	Surface

GD: Dried Gaplek; GS0: Gaplek soaked for 0 hour; GS48: Gaplek soaked for 48 hours