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Identification of yeasts in different rubber leaf (*Hevea brasiliensis*) clones and their effects on the physical properties of fermented glutinous rice tapai

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Abstract

Tapai is one of the most popular traditional desserts in Malaysia and other Asian countries. Traditionally, tapai is wrapped in a rubber leaf to enhance the smell and increase its palatability. The study focused on identifying the yeasts present before and after the production of glutinous rice tapai wrapped in different rubber leaves clones, namely RRIM 2025, RRIM 2002, PB 260 and PB 350. The identification of the yeast was carried out using API 20C AUX test strips for all rubber leaves clones, glutinous rice tapai wrapped in RRIM 2025, RRIM 2002, PB 260, PB 350 and in a container (control). The results showed that *Crytococcus laurentii*, *Rhodotorula mucilaginosa* 2*, Candida famata, Rhodotorula minuta* were present in rubber leaf clones. While the yeasts that had been identified in tapai wrapped in rubber leaf were *Candida guilliermondii*, *Rhodotorula mucilaginosa* 2, *Candida parapsilosis* and *Trichosporon mucoides* and only *C. guilliermondii* was found in the container. The physical properties of the tapai that are wrapped in rubber leaves have a difference in texture, pH value and total soluble solids content compared to the control sample. The tapai sample wrapped in RRIM 2025 and RRIM 2002 had a high total soluble solid content of 45.8±0.14% and 45.78±0.16% °Bx, respectively. Meanwhile, the control sample has the highest pH value and the hardest rice kernels, which were 4.71±0.05 and 218.19±25.39 N, respectively. The results showed that the different yeasts present in the rubber leaf may cause changes in the physical properties of glutinous rice tapai.

1. Introduction

The use of plastics or styrofoams as food wrappers and containers is growing from year to year and can inadvertently endanger health (Harijati *et al*., 2013). This is because the packaging may contain acrylonitrile components that act as a genotoxic agent by inducing breakage of the DNA strand and non-disjunction of the sex chromosome in spermatogenesis (Xu *et al*., 2003). In order to prevent such dangerous effects of plastic or styrofoam, conventional local practices use leaves as food wrapping or packaging or containers. Traditionally, many leaves can be used as food wrappers, including rubber tree leaves, banana leaves, mango leaves and cashew leaves in tapai preparation. Wrapping the tapai in rubber tree leaves will incorporate its valuable components into the product or give impact to the aroma of the product (Kabuo *et al*., 2013).

The rubber tree (*Hevea brasiliensis*) is a member of the Euphorbiaceae family, with a trunk circumference approximately 50 cm to 1 m above the ground (Valognes *et al*., 2011). Rubber tree or *H. brasiliensis* is a major commercial source of natural rubber that is used extensively in manufacturing high-quality rubber products (Fenghua *et al*., 2013). Rubber trees have become a development instrument for developing countries, and a good way to fight against deforestation and soil erosion, critical problems in tropical countries. A rubber clone is considered stable if its performance

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across environments does not deviate considerably from the average performance of a standard clone population (Gonçalves *et al*., 2003). Clones of rubber trees such as RRIM 2025, RRIM 2002, PB 260 and PB 350 were developed to produce a better homogeneous and high productivity in plantations which allow small farmers to get high-performance clones for the high yield of rubber (Valognes *et al*., 2011).

Tapai is one of the popular traditional desserts in Malaysia and among other Asian countries which is prepared by fermenting glutinous rice (*Oryza sativa* glutinous) or using cassava tuber (*Manihot utilisima*) (Abdul Raji *et al*., 2017). Tapai is produced by inoculating a carbohydrate source with the required microorganisms in the starter culture, with a soft texture, a sweet, sour taste and an alcoholic taste (Atmodjo, 2006). The fermentation in tapai involves the conversion of starch (sugar) from the glutinous rice into alcohol via fermentation by yeast, moulds and other bacteria. Steinkraus (2002) reports on fermentation using microorganisms is one of the oldest transformation methods used to preserve and enhance the flavour, aroma and nutritive values of food. Generally, there are three main groups of microorganisms which are yeasts, moulds, and bacteria found in traditional 'ragi'. Moulds including *Aspergillus oryzae, Rhizopus oryzae, Amylomyces rouxii* or Mucor species, and yeasts including *Saccharomyces cerevisiae*, and *Saccharomycopsis fibuliger, Endomycopsis burtonii* and others, along with bacteria (Pediococcus sp, Bacillus sp, Lactobacillus sp) were commonly found in the 'ragi' (Gandjar, 2003; Rahayu, 2003). The presence of different microorganisms could result in different physical properties between tapai that are unwrapped and wrapped in rubber leaf (Law *et al*., 2011). Traditionally, tapai is wrapped in rubber leaf to enhance the odour and increase its palatability. However, there is a lack of information on what possible growth of microorganisms is present after its production wrapped in rubber leaf. Different clones of rubber leaf contain several of the microorganisms that may take place in the fermentation of tapai.

In order to identify potential microorganisms present in the rubber leaf and after the production of tapai, the isolation and identification of yeasts were carried out before and after the production of tapai glutinous rice. This is because yeasts play important roles in tapai fermentation and can lead to changes in the physical properties of tapai. The aim of the study was, therefore, to isolate and identify yeasts present in four different rubber leaf clones, namely RRIM 2025, RRIM 2002, PB 260 and PB 350, and to assess the physical properties of the wrapped and unwrapped tapai.

2. Materials and methods

2.1 Materials

Rubber tree (*H. brasiliensis)* leaf was obtained from Tok Dor Nursery, Jerteh, Terengganu. Leaves from four clones (RRIM 2025, RRIM 2002, PB 260 and PB 350) were collected. Leaves with a diameter of 20.0±0.5 cm were selected to ensure that glutinous rice tapai could be fermented evenly. The leaves were cleaned under running water and dried using a clean cloth. The rubber leaf was stored in a chiller at 4^{o}C prior to usage. Table 1 illustrates the description of rubber leaf clones that were picked from Tok Dor Nursery, RISDA Jerteh, Terengganu.

2.2 Preparation of glutinous rice tapai

Approximately 200 g of glutinous rice were washed and soaked in 500 mL water for 2 hrs, and then steamed for 15 mins until the glutinous rice was soft and sticky. The steamed glutinous rice was then cooled to room temperature (25°C to 30°C). After that, 2.5 g of powdered ragi (starter culture) was sprinkled onto it. The inoculated substrate was divided into small portions (25 g) and it was wrapped in rubber leaves and half of the portion was unwrapped and put in the container. Then, the fermentation process continued for 24 to 48 hrs at ambient temperature (25°C to 30°C) in the fermentation chamber. After the product has had a watery and alcoholic odour, the fermentation was complete and kept chilled at 4°C.

2.3 Microbiological analyses

2.3.1 Isolation of yeasts

All four clones of rubber leaves and the glutinous rice were analysed for the presence of yeast before and after the glutinous rice tapai production.

A sample of 25 g (different clones of rubber leaf or tapai) was homogenised with 225 mL of peptone water in the stomacher bag for 2 mins at normal speed. Serial dilution was obtained by transferring 1 mL of the sample (dilution 10^{-1}) to 9 mL of buffered peptone water until 10^{-4} dilution. A volume of 0.1 mL of the dilutions (dilution 10^{-2}, dilution 10^{-3} and dilution 10^{-4}) was pipette into Malt Extract Agar (MEA). The spread-plate method was then performed using an L-spreader and then incubated for 24 -72 hrs at 28°C. The colony was selected randomly from the plate and streaked again on fresh MEA agar plates. The procedure continued with the identification of yeasts using the Analytical Profile Index (API) 20C AUX test strips.

Table 1. Rubber leaf from different clones that were collected from RISDA, Tok Dor, Terengganu

Rubber leaf clones	Picture	Description
PB 260		Diameter 22.5±4.0 cm Have bigger size than the other clones Do not have a smooth structure
PB 350		Diameter 17.5±3.0 cm Have smooth structure Oval in shape
RRIM 2025		Diameter 19.0±2.0 cm Shape: longer and round at the tip of the leaf Do not have a smooth structure
RRIM 2002		Diameter 17.5±3.0 cm Shape: long and round at the tip of the leaf Have smooth structure

2.3.2 Identification of yeasts

A portion of the yeast colony was collected by subsequent touch using sterile swab cotton. A turbidity suspension equal to 2 McFarland with 2 mL of 0.85% NaCl was prepared. API C Medium was opened and then, approximately 100 µL suspension was transferred. The medium was gently homogenised using the pipette. The cupules of the API 20 C AUX test strips were filled with suspension in ampoule API C Medium. The lid was placed on the tray and was incubated at 29°C for 48 – 72 hrs. The turbidity of each cupule was compared to 0 cupules. It indicated positive results when there were more turbid than 0 cupules (Chay *et al*., 2017).

2.3.3 Morphology test

The presence of the hyphae or pseudohyphae was determined using yeast extract tween solution under a compound microscope for 48 hrs and 72 hrs. Firstly, the yeasts colony was suspended into the yeasts extract tween solution which was incubated for 3 hrs at 37°C. The glutinous rice tapai samples were then incubated for 72 hrs at 25°C. The presence of hyphae or pseudohyphae indicated a positive result.

2.4 Physical analysis of glutinous rice tapai

2.4.1 Texture profile analysis

A double arm texture analyser was used (TA.HD plus) (Stable Micro System, USA) to analyse the texture of the tapai, using HDP/BSW; blade set with warner bratzler. Tapai sample was subjected to post-test speed (10 mm/s), with test speed (2 mm/s), and the distance was 25 mm, having a load cell of 100 kg, using the P36 m probe. The texture analyzer calibrated its height and force for 3 kg. The sample was measured for its hardness by applying a maximum force (Wan Mohamad Din *et al*., 2020).

2.4.2 Colour analysis

Colour (L*a*b* colour system, where L (lightness), a (redness) and b (yellowness)) of the samples were determined using Colourimeter (Chroma Meter, Konica Minolta Sensing, Inc., Japan). The Hunter L* a* b* was calibrated on the white tile first. The colourimeter was placed onto the fermented glutinous rice tapai. Next, the reading of L*, a*, b* value was taken and recorded (Wan Mohamad Din *et al*., 2020).

2.4.3 Total soluble solids analysis

In order to measure the total soluble solids of the glutinous rice tapai, a refractometer of 0 to 85% °Bx (Refractometer, Milwakee) was used. Firstly, the refractometer was calibrated using distilled water. Then, the sample was poured on the refractometer, and the reading of the sample was recorded. The formation of bubbles was avoided for accurate measures.

2.4.4 pH analysis

Firstly, the pH meter was calibrated using pH and pH 9 buffered solution. Preparation was done by finely grinding 5 g of samples and homogenised them in 20 mL of distilled water. Then, the sample was poured into a clean beaker. Next, the pH value of the sample was taken and recorded (Waterproof Portable Meter, Eutech Instruments, Singapore).

2.5 Statistical analysis

The results were expressed as mean±standard deviation. The significant difference at ($p<0.05$) was produced by one-way variance analysis (ANOVA) and a Fisher's Least Significant Difference (LSD) test (Zainol *et al*., 2018).

3. Results and discussion

3.1 Microbiological analysis

3.1.1 Isolation of yeasts

3.1.1.1 Isolation of yeasts from rubber leaf

Each of the yeast species was identified based on their differences in colour, size and shape of the colony. Table 2 shows the list of yeasts isolated from the rubber leaf before the production of glutinous rice tapai.

3.1.1.2 Isolation of yeasts from glutinous rice tapai wrapped in four different clones of rubber leaves and container

Table 3 shows the description of yeasts isolated from tapai wrapped in rubber leaf and containers after the fermentation process. There were several differences in the amount of yeast found in the different rubber leaves

Table 2. Yeasts isolated from different rubber leaf clones (RRIM 2025, RRIM 2202, PB 350 and PB 260)

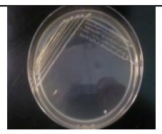
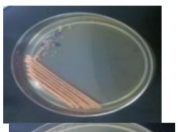
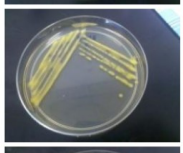
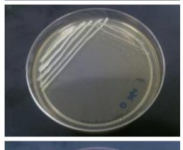
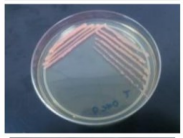
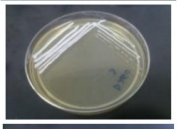
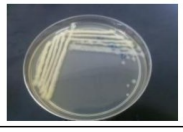
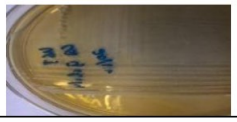
Rubber leaf Clones	Colour of colony	Morphology on Plate	Size of Colony	Shape of Colony
RRIM 2025	Orange		Small	Circle
RRIM 2025	Orange-pinkish		Large	Circle
RRIM 2002	Pinkish Yellow		Small	Circle
RRIM 2002	Milky- white		Medium	Circle
RRIM 2002	Pinkish Yellow		small	Circle
PB 350	Pale pinkish		Small	Circle
PB 350	Pale-yellow		medium	Circle
PB 260	Milky white		Small	Circle
PB 260	Orange- pink		Medium	Circle
PB 260	White		Small	Circle
PB 260	Yellow		medium	Circle

Table 3. Descriptions of yeasts isolated from glutinous rice tapai wrapped in rubber leaf and container

Rubber leaf Clones	Colour of Colony	Morphology on Plate	Size of Colony	Shape of the Colony
RRIM 2025	Creamy white		Medium	Circle
RRIM 2025	Pink		Large	Circle
RRIM 2025	Pinkish Yellow		Medium	Circle
RRIM 2025	Creamy white		Large	Circle
RRIM 2002	Pink		Large	Circle
RRIM 2002	White		Medium	Circle
PB 350	White		Large	Circle
PB 260	White		Medium	Circle
Container	White		Medium	Circle

and also in the container and the glutinous rice tapai itself.

3.1.2 Identifications of yeasts

3.1.2.1 Identifications of yeast in rubber leaf

Table 4 indicates the presence of *C. laurentii* in the RRIM 2025, *R. mucilaginosa* 2 and *C. famata* in the RRIM 2002, *C. famata* and *C. laurentiiin* the PB260, and *R. minuta* and *R. mucilaginosa* 2 in the PB 350, respectively. The results also show that rubber leaf clones RRIM 2025 and PB 260 possessed low discrimination yeast which was *C. lauretii*. The proposed complementary test for the yeast profile 6 7 7 7 7 7 3 and 6 7 5 5 7 7 7 3, for which the rubber leaf yeasts RRIM 2025 and PB 260 respectively contained both dilactate A and antidion R, was proposed. The yeasts believed to have been detected by these complementary tests for both the dilactate A and antidion R profiles were *C. humicola* (100% and 71% compatibility), while the yeasts *T. mucoides* (100% compatibility) for both complementary dilactate A and antidion R tests. Thus, there are possibilities that the yeasts are *C. humicola* and *T. mucoides* exist in the rubber leaf clones.

R. minuta is believed to act as a protective agent against the toxic effect of aflatoxin on rubber leaves. This is supported by Batt and Tortorello (2014), *R. minuta* was applied as a biocontrol agent of postharvest mango anthracnose and as a protective agent against the toxic effect aflatoxin. *Candida famata* was usually found in leaves as it is known to thrive in organic materials. The presence of *C. famata* was pleasantly needed in the fermentation of glutinous rice tapai due to its ability to improve the production of riboflavin during fermentation (Abbas and Sibbirny, 2012). The *C. lauretii* that exist in the rubber leaf may play an important role in fermenting the glutinous rice tapai. Machado and Linardi (1990) cited that yeasts from genera Candida, Cryptococcus, Debaromyces, Trichosporum and Rhodotorula have the abilities to produce amylase which could improve the fermentation performance. The reason that *Rhodotorula mucilaginosa* 2 is present in the rubber leaf clones because it is usually present in nature and usually found in a product-based environment. This can be supported by Tuan and Costa (2008), who stated that the habitat of the *R. mucilaginosa* 2 was widely found in nature and can be isolated from environmental sources and products.

Table 4. Identification of yeasts using API 20AUX test strips in different rubber leaf clones

Rubber leaf Clones	Significant Taxa	Identification (%)	Descriptions
RRIM 2025	*Cryptococcus laurentii*	46.1	Low discrimination
RRIM 2002	*Rhodotorula mucilaginosa* 2	99.9	Excellent identification
	Candida famata	86.9	Acceptable identification
PB 260	*Candida famata*	85.6	
	Cryptococcus laurentii	73.6	Low discrimination
PB 350	*Rhodotorula minuta*	91.1	Good identification
	Rhodotorula mucilaginosa 2	95.5	Good identification

Table 5. Identification of yeasts using API 20C AUX test strips in glutinous rice tapai wrapped in different rubber leaf clones and container

Rubber leaf Clones	Significant Taxa	Identification (%)	Descriptions
RRIM 2025	*Candida guilliermondii*	81.0	Acceptable identification
	Rhodotorula mucilaginosa 2	98.2	Good identification
	Candida parapsilosis	98.4	Good identification
RRIM 2002	*Candida guilliermondii*	71.6	Excellent identification to the genus
	Rhodotorula mucilaginosa 2	98.2	Good identification
PB 260	*Candida parapsilosis*	99.2	Good identification
PB 350	*Trichosporon mucoides*	93.2	Good identification
Container	*Candida guilliermondii*	99.7	Very good identification

3.1.2.2 Identification of yeasts from glutinous rice tapai wrapped in rubber leaf and container

Table 5 depicts that at least three yeasts were present in glutinous rice tapai that were wrapped in rubber leaf clones RRIM 2025, which were *C. guilliermondii*, *R. mucilaginosa* 2 and *C. parapsilosis*. Yeasts *R. mucilaginosa* 2 and *C. guilliermondii* were found in glutinous rice tapai wrapped in rubber leaf clones RRIM 2002. *C. parapsilosis* and *T. mucoides* were found in glutinous rice tapai that were wrapped with rubber leaf clones PB 260 and PB 350. On the other hand, *C. guilliermondii* was found in glutinous rice tapai packed in containers. Tables 4 and 5 show that all the yeast confirm their existence in rubber leaf was no longer present in glutinous rice tapai wrapped in rubber leaves except for *R. mucilaginosa* 2.

The absence of these microorganisms in glutinous rice tapai was suspected to be due to the physical properties of the tapai itself. The low pH of the tapai can eliminate enteropathogen, coliforms and spoilage organisms in the tapai (Chiang *et al*., 2006). However, the presence of *R. mucilaginosa* 2 in glutinous rice tapai could indicate that the environment of the sample is favourable for it and not possible since *R. mucilaginosa* 2 is reported to be present in other fermented products. The survival of the *R. mucilaginosa* in tapai despite the high pH indicates that these yeasts could survive in extreme conditions of the gastrointestinal tract (Silva *et al*., 2004). Yet, it is still uncertain whether Rhodotorula is capable of passing from the gastrointestinal tract into the bloodstream. However, *R. mucilaginosa* was also suspected to be found due to contamination from the air environment. This is supported by Viljoen and Greyling (1995), *R. mucilaginosa* and *R. glutinis* previously referred to as the species of common air contaminants or natural contaminants in cheese. The presence of *C. guilliermondii* in glutinous rice tapai wrapped in rubber leaf and containers, it was suspected that comes from the starter culture (ragi) itself. This is because *C. guilliermondii* was used safely as food additive organisms for fermentation in the production of citric acid. Similarly, *C. parapsilosis* was also suspected to originate from the ragi itself. Kofli and Mohd Dayaon (2010), stated that the yeasts that are found in Sarawak tape' that are wrapped in banana leaves are *Cryptococus humicola* and *Candida glabrata*.

3.2 Physical analysis

3.2.1 Texture profile

Table 6 depicts the significant difference in texture between tapai in containers and other tapai that are wrapped in rubber leaf. Interestingly, there were significant differences ($p<0.05$) in texture between the RRIM 2025 and RRIM 2002 clones with PB 260 and PB 350. Glutinous rice tapai in the container showed significantly ($p<0.05$) harder texture properties (218.19 N) than that of tapai wrapped in rubber leaves. It is believed that the microorganisms present in the rubber leaf play an important role in the contribution of the texture to the wrapped tapai, since there was only one yeast present in the container. Fermentation processes usually change the texture of the product due to microorganisms that hydrolyse the sugar as it is the medium for the microorganism to grow (Dung *et al*., 2005). This can therefore be seen in the high number of yeasts present in RRIM 2002 and RRIM 2025 rubber

Table 6. Hardness value, pH, total soluble solid and colour profiles glutinous rice tapai wrapped in different rubber leaf clones and container.

Sample	Hardness (N)	pH	TSS (°Bx)	Colour		
				L*	a*	b*
Container	218.19±25.39[a]	4.71±0.67[a]	45.31±1.29[b]	78.22±5.13[b]	-0.94±0.06[a]	14.93±0.98[a]
RRIM 2025	81.82±2.90[c]	4.29±0.56[b]	45.80±3.36[a]	79.10±2.73[ab]	-1.12±0.05[a]	15.56±2.21[a]
RRIM 2002	68.16±3.48[c]	4.28±0.31[b]	45.75±3.68[a]	80.72±4.87[a]	-1.18±0.06[a]	15.16±1.28[a]
PB 350	139.03±22.48[b]	4.20±0.27[b]	45.67±2.95[a]	80.97±6.47[a]	-1.29±0.07[a]	15.20±1.78[a]
PB 260	134.87±27.56[b]	4.26±0.62[b]	42.67±5.55[c]	77.76±2.98[b]	-0.55±0.03[b]	15.54±3.11[a]

TSS, total soluble solid. Values are expressed as mean±SD. Values with different superscripts within the same column are significantly different ($p < 0.05$).

leaf clones that other rubber leaf clones can reduce the weight and affect the texture of glutinous rice tapai.

3.2.2 Colour profile

Table 6 also reveals that there is no significant difference in the colour profile of glutinous rice tapai in containers and all tapai wrapped in rubber leaf clones except tapai in the container and wrapped in PB 350 found to be higher than other tapai samples. The findings indicate that the colour of the tapai is grey and yellow. However, Ramos *et al*. (2011) reported that yeasts *R. mucilaginosa* can produce pink or red pigments and can cause discolouration to the foods. *R. mucilaginosa* was produced in the tapai that wrapped in rubber leaf RRIM 2025 and RRIM 2002 as indicates in the results, but it still does not give any significant differences in values of a*.

3.2.3 Total soluble solids

Table 6 illustrates that there were significant differences in total soluble solids between tapai wrapped in rubber leaf and tapai in the container. There are also significant differences in °Bx values between tapai wrapped in rubber leaf clone PB 260 compared to other clones. The highest °Bx was found in tapai that was wrapped in rubber leaf RRIM 2025 which was 45.8 and there are no significant differences in °Bx value between tapai that wrapped in RRIM 2025, RRIM 2002 and PB 350. Meanwhile, tapai that wrapped in rubber leaf clones PB 260 indicates the lowest Bx° value, which was 42.67 and tapai in the container has a significant difference between all the tapai that was wrapped in rubber leaf.

The presence of the yeasts was able to hydrolyse more carbohydrates in simpler forms than the yeast that was presented alone (*C. parapsilosis*) in PB 260, therefore unable to generate a high °Bx value than other tapai, wrapped in other rubber leaves with several forms of yeasts. In contrast, tapai in containers, also containing one type of yeast, *C. guilliermondii* are usually used as food additives. Azmi *et al*. (2010) suggested that different yeasts strain capable of hydrolysing carbohydrate but at different rates. Previously, *ragi* tapai had been reported to produce the highest concentration of glucose rather than other yeasts strain such as *S. cerevisiae* and *C. tropicalis* that were maintained at low glucose concentration which is below 7 g/L of glucose at 72 hrs fermentation. Thus, it was the reason for the high production of glucose concentration in tapai which was using ragi as starter cultures.

Gandjar (2003) stated that the declines or low level of sugar could be due to the activity of mould Mould on the tapai has strong amylolytic properties and can degrade carbohydrates into simple sugars from glutinous rice which is then broken down by yeast into alcohol compounds. Cronk *et al*. (1977) reported that total soluble solids of tapai usually decreased by 50% in 192 hrs. Thus, there was a decrease in the total soluble solid content after 72 hrs fermentation.

3.2.4 pH analysis

Tapai in a container exhibited a significantly ($p<0.05$) high pH value (4.71) compared to other tapai wrapped in rubber leaf (Table 6). However, there are no significant differences in pH between the tapai that are wrapped using different rubber leaf clones. This result also shows that the microorganism presented before the production of tapai, which affects the final production of tapai in the rubber leaf itself, suggesting different microorganisms were presented in tapai in containers and tapai that are wrapped in rubber leaf since there are not many yeasts found in tapai in containers rather than tapai that are wrapped in rubber leaf. The decrease in the pH value could be also caused by the conversion of hydrogen ions. Akin *et al*. (2008) reported that the pH decrease is assumed to be correlated to nitrogen consumption. It is known that during the fermentation, the consumption of nitrogen by yeasts produces H+ ions which were released in solution. Since there is only one type of yeast present in tapai fermented in containers, it cannot release as many H+ ions as the other yeasts that numbers in other tapai wrapped in rubber leaf. The reasons for the reduced number of pH value in the tapai wrapped in rubber leaf which is in concert with a study by Jakobsen and Narvhus (1996), who stated the yeasts

produced can support the starter culture function and inhibit the spoilage organisms. Thus, the yeasts presented before the fermentation of tapai could support the starter culture function in increasing the fermentation performances. The different types of wrapper can bring different microorganisms that later can affect the physical attributes in the final production. Yeasts that were present before the fermentation in the rubber leaf were suspected of supporting starter cultures in increasing the hydrogen ions and improving the fermentation performances.

4. Conclusion

This study revealed that there are different types of yeast can be found in different clones of rubber leaves. *Crytococcus laurentii* was found in RRIM 2025 and PB 260, *Rhodotorula mucilaginosa* 2 was found in RRIM 2002 and PB 350, *Candida famata* was found in RRIM 2002 and PB 260, and *R. minuta* was found in PB 350. *C. lauretti*, *C. famata* and *R. minuta* were found in rubber leaf due to their habitat and some are due to application in protecting the rubber leaf. The presence of the yeast helps to produce a better glutinous rice tapai. The physical properties of the tapai that are wrapped and unwrapped have a difference in texture, pH value and Total soluble solids content. The existence of yeasts prior to the start of the fermentation process is believed to promote starter cultures to enhance fermentation efficiency.

Conflict of interest

The authors declare no conflict of interest.

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