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Fermentation of Tempoyak Using Isolated Tempoyak Culture

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Abstract: The present study reported on the isolation of tempoyak inoculum, pasteurization of durian and tempoyak and microbiological and chemical changes in for pasteurized durian, pasteurized durian inoculated with tempoyak inoculum and natural tempoyak. The isolated dominant lactic acid bacteria (LAB) was grown in MRS broth and inoculated into pasteurized durian. The isolated tempoyak inoculum has the LAB properties i.e. the ability to grow in MRS agar, plate count agar and potato dextrose agar, catalase-negative, gram-positive, rod shaped and may occur in chains. The growth of tempoyak inoculum in MRS broth achieved stationary phase after 24 h. Fermentation of three samples of tempoyak were studied i.e. natural tempoyak (NT), pasteurized durian inoculated with tempoyak inoculum (PDWI) and pasteurized durian (PD). For PDWI, the final LAB count achieved was $\log 9.1 \text{ cfu g}^{-1}$, which is slightly lower than in NT. After 6 days of fermentation, the final pH value of PDWI is 4.02 and lactic acid content is 2.75%. Meanwhile the pH value of NT was 4.14 and the lactic acid content was 2.36%. The lower acidity in NT might be due to the presence of heterofermentative lactic in the pasteurized durian. D_{50} and F_{50} for pasteurization of durian were 4.5 and 32.5 min, respectively. For pasteurization of tempoyak, D_{50} and F_{50} were 4.5 and 30.2 min, respectively. Pasteurization of durian and tempoyak at 60°C and 70°C is not possible because durian and tempoyak became viscous, due to evaporation of water.

Key words: Durian, tempoyak, fermentation, pasteurization, lactic acid bacteria

Introduction

Tempoyak is a fermented condiment prepared from durian (*Durio zibethinus*) pulp. Tempoyak is normally prepared from excess, poor quality or over-ripe durian (Gandjar, 2000). It is prepared by mixing durian pulp with or without salt and placing in a sealed container (Merican, 1977). Usually low amount salt is added, approximately 1.3% (Suan, 1996), for making tempoyak to add flavour, to inhibit pathogen as well as to stimulate the growth of lactic acid bacteria. The fermentation process will take about 7 days. The pH value of tempoyak ranging from 3.8-4.6 (Merican, 1977). Therefore tempoyak has sour taste, salty and has distinct durian smell (Gandjar, 2000). It is usually eaten freshly or used to cook other dishes such as sambal, curry, etc. Unfortunately, in Malaysia, tempoyak is only available during durian season at the wet market or night market, as it has not been commercialized in a large scale yet.

Usually tempoyak has long shelf life because it is preserved by lactic acid, which is produce by lactic acid bacteria and the salt, which is added during processing will inhibit undesirable competitors of the lactic, e.g. proteolytic bacteria and aerobic and anaerobic sporeformers (William and Dennis,

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1988). Earlier study showed that LAB are the predominant microorganisms in tempoyak (purchased from night market) and *Lactobacillus plantarum* was the predominant group in LAB flora (Leisner, 2001).

Tempoyak is one of the popular Malaysian traditional fermented foods but until now very little research has been reported about it. So far, there is no study on isolation of tempoyak culture, pasteurization of durian/tempoyak and fermentation of tempoyak using tempoyak inoculum. Besides contributing new knowledge, the results from this project are valuable in producing standard commercial tempoyak with uniform and high quality. The isolated tempoyak inoculum also can be studied further for its properties especially the microbial inhibitory of its lactic acid.

The objectives of the present study are to isolate tempoyak inoculum, to study tempoyak fermentation in inoculated and non-inoculated durians and to study the pasteurization of durian and tempoyak.

Materials and Methods

Local durians were purchased from the plantation in Segamat, Johor, Peninsular Malaysia. The flesh of durian was separated from the seed, mixed with mixer and placed in a clean container. The durian pulp was then mixed using food mixer and was kept in freezer until use. Microbiological media were purchased from Oxoid and other chemicals were of analytical grade.

Isolation of Tempoyak Inoculum

The fresh durian pulp was mixed with 1% of salt in a sealed container. The durian was left to ferment for 7 days. LAB was plated on MRS agar everyday by streak plate method and incubated under anaerobic condition (candle jar) at room temperature for 7 days for purposely observation. The DB was then examined microscopically and tested for catalase reaction with H₂O₂.

Streak Plate Method

About 25 g of tempoyak was mixed with 225 mL sterile 0.85% (w/v) saline water in duplicate. The mixture was shaken to distribute organisms uniformly. Serial dilution was carried out until the dilution factor of 10⁻⁸. 0.1 mL of samples was streak on MRS agar. Incubation was done under anaerobic conditions in candle jar at room temperature for 1 to 4 days.

Determination of Growth Curve of Tempoyak Inoculum

One pure colony of dominant bacteria (DB) at day 7 was transferred from MRS agar to 100 mL of MRS broth. Incubation was done under anaerobic conditions in candle jar at room temperature. LAB counts were carried every 2 h for 30 h. LAB count versus time was plotted to determine the growth curve of dominant bacteria. the objective of this experiment is to determine the time needed to reach the initial stationary phase (or the end of log phase) for this bacteria.

Preparation of Inoculum

One colony of dominant bacteria (DB) from pure culture was transferred to 100 mL of MRS broth and incubated under anaerobic condition at room temperature for 24 h (the time needed for the population to reach stationary phase as determined earlier)

Pasteurization of Durian

Six hundred gram of frozen durian pulp was thawed and divided into three portions and was pasteurized at 50, 60 and 70 °C for 30 min, respectively. Samples for determining total plate counts (TPC), yeast and mould count (YMC) and lactic acid bacteria (LAB) counts were taken every 5 min

in duplicate. The timing was counted when the internal temperature of durian reached 50, 60 and 70°C, respectively.

The data obtained were plotted as log bacteria count versus time (min). At a certain temperature, D value is calculated by fitting a linear regression to the above plot was fitted to the data. The linear equation ($y=mx+c$) obtained is used to calculate D value. D value is the value of x when y is equal to 1 (90% of the population killed or 1 log cycle). F value at a certain temperature were calculated using the following formula:

$$\begin{aligned} \text{F value: } F_0 &= D_r (\log a - \log b), \\ \text{where } D_r &= \text{D value of a population of microorganism} \\ a &= \text{the number of cells in the initial population} \\ b &= \text{the number of cells in the final population} \end{aligned}$$

Preparation of Tempoyak Samples

Three samples of tempoyak were used i.e. natural tempoyak (NT), pasteurized durian inoculated with tempoyak inoculum (PDWI) and pasteurized durian without any inoculum added (PD). To prepare these tempoyak samples, the starting material (durian flesh) must be homogeneous to avoid any difference in microflora. Durian flesh was mixed with food mixer and then divided onto three portions. For preparing NT samples, the homogeneous durian flesh was kept in a closed bottle and left to ferment at room temperature. For preparing PDWI samples, the homogeneous durian flesh was pasteurized, then mixed with the isolated tempoyak inoculum before it was fermented in a closed bottle at room temperature. For preparing PD samples, the the homogeneous durian flesh was pasteurized and left to ferment at room temperature.

Total Plate Count (TPC)

Twenty five gram of tempoyak was mixed with 225 mL sterile 0.85% (w/v) saline water in duplicate. The mixture was shaken to distribute organisms uniformly.

Serial dilution was carried out until the dilution factor of 10^{-8} . Petri plates and tubes and bottles were labeled with the sample number, dilution, date and media. Sample of 0.1 mL was pipetted and spread on PCA. Incubation was done at 35°C over a period of 48 ± 3 h under aerobic and anaerobic condition. Plates containing 25–250 colonies were counted.

Lactic Acid Bacteria Count (LABC)

DeMan, Rogosa, Sharpe Agar (MRS) was used to grow LAB. The procedure used was the same as in TPC. Incubation was carried out under anaerobic conditions in candle jar at room temperature for 24 to 48 h. Plates containing 25–250 colonies were counted.

Yeast and Mould Count (YMC)

PDA was used to grow yeast and mould. The procedure used was the same as in TPC. Incubation was carried out at room temperature under aerobic and anaerobic condition. Plates containing 25–250 colonies were counted at the end of 5 days incubation.

Determination of Lactic Acid Content

Total acidity was expressed as % lactic acid. Tempoyak (10 g) was mixed with 10 mL of distilled water into an Erlenmeyer flask and boiled to drive off the CO_2 . The flask was then cooled and 5 drops of 1% phenolphthalein was added to the sample. The sample was titrated to the first pink colour with 0.1N NaOH. Percentages of lactic acid is calculated as follows:

$$\begin{aligned} \% \text{ lactic acid} &= \frac{\text{mL of alkali} \times \text{normality of alkali} \times 9}{\text{Weight of sample in g}} \end{aligned}$$

Determination of pH

About 10 to 20 mL of distilled water was added to 100 g of sample. Temperature of the prepared paste was adjusted to 25°C. The electrodes were immersed in the sample and the pH reading was taken after allowing the meter to stabilize for 1 min.

Pasteurization of Tempoyak

Tempoyak was pasteurized in water bath with the internal temperature at 50°C for 30 min. The LAB count, yeast and mould count and total plate count was carried out for every 5 min internal in duplicate. The data obtained were plotted as log bacteria count versus time (min). D values and F values were calculated as mentioned earlier in the subtopic of pasteurization of durian.

Results and Discussion

Isolation of Tempoyak Culture

Daily observation of tempoyak colony in MRS agar plate (day 1–7) shows that the isolated dominant bacteria started to be dominant on day-3. The isolated dominant bacteria was Gram-positive, rod shape and form chains. Besides that, it was catalase negative and it can grow in anaerobic condition at room temperature.

Most probably these isolated dominant bacteria was LAB. MRS agar is generally used for cultivation of Lactobacilli and other lactic acid bacteria. A work carried out by Leisner *et al.* (2001) also showed that lactic acid bacteria (LAB) were the predominant microorganisms in tempoyak, ranging from log 8.4 to log 9.2 cfu g⁻¹ and no other microorganisms were present to such a level.

It is not surprising that LAB are present in high levels in tempoyak due to the chemical composition of the durian fruit with total sugar of 15–20% (Ketsa and Daengkanit, 1998) and saccharose of 17% which may favour LAB and other saccharolytic microorganisms.

Growth Curve of Dominant Bacteria

Figure 1 shows the growth curve of the isolated dominant bacteria from tempoyak in MRS broth at room temperature. The isolated dominant bacteria are at lag phase for the first 6 h in MRS broth (under anaerobic condition) and achieved log phase and stationary phase at 8 h–24 h, respectively.

During lag phase, the growth usually does not begin immediately but only after a period of time. It is because when bacteria is adapting to new environmental condition, enzymes need to be synthesized to utilize the nutrients present and cells are increased in size before the division can occur (Garbutt, 1997).

During log phase, after 6 h time, the growth increase drastically. However, the rapid increase in numbers does not occur indefinitely and the population passes into the stationary phase after 24 h of incubation.

The growth curve obtained are as expected for any microbial growth which consists of four phase, i.e., lag, log or exponential, stationary and death. However, the lag phase was not noticeable from the figure.

Pasteurization of Durian

Initially, pasteurization was carried out at 60°C and 70°C the timing was started only when the internal or the center of durian reaches those temperatures. However, pasteurizing durian at 60 and 70°C even at 0 min resulted in no total plate count (TPC), yeast and mould count (YMC) and lactic acid bacteria (LAB) growth.

Table 1: D₅₀ and F₅₀ values of durian microbial population grown on PCA, PDA and MRS agar.

Media	D ₅₀ (min)	F ₅₀ (min)
PCA	4.5	32.5
PDA	4.3	31
MRS agar	4.6	32.9

This shows that the microbes in durian was susceptible to heat treatment. Besides that the durian became viscous and evaporation of water starts to occur at those temperatures. Pasteurization will decrease the water activity of the durian as the water evaporated and will increase the viscosity of durian. Thus, pasteurization has to be carried out with cautious and the correct period of pasteurization is crucial. Because of this problem, lower temperature was used to study pasteurization of durian, i.e. 50°C.

Figure (2-4) shows TPC, YMC and LABC for pasteurization of durian at 50°C. The initial bacteria count was very similar in PDA, PCA and MRS agar. As expected, the TPC, YMC and LABC decreased with time. For all media, bacteria decreased from log 7.1 cfu g⁻¹ to 0 within 30 min.

Table 1 shows the calculated D values and F values for TPC, YMC and LABC. F values and D values of durian is lower compared to sauerkraut (73.9°C) and cucumber (75°C). This fact is expected as durian, sauerkraut and cucumber have different physical properties that will affect their thermal properties as well as evaporation of water from the product.

Total Plate Count (TPC)

Figure 5 shows the TPC of the tempoyak prepared from pasteurized durian and inoculated with tempoyak inoculum (PDWI), pasteurized durian (control - PD) and natural fermented durian (NFT).

As expected, pasteurized durian showed no growth throughout the 6-day fermentation. It is expected that most of the microbes will be destroyed after pasteurization. TPC for PDWI ranges from log 1 to log 8.9 cfu g⁻¹. There was a drastic increase from day 0 to day 5 and began to decline afterwards. Formation of gas only appeared after 2 days of fermentation. Meanwhile TPC for NFT ranges from log 7.35 to log 9.55 cfu g⁻¹. The bacteria showed drastic increase from day 0 to day 4 but start to decline afterwards. The initial TPC for NFT (log 7.35 cfu g⁻¹) was higher than PDWI (log 1 cfu g⁻¹). This is expected as the natural microorganisms were destroyed by pasteurization in PDWI.

The bacteria in PDWI increased much rapidly than in NFT but the total number of bacteria in PDWI is lower than in NFT. The lag phase was not noticeable in PDWI.

The TPC plates showed uniform colonies throughout the 6-day fermentation, both under aerobic and anaerobic condition. However there was some difference in colony size and colony color. The bacteria tested was catalase negative, gram positive, rod shape and form chain. Leisner *et al.* (2001) had suggested that the small, white, catalase negative colonies carried out from tempoyak, which able to grow under aerobic conditions on PCA plate were actually LAB.

Yeast and Mould Count (YMC)

Figure 6 shows the YMC of the tempoyak prepared from pasteurized durian and inoculated with tempoyak inoculum (PDWI), pasteurized durian (control - PD) and natural fermented durian (NFT). YMC and TPC of the three samples have a very similar trend. This could be to the absence of any antibiotics in the PDA, resulting in the growth of lactic acid bacteria (able to grow at low pH) as well in PDA. YMC for PDWI ranges from log 1 to log 9.0 cfu g⁻¹. Drastic increase started from day 0 to day 5 and began to decline afterwards. As expected, for control durian (PD), there was no bacteria growth on PCA. On the other hand, for NFT, TPC ranges from log 8.05 to log 9.45 cfu g⁻¹. The bacteria showed little change from day 0 to day 6. As expected the initial YMC for PDWI was very low as compared to NFT, because of pasteurization effect.

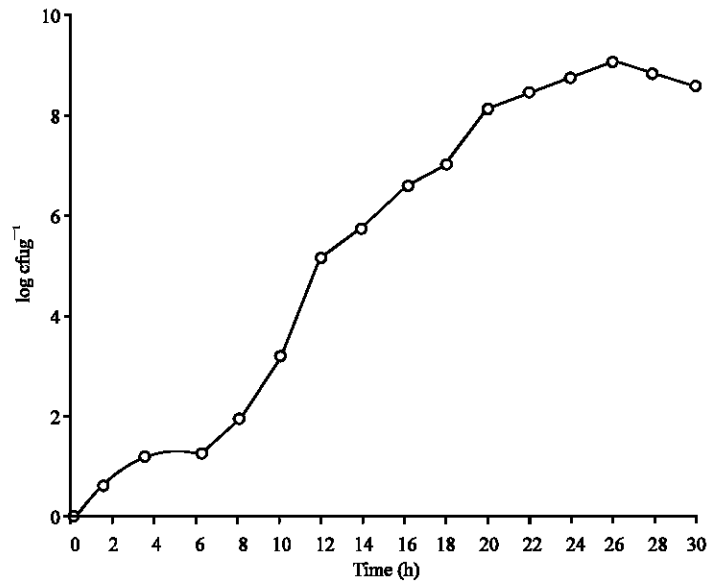


Fig. 1: The growth curve of the isolated dominant bacteria from tempoyak in MRS broth at room temperature

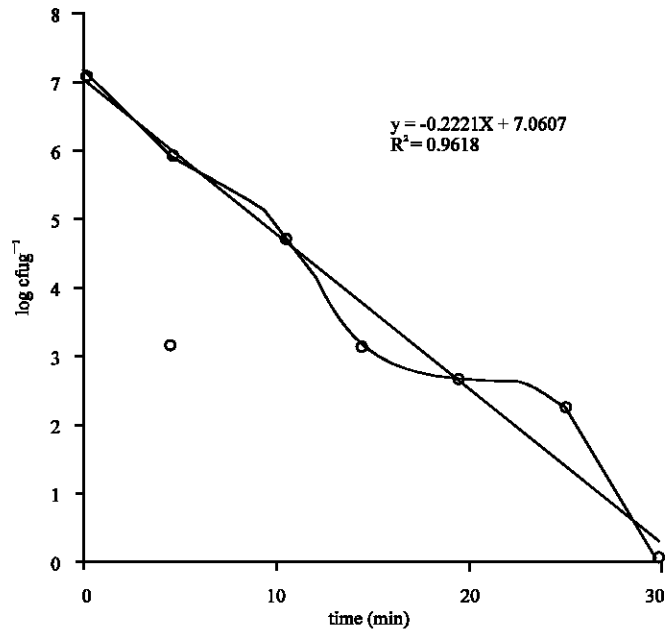


Fig. 2: Total plate count for pasteurization of durian at 50°C

The PDA plates, uniform colonies throughout the 6 day fermentation, like PCA plate. The microorganism on PDA plates can grow under aerobic and anaerobic condition but it is much smaller compared to yeast and mould. Besides that, the bacteria was proved to be catalase negative, gram positive, rod shape and form chains. In addition, the colonies from PDA plates can grow on MRS agar under anaerobic condition. This shows that the microorganisms on PDA plates are the same microorganism grown on MRS agar.

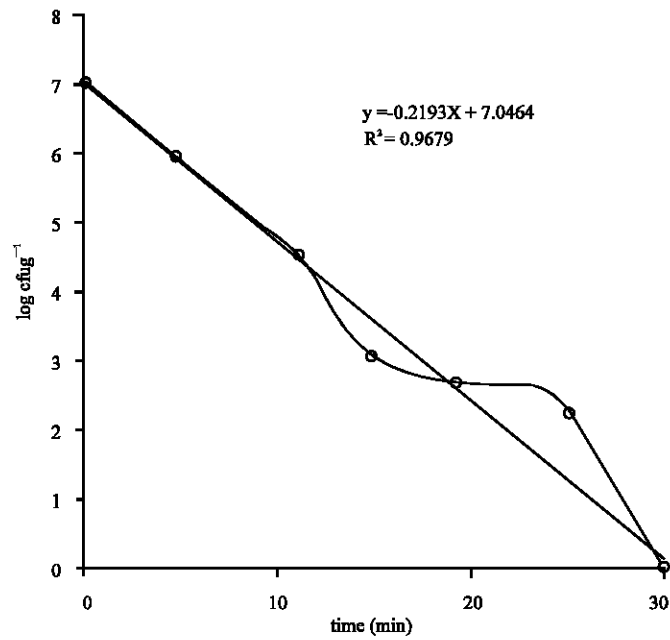


Fig. 3: Lactic acid bacteria count for pasteurization of durian at 50°C

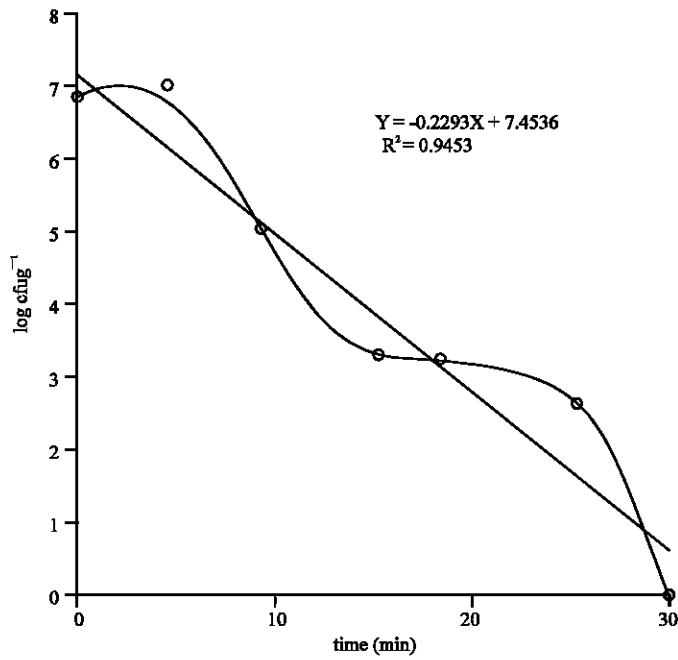


Fig. 4: Yeast and mould count for pasteurization of durian at 50°C

Figure 7 shows the LABC of the tempoyak prepared from pasteurized durian and inoculated with tempoyak inoculum (PDWI), pasteurized durian (control - PD) and natural fermented durian (NFT).

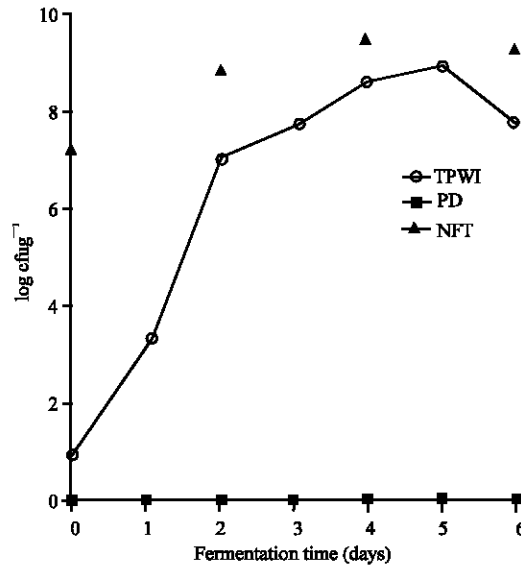


Fig. 5: Total plate count of PDWI, PD and NFT throughout the 6-day fermentation

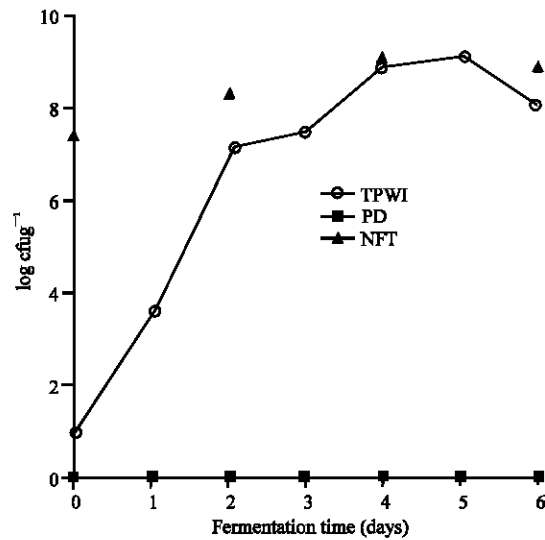


Fig. 6: Yeast and mould count of PDWI, PD and NFT throughout the 6-day fermentation

LABC, TPC and YMC have very similar trend. LABC for PDWI ranges from log 1 to log 9.1 cfu g⁻¹. From day 0 to day 5, there was a drastic increased. However, LABC decline on the 6th day. For control durian (PD), there was no bacteria growth on PCA as expected. For NFT, LABC ranges from log 7.50 to log 9.25 cfu g⁻¹.

The MRS plates also showed uniform colonies for 6 days during the fermentation as for PCA and PDA plates. This microorganism is facultative anaerobic but grows better under anaerobic condition. The bacteria are catalase negative, gram positive, rod shape and form chain.

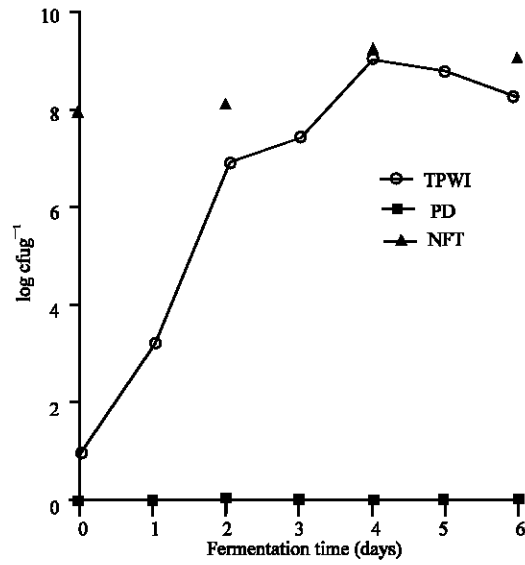


Fig. 7: Lactic acid bacteria count of PDWI, PD and NFT throughout the 6-day fermentation

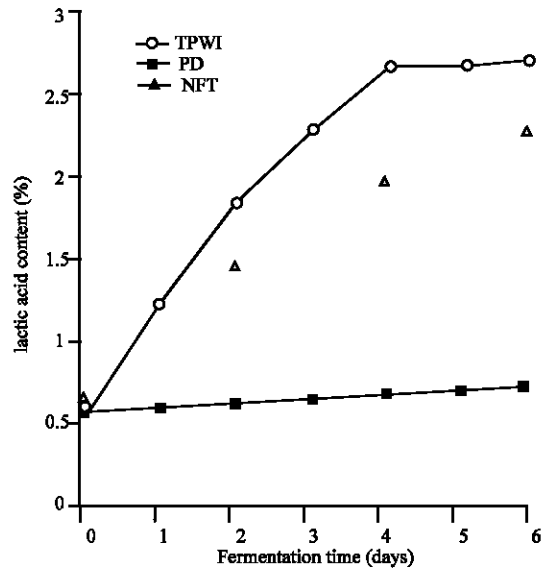


Fig. 8: Lactic acid content of PDWI, PD and NFT throughout the 6-day fermentation

These results shows that the tempoyak culture is able to grow equally well in MRS agar, PCA and PDA. To ensure only yeast and mould will grow in PDA, some antibiotics should be added as well or alternatively other medium such as dichloran Rose Bengal chloramphenicol.

Lactic Acid Content (LA)

Figure 8 shows the LA of the tempoyak prepared from pasteurized durian and inoculated with tempoyak inoculum (PDWI), pasteurized durian (control - PD) and natural fermented durian (NFT).

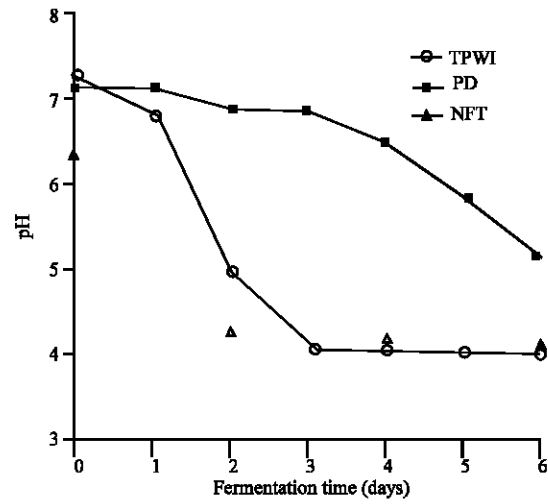


Fig. 9: pH values of PDWI, PD and NFT throughout the 6-day fermentation

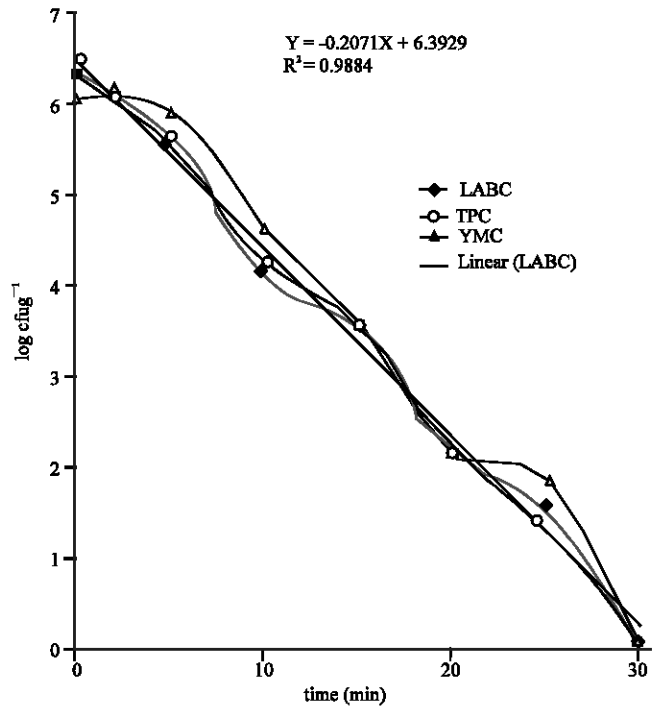


Fig. 10: Total plate count, yeast and mould count and lactic acid bacteria count for pasteurization of tempoyak at 50°C

In general, the highest LA was achieved by PDWI, followed by NFT and PD. LA for PDWI ranges from 0.61 to 2.75%. Drastic increase of LA start from day 0 to day 4 and LA is constant afterwards. LA in NFT increased from 0.71 to 2.36%. For control durian (PD), there was little increase in LA ranges from 0.588 to 0.758%.

The increasing of LA was in line with the growth rate of lactic acid bacteria. The total acidity of 3.6% in the final product expressed as acetic acid had been observed by Merican (1977). Meanwhile Leisner *et al.* (2001) showed that the total acidity of 2.70%, which is expressed as lactic acid (1.89%) and acetic acid (0.81%).

PDWI shows the highest LAC after 6 days of fermentation if compare to NFT although the LAB in NFT is higher than in PDWI. This might be because the isolated tempoyak inoculum are homofermentative lactic and produce mainly lactic acid. Meanwhile, for NFT, there are a mixture culture by heterofermentative and homofermentative lactic. Heterofermentative lactic produce lactic acid plus appreciable amounts of ethanol, acetate and carbon dioxide, thus, reduced the production of lactic acid.

pH

Figure 9 shows the pH of the tempoyak prepared from pasteurized durian and inoculated with tempoyak inoculum (PDWI), pasteurized durian (control - PD) and natural fermented durian (NFT). The pH value for PDWI dropped from 7.28 to 3.98. The drastic decrease of pH only start from day 1 to day 3 and became constant afterwards. For control durian (PD), pH was decreasing from 7.18 to 5.14. Although the pH was decreased, we noticed that PD did not have sour taste like tempoyak. For NFT, pH ranges from 6.54 to 4.14 after 6 days of fermentation. The drastic decrease occurred from day 0 to day 2 and afterwards there was very little changes.

Leisner *et al.* (2001) reported that the initial pH of durian was 6.7. The final pH values of 3.8 – 4.6 in tempoyak have been observed (Merican, 1977; Steinkraus, 1996). The final pH value for PDWI was 4.0 which is in the range reported by Merican (1977) for tempoyak.

The pH values of NFT, is in agreement with LABC, but is not proportional with lactic acid content. The drastic decrease of pH after day 0 might be due to product other than lactic acid such as acetic acid and carbon dioxide produced by heterofermenters LAB.

Pasteurization of Tempoyak

Figure 10 shows total plate count (TPC), yeast and mould count (YMC) and lactic acid bacteria count (LABC) for pasteurization of durian at 50°C. As expected, the TPC, YMC and LABC decreased with time. Bacteria decreased from log 6.3 cfu g⁻¹ to log 0 cfu g⁻¹ for TPC, from log 6.3 to log 0 cfu g⁻¹ for LABC and from log 6 to log 0 cfu g⁻¹ for YMC within 30 min.

LAB needed a period of 4.8 min for the bacteria to pass through a log cycle (90% of the population killed). Therefore the time needed to kill a population of cell or spores, F value, is 30.2 min. From the D values and F values obtained, this shows that pasteurization of tempoyak could be achieved by heating at 50°C for 30.2 min.

Conclusions

In conclusion, the isolated dominant bacteria in tempoyak was Gram-positive, rod shape, form chains, catalase negative and facultative bacteria. The growth of tempoyak inoculum in MRS broth achieved stationary phase after 24 h. D₅₀ and F₅₀ for pasteurizing durian are 4.5 and 32.5 min, respectively. Meanwhile, D₅₀ and F₅₀ for pasteurizing tempoyak are 4.8 and 30.2 min, respectively. For PDWI, the final LAB count achieved was log 9.1 cfu g⁻¹, which is slightly lower than in NT. After 6 days of fermentation, the final pH value of PDWI is 4.02 and lactic acid content is 2.75%. Meanwhile the pH value of NT was 4.14 and the lactic acid content was 2.36%. The lower acidity in NT might be due to the presence of heterofermentative lactic in the pasteurized durian. D₅₀ and F₅₀ for pasteurization of durian were 4.5 and 32.5 min, respectively. For pasteurization of tempoyak, D₅₀ and F₅₀ were 4.5 and 30.2 min, respectively. Pasteurization of durian and tempoyak at 60°C and 70°C is not possible because durian and tempoyak became viscous, due to evaporation of water.

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