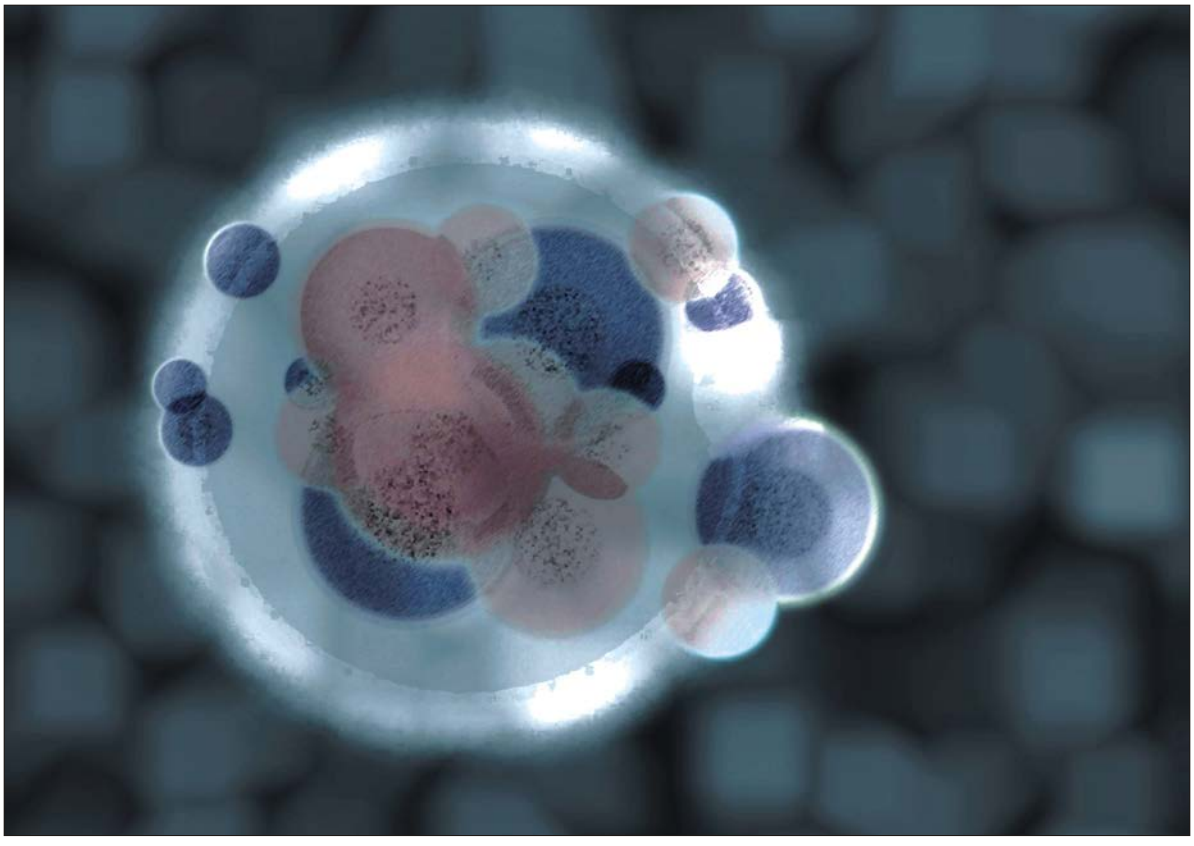

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 4 (5): 650-653, 2004
ISSN 1727-3048

Effect of Salt on Tempoyak Fermentation and Sensory Evaluation

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Abstract: This project looks into the effect of addition of salt in tempoyak formulation on tempoyak fermentation and its acceptability. In this study, tempoyak with 0, 1, 2 and 3% salt, respectively, were analyzed in terms of total plate count, lactic acid bacteria count, lactic acid content, pH and acceptability by sensory panelists. The fermentation was carried out for 10 days at room temperature. Generally, total plate count and lactic acid bacteria count shows a similar trend where initially there was an increase to a maximum in microbial count and then a decrease afterwards. Tempoyak with 1% salt showed the highest total plate count and lactic acid bacteria count, followed by tempoyak at 2, 0 and 3%, respectively. For lactic acid content, tempoyak with 1% salt showed the highest lactic acid content, followed by tempoyak with 2, 0 and 3% salt, respectively. The final pH obtained in all tempoyak samples is 3.96 to 4.08. For sensory evaluation, tempoyak with 2% of salt was most preferable by the panelists. Statistical analysis shows a significant difference among tempoyak with 0, 1, 2 and 3% of salt for all the attributes, except for salty attribute.

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

INTRODUCTION

Tempoyak is a fermented condiment prepared from durian (*Durio zibethinus*) flesh or aril. Tempoyak is normally prepared from excess, poor quality or over-ripe[1]. Tempoyak can be prepared with or without salt. Usually low amount of salt (1.3%)[2] is added to support the growth of lactic acid bacteria, in addition to other saccharolytic microorganisms such as yeast. However, the salt cannot be added in excess because it may make tempoyak very salty. Tempoyak is prepared by mixing the durian pulp with salt and left to ferment at room temperature for 3-7 days. Tempoyak is usually eaten fresh with rice or added into the cooking dishes[1]. It has the distinctive durian smell and a creamy yellow colour. The taste of tempoyak is sour and salty, with sour being the dominant taste[1]. Nowadays, tempoyak is hardly sold in supermarket. It is normally found in night market or wet market during durian season only because it is still treated as a cottage industry. The total acidity of tempoyak is around 3.6% as acetic acid and its final pH values is 3.8-4.6[3,4]. Suan[2] reported that tempoyak has 2.0% ash, 67% moisture, 4.5% total sugar, 2.5% crude fibre and 1.4% fat.

Until now, very little study has been done on tempoyak. Recent study reported that analysis of tempoyak samples obtained from various night markets in Malaysia showed that Lactic Acid Bacteria (LAB) are the dominant microorganisms in tempoyak, with *Lactobacillus plantarum* as the predominant group[5].

This objective of this project was to study the effect of different levels of salt on tempoyak fermentation with 0, 1, 2 and 3% of salt in tempoyak, respectively, in terms of total plate count, lactic acid bacteria count, lactic acid content, pH and sensory evaluation. The data obtained hopefully, will give valuable information on tempoyak fermentation. With this information in hand, it is hoped that tempoyak will become a commercial product in the future.

MATERIALS AND METHODS

Durian were purchased from a plantation in Segamat, Johor, Malaysia. Microbiological media were purchased from Merk and other chemicals are of analytical grade.

Preparation of tempoyak samples: Four preparations of tempoyak with different level of salt were prepared i.e., without salt, 1, 2 and 3% salt. The durian pulp was mixed with salt using a food mixer before it was placed in an air tight container at room temperature for ten days. The analysis was carried out every two days in terms of Total Plate Count (TPC), Lactic Acid Bacteria Count (LABC), lactic acid content, pH reading. All microbiological analysis were carried out using pour-plate method.

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Sensory evaluation was carried out only once after ten-day fermentation. All analysis were carried out in duplicate.

Total Plate Count (TPC): TPC was carried out using plate count agar (PCA). Tempoyak samples of 25 g was used and serial dilution was done from a dilution factor 10^{-2} until 10^{-9}. Then 1 mL sample from each dilution was pipetted into petri plates in duplicate. Then around 15 mL of PCA was poured into petri plates containing the diluted samples. The plate content was mixed by gently swirling clockwise and then counter clockwise. After the agar solidify, the plates were inverted and placed in the incubator. The plates were incubated at 35°C over a period of 24±3 h. Plates containing colonies between 25-250 were counted.

Lactic Acid Bacteria Count (LABC): DeMan, Rogosa, Sharpe agar (MRS agar) was used to determine LABC. The procedures used were the same as in TPC. The incubation was carried out under anaerobic conditions in a candle jar at room temperature for 24-48 h. Plates containing 25-250 colonies were counted.

Determination of lactic acid content: Lactic acid content was determined by weighing 10 g of tempoyak or durian sample into an Erlenmeyer flask and 10 mL of distilled water was added. Sample was boiled to drive off the CO_2 and cooled. Then 5 drops of 1% phenolphthalein were added to the sample. The sample was titrated with 0.1N NaOH to pink colour. Total acidity content was expressed as % lactic acid.
% lactic acid is calculated as follows:

$$\%\,\text{lactic acid} = \frac{\text{mL of alkali x normality of alkali x9}}{\text{Weight of sample ing}}$$

Determination of pH: Ten to 20 mL of distilled water was added to 100 g of sample. The 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.

Sensory evaluation: Four tempoyak samples i.e., tempoyak with 0, 1, 2 and 3% of salt after 10 day fermentation were evaluated. Thirty Malay panelists were selected randomly. Each panelist was given 20 g plain rice served with 10 g tempoyak.

RESULTS AND DISCUSSION

Total Plate Count (TPC): Figure 1 shows TPC of tempoyak in different levels of salt during fermentation. TPC for all tempoyak samples ranges from log 7.25 to log 9.55 cfu g^{-1}. Generally, TPC for all tempoyak at different salt levels showed the same trend. There was an increase in TPC from day 0 until it reaches maximum at day 4 (for all the tempoyak) to day 6 before it starts to decline afterwards. The trend obtained is 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 Fig. 1. Tempoyak with 1% salt showed the highest TPC, followed by tempoyak at 2, 0 and 3%, respectively. The low TPC in tempoyak with 3% salt might be due to the intolerance of microorganism to salt.

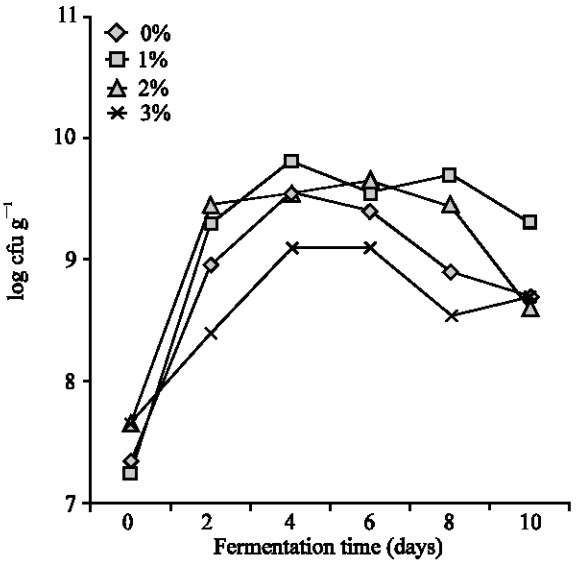

Fig. 1: Total plate count of tempoyak with different levels salt during fermentation

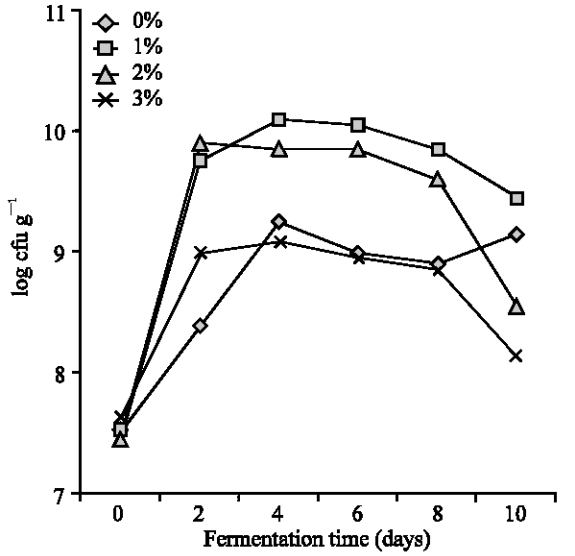

Fig. 2: Lactic acid bacteria count of tempoyak with different levels of salt during fermentation

Lactic Acid Bacteria Count (LABC): Figure 2 shows LABC of tempoyak with different levels of salt during fermentation. The results showed that LABC for all tempoyak samples ranges from log 7.45 to log 10.10 cfu g^{-1}.

Generally, LABC for all tempoyak at different salt level shows almost the same trend. There was an increase in LAB from day 0 until it reaches maximum at day 2 (for tempoyak with 2 and 3% salt) or day 4 (for tempoyak 0 and 1% salt). Tempoyak with 1% salt showed the highest LABC, followed by tempoyak with 2, 0 and 3% salt, respectively. This shows that tempoyak with 1 and 2% of salt are more favourable for LAB growth compared to tempoyak with 3% of salt. According to Pederson and Albury[6], a low salt concentration of 1% is more advantageous to the growth of the heterofermentative lactic whereas a high concentration of 3.5% is more detrimental to their growth than to the growth of the homofermentative lactic in sauerkraut.

TPC also shows the same phenomenon. This could be due to the fact that both PCA and MRS agar supporting the growth of the same microorganism i.e., LAB. According to Leisner *et al.*[5], nearly all the colonies of tempoyak on PCA plates were LAB. LAB constitutes the predominant element of the microbial flora in final products of tempoyak.

The presence of LAB in high levels in these products might be due to the chemical composition of the durian fruit with 15-20% total sugar[7] and 17% saccharose which may favour the growth of LAB and other saccharolytic microorganisms[5].

Lactic acid content: Figure 3 shows the lactic acid content in tempoyak with different level of salt during fermentation. Generally, the lactic acid content is either increasing or constant from the day 0 to day 10. Tempoyak with 1% salt showed the highest lactic acid content, followed by tempoyak with 2, 0 and 3% salt, respectively.

The initial content of lactic acid ranges from 0.71 to 0.89% while the final lactic acid content ranges from 2.8 to 3.64% for all tempoyak.

The highest lactic acid content up to 3.65% is showed by 1% salt concentration tempoyak. The lactic acid content is in agreement with LABC. The increase in LABC will lead to the increase in lactic acid content.

This result is in agreement with previous study[4,5]. The total acidity of 3.6% in tempoyak expressed as acetic acid has been observed by Merican[4]. Meanwhile Leisner *et al.*[5] reported that the total acidity of tempoyak was 2.7%, which expressed as lactic acid (1.89%) and acetic acid (0.81%).

pH: Figure 4 shows the pH changes in tempoyak with different level of salt during fermentation. For all

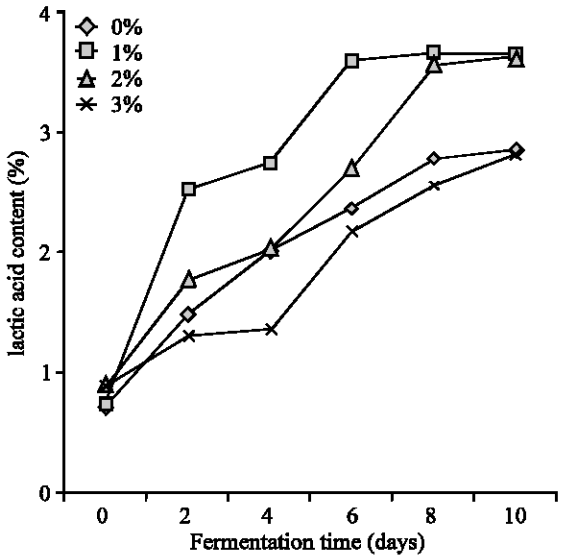

Fig. 3: Lactic acid content of tempoyak with different levels of salt during fermentation

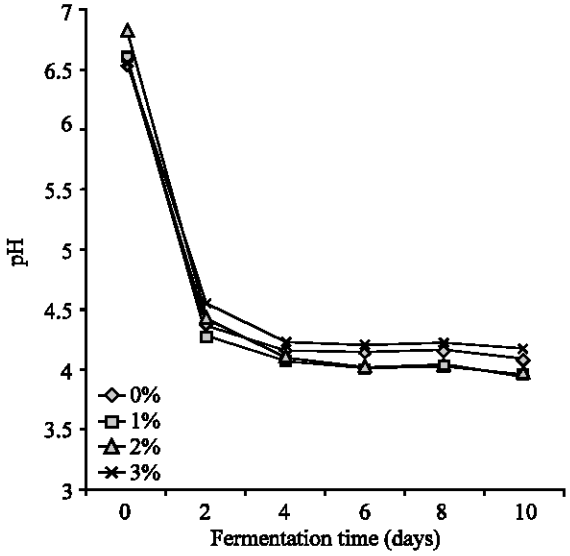

Fig. 4: pH of tempoyak with different levels of salt during fermentation

tempoyak samples, there was a drastic decrease in pH value from day 0 to 2, followed by little pH changes afterwards.

The initial pH ranges from 6.62 to 6.83 and the final pH ranges from 3.96 to 4.08 for all tempoyak. Tempoyak pH ranges from 3.96 to 4.24 after day 2. There results are in agreement with those of Leisner *et al.*[5] whom reported that the initial pH of durian was 6.7 and those of Merican[4] whom reported that the final pH values of 3.8-4.6 in tempoyak.

From the results, a pH change was in agreement with LABC but is not proportional with lactic acid

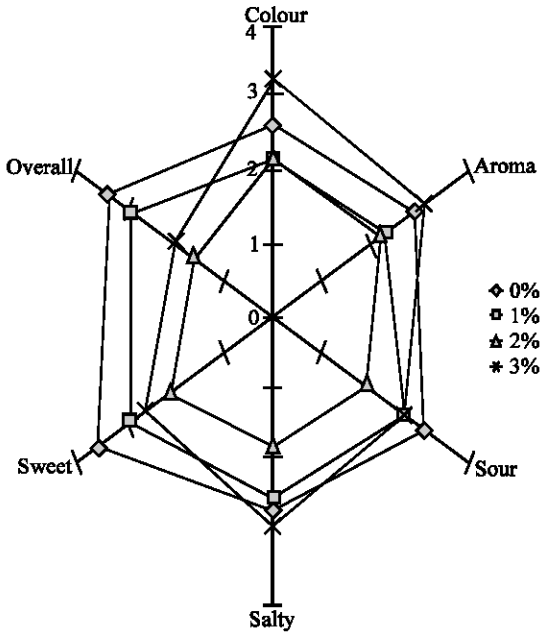

Fig. 5: The acceptability of tempoyak (mean score) after 10-day fermentation with different levels of salt

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.

Sensory evaluation: Figure 5 shows the acceptability (mean score) of tempoyak after 10-day fermentation with different level of salt. Sensory evaluation was done based on the panelists' preference to the taste of tempoyak samples, where scale ranges from 1 (most preferable) and 7 (most unpreferable). The five attributes that were tested are sour, salty, sweet, colour and aroma.

Based on the total mean score, tempoyak with 2% salt was found to be the most preferable by the panelists. For all attributes tested. For overall and sweet attributes, panelists seems to prefer tempoyak which contains 2 and 3% of salt, followed by 1 and 0% tempoyak, respectively.

ACKNOWLEDGMENT

The authors acknowledge that this work was supported by a short term grant from Kolej Universiti Sains dan Teknologi Malaysia (Project No. 54111).

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