

Effect of Traditional Fermentation as a Pretreatment to Decrease the Antinutritional Properties of Rambutan Seed (*Nephelium lappaceum* L.)

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Abstract. The seed of Rambutan (*Nephelium lappaceum* L.) mostly remain as a waste material annually in large amount because it is slightly bitter, cause of bitterness in seeds of rambutan are traces of alkaloid, tannin, saponin and polyphenol content . There should be a proper method for removing bitterness from the seed to produce almond like snack item. Taking this issue into consideration, this study attempts to determine the effect of fermentation on reduction the levels of antinutritional factors and bitterness in kernel. This indicates that their deleterious and antinutritional effects could partly be removed by fermentation as a pretreatment. Chemical composition of fermented seeds was considered for 0,1,2,3 to 10 days. Effect of fermentation period were analyzed for chemical composition and reduce cause of bitterness, Regression results showed that fermentation significantly ($p \leq 0.05$) increased the lactic acid, acetic acid, fermentation index, lipid content and color(a*,b*), however decreased moisture content, pH, crude protein, total phenol content, saponin content and tannin content.

Keywords: Rambutan, bitterness, fermentation

1. Introduction

Rambutan (*Nephelium lappaceum* L.) is one of the famous fruits that mostly can be found in Southeast Asia which belongs to the family of Sapindaceae [1]. The Rambutan seeds mostly considered as a discarded material because it is slightly bitter [2]. Now seeds are thrown away yearly in huge amount although this is potentially valuable source of nutrition [3]. Reason of bitterness in seeds of rambutan is traces of alkaloid, tannin, saponin and phenolic content as ellagic acid corillagin and gerannin [4], [5]. Seed is 2.53 g (9.5 %) approximately *Nephelium lappaceum* has a high nutritional value for instance it has high amount of fat (between 14% and 41%) [6]. Mineral content of seeds are also a valuable source of Calcium, Zinc, Iron, Mg and Mn. Having this desirable attribute, there is a need to find an appropriate way to remove bitterness from the seed in order to develop almond like snack product that can be consumed as valuable nut. Therefore, fermentation is one of the preliminary techniques to reduce the amount of tannin, saponin and total phenol content as a cause of bitterness in the seed in this study. However, this process is required as an additional improvement to achieve an absolutely safe product.

2. Method and Material

2.1. Row Material, Chemicals and Reagent

Freshly harvested rambutan fruit were purchased from MARDI Serdang, Malaysia. Leaves of Banana as well as wooden box were used to undertake the fermentation. Gallic acid, sodium carbonate, folin-ciocalteu reagent, Ethanol, NaOH, petroleum ether, Hydrochloric acid, and methanol, all these chemicals used were analytical grade bought from Merck and Fisher.

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2.2. Rambutan Seed Fermentation Process

Freshly harvested rambutans (Anak Sekolah-R191) 50 kg were fermented by using wooden box (38*30.5*30cm) during 10 days; seeds were covered in the box with fresh banana leaves. Every 24 hours 60 samples were removed from the box and were kept in the refrigerator at -40°C for further analysis.

2.3. Preparation of Plant Extracts and Phenolic Content Determination

The seed of rambutan dried and powdered was mixed with ethanol [1:10 (w/v)] with stirring, the suspension was filtered using a 114 Whatman paper and the collected solvent was concentrated using a rotary evaporator. Total phenolic content was determined using the Folin-Ciocalteu method, according to Miliuskas et al, [7]. A 1-ml of the ethanolic extract was added to 5 ml of Folin reagent then 4 ml of 7.5% Na₂CO₃ solution was added to the mixture, the absorbance was recorded at 765 nm. The phenolic content in a sample was expressed in mg/g of extract, gallic acid equivalents (GAE).

2.4. Chemical Assessments

Five grams of ground seeds were homogenized in 45 ml distilled water, the mixture was filtered with Whatman no #4, then the pH was measured a pH-meter [8]. And the additional 25 ml aliquot was titrated with 0.01N NaOH to an end point pH of 8.1. The values were reported as meq of sodium hydroxide per 10 g of dry seed.

2.5. Moisture Content

The moisture content was measured according to [9].

2.6. Color Test (L*, a*, b*)

The ground rambutan seeds were placed into a plastic box and measurement was done nine times repeatedly. The color of the ground seeds was evaluated for L* (light–dark spectrum, 0 (black) to 100 (white)), a* (green–red spectrum -60 (green) to +60(red)) and b* (blue–yellow spectrum, 60 (blue) to +60 (yellow)) values.

2.7. Seed Texture

For measuring seed texture analyzer (Stable Microsystems TA.XT TEE32, UK) was used. The analyzer consist of a cylindrical probe (2 mm), speed of 0.5 mm and a penetration distance of 2 mm. Trigger force was set to auto (5 g) with data acquisition rate of 40 Hz. This measurement was performed in nine replicate.

2.8. Determination of Fermentation Index

Ground seeds (0.5g) were homogenized in methanol: hydrochloric acid (97:3, 50 mL) solution, fermentation index was determined using the method of Gourieva and Tserevinov [10]. After 16 h the supernatant was read at absorbance of 460 and absorbance of 530 using a V-1100 spectrophotometer then the fermentation index was calculated by the ratio of A₄₆₀ to A_{530nm}.

2.9. Crude Protein and Lipid Content

Crude protein and lipid content were measured by kjeldahl and soxhelet methods according to [9].

2.10. Saponin and Tannin Content

The saponin content was determined using the method of Hudson and El-Difrawi [11]. 10g of the defatted sample was added 20 ml of 20% ethanol, 2 ml of diethyl ether was added and shaken strongly. The ether discarded, the process of purification was continued until the pH of colorless solution reach to 4.5. Afterward 6 and 3 ml of n-butanol were added to the solution, then the combined butanol extract was washed using 5% aqueous NaCl and evaporated, which was weighed (Saponin content = weight of sample before extraction – loss in weight after extraction).

The method described by [12] Joslyn (1970) was used 2 g of the sample containing 50 ml distilled water and heated to 60 °C. It was filtered and the residue discarded. 10 ml of 4% copper acetate solution was added to the filtrate and boiled for 10 min. The residue was dried using filter paper and the dried sample from filter paper into a crucible. The weight was recorded as W. The crucible was incinerated in a muffle furnace at

550 °C and then reweighed as W1. The difference between the weight of sample before and after incineration shows the tannin content.

3. Result and Discussion

3.1. Changes in Total Polyphenols Content, Saponin and Tannin

3.1.1. Total poly phenol content

Polyphenols impart an astringent and bitter taste to the seed, in this study total poly phenol content (TPC) of the seed ranged from day 0 as a control (36.53mg/g) and day 10 as a last day of fermentation (25.8 mg/g). It means that fermentation causes a positive effect on the reduction of bitterness in the seed of rambutan approximately 30%. This may be due to the diffusion of polyphenols in cell liquids from their cells and are oxidized enzymatically by the polyphenol oxidase [13]. Fig. 1 shows decreasing amount of TPC before and after fermentation.

An independent sample t-test was performed to examine the difference between total phenol content in the day 0 and the day 10. The results indicate that the mean day zero (M= 36.53, SD = 1.63) were significantly different than the mean of the tenth day (M= 25.800, SD= 0.458), t value =11.01, p = 0.000). The effect of size calculated using eta squared, was 0.10. This indicates that there is a large difference in mean test scores between the day 0 and the day 10. Therefore, it can be concluded that, TPC was significantly decreased from day zero to day 10 as shown in Table 1.

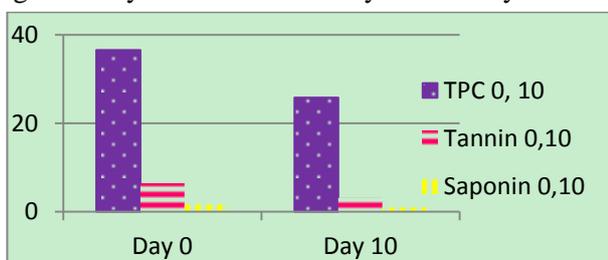


Fig.1: Amount of TPC, Tannin and Saponin in day 0 and day 10th of fermentation

Table 1: Independent Two Sample T-Test for TPC, Tannin and Saponin

	Day	Mean	Std.	T value	P value
TPC	0	36.53	1.63	11.01	0.000
	10	25.800	0.458		
Tannin	0	6.480	0.226	20.26	0.000
	10	3.020	0.191		
Saponin	0	1.64	0.0651	8.09	0.001
	10	0.997	0.122		

3.1.2. Tannin and saponin

As can be seen from Fig. 1, the amount of tannin in the seed ranged between 6.48 from last day to 3.12 in day zero, in addition saponin decreased from 1.64 (day 10) to 0.99(day 0). In fact fermentation causes decrease the tannin and saponin contents of the seed. This reduction may be due to some enzymatic reaction in addition microorganism breakdown the carbon and nitrogen sourced and use for production energy and their activity during fermentation [14], [15]. An independent sample t-test was performed to examine the difference between tannin and saponin content in the day 0 and the day 10. The results indicated that the mean day zero of tannin content (M= 6.480, SD= 0.226) and saponin (M = 1.64, SD = 0.0651) were significantly different than the mean of the tenth day for tannin (M= 3.020, SD= 0.191), t value = 20.26, p = 0.000) and saponin (M=0.997, SD= 0.122), t value = 8.09, p = 0.001). The effect of size calculated using eta squared for tannin was 0.99 and for saponin was 0.94. This indicates that there is a large difference in mean test scores between the day 0 and the day 10. Consequently it can be determined that, tannin content and saponin content were significantly decreased from the day zero to the day 10 as shown in Table 1.

3.2. Hardness, Lipid Content and Protein Content

The hardness of the seed was investigated in the day 0 and day 10 (1402.78 to 1525.46) and compared with two different nuts (walnut 1580 & almond 1261 N). The hardness of the seed increased due to the drain the mucilage from the seed, although the hardness is acceptable between the range for almond and walnut (see Fig. 2 and Fig. 3).

As Fig. 4 shows the amount of lipid content from day 0 to day 10 was 10.27% and 12.92%. This measurement experienced a gradual increase for lipid in which the findings are consistent with [16]. The increase of lipid content may be due to micro-organism using up carbohydrate and converting it to fatty acid.

Simple regression showed that lipid content had a positive correlation with time; it means that with increasing the time of fermentation lipid content increased (see Fig. 4). Amount of protein content decreased during fermentation might be due to the fact that microorganism utilized the nitrogen for their activities (see Fig. 5).

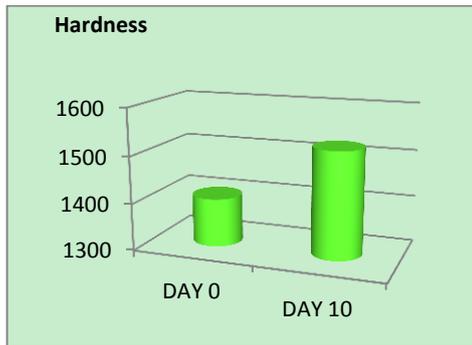


Fig. 2: Hardness of rambutan seed in day 0 & 10

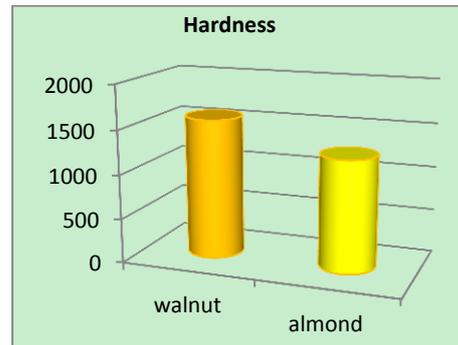


Fig. 3: Hardness of almond & walnut

Table 2: Two-Sample T-Test for Protein

	Day	Mean	Std.	T value	p value
Protein content	0	7.775	0.444	3.78	0.019
	10	5.828	0.774		

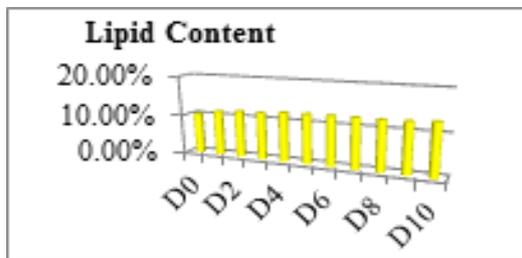


Fig. 4: Variation in lipid content during fermentation

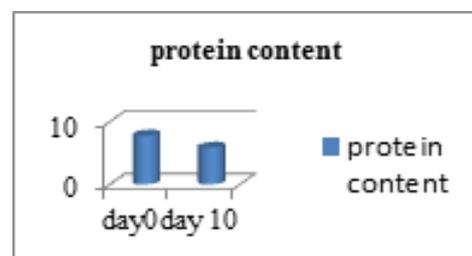


Fig. 5: Variation in protein content

Lipid content equation= $0.113 + 0.00160 \text{ time}$

R-Sq = 96.8%

An independent sample t-test was performed to observe the difference between protein content in day 0 and day 10. The results indicated that the mean day zero for protein content (M=7.775, SD= 0.444) was significantly different than the mean of the tenth day (M=5.828, SD=0.774), t value = 3.78, p = 0.019). The effect of size calculated using eta squared was 0.78. This indicates that there is a large difference in mean test scores between the day 0 and the day 10. Thus it can be determined that, protein content was significantly decreased from day zero to day 10 as shown in Table 2.

3.3. Fermentation Index, pH and Titratable Acidity (TA)

Fermentation index is based on the change in the color of the cotyledon during fermentation. Fig. 6 shows that the fermentation index increased gradually from day zero (0.12) to the last day (1.21) of the fermentation.

Initially the ethanol produced from original microorganism is converted into the acetic acid and lactic acid by oxidation [17]. Acetic acid is so volatile although lactic acid is not volatile, it remains into the beans and increased the acidity of the seed. Duration and temperature affect the pH and titratable acidity thus influences the microbial enzymes activities. The pH and titratable acidity of seed from different days of

fermentation showed the pH at 0 day was really higher which is around 7 then gradually decreased until the pH reached at around 3.8. The adhering pulp becomes liquid and micro-organisms will produce acetic acid lactic acid and ethanol. These findings are consistent with [18] [19] (see Fig. 6).

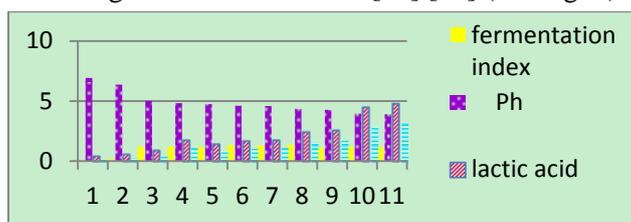
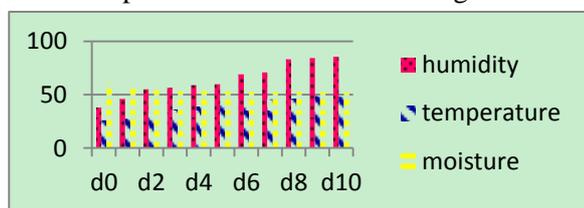


Fig. 6: Variation of fermentation index, pH, lactic acid and acetic acid during fermentation.

$$\begin{aligned}
 \text{FI} &= 0.0542 + 0.145 \text{ time} & \text{R-Sq} &= 96.8 & \text{pH} &= 5.57 - 0.174 \text{ time} & \text{R-Sq} &= 92.6\% \\
 \text{Lactic Acid} &= 0.00071 + 0.00399 \text{ time} & \text{R-Sq} &= 86.5\% & \text{Acetic acid} &= 0.047 + 0.266 \text{ time} & \text{R-Sq} &= 86.6\%
 \end{aligned}$$

3.4. Changes in Moisture Content

Fig. 7 indicates moisture content decreased gradually during fermentation from day zero until the last day of fermentation (61% to 52%) and could be the reason for the decrease in pulp volume per seed due to water evaporation and inversion of sugar.



$$\begin{aligned}
 \text{Moisture} &= 0.611 - 0.00876 \text{ time} & \text{R-Sq} &= 95.0\% \\
 \text{Humidity} &= 0.410 + 0.0467 \text{ time} & \text{R-Sq} &= 96.5\% \\
 \text{Temperature} &= 27.7 + 2.40 \text{ time} & \text{R-Sq} &= 94.9\%
 \end{aligned}$$

Fig. 7: Variation in moisture content temperature and humidity during fermentation

The color of fermented seeds can be an important quality parameter, which has a direct influence on the acceptability of the developed product. $L^*a^*b^*$ parameters of fermented seed are presented in Fig. 8. There was a general increase in a^* , b^* , L^* parameters indicating changes during fermentation. Fermentation could have been caused by the oxidation of some organic compounds, such as phenolic compounds.

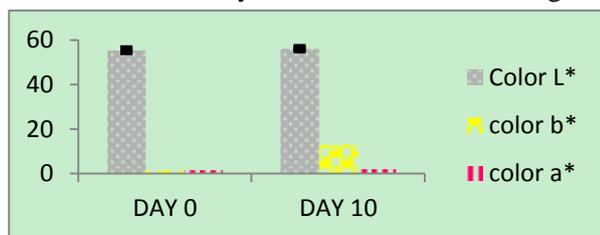


Fig. 8: Changes in L^* , a^* , b^* during fermentation

	Day	Mean	Std.	T value	p value
Color L^*	0	55.372	0.806	-2.11	0.051
	10	56.028	0.464		
Color a^*	0	1.597	0.114	-7.22	0.000
	10	2.023	0.136		
Color b^*	0	1.487	0.865	-37.80	0.000
	10	12.927	0.277		

An independent sample t-test was completed to examine the difference between color (L^* , a^* and b^*) in the day 0 and day 10. The results revealed that the mean day zero of L^* ($M=55.372$, $SD=0.806$) was not significantly different than the mean of the tenth day ($M=56.028$, $SD=0.464$), t value $=-2.11$, $p=0.051$). The effect size calculated using eta squared, was 0.21. This indicates that there is not any a large difference in mean test scores between the day 0 and the day 10. Consequently it can be determined that, color L^* was not significantly difference from the day zero to the day 10 as shown in Table 3. Although for color a^* in day 0 ($M=1.597$, $SD=0.114$) color b^* ($M=1.487$, $SD=0.806$) was significantly different than the mean day of the tenth day for color a^* ($M=2.023$, $SD=0.136$) t value -7.22 , $p=0.000$ and color b^* ($M=12.927$, $SD=0.277$) t value -37.80 , $p=0.000$. eta squared were 0.75 (color a^*) and 0.98 (color b^*) this data showed that color a^* and b^* were a significant large difference from the day zero to the day 10 (see Table 3).

4. Statistical Analysis and Conclusion

Results were expressed as the effect of time (predictor) of fermentation on different responses was investigated by Regression with three replication. The relationship between responses are studied by correlation with 95% CI. Independent two sample T- test was used for Statistical comparisons between groups were performed for observations (tannin, saponin, total phenol content, protein and color L*, a*, b*) differences were considered significant at $p < 0.05$.

Seeds of rambutan discarded annually in a large amount, because it is marginally bitter, consequently it should apply a method to remove bitterness from the seeds. Traditional fermentation had a positive effect on decrease saponin content (60%) and total phenol content (30%) and tannin content (approximately 50%) of seed of rambutan in 10th day ($p < 0.05$). The color (a*,b*) was increased after fermentation although (L*) was not significantly difference from the day zero to the day 10, Lipid content increased gradually during fermentation but protein content decreased in this process.

5. References

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