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Research Article
Effect of Length of Soaking and Fermentation Using Palm Juice on Nutrient Content of Tamarind Seeds

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Abstract
Background and Objective: Tamarind seed is an underutilized byproduct of tamarind fruit processing industry. It has rich nutrient contents but its utilization as feed is limited due to its association with a hard and non-destructive seed husk structure and the presence of anti-nutrient factors such as tannins and antitrypsin. Pre-treatment is needed to reduce or partially remove the anti-nutrients in order to increase its utilization. This study was conducted to determine the best treatment combination of soaking and fermentation using palm juice with tamarind seed which is yet unknown. **Materials and Methods:** Tamarind seeds were collected and sorted using a floating test. Selected seeds were then strained, dried and soaked in clean water. The study used a randomized complete design with a 3×3 factorial pattern and 3 replications. First factors was length of soaking (W0 = 0 days, W1 = 2 days and W2 = 4 days, second factor was level of palm juice (L0 = 0% of palm juice, L1 = 20% of palm juice and L2 = 40% of palm juice) and third factor was length of fermentation (F0 = 0 h, F1 = 36 h, F2 = 72 h and F3 = 108 h). Parameters measured were dry matter, crude protein, crude fat, crude fiber, Ash, Ca, P and tannin content. **Results:** The results showed that there was interaction between length of soaking, level of palm juice and length of fermentation. Soaking and fermentation using palm juice can improve the nutrients and reduce tannins of tamarind seed by 82.87%. **Conclusion:** The best nutritional value and lowest tannin content in tamarind seeds was achieved with 2 days of soaking and 20% palm juice at 72 h fermentation.

Key words: Crude protein, fermentation, palm juice, soaking, tamarind seed

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tamarind seed is a byproduct of tamarind pulp processing that is mostly underutilized and discarded as waste. The seed has potential as feed in livestock since it has a rich nutrient content of crude protein (131.3 g kg^{-1}), crude fiber (67.1 g kg^{-1}) crude fat[1] (48.2 g kg^{-1}) and fatty acids[2]. The utilization of tamarind seed as feed is limited due to its association with a hard and non-destructive seed husk structure and the presence of anti-nutrient factors such as tannins (56.2 g kg^{-1} of DM) and antitrypsin (10.8 g kg^{-1} of DM)[1], which is mostly located in the testa or seed husk[1]. Tannins tend to bind to other compounds such as proteins and carbohydrates to form complex compounds that are difficult to breakdown or digest. Pre-treatment is needed to reduce or partially remove the anti-nutrients in order to increase its utilization.

Several processes were applied in pre-treatments such as soaking, boiling and dehusking[3]. Their combination can affect the chemical, physical and functional properties of koro sword bean flour and reduce the phytic acid level[3]. Soaking in water and fermentation were used to increase nutrient content in tamarind seed[4].

Fermentation is a process of enzyme reactions generated by microorganisms to change the physical and chemical complex organic materials such as proteins, carbohydrates and fats into simpler molecules[5]. Fermentation can preserve, eliminate odors, increase digestibility, increase flavor, eliminate anti-nutrients and toxins that are usually present in raw foods[6], increase the content of crude protein and lower crude fiber content[7].

Efforts to improve the quality of the fermentation process include the addition of materials containing high soluble carbohydrates[8]. One of the potential local sources of carbohydrates is the juice derived from palm trees (*Borassus flabellifer*, Linn) or siwalan trees. According to Cahyaningsih[9], fresh juice from sap tapping of palm trees develops organic acid formation and spontaneous fermentation occurs which causes the pH to decrease to 3.28 in 72 h of fermentation.

Palm juice contains amylolytic bacteria such as *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides*, *Lactobacillus fermentum.* It also contains lactic acid bacteria (LAB) which is only found in fresh palm juice up to 24 h after spontaneous fermentation at room temperature. The highest value of LAB at 24 h fermentation is 7.1×103 CFU mL^{-1}, with the pH of the juice of 4.1. Composition of fresh palm juice is 1.04% of protein, 0.19% of fat, 76.86% of sucrose, 1.66% of glucose, 3.15% of minerals, 0.861% of calcium, 0.052% of phosphorus[10] and 11.01 mg/100 g of iron[10]. Utilization of 30% palm juice after approximately 2 h tapping in tamarind seed fermentation for 21 days did not affect nutrient quality[11] but decreased tannins by ±8.6%[12]. The aim of this study was to determine the best treatment combination of soaking and fermentation using palm juice with tamarind seed which is yet unknown.

MATERIALS AND METHODS

Preparation of tamarind seed: The tamarind seeds were collected from a 2016 crop yield from Soe Regency, East Nusa Tenggara, Indonesia. The seeds were sorted using a floating test. Selected seeds were then strained, dried and soaked in clean water with a 1:2 ratio over a period of time according to the treatment's soaking period.

Preparation of palm juice: Palm juice was collected from the Lasiana area in Kupang City. The juice was incubated in an open container for ±24 h at room temperature.

Fermentation of tamarind seed: The soaked tamarind seeds were drained then mixed with the prepared palm juice. The amount of palm juice added was determined by the palm juice treatment. The mixture of tamarind seeds and palm juice was fermented in anaerobic conditions for a length of time according to the fermentation treatment. After the fermentation process, samples were sent to the laboratory for further analysis of dry matter, crude protein, crude fat, crude fiber, Ash, Ca, P and tannin content. The proximate analysis was determined according to AOAC[13] methods. The analysis was conducted at the Nutrition and Feeding Laboratory of Kupang State Agricultural Polytechnic Laboratory.

Statistical analysis: The study used a randomized complete design with a 3×3 factorial pattern and 3 replications. The first factor was length of soaking (W0 = 0 days, W1 = 2 days and W2 = 4 days, the second factor was level of palm juice (L0 = 0% of palm juice, L1 = 20% of palm juice and L2 = 40% of palm juice) and the third factor was length of fermentation (F0 = 0 h, F1 = 36 h, F2 = 72 h and F3 = 108 h). Data analyses were conducted using IBM SPSS statistics v23. Differences between treatments were tested using Duncan's Multiple Range Test (DMRT) at 1% level of significance.

RESULTS AND DISCUSSION

Dry matter: The dry matter content of processed tamarind seeds is presented in Table 1. Statistical analysis showed a highly significant ($p<0.01$) interaction between length of

Table 1: Effect of treatments on dry matter of tamarind seed

		Soaking (days)		
Palm juice (%)	Fermentation (h)	0	2	4
0	0	92,19^{aA}	43.49^{xB}	44.23^{xC}
	36	91.39^{bD}	59.31^{oE}	54.64^{qrF}
	72	90.64^{cG}	54.23^{rH}	56.96^{pI}
	108	87.82^{dJ}	63.37^{mK}	56.86^{pL}
20	0	83.92^{gM}	41.82^{yN}	41.25^{yO}
	36	85.02^{fP}	62.90^{mQ}	48.78^{uR}
	72	86.27^{eS}	52.12^{sT}	49.84^{tU}
	108	84.87^{fV}	50.32^{tW}	45.76^{wX}
40	0	79.96^{hM}	45.76^{wN}	43.53^{xO}
	36	78.86^{iP}	66.26^{kQ}	60.65^{nR}
	72	75.48^{jS}	50.36^{tT}	62.87^{mU}
	108	65.11^{lV}	55.35^{qW}	46.86^{vX}

SEM 0.042

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated superscripted lower case letter on the same column indicated highly significant ($p<0.01$)

Table 2: Effect of treatments on crude protein of tamarind seed

		Soaking (days)		
Palm juice (%)	Fermentation (h)	0	2	4
0	0	14.93^{lmA}	16.38^{abcdB}	16.81^{aB}
	36	15.32^{ijklmC}	15.76^{defghijD}	15.77^{defghijD}
	72	16.11^{bcdefgE}	16.80^{aF}	16.18^{abcdeF}
	108	15.77^{defghijE}	16.56^{abcHF}	15.49^{fghijklmF}
20	0	15.22^{jklmG}	15.63^{efghijklH}	15.32^{ijklmH}
	36	15.04^{klmI}	15.39^{ijklmJ}	15.43^{ghijklmJ}
	72	15.82^{defghijK}	15.64^{efghijkL}	15.64^{efghijkL}
	108	16.09^{bcdefghK}	16.31^{abcdeL}	15.22^{jklmL}
40	0	15.41^{hijklmM}	15.29^{ijklmN}	15.22^{jklmN}
	36	14.22^{nO}	14.85^{mP}	15.26^{jklmP}
	72	15.65^{efghijkQ}	15.97^{cdefghiR}	15.18^{jklmR}
	108	15.27^{jklmQ}	15.1367^{jklmR}	16.72^{abR}

SEM 0.034

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated superscripted lower case letter on the same column indicated highly significant ($p<0.01$)

soaking, level of palm juice and length of fermentation on dry matter content of fermented tamarind seed using palm juice. The highest dry matter content was found in tamarind seeds with no treatment. Application of 4 days soaking and 20% palm juice without fermentation decreased the dry matter content of tamarind seed for 55%.

However, the dry matter content partially different between 0, 2 and 4 days of soaking duration. There was no difference between 20 and 40% of palm juice but different with 0% of palm juice. The dry matter content were also has no differences between lengths of fermentation. Economically, it is recommended to use 20% palm juice at 72-108 h fermentation.

Dry matter content in unprocessed tamarind was the highest and it was the lowest the longer the soaking and the higher the use of palm juice fermentation level. The high value of dry matter content occurred at day 0 of soaking, followed by days 2 and 4. This was possibly due to a longer soaking time to soften the seed husk since the water enters the tamarind seed cell structure easily and increases the water content but decreases the dry matter. In addition, soaking causes the pH to decrease due to the presence of lactic acid bacteria which will annihilate pathogenic bacteria in fermentation materials.

The average value of dry matter content of tamarind seeds in this research was 62.75% lower than the results of Teru[14] who reported that the dry matter content of dehusked tamarind seed was 89.14 and 89.89% with soaking for a night, dehusked tamarind seed with spontaneous bioconversion was 90.02%[15] and tamarind seeds fermented with 30% palm juice for 21 days was 72.89%[11]. The low content of dry matter in this research may have been caused by the soaking process using water and fermentation with palm juice hence the high water content of the material lowered the dry matter content.

Crude protein: Table 2 shows the DMRT result that the crude protein content of tamarind seed was increased by 12.59% and found in soaking for 2 and 4 days at 72 h fermentation.

This is because soaking and fermentation treatments cause tannin dissolution as a protein binding compound so that the proteins become more available. This is in line with Riadi[5] who found that fermentation is a process that occurs through enzyme reactions generated by microorganisms to transform both physical and chemical complex organic materials such as proteins, carbohydrates and fats into simpler molecules. The average value of crude protein content in soaking and fermentation treatment is 15.40-15.81% higher than the result of the research by Ue *et al.*[11] which is 13.25-13.43% using sliced tamarind seed and 20% palm juice with 21 days of fermentation. This result indicates that tamarind seed processing by soaking and fermentation with palm juice was preferable in this research.

Crude fiber: Based on Table 3, the lowest crude fiber was found in tamarind seed without treatment but the value cannot be stated as the best because the cell walls were still present in the form of complex compounds and did not decompose. Soaking and fermentation using palm juice increases the water imbibition into plant cells, which loosened the bond complex into simple compounds, indicated by a high increase of crude fibers but in free form.

This finding was in line with Despal *et al.*[16] who reported that the processing of feed ingredients by spontaneous fermentation or using water-soluble carbohydrate will produce epiphytic lactic acid bacteria (BAL) that ferments the water soluble carbohydrate in the plant to lactic acid and a small part converted to acetic acid which will lower the pH of the substrate and inhibit the development of harmful pathogenic microorganisms. The presence of acetic acid which is corrosive, softens the texture of tamarind seeds and loosens the binding of complex compounds to become simple compounds. The mean crude fiber in this study is lower than the research results of Ue *et al.*[11] which was 10.00%.

Calcium: Based on Table 4, the highest calcium content was 0.71%, found in the treatment of 4 days of soaking, 20% of

Table 3: Effect of treatments on crude fiber of tamarind seed

		Soaking (days)		
Palm juice (%)	Fermentation (h)	0	2	4
0	6.75^{nA}	8.05^{ghijB}	7.31^{mC}	
	36	8.42^{efghD}	9.51^{abE}	7.75^{ijklmF}
	72	6.59^{nD}	8.54^{defgE}	8.67^{cdefF}
	108	8.45^{defghG}	7.67^{ijklmH}	7.54^{jklmI}
20	0	6.76^{nJ}	10.02^{aK}	7.5^{jklmL}
	36	7.67^{ijklmM}	7.82^{ijklmN}	7.93^{hijklO}
	72	8.24^{fghiM}	8.19^{fghiN}	9.02^{bcdO}
	108	7.36^{lmP}	8.48^{defghQ}	9.79^{aR}
40	0	8.43^{efghJ}	7.71^{ijklmK}	8.03^{ghijkL}
	36	8.46^{defghM}	8.43^{efghN}	7.69^{ijklmO}
	72	8.47^{defghM}	8.18^{fghiN}	7.45^{klmO}
	108	8.73^{cdefP}	9.11^{bcQ}	8.91^{cdeR}

SEM 0.029

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated superscripted lower case letter on the same column indicated highly significant (p<0.01)

Table 4: Effect of treatments on calcium content of tamarind seed

		Soaking (days)		
Palm juice (%)	Fermentation (h)	0	2	4
0	0	0.41^{cdeA}	0.48^{bcdeB}	0.43^{cdeA}
	36	0.38^{deA}	0.55^{abcdB}	0.50^{bcdeA}
	72	0.37^{deA}	0.60^{abcB}	0.41^{cdeA}
	108	0.45^{cdeA}	0.47^{bcdeB}	0.39^{deA}
20	0	0.38^{deA}	0.34^{eB}	0.71^{aA}
	36	0.53^{abcdeA}	0.64^{abB}	0.45^{cdeA}
	72	0.42^{cdeA}	0.53^{abcdeB}	0.36^{deA}
	108	0.44^{cedA}	0.45^{bcdeB}	0.42^{cdeA}
40	0	0.51^{bcdeA}	0.53^{abcdeB}	0.38^{deA}
	36	0.56^{abcdA}	0.54^{abcdB}	0.38^{deA}
	72	0.50^{bcdeA}	0.49^{bcdeB}	0.45^{bcdeA}
	108	0.47^{bcdeA}	0.65^{abB}	0.51^{bcdeA}

SEM 0.009

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated superscripted lower case letter on the same column indicated highly significant (p<0.01) palm juice and 0 h fermentation. Ca content of tamarind seed had no difference between the level of palm juice and the length of fermentation. This indicates that the soaking and fermentation using palm juice caused the complex binding to loosen since many nutrients dissolved including minerals such as calcium and phosphorus. The average value of calcium obtained in this study was higher than Wunu *et al.*[17] and Yusuf *et al.*[18] that is 0.23 and 0.10%, respectively. These differences are possibly due to different ways of processing the tamarind seeds that were used, which included dehusked tamarind seeds with the addition of soluble carbohydrates and fermentation.

Phosphorus: Table 5 shows the effect of treatments on Phosphorus Content of Tamarind Seed. The result of statistical analysis shows that there is high interaction ($p<0.01$) between length of soaking, the usage of palm juice and length of fermentation on P content of tamarind seed. Based on DMRT, the highest phosphorus content was found in the treatment of 2 days soaking, 20 and 40% of palm juice and 108 h fermentation.

This result is possibly due to the process of soaking and fermentation using palm juice which caused the complex binding to loosen since many nutrients dissolved including minerals such as calcium and phosphorus. The average value of phosphorus content obtained in this study was higher than Wunu *et al.*[17] and Yusuf *et al.*[18] that was 0.031 and 0.041%, respectively. These differences were caused by the different ways of processing the tamarind seeds, which included dehusked tamarind seeds with the addition of soluble carbohydrates and fermentation.

Tannin: Tannin content of tamarind seed processed by soaking and fermentation using palm juice are presented in Table 6. Based on DMRT, there is a significant ($p<0.01$)

Table 5: Effect of treatments on phosphorus content of tamarind seed

		Soaking (days)		
Palm juice (%)	Fermentation (h)	0	2	4
0	0	0.076^{lmA}	0.090^{efgB}	0.084^{ghijkC}
	36	0.093^{defD}	0.094^{deE}	0.090^{efgF}
	72	0.080^{klDG}	0.075^{lmEH}	0.083^{hijkFI}
	108	0.068^{oDJ}	0.087^{ghiEK}	0.108^{aFL}
20	0	$0.085^{ghijkAM}$	0.082^{hijkBN}	0.097^{cdCO}
	36	0.111^{aDP}	0.081^{jklEQ}	0.070^{noFR}
	72	$0.085^{ghijkDGS}$	0.076^{lmEHT}	$0.085^{ghijkFIU}$
	108	0.087^{ghiDJV}	$0.083^{hijkEKW}$	0.073^{mnoFLX}
40	0	0.084^{hijkMY}	0.101^{bcNZ}	0.102^{bOAa}
	36	$0.083^{hijkPAb}$	0.055^{pQAc}	0.074^{mnRAd}
	72	$0.083^{hijkPSAe}$	$0.088^{fghQTAf}$	$0.085^{ghijkRUAg}$
	108	$0.082^{hijkPVAh}$	$0.086^{ghjiQWAi}$	$0.082^{ijkRXAj}$
SEM 0.000				

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated superscripted lower case letter on the same column indicated highly significant ($p<0.01$)

Table 6: Effect of Treatments on tannin content of tamarind seed

		Soaking (days)		
Palm juice (%)	Fermentation (h)	0	2	4
0	0	2.47^{aA}	0.92^{mB}	0.92^{mC}
	36	2.19^{bD}	1.10^{ijE}	1.16^{hiF}
	72	1.41^{gG}	1.48^{nfH}	0.60^{rI}
	108	2.05^{cJ}	0.93^{mK}	0.54^{rL}
20	0	2.51^{aM}	0.82^{nN}	1.03^{klO}
	36	2.17^{bP}	1.19^{hQ}	1.08^{jkR}
	72	1.59^{eS}	0.84^{nT}	0.74^{opU}
	108	1.36^{gV}	0.78^{noW}	1.01^{lX}
40	0	1.90^{dY}	0.71^{pqZ}	0.82^{nAa}
	36	1.61^{eAb}	1.59^{eAc}	0.67^{qAd}
	72	1.37^{gAe}	1.10^{ijAf}	1.01^{klAg}
	108	0.92^{mAh}	1.42^{fgAi}	0.43^{sAj}
SEM 0.04				

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated superscripted lower case letter on the same column indicated highly significant ($p<0.01$)

interaction between length of soaking, level of palm juice and length of fermentation on tannin content. The best quality of tamarind seed in this study based on the lowest tannin content was found at 4 days of soaking, with 40% palm juice and 108 h fermentation.

The average tannin content found in this research decreased by 82.25-82.47%. The highest value of tannins found in tamarind seeds was without the soaking and fermentation process and the lowest was in fermented tamarind seed using palm juice with a soaking treatment of 4 days.

The high content of tannins in tamarind seeds without processing was probably due to the intact seeds. Tamarind seeds processed with soaking and fermentation in palm juice caused exfoliation of the husk and this indicated the tannin was loosened or dissolved in the soaking water or palm juice. In fact, this induces the tannin structure in the husk to break down, resulting in nutrients in the form of complex compounds being separated into simple compounds.

This finding was in line with the report from Suliantri and Rahayu[6] that fermentation can preserve, increase digestibility and flavor, as well as eliminate smell, anti-nutrients and toxins that are usually present in raw foods. Thus, Panigrahi *et al.*[1] stated that tamarind seeds have anti-nutrient substances in the form of tannins (56.2 g kg^{-1} of dry matter (DM)) and antitrypsin (10.8 g kg^{-1} of DM) which is mostly located in the testa or husk. Based on the results of this study, it is indicated that the treatment of tamarind seeds by soaking and fermentation using up to 40% palm juice can increase the nutrient content and reduce the tannin content. There was no difference in the utilization of 20 and 40% palm juice in this treatment; therefore, it is recommended to use 20% palm juice in fermentation of tamarind seed.

CONCLUSION

There is an interaction between length of soaking, palm juice level and length of fermentation on the nutrient and tannin content of tamarind seeds. The best nutritional value and lowest tannin content in tamarind seeds was achieved with 2 days of soaking and 20% palm juice at 72 h fermentation. It is recommended to determine the alternative combination to know the tamarind seed digestibility in livestock, especially pigs.

SIGNIFICANCE STATEMENTS

This study discovered that processing tamarind seeds by soaking and fermentation using palm juice increased the nutritional content and reduced the anti-nutrient tannin. The nutritional content of fermented tamarind seeds with 20 and 40% palm juice were not different. Using 20% palm juice in tamarind seed fermentation is sufficient. This study will help the researchers to carry out further research about the digestibility of tamarind seed fermented by palm juice

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