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Enriching nutritive value of tamarind seeds by *Saccharomyces cerevisiae* fermentation

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Abstract

The goal of this experiment is to enhance the nutritive value and reduce tannin concentration of tamarind seeds collected from semiarid parts of Indonesia by yeast fermentation. The tamarind seeds were first dried in sun light and powdered using pulverizer. The kernel flour was used for yeast fermentation for 12 and 24 hours with 5 repetitions. The crude fiber, amino acids, fatty acids and tannins of pure kernel flour and fermented kernel flour were compared. Data were analyzed by using Analysis of Variance (ANOVA). The proximity analysis showed that tamarind seeds kernel flour contains crude protein 16.2%, fats 7.06 %, crude fiber 17.7 %, Nitrogen Free Extract (NFE) 57.88% and ash 1.16%. HPLC analysis of crude protein showed the presence of 16 types of amino acids (13.3 mg/100g protein). Gas chromatography was used for finding the composition of fatty acids (74.1 mg/100g fats) and tannin concentration (300mg/100g of kernel flour). The fermentation increased the crude protein and amino acids content and lowered the concentration of fatty acids and tannins. Fermentation with *Saccharomyces cerevisiae* in kernel flour for 12 hours is comparatively more effective than 24 hours to lower tannins content and improve nutritive value.

Keywords: Tannins, tamarind seeds, *Saccharomyces cerevisiae*, fermentation

Introduction

Central part of Indonesia has a semiarid climate which is rich in various agriculture wastes and has a potential food source for livestock, but effective utilization cannot be done without preprocessing the nutritive values. Tamarind seed is one type of agriculture wastes that has potential as protein sources in this region. The production of tamarind seeds in the region can reach up to 30,000 kg/year (Shaun Ferris et al 2009) and has a crude protein content of 16-20% (Sabu et al. 2005). Yet, it cannot be taken as a potential food source due to its sour taste. The seed shell is made up of astringent compounds that containing tannins which can reach up to 0.7% (Pugalenthi et al. 2004). Tannins may inhibit digestion and absorption of nutrients, causing constipation and digestive tract disorders of monogastric animals (Pugalenthi et al. 2004; De Caluwé et al. 2010). Therefore, the processing method which can be capable of optimally eliminating tannins in tamarind seeds is needed. Only physical processing cannot eliminate tannins (Pugalenthi et al. 2004) and there is a need to couple microbial fermentation to reduce its concentration (Liang et al. 2008). Tannins can be degraded using tannase enzyme related by *Saccharomyces cerevisiae* (Mondal et al. 2001a; Rodriguez-Duran et al. 2011; Mandal 2012; Mohan & Arunkumar, 2014). Fermentation with *Saccharomyces cerevisiae* has proven to increase the nutritive value of soy (Hassaan et al. 2015). Tamarind seed flour was fermented using *Rhyzopus oliogosporus* for 12-24 hours for enhancing nutritive value (Wea et al 2012). Aguilera-Carbo et al. in 2008 reported that *Saccharomyces cerevisiae* can produce tannase enzyme.

The current study was conducted to reduce tannin content using *Saccharomyces cerevisiae* in solid state fermentation with tamarind seed flour for 12 to 24 hours. The exponential phase of *Saccharomyces cerevisiae* starts after 12 hours and accelerates up to 24 hours and reaches death phase at 48 hours. The tannins concentration along with crude protein, amino acids and fatty acids were measured.

Materials and Method

Preparing the Materials

Fresh tamarind seeds weighing 30 kg were taken from the central part of Indonesia where it has the semiarid climates. The seeds were cleaned and dried under the sun for two days.

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A total of 6 kg of sun-dried tamarind seeds were drawn at random as sample for this study.

Research Method

This study was conducted in accordance with the complete randomized procedure design consisting of three treatments and five repetitions. All three treatments applied were as follows:

Treatment Code	Treatment procedure
F0	Pulverized tamarind seeds kernel flour up to 0.6 to 1 mm particle size.
F12	Fermented tamarind seed kernel flour with 0.3% w/w of *Saccharomyces cerevisiae* for 12 hours
F24	Fermented tamarind seed kernel flour with 0.3% w/w of *Saccharomyces cerevisiae* for 24 hours

The sun dried tamarind seeds weighing 6 kg were sorted into two groups, 2 kg for F0 treatment and the other 4 kg for F12 and F24 treatment. For F0 treatment, a low speeded milling machine was used to deshell the tamarind seeds. The raw kernels of tamarind seeds were cleaned to remove any shell parts. Further grinding is done using flour milling machine to until it reaches an average size of 0.6-1 mm. 250 g of flour was divided into five parts of 50 g each and was used for the study. The five parts were added to five 250 g aluminum container and wrapped with aluminum foil, stored in laboratory for analysis of crude protein, fat, crude fiber, Ca, P, amino acids, fatty acids and tannins.

The remaining 4 kg of raw sun-dried tamarind seeds were first roasted in a skillet pan at 80^0C for 15 minutes. The tamarind seeds were removed from the skillet as soon as they appear blackish brown with cracked shells and peanut aroma. The roasted tamarind seeds were then cooled in the open air for about 15 minutes and were powdered similar to F0 treatment. 500 g of flour was taken for F12 and F24 treatment units. They were divided into ten units each having 50g powder. Furthermore, the first five units were chosen randomly for the F12 treatment (12 hour fermentation) and the other five units were taken for the F24 treatment (24 hour fermentation). The container used to fill all ten treatment units is an aluminum bowl with 250 g capacity.

Saccharomyces cerevisiae fermentation

The yeast was purchased from local market of Kupang, Indonesia. The cell count was made in terms of Colony Formation Units (CFU per gram) and was found to be $10.05x10^{10}$ CFU of *Saccharomyces cerevisiae*/g. 1.5 g was dissolved in 300 ml of distilled water to form a homogeneous culture of *Saccharomyces cerevisiae*. The culture media was then divided into two equal parts (150.75 ml for each). The first part was selected for the F12 treatment and second part for F24 treatment. The first part (F12) as well as the second part (F24) is further divided into five units, added to corresponding aluminum bowls, wrapped and incubated for 12 hours and 24 hours at 35^0C respectively.

The fermentation process for F12 treatment was stopped after 12 hours of incubation. The aluminum bowls were directly kept in a preheated oven at 60^0C for one hour to halt *Saccharomyces cerevisiae* growth. After cooling, the samples were sent to laboratory for further analysis of crude protein, fat, crude fiber, Ca, P, amino acids, fatty acids and tannins. The same procedure was repeated for F24 treatment after 24 hours incubation.

The total amount of *Saccharomyces cerevisiae* culture to be added to known quality of roasted tamarind flour was based on the literature (Umiyasih & Anggraeny, 2008, Wea et al. 2012). Care should be taken in such a way that the final solid state medium should be soft and should not be loose or sticky. The entire process adopted in this study was given in Figure 1 (van der Stege et al (2010).

Variables being studied in this research consist of the following:

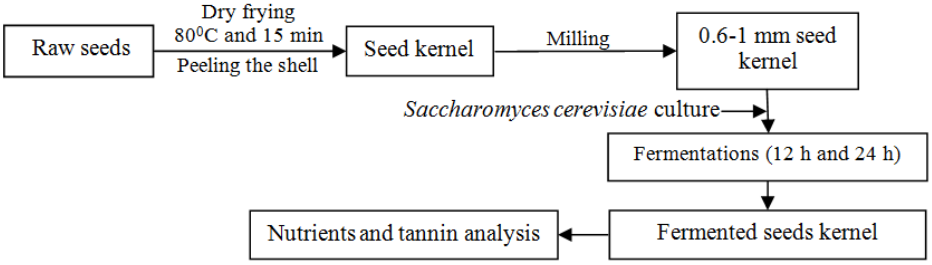

Figure 1: Briefly stages of tamarind seeds processing

1. Nutrients: Crude Protein (CP), fats, Crude Fiber (CF), (Analyzed using proximate analysis (AOAC*)); Gross Energy (GE) (Bomb Calorimetry, NS170); Amino acids (HPLC (Eclipse Plus-C18 Column,. Agilent Technologies)) and fatty acids (Gas Chromatography (AOAC)).
2. Minerals: Calcium and Phosphorus were analyzed using AAS (AOAC).
3. Tannin content was analyzed using spectrometric method (AOAC) (Khasnabis et al. 2015).

(*AOAC - Association of Analytical Communities)

Analysis was done in Integrated Laboratory of Bogor Agricultural University, Bogor, Indonesia.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was applied to the tannin concentration and the data from proximity analysis and the data analysis was done using SPSS version 19.0.

Results and Discussion

Nutrients

Calcium, Phosphorus, carbohydrate percentage, amino acids, fatty acids and proximate analysis (crude protein, fats, crude fiber, Nitrogen Free Extract (NFE) and Ash), energy content were analyzed in the Integrated Laboratory of Bogor Agricultural University, Bogor, Indonesia. The results of proximate analysis are shown in Table 1.

Table 1 Proximate analysis of tamarind seeds(*)

Content	**F0**	***F12***	***F24***
Moisture	8.5	*28.8*	*28.4*
Dry Matter (%)	91.5	71.2	71.6
Dry Matter Composition (91.5 g)			
Organic Matter (%)	97.5	97.0	97.0
Ash (%)	1.16	2.96	2.90
Others (%)	1.34	0.04	0.1
Proximity Analysis (Dry Matter - 91.5 g)			
Crude Protein (CP) (%)	16.2	18.75	19.5
Fat (%)	7.06	6.14	6.63
Crude Fiber (CF) %	17.7	9.54	9.01
Nitrogen Free Extract (NFE) %	57.88	62.61	61.96
Ash (%)	1.16	2.96	2.90
Composition with carbohydrates (Dry Matter 91.5 g)			
Crude Protein (CP) (%)	16.2	18.75	19.5
Fat (%)	7.06	6.14	6.63
Carbohydrates (%)	75.58	72.15	70.97
Ash (%)	1.16	2.96	2.90
Ash Analysis in Dry Matter - 91.5 g			
Ash (g)	1.06	2.70	2.65
Approximate Ash Composition (g)			
Calcium (g)	0.72	0.72	0.72
Phosphorus (g)	0.30	0.31	0.31
Others (g)	0.04	1.67	1.62
Energy			
Gross Energy (MJ/kg)	18.0	19.1	19.3

% NFE = % DM - (% fat+ % CP + % ash + % CF); where: NFE = nitrogen free extract; DM = dry matter; Fat = Crude lipid ; CP = Crude Protein; CF = Crude Fiber

Table 2: ANOVA Table

		Sum of Squares	**DF**	**Mean Square**	**F**	**P**
Dry Matter	Between Groups	1348.144	2	674.072	1150.215	.000
	Within Groups	7.032	12	.586		
	Total	1355.177	14			
Organic Matter	Between Groups	.659	2	.330	8.041	.006
	Within Groups	.492	12	.041		
	Total	1.151	14			
Crude Protein	Between Groups	29.901	2	14.951	65.148	.000
	Within Groups	2.754	12	.229		
	Total	32.655	14			
Fat	Between Groups	2.129	2	1.064	7.271	.009
	Within Groups	1.757	12	.146		
	Total	3.886	14			
Crude Fiber	Between Groups	237.427	2	118.714	30888.203	.000
	Within Groups	.046	12	.004		
	Total	237.473	14			
Carbohydrates	Between Groups	58.141	2	29.071	81.216	.000
	Within Groups	4.295	12	.358		
	Total	62.436	14			
Nitrogen Free Extract (NFE)	Between Groups	65.271	2	32.635	97.135	.000
	Within Groups	4.032	12	.336		
	Total	69.302	14			
Ca	Between Groups	.000	2	.000	.014	.986
	Within Groups	.006	12	.000		
	Total	.006	14			
P	Between Groups	.001	2	.000	1.072	.373
	Within Groups	.004	12	.000		
	Total	.005	14			
Energy (MJ/kg)	Between Groups	5.137	2	2.569	234.577	.000
	Within Groups	.131	12	.011		
	Total	5.269	14			

DF: Degree of Freedom

The experimental results were found to be statistically significant based on P-value (P<0.05). The crude protein content for F12 treatment increased with fermentation up to 2.55 % when compared with F0 treatment and similar increase in protein content during fermentation was also reported in literature (Vadivel and Pugalenthi 2007; Pugalenthi et al. 2004).

It is observed that the total fat content decreased up to 0.95% for F12 treatment when compared with F0 treatment. After 12 hours there is an increase of 0.49% fat content. This may be due to the consumption of unsaturated fatty acids by *Saccharomyces cerevisiae* during the lag phase (Andreasen & Stier 1954) and increase was observed due to release of saturated fatty acids in exponential phase after 12 hours of fermentation. The content of crude fiber decreased from 9.54% to 9.01% for 12 h and 24 h fermentation. This may be due to the increase in consumption of carbohydrates by *Saccharomyces cerevisiae* during exponential phase.

Composition of Amino Acids (AA)

The composition of amino acids were reported in Table 2.

Table 2. Composition of Amino Acids of Tamarind Seeds

Contents	F0	*F12*	*F24*
Total AA(mg/100g protein	*13.3*	14.0	13.9
Aspartic acid	1.59	1.90	1.88
Glutamic acid	2.44	2.63	2.71
Serine	0.78	1.02	0.93
Histidine	0.33	0.30	0.30
Glysine	0.82	0.70	0.71
Threonine	0.46	0.54	0.52
Arginine	1.04	0.98	0.96
Alanine	0.61	0.69	0.67
Tyrosine	0.58	0.63	0.63
Methionine	0.15	0.12	0.12
Valine	0.69	0.69	0.69
Phenylalanine	0.75	0.81	0.80
Iso-leusine	0.74	0.79	0.81
Leucine	1.18	1.30	1.30
Lysine	1.18	0.94	0.91
Total Essential Amino Acids (EAA) (mg/100g)	6.06	6.12	6.08
Total Non EAA (mg/100g)	7.28	7.92	7.86

It is evident from the amino acids profile that there is considerable increase in the protein content during fermentation (Table 2). It is assumed that increase of the protein content occurred due to increased fraction of Non-Protein Nitrogen (NPN) or due to increase in protein denaturation. The amino acid concentration significantly reduced after 24 hours. It can be assumed that the 24 hour fermentation tend to damage lot of protein fractions and NPN. The decrease of the concentration of lysine appears to contribute significantly to the decrease in the concentration of essential amino acids after 24 hours of fermentation. Total concentration of non-essential amino acids increased by 2.34-7.97% for 12 and 24 hour fermentations and this may be release by yeast during exponential growth.

Composition of Fatty Acids

The results of the composition of fatty acids are shown in Table 3.

Table 3. FFA and Tannin contents of tamarind seeds

Content	F0	*F12*	*F24*
Total Fat %	7.06	4.23	4.19
Total FA(mg/100g fat)	*74.1*	54.9	45.3
Caprilic. C8:0	0.05	0.09	0.17
Lauric. C12:0	0.48	0.02	0.03
Myristic. C14:0	0.36	0.09	0.11
Pentadecanoic. C15:0	0.03	0.04	0.06
Palmitic. C16:0	5.72	6.09	8.08
Palmitoleic. C16:1	0.03	0.03	0.06
Heptadecanoic. C17:0	0.08	0.11	0.14
Stearic. C18:0	3.56	4.40	5.08
Oleic. C18:1n9c	18.0	10.5	10.1
Elaidic. C18:1n9t	0.03	0.03	0.06
Linoleic.C18:2n9c	35.9	21.2	9.39
Linolenic. C18:3n3	nd	Nd	0.05
Arachidic. C20:0	1.79	2.35	2.47
Eicosenoic. C20:1	0.82	0.60	0.48
Eicosenoic. C20:2	0.13	0.07	0.04
Eicosapentanoic. C20:5n3	0.05	0.05	0.04
Heneicosanoic. C21:0	0.03	0.06	0.08
Behenic. C22:0	2.38	3.65	3.81
Erucic. C22:1n9	nd	0.03	0.02
Decosahexaenoic. C22:6n3	nd	0.06	0.07
Decosenoic. C22:2	nd	Nd	nd
Tricosanoic. C23:0	0.12	0.19	0.23
Lignoceric. C24:0	4.55	4.72	4.77
Total Saturated Fatty Acids (SAFA)	19.5	23.2	25.7
Total Unsaturated Fatty Acids (UFA)	53.9	31.8	19.6
Tannin (mg/100g)	300	266	289.3

Table 3 shows that the concentration of fatty acids decreased by 25-38% during fermentation. The concentration of saturated fatty acids (SAFA) increased by 18.8% to 32.1% for 12 and 24 hours of fermentations.

The concentration of unsaturated fatty acids (UFA) decreased at 12 and 24 hours of fermentation. Table 3 also shows that the UFA group experienced higher decrease than in SAFA group due to the fermentations This may be due to the consumption of UFA by yeast during the fermentation process (Walenga 1975).

Tannin Content

The effect of fermentation on decrease in tannin concentration was statistically significant (P <0.05) as per ANOVA (Table 2). Table 3 shows that there is a decline in tannins concentration to 11.4% and 3.5% for 12 and 24 hours of fermentations respectively when compared with pure tamarind seed kernel. The decline of the tannins was high for F12 treatment then F24 treatment. This proves that *Saccharomyces cerevisiae* produces more tannage enzyme and consumes more tannin in lag phase when compared with exponential phase.

Conclusion

Fermentation with *Saccharomyces cerevisiae* for 12 hours is generally capable of lowering the concentration of tannins and fixing nutrient higher than the 24 hour fermentation. The nutritive value is also found to be considerably significant due to increase in protein concentration.

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