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The Phytochemical and Antioxidant Characteristics of Fermented Jackfruit (*Artocarpus heterophyllus* L.) Leaves Using Single and Mixed Starter Culture

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Abstract: The phytochemical and antioxidant properties of fermented jackfruit leaves, using yeast and acetic acid bacteria, individually or in combination during 6 days of fermentation were investigated in the present study. Changes in pH, total reducing sugar, ethanol, acetic acid, total phenolics and flavanoids content were examined. A number of antioxidant activities such as ferric reducing antioxidant power (FRAP) and DPPH free radical scavenging activities were comparatively tested to determine the differences in the respective properties during the fermentation process. Results obtained demonstrated that there were differences in the phytochemical and antioxidant properties of fermented jackfruit leaves, depending on the fermentation starters. Fermented leaves using acetic acid bacteria alone, exhibited higher total reducing sugar (6.89 mg glucose/mL) compared to others. The ferric-reducing activities of fermented jackfruit leaves showed an increasing trend in the yeast fermentation process (5.96-10.12 mg ascorbic acid equivalent/mL). Generally, all extracts of fermented jackfruit leaves exhibited stronger DPPH radical scavenging activity except in the total phenolics and flavonoids content, whereby it showed a decrease trend throughout the fermentation process.

Key words: Jackfruit leaves, acetic acid bacteria, yeast, fermentation, antioxidant.

1. Introduction

Jackfruit (*Artocarpus heterophyllus* L.) tree is widely grown in India, Bangladesh and several parts in Southeast Asia, mainly in Thailand, Vietnam and Malaysia. Jackfruit leaves are abundant and commonly used by farmers as an animal feed. In some native area in India, the leaves were useful to cure fever, healing wounds, boils and ulcers [1]. Recently, jackfruit leaves have gained attention among scientists due to the health benefits claimed by the traditional medicine practitioners.

Generally, the antioxidant activity in plants is due to the presence of phenolic and flavonoid compounds, which offer various health benefits for human being. Previous studies on the antioxidant properties of jackfruit leaves extracts revealed that all the extracts showed positive antioxidant activities when tested in different *in vitro* assay (DPPH, FRAP and ABTS) [2] while ethanol and n-buthanol jackfruit leaves extract exhibited better antioxidant activity when assayed using DPPH and Fe^{+} chelating activity method [3]. In addition, Wang et al. [4] discovered several phenolic compounds that showed inhibitory activity against selected tested cancer cell.

Fermentation has been an effective and valuable method to preserve food and beverages for longer shelf life, improved flavour and retaining the nutritional properties. Moreover, fermentation also enhances the antioxidant properties due to the hydrolysis of plants cell wall by microorganisms, which lead to the secretion of assorted antioxidant compounds [5]. To the best of our knowledge and based on the cited literature, this is the first work on evaluating the potential of jackfruit leaves as a fermentation substrate. In this fermentation process,

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the jackfruit leaves were subjected to pure yeast, acetic acid bacteria and in combination of these two cultures for 6 days and the phytochemical and antioxidant properties of the fermented leaves were determined.

2. Materials and Methods

2.1 Jackfruit Leaves Preparation and Fermentation Process

Leaves of *Artocarpus heterophyllus* L. were collected from a local jackfruit plantation in Lanchang, Pahang. The leaves were thoroughly washed before air dried (45 °C) and ground to produce powder. The dried powder was packed in the sealed container and stored in a chiller (5 °C) until further use.

Two types of microorganisms selected from the Collection of Functional Food Cultures (CFFC), MARDI were yeast (*Dekkera bruxellensis*) and acetic acid bacteria (*Gluronacetobacter* sp.). The jackfruit leaves powder (10 g) was inoculated individually with yeast and acetic acid bacteria at a concentration of 5% (w/v) with the colony count of 1×10^8 cfu/mL, respectively for duration of 6 days at 30 °C. The mixed cultures fermentation was conducted by mixing the acetic acid bacteria and yeast mother stock with the colony count of 1×10^8 cfu/mL at the ratio of 1:1 for the same period of fermentation time. Non fermented jackfruit leaves powder suspension was prepared as a control. During fermentation, sampling was performed at day 1, 2, 3, 4 and 6. The supernatant was collected after centrifuged the jackfruit leaves suspension at 10,000 rpm for 10 minutes and subjected to pH, Brix, total reducing sugar content and antioxidant properties analysis.

2.2 Chemicals

Solvents and chemicals were of analytical grade purchased from local suppliers. All standards such as ascorbic acid, gallic acid and quercetin were acquired from Sigma Aldrich, USA. UV-Vis spectrophotometer (Agilent, Varian 50 Conc, France) was used to measure the absorbance for total reducing sugars and antioxidant analyses.

2.3 pH, Total Soluble Solids (Brix) and Total Reducing Sugar

The pH measurement was made using a pH meter (Mettler Toledo, Model: Seven Easy GMBH 8603, Switzerland) and the total soluble solids of all samples were measured using a refractometer (Atago, PAL-3, Japan) whereby the results were expressed in Brix value. Total reducing sugars were estimated using the dinitrosalicylic acid (DNS) method developed by Miller [6]. Pure glucose was used as sugar standard.

2.4 Total Phenolics Content (TPC) and Total Flavonoids Content (TFC)

TPC was determined using the Folin-Ciocalteu method according to Singleton and Rossi [7]. Gallic acid was used as standard and results were expressed in mg gallic acid equivalent (mg GAE mL^{-1}). TFC was determined using aluminium chloride method adopted from Jia et al. [8]. Standards of quercetin in the concentration range of 0 to 500 $mg \cdot mL^{-1}$ were run, from which the standard curve was plotted.

2.5 DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Free Radical Scavenging Assay

DPPH radical scavenging activity was carried out based on the modified method proposed by Thaipong et al. [9]. The measurement was based on bleaching effect on purple-coloured methanol solution of DPPH. Briefly, the sample extract (0.15 mL) was allowed to react with 2.85 mL of DPPH methanolic solution for 30 min in dark condition. Ascorbic acid was used as the standard and results were expressed as mg ascorbic acid equivalents per mL of sample (mg AAE mL^{-1}).

2.6 Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay was determined according to the method proposed by Benzie and Strain [10] with some modifications. The FRAP working reagent was

produced by mixing 25 mL acetate buffer, 2.5 mL 10 mM TPTZ solution and 2.5 mL 20 mM $FeCl_3 \cdot 6H_2O$ solution and heated for 10 minutes at 37 °C prior to usage. The sample (150 µL) was allowed to react with 2,850 µL of the working reagent for 30 minutes in dark condition. The reaction mixture absorbance was then recorded using UV-Vis spectrophotometer at 593 nm. Results were expressed as mg ascorbic acid equivalents per mL of sample (mg AAE mL^{-1}).

3. Results and Discussion

Different starter culture inoculated in the jackfruit leaves powder exhibited different pH behaviour, total soluble solid and total soluble sugar content during fermentation process (Table 1). Generally, the mixed cultures fermentation showed the drastic reduction of pH value when compared to pure yeast and acetic acid fermentation after 6 days fermentation. The lowest pH value (2.95) was recorded in the mixed cultures fermented jackfruit leaves whereas the pH value for yeast and acetic acid bacteria fermented samples were 5.38 and 3.33, respectively. The declination of pH value in all fermentation processes could be due to the rapid growth of yeast and acetic acid bacteria that utilized sugar as carbon source and convert it into other metabolites that contributing to the acidity of fermented samples. The combination of these two cultures fermentation may provide the synergic effect, whereby the yeast will produce the ethanol for enhancing acetic acid production by acetic acid bacteria. Total soluble solid was measured using refractometer to estimate the content of sugar, soluble organic acids, soluble phenolic acid and other soluble solid compound. Generally, the total soluble solid content in both single culture fermentation processes were slightly drop as the fermentation time increased. At the end of the fermentation process, the total soluble solid value was found to be lowest (9.28 Brix) in the yeast fermentation process compared to others.

Total reducing sugar in yeast and mixed cultures fermentation also showed a decreased trend at the end of the fermentation. However, acetic acid bacteria fermentation exhibited an inverted trend, whereby it produced higher amount of reducing sugar after 6 days of fermentation. A drastic reduction of reducing sugar (from 4.98 to 1.57 mg glucose per mL,) was shown in the yeast fermentation process, most probably due to the sugar uptake by yeast for production of other metabolites and active compounds. Galafassi et al. [11] has reported that the yeast *Dekkera* sp. can utilize various sugars such as glucose, fructose and arabinose during fermentation. In contrast, the increment of total reducing sugar in acetic acid fermentation sample may due to the breakdown of complex sugar present in the jackfruit leaves into simple sugars like glucose and fructose *via* bacterial hydrolysis action. For further confirmation, it is important to conduct the sugar composition analysis for both fermented and non fermented jackfruit leaves product in the next study.

Total phenolics and flavonoids in all fermented samples throughout 6 days of fermentation were summarized in Fig. 1. As illustrated in Figs. 1a and 1b, all fermented jackfruit leaves exhibited a decrease trend of TPC and TFC. Particularly, the mixed cultures fermented jackfruit leaves showed a drastic reduction of total phenolics content (from 1.70 to 0.78 mg GAE mL^{-1}) as compared to other samples. The data also revealed total flavonoid losses were 31.36%, 55.0% and 34.0% for yeast, acetobacter and mixed culture fermentation, respectively. Based on the findings, we hypothesized that the presence of microorganisms (yeast and acetic acid bacteria) utilized polyphenol compounds in jackfruit leaves for their growth to produce other metabolites that contributing to the improvement of taste, aroma and other functional bioactive metabolites during fermentation.

DPPH method is based on free radical scavenging activity of specific compound or extracts, indicates the ability of antioxidant to suppress lipid oxidation [12]. The DPPH free radicalis are stable with a dark purple colour which will become brownish or colorless when

Table 1 Changes of pH, total soluble solids and total reducing sugar in fermented jackfruit leaves during 6 days fermentation.

Fermentation time (day)	Yeast fermentation			Acetic acid bacteria fermentation			Mixed culture fermentation		
	pH	Total soluble solid (^{o}Brix)	Total reducing sugar (mg glucose mL^{-1})	pH	Total soluble solid (^{o}Brix)	Total reducing sugar (mg glucose mL^{-1})	pH	Total soluble solid (^{o}Brix)	Total reducing sugar (mg glucose mL^{-1})
Control	5.70 ± 0.00	10.5 ± 0.10	4.98 ± 0.02	5.76 ± 0.02	10.47 ± 0.06	4.98 ± 0.02	5.76 ± 0.02	10.47 ± 0.06	4.82 ± 0.01
1	4.35 ± 0.39	9.77 ± 0.05	2.92 ± 0.01	3.76 ± 0.01	10.47 ± 0.06	19.71 ± 0.66	4.02 ± 0.00	13.40 ± 0.06	1.59 ± 0.01
2	4.11 ± 0.00	9.65 ± 0.05	2.19 ± 0.06	3.37 ± 0.01	10.30 ± 0.00	8.50 ± 0.91	3.33 ± 0.00	11.70 ± 0.06	1.73 ± 0.07
3	4.36 ± 0.07	9.53 ± 0.12	1.50 ± 0.01	3.35 ± 0.00	10.40 ± 0.00	8.03 ± 0.18	3.10 ± 0.00	11.20 ± 0.00	1.75 ± 0.09
4	5.33 ± 0.15	9.53 ± 0.05	1.57 ± 0.01	3.33 ± 0.00	10.17 ± 0.06	7.43 ± 0.61	2.95 ± 0.01	11.30 ± 0.00	1.81 ± 0.08
6	5.38 ± 0.04	9.28 ± 0.04	1.57 ± 0.05	3.33 ± 0.00	10.17 ± 0.06	6.88 ± 0.07	2.95 ± 0.01	10.90 ±0.00	1.77 ± 0.04

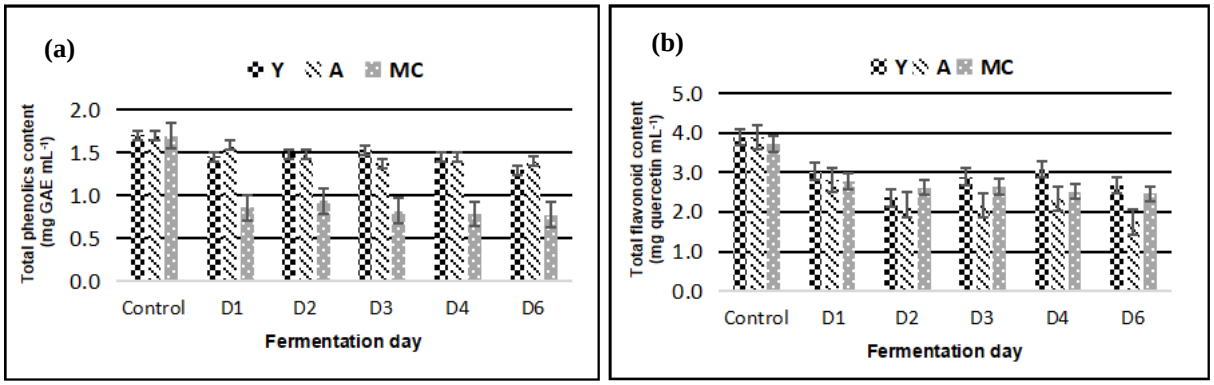

Fig. 1 Total phenolics content, TPC (a) and total flavonoids content, TFC (b) of jackfruit leaves during 6 days of fermentation. Values are the mean of triplicates ± SD. Y—yeast, *Dekkera bruxellenxis*, A—acetic acid bacteria, *Gluronacetobacter* and MC—mixed culture.

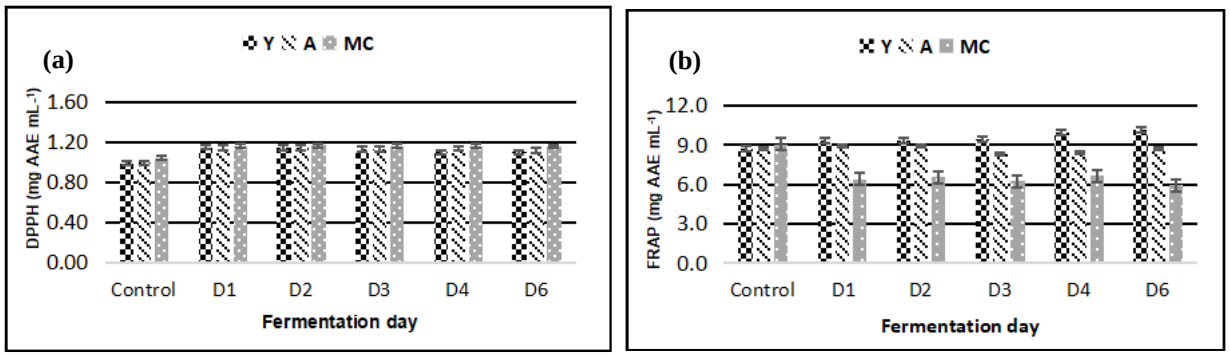

Fig. 2 Radical scavenging activity of fermented jackfruit leaves by (a) DPPH, (radical scavenging activity, %RSA) and (b) Ferric-reducing antioxidant power (FRAP) during 6 days of fermentation. Values are the mean of triplicates ± SD. Y—yeast, *Dekkera bruxellenxis* and A—acetic acid bacteria, *Gluronacetobacter* and MC—mixed culture.

scavenged with antioxidants. As illustrated in Fig. 2a, the scavenging activity against DPPH radical for all fermented leaves samples was slightly increased compared to non fermented (control) sample. Mixed culture fermented leaves gave the highest DPPH free radical scavenging activity (1.16 mg AAE mL^{-1}) after 2 days of fermentation and then remained the same antioxidant activity even subjected to 6 days of fermentation periods.

In contrast with DPPH, the FRAP assay (Fig. 2b) exhibited a different trend between all the samples. The FRAP value was found to be the lowest for mixed cultures samples, whereby it showed a drastic drop after 6 days of fermentation (5.96 mg AAE mL^{-1}) when compared to control sample (9.05 mg AAE mL^{-1}). However, the FRAP value for yeast fermented leaves showed an increasing trend during the fermentation period and exhibited approximately 10.12 mg AAE mL^{-1} sample after 6 days of fermentation.

4. Conclusions

In general, single and mixed cultures fermentation on jackfruit leaves have exhibited different behaviour changes in terms of pH value, total soluble solids, total reducing sugar and antioxidant properties. The differences in antioxidant activities were highly related to the specific metabolites produced by different microorganisms which contribute to different antioxidant mechanism as observed in this study. Further investigation will be conducted to identify the types of metabolites produced in single and mixed cultures fermentation of jackfruit leaves.

References

[1] Gupta, A. K., and Tandon, N. 2004. *Review on Indian*

Medicinal Plants. New Delhi, India: Indian Council of Medical Research, 182-200.

[2] Loizzo, M. R., Tundis, R., Chandrika, U. G., Abeysekera, A. M., Menichini, F., and Frega, N. G. 2010. "Antioxidant and Antibacterial Activities on Foodborne Pathogens of *Artocarpus heterophyllus* (*Moraceae*) Leaves Extracts." *Journal of Food Science* 75 (5): 291-5.

[3] Omar, H. S., El-Beshbishy, H. A., Moussa, Z., Taha, K. F. and Singab, A. N. B. 2011. "Antioxidant Activity of *Artocarpus heteropyllus* Lam (Jackfruit) Leaf Extracts: Remarkable Attenuations of Hyperglycemia and Hyperlipidemia in Streptozotocin-Diabetic Rats." *The Scientific World Journal* 11: 788-800.

[4] Wang, X. L., Di, X. X., Shen, T., Wang, S. Q., and Wang X. N. 2017. "New Phenolic Compounds from the Leaves of *Artocarpus heterophyllus*." *Chinese Chemical Letters* 28: 37-40.

[5] Hur, S. J., Lee, S. Y., Kim, Y-C., Choi, I., and Kim, G-B. 2014. "Effect of Fermentation on the Antioxidant Activity in Plant-Based Foods." *Food Chemistry* 160: 246-356.

[6] Miller, G. A. I. L. 1959. "Use of Dinitrosalicylic Acid for Detection of Reducing Sugars." *Analytical Chemistry* 31: 3.

[7] Singleton, V. L., and Rossi, J. A. 1965. "Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents."*Am. J. Enol. Vitic.* 16: 144-58.

[8] Jia, Z. S., Tang, M. S., and Wu, J. M. 1999. "The Determination of Flavanoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals." *Food Chemistry* 64: 555-9.

[9] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., and Byrne, D. H. 2006. "Comparison of ABTS, DPPH, FRAP and ORAC Assays for Estimating Antioxidant Activity from Guava Fruit Extracts." *J. Food Comp Anal.* 19: 669-75.

[10] Benzie, I. F. F., and Strain, J. J. 1996. "The Ferric Reducing Ability of Plasma (FRAP) as a Measure of 'Antioxidant Power': the FRAP Assay." *Analytical Biochem* 239: 70-6.

[11] Galafassi, S., Merico, A., and Pizza, F. 2011. "*Dekkera/Brettanomyces* Yeast for Ethanol Production from Renewable Sources under Oxygen-Limited and Low pH Conditions." *J. Ind. Microbiol Biotechnol* 38 (8): 1079-88.

[12] Jayanta, K. P., Sameer, K. S., and Manas, R. S. 2016. "Biochemical Composition and Antioxidant Potential of Fermented Tropical Fruit Juices." *Agro. food Ind. Hi-Tech.* 27 (4): 29-33.