Effect of Yeast and Acetic Fermentation on Phytochemical and Antioxidant Properties of Jackfruit Pulp (*Artocarpus heterophyllus* L.)

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Abstract

Jackfruit (*Artocarpus heterophyllus* L.) is one of the important tropical fruits in Malaysia, mainly used for fresh consumption because of its good source of energy, deliciously sweet in taste, proteins, dietary fiber, minerals and vitamins. To date, there is lack of fermentation study reported on jackfruit. Hence, the aim of this work is to evaluate the phytochemical content and antioxidant properties of fermented jackfruit which was subjected to acetic acid bacteria and yeast fermentation individually for a duration of 7 days. The changes in the pH value, brix value, total reducing sugar content, total phenolic and total flavonoid content were examined for both yeast and acetic acid fermented jackfruit samples. Few antioxidant activities such as ferric reducing antioxidant power (FRAP) value and 2,2-diphenyl -1-picrylhydrazyl (DPPH) free radical scavenging activity were also analyzed to determine the differences in the respective properties of jackfruit pulp after being subjected to yeast or acetic acid fermentation. There is a slight change on brix value and DPPH free radical scavenging activity (31.05-33.17% radical scavenging activity) for both yeast and acetic acid fermented samples. However, acetic acid fermented jackfruit exhibited higher total reducing sugar content (23.24 mg glucose/mL) and total phenolics content when compared to yeast fermented jackfruit sample. Both yeast and acetic acid fermented jackfruit samples showed higher FRAP value (0.435 -0.48 mg ascorbic acid/mL) than unfermented jackfruit sample (0.372 mg ascorbic acid/mL) except for total flavonoid content which exhibited a decreasing trend after fermentation.

Keywords jackfruit, fermentation, antioxidant activity, acetic acid, yeast

INTRODUCTION

Jackfruit is a non-seasonal fruit in Malaysia which is known by the botanical name of *Artocarpus heterophyllus* L. and it is a part of the *Moraceae* family. The most popularly grown of jackfruit varieties in Malaysia are Tekam Yellow, Mastura and Mantin. Young fruits are commonly cooked and served as vegetables whereas the pulp of ripe fruit is usually eaten fresh or used in the productions of food products. Many products could be made from jackfruit pulp such as jam, chips, juice, jelly and biscuits.

Generally, 100 g of jackfruit pulp contains 72.0 g of moisture, 1.9 g of protein, 0.4 g of fat, 16.0 g of carbohydrate and 3.6 g of fiber [1]. Jackfruit pulp is rich in phytochemicals such as phenolics and flavanoids which are essential for good antioxidant properties [2]. Several phytochemical studies have shown that jackfruit contains useful compounds such as flavonoids, sterols and prenylflavones which may have been responsible for various pharmacological properties [3, 4, 5, 6]. Nevertheless, the market demand for jackfruit still poor compared to other tropical fruits such as pineapples, mango and papaya. This is due to

the wide variation of fruit quality and common belief that excessive consumption of jackfruit will lead to certain digestive disorders [7].

Due to the lack of postharvest knowledge and less shelf life, fresh fruits cannot be stored for longer time. The shelf life of jackfruit can be improved and nutrient content can be preserved by the application of the fermentation process. Substances in fermented foods have been found to have antimicrobial properties and protective effect against cardiovascular diseases and development of cancer [8]. Antioxidants are compounds that are able to delay or inhibit oxidation by scavenging free radicals in the body. Research interest in naturally occurring antioxidant in fruits among scientist and nutritionist has increased remarkably as they may reduce the risk of chronic diseases including heart disease and certain cancers. According to Harris & Brannan [9], the antioxidant capacity of fruit pulp was influenced and highly related with the ripening stage of the fruits, whereby the ripened fruits showed lower antioxidant power compared to the young fruits. Faria et al. [10] reported that jackfruit contains bioactive compounds such as carotenoids which may serve as a good source of antioxidants and provide health benefits.

To the best of our knowledge, there are no reported studies on jackfruit fermentation using yeast and acetic acid bacteria for value added purpose. Therefore, the aim of this study was to evaluate the changes in phytochemical content and antioxidant properties of fermented jackfruit which was subjected to individual fermentation by acetic acid bacteria and yeast for a duration of 7 days.

EXPERIMENTAL

Materials

a. Fruit

The fully ripened jackfruits (Tekam Yellow) were collected from Agriculture Park in Lanchang, Pahang. The pulp was separated from the seeds and thoroughly cleaned with filtered water before minced into smaller chunks and oven dried (45^{o}C) to produce fruit powder. The dried powder were packed in the aluminium bag and stored in a chiller (5^{o}C) until further process and analysis.

b. Chemicals

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

Fermentation Process

Two types of microorganism were used in the production of fermented jackfruit pulp samples, which were yeast (*Dekkera bruxellensis*) and acetic acid bacteria (*Gluronacetobacter sp.*), obtained from Collection of Functional Food Cultures (CFFC), MARDI. A jackfruit pulp suspension was prepared at the concentration of 5% (w/v) and inoculated with yeast and acetic acid bacteria with the colony count of 1 x 10^8 cfu/mL, individually for a duration of 7 days at 30°C. Non-fermented sample was set up as a control. During fermentation, sampling was performed at 1-day time interval to analyze its pH, Brix, total reducing sugar content and antioxidant properties.

Phytochemical and Antioxidant Analyses

a. pH and total soluble solids (Brix)

The pH measurements were made using the pH meter (Mettler Toledo, Model: Seven Easy GMBH 8603, Switzerland) and standard buffer solutions of pH 4.0 and 7.0 were used as reference. The total soluble solids of both fermented and non fermented samples were measured using refractometer (Atago, PAL-3, Japan). Results were expressed in Brix value.

b. Total reducing sugar content

Total reducing sugars were estimated using dinitrosalicylic acid (DNS) method developed by Miller [11]. Pure glucose was used as sugar standard.

c. Antioxidant analyses

i. Total phenolic content (TPC)

Total phenolic content (TPC) in both fermented and non fermented samples were determined using Folin-Ciocalteu method. Briefly, 1 mL sample was allowed to react with 5 mL of diluted Folin-Ciocalteu reagent for 5 minutes. Then, 4 mL of 7.5% sodium carbonate solution was added to the mixture and kept in dark condition for 2 hours at room temperature (27^{o}C). Absorbance was recorded at 765 nm using UV-Vis spectrophotometer and gallic acid was used as a standard. The TPC was expressed in mg gallic acid equivalent (mg GA/mL). [12].

ii. Total flavonoid content (TFC)

Total flavonoid content was determined using aluminium chloride method adopted from Jia et al. [13] with minor modification. The quercetin was used as a standard. Firstly, 1 mL sample was added with distilled water (4 mL) and subsequently mixed with 0.3 mL of 5% $NaNO_3$ solution. The $AlCl_3$ (0.3 mL) solution was added to the mixture 5 minutes later. The mixture was thoroughly mixed and allowed to stand for 6 minutes before adding 2 mL of 1M NaOH solution. After that, the distilled water was added to bring the final volume to 6 mL. Absorbance was measured at 510 nm using UV-Vis spectrophotometer and the results were expressed as mg quercetin equivalent per mL of sample (mg QUE/mL).

iii. 2,2 diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

Determination of antioxidant activity through DPPH scavenging system was carried out according to method proposed by Thaipong et al. [14] with slight modification. The stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and stored at -20^{o}C before used. The working solution was prepared by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance at 1.1 ± 0.02 units at 515 nm using the UV-Vis spectrophotometer. The sample extract (0.15 mL) was allowed to react with 2.85 mL of DPPH methanolic solution for 30 min in dark condition. Percentage of radical scavenging activity (% RSA) was calculated in the following way:

$$\% \text{ RSA} = (A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}} \times 100$$

Where, control is solution without sample extract and A = absorbance at 515nm.

iv. Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to Benzie and Strain [15] with some modifications. The stock solutions of 300 mM acetate buffer at pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution was prepared. The FRAP working reagent was produced by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3.6H_2O$ solution. The reagent was incubated for 10 minutes at 37°C prior to usage. The sample extract (150 µL) was allowed to react with 2850 µL of the working reagent for 30 minutes in dark condition. Absorbance of was measured using UV- Vis spectrophotometer at 593 nm. Ascorbic acid was used as the standard and results were expressed as mg ascorbic acid equivalents per mL of sample (mg AAE/ mL)

RESULTS AND DISCUSSION

Phytochemical analysis of fermented jackfruit pulp

The initial pH for yeast and acetic acid fermentation on jackfruit pulp were 5.1 and 5.76 respectively. As shown in Table 1, it was notable that the pH decreased gradually along the fermentation period in both yeast and acetic fermentation. This is expected due to the yeast and bacteria growth during fermentation requiring consumption of sugars and production of other metabolites such as alcohol and organic acids. The pH of growth media plays an important role in fermentation process, depending on the type of microorganisms used. The optimum pH can induce the degradation of cell wall releasing phenolic compounds and affect the antioxidative activity [16]. Generally, the lowest pH value was observed after 7 days of fermentation, which were 3.47 and 3.08 for yeast and acetic fermentation, respectively.

Brix value was measured to estimate the total soluble solid content, which include the estimation of sugar content, soluble organic acids, soluble phenolic acid and other soluble solid compounds such as proteins and minerals, present in both fermented and non fermented sample. The reduction of total reducing sugars content was observed throughout the yeast fermentation mainly because of the conversion of sugars during fermentation into ethanol production. According to Galafassi et al. [17], the yeast *Dekkera sp.* can utilize different sugar such as glucose, fructose and arabinose. Rozpedowska et al. [18] have described the growth of *Dekkera* sp. will contribute to production of substantial amounts of ethanol under aerobic condition. Furthermore, Blomqvist et al. [19] had reported that the growth rate and ethanol yield of the yeast was higher when using glucose as carbon source in comparison to maltose. Generally, the lowest total soluble solids and reducing sugar were observed in yeast fermented jackfruit at the end of the fermentation process with 10.88 °Brix value and 6.82 mg glucose/mL respectively.

During the acetic acid fermentation process, total soluble solids content in acetic acid fermented samples increased slightly from 13.0 to 13.85 °Brix, whereas the reducing sugars content showed a drastic increasing trend. The increment of total reducing sugar in acetic acid fermentation sample may due to the breakdown of complex sugar present in the jackfruit pulp into simple sugars such as glucose and fructose *via* microbial hydrolysis action. Basically, the decrease in total soluble solids corresponded with the decreasing trend in total reducing sugar contents of the sample, however, there was a slight change of total soluble solids observed in fermented jackfruit pulp samples, mainly because total soluble solids also represent other soluble solids substances presents in sample such as organic acids, proteins, and minerals, which may increase during acetic acid fermentation.

This current work demonstrated good fermentative potential of jackfruit either for yeast or acetic acid bacteria fermentation. Thus, further exploitation of this fruit for targeting functional fermented beverage and nutraceutical products are possible.

Table 1 Changes of pH, total soluble solids (°Brix) and total reducing sugar content in fermented jackfruit pulp during 7 days of fermentation

Fermentation time (day)	Yeast fermentation			Acetic fermentation		
	pH	Total soluble solids (°Brix)	Total reducing sugar (mg glucose/ml)	pH	Total soluble solids (°Brix)	Total reducing sugar (mg glucose/ml)
Control	5.1 ± 0.01	13.00 ± 0.10	14.41 ± 0.00	5.76 ± 0.02	13.00 ± 0.10	14.41 ± 0.00
1	4.72 ± 0.03	12.65 ± 0.27	13.78 ± 0.40	3.35 ± 0.02	13.73 ± 0.08	25.24 ± 1.11
2	3.79 ± 0.01	12.28 ± 0.57	12.06 ± 0.05	3.17 ± 0.05	13.67 ± 0.08	22.74 ± 0.16
3	3.77 ± 0.00	12.13 ± 0.37	8.22 ± 0.05	3.13 ± 0.03	13.53 ± 0.05	20.94 ± 0.15
4	3.86 ± 0.21	11.55 ± 0.18	7.91 ± 0.17	3.21 ± 0.03	13.60 ± 0.11	20.78 ± 0.29
5	3.49 ± 0.02	11.20 ± 0.11	6.74 ± 0.02	3.03 ± 0.00	13.02 ± 0.04	19.72 ± 0.04
6	3.47 ± 0.02	10.90 ± 0.13	6.45 ± 0.16	3.06 ± 0.13	13.47 ± 0.05	22.81 ± 0.28
7	3.47 ± 0.02	10.88 ± 0.08	6.82 ± 0.05	3.08 ± 0.07	13.85 ± 0.16	23.24 ± 0.18

Antioxidant properties of fermented jackfruit pulp

a. Total phenolic (TPC) and total flavonoid (TFC) content

Antioxidant properties of fermented jackfruit pulp were carried out in term of total phenolic, flavonoid and DPPH free radical scavenging activity are summarized in Figures 1 & 2. Phenolic compounds are known as good antioxidants because of their potential to scavenge free-radical and active oxygen species such as singlet oxygen, superoxide free radicals and hydroxyl radicals [20]. Based on the findings, it was discovered that acetic acid fermentation had a positive effect on TPC in jackfruit pulp. The TPC observed increased up to 0.15 mg GA/ml in acetic acid fermented jackfruit pulp after 7 days of fermentation, whereas a slight increment (0.11 mg GA/ml) was noted in yeast fermented jackfruit pulp (Figure 1A). During fermentation, microbial enzyme was secreted by the microorganism and capable to hydrolyse complex polyphenols into small molecules (also known as bioactive metabolite) which may contribute to the functional effect. Thus, the increment of phenolic compounds observed in both microbial fermentations was a result of the breakdown of polyphenol present in jackfruit pulp *via* microbial hydrolysis action.

Flavonoids are a group of phytonutrients that is responsible for natural colours in the fruits and vegetables belonging to polyphenols family. Various scientific findings have reported on the vast health benefits of flavonoids present in fruits and vegetable. Numerous clinical studies have shown that flavonoids such as quercetin, catechin, flavanones and isoflavones can contribute to multiple health benefits such as prevention of cardiovascular disease, osteoporosis and lung cancer [21]. In this study, the results of TFC evaluated in both yeast and acetic acid fermented samples were observed a decreasing trend during the first 3 days fermentation period, however, the TFC was noted a fluctuating trend of increment on day 4 onwards (Figure 1B**)**. Generally, after 7 days fermentation period, the TFC was reduced slightly from 0.08 to 0.05 and 0.06 quercetin equivalent (mg/mL) in yeast and acetic acid

fermented jackfruit pulp samples respectively. Microorganisms can produce a wide range of metabolites throughout fermentation process. Thus, we expect that during fermentation, some flavonoids compound have been utilized by the microbes to produce other active metabolites through their enzymes secretion, which may contribute to other bioactivities effect in fermented fruit.

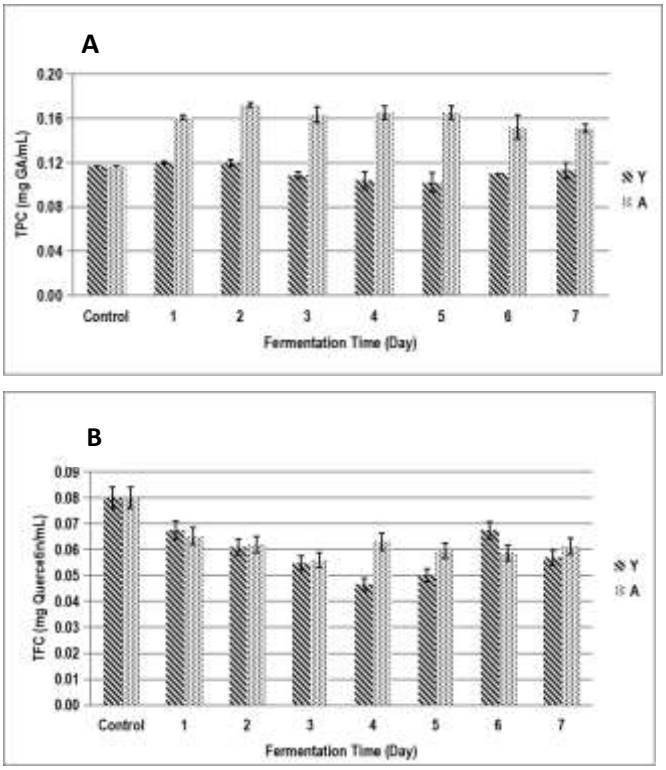

Figure 1 Total phenolic content, TPC (**A**) and total flavanoid content, TFC (**B**) during 7 days of fermentation.Values are the mean of triplicates ± SD.
Y- yeast, *Dekkera Bruxellenxis* and A- acetic acid bacteria, *Gluronacetobacter*.

b. DPPH and FRAP assay

The DPPH method, a reliable general assessment, was used widely to investigate the free radical scavenging effects of antioxidants compound presents in sample. The free radical scavenging effects of both fermentated samples were presented in Figure 2A. The results indicated that acetic acid fermented jackfruit pulp exhibited higher DPPH clearance (33.2%) in comparison to non fermented (31.8%) and yeast fermented jackfruits (31.0%). Compared to this current work, Jayanta et al. [22] obtained lower DPPH free radical scavenging activity (10.0- 20.0%) during fermentation of jackfruit with *Sacchromyces cerevisiae* for 15 days. As mentioned earlier, the flavonoid content was not prominent during the fermentation. Thus, this current DPPH results obtained suggested that the improvement of DPPH scavenging ability could be due to the presence of phenolic acids. Similar to our findings, other researchers also found that the DPPH free radicals scavenging activity was highly related to phenolic acid compounds present in the plants [23, 24].

Other than DPPH assay, the FRAP assay is also commonly used to evaluate the antioxidant power because it is simple, rapid and highly sensitive [25]. On top of that, the reaction was consistent and linearly related to molar concentration of the antioxidants. Higher FRAP values indicated higher antioxidant capacity because FRAP value was based on reducing ferric ion, where antioxidants act as the reducing agent. The reducing power in

fermented jackfruit pulp increased in both fermentation process is shown in Figure 2B. After 7 days of fermentation, yeast fermented jackfruit pulp exhibited higher FRAP value (0.48 ascorbic acid equivalent, mg/mL) than acetic acid fermented sample (0.44 ascorbic acid equivalent, mg/mL). This current work indicates that jackfruit pulp has a good reducing power and both types of fermentation process were able to improve the antioxidant activity of the jackfruit.

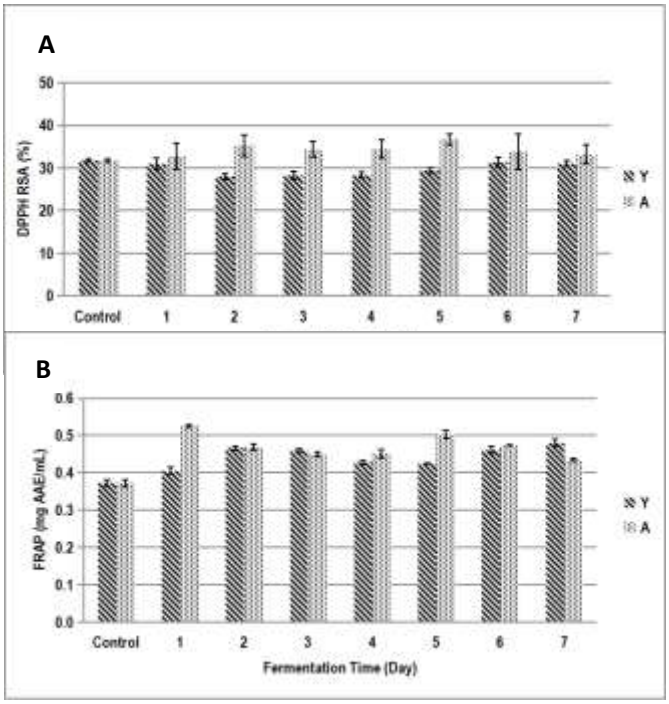

Figure 2 Radical scavenging activity of fermented jackfruit pulp by (**A**) DPPH, (radical scavenging activity, %RSA) and (**B**) Ferric-reducing antioxidant power (FRAP) during 7 days of fermentation. Values are the mean of triplicates ± SD. Y- yeast, *Dekkera Bruxellenxis* and A- acetic acid bacteria, *Gluronacetobacter*.

CONCLUSION

Generally, mono-culture fermentation process has positive influence on phytochemical properties, total phenolic content and antioxidant activities. However, the flavanoids content in jackfruits were slightly reduced when subjected to yeast or acetic acid fermentation. Advanced study will be conducted to determine the type of organic acids, phenolic acids and flavonoids presents in the fermented jackfruit pulp samples which indirectly contributing to the increment of antioxidant properties after fermentation process. This preliminary study have indicated that jackfruit could be a highly promising raw material for the development of functional food product through bioprocessing strateg

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