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MODULATION OF PHENOLICS SUBSTANCES AND ANTIOXIDANT ACTIVITY IN *MANDAI CEMPEDAK* BY UNSALTED SPONTANEOUS AND *Lactobacillus casei* INDUCED FERMENTATION

[Modulasi Komponen Fenolik dan Aktivitas Antioksidan pada Mandai Cempedak yang Difermentasi Spontan tanpa Garam dan Diinduksi Lactobacillus casei]

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ABSTRACT

Cempedak (Artocarpus champeden) is a tropical fruit whose inner-skin contains high polyphenolic-based antioxidant. Traditionally, the inner-skin is soaked in brine to induce spontaneous lactic acid bacteria (LAB) fermentation. This research aimed to investigate the changes of polyphenolics substances and antioxidant activity during the course of spontaneous and *Lactobacillus casei* induced fermented at optimum (37°C) and sub-optimum (8°C) temperatures. Phenolic substances were spectrophotometrically measured with gallic acid (GAE), tannic acid (TAE), and catechin (CE) as the standards for the respective groups. The 50% maximum inhibitory concentration (IC_{50}) for DPPH reduction was measured. A thin layer chromatography (TLC) using different solvents was utilized to qualitatively show differences of substances extracted from the unfermented and fermented *mandai cempedak*. The retention factor (Rf) values for TLC spots were measured after the plates were exposed under 366 and 254 nm of UV lamp. Fermentation increased phenolic substances release from the inner skin of *cempedak* which positively modulated the potential antioxidant activity. Sub-optimum temperature fermentation reduced total phenolic-based antioxidant. Ethyl acetate or combinations of n-hexane and ethyl acetate gave better separation in TLC plate. Differences in stain patterns were exhibited by *mandai cempedak* before and after *L. casei* induced fermentation. In summary, *L. casei* induced fermentation was more effective at optimum temperature to increase phytochemical substances of *mandai cempedak*, while spontaneous fermentation showed to be more effective after 11 and 13 days at sub-optimum temperature.

Keywords: antioxidant activity, fermentation, *mandai cempedak*, phytochemicals, TLC

ABSTRAK

Cempedak (Artocarpus champeden) adalah buah tropis yang kulit bagian dalamnya mengandung antioksidan berbasis polifenol dengan konsentrasi yang cukup tinggi. Secara tradisional, kulit bagian dalam direndam dalam air garam untuk menginduksi fermentasi spontan bakteri asam laktat (LAB). Penelitian ini bertujuan untuk mengetahui perubahan komponen polifenolik dan aktivitas antioksidan selama fermentasi spontan dan terinduksi L. casei pada kondisi optimum (37°C) dan sub optimum (8°C). Senyawa-senyawa fenolik diukur secara spektrofotometri dengan asam gallic (GAE), asam tanat (TAE), dan katekin (CE) sebagai standar. Konsentrasi penghambatan setengah dari maksimal (IC_{50}) untuk reduksi DPPH diukur secara spektrofotometri. Kromatografi lapis tipis tipis (TLC) digunakan untuk secara kualitatif menunjukkan perbedaan senyawa-senyawa yang diekstraksi dari mandai cempedak yang tidak difermentasi dan difermentasi dengan pelarut yang berbeda. Nilai faktor retensi (Rf) diukur untuk masing-masing senyawa yang berhasil dipisahkan di bawah sinar UV dengan panjang gelombang 366 dan 254 nm. Fermentasi meningkatkan pelepasan zat fenolik dari matriks buah yang secara positif memodulasi aktivitas antioksidan potensial. Fermentasi suhu suboptimal mengurangi total antioksidan berbasis fenol. Etil asetat atau kombinasi n-heksana dan etil asetat memberikan pemisahan yang lebih baik pada pelat KLT. Perbedaan pola noda diperlihatkan dari mandai cempedak sebelum fermentasi dan setelah fermentasi dengan induksi L. casei. Sebagai kesimpulan, L. casei menginduksi fermentasi lebih efektif pada suhu optimal, sedangkan kultur spontan lebih efektif setelah 11 dan 13 hari pada fermentasi sub optimum.

Kata kunci: *aktivitas antioksidan, fermentasi, fitokimia, mandai, TLC*

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INTRODUCTION

Cempedak fruit (*Artocarpus integer* (Thunb.) Merr.) becomes a functional food source cultivated and easily obtained in Kalimantan. The appearance of the fruit is in resemblance to jackfruit, but has a stronger and unique aroma. The inner-skin is prevalently processed into fermented product called *mandai cempedak*. Bakar *et al.* (2015) reported that *cempedak* skin contains total phenolics, flavonoids, carotenoids, and antioxidant activity that are higher in concentrations than those in the flesh and seeds. *Mandai cempedak* fermentation is accomplished by soaking the product in brine for a certain period of time. To be effective and to reduce contamination, spontaneous fermentation uses high concentration of salt of approximately at 15-25% w/v (Rhee *et al.,* 2011). However, the addition of salt may limit consumer acceptance of the product and may negatively affect consumer health. Furthermore, the use of salt in spontaneous fermentation of *mandai cempedak* may reduce the potential utilization of derivative products in the food industry. The use of low concentrations of salt or no salt added may contribute to reduce the health risk of high-salt (Nur, 2009). Hygienic processing was proven to reduce the requirement of salt induced spontaneous LAB fermentation as exhibited by Rahmadi *et al*. (2017).

Lactobacillus casei was selected as the pilot culture for induced *mandai cempedak* fermentation due to the availability in the form of commercial culture. Further, *L. casei* is one of the most studied LAB. Therefore, the performance of the LAB in *mandai cempedak* can be better related to fermentation profiles of *L. casei* reported in other research. Rahmadi *et al.* (2017) reported that isolate of *L. casei* may be used to induce fermentation in *cempedak* mandai.

To produce a derivative product, *i.e.* local flavor, that is also rich in antioxidants, fermentation and further processing of *mandai cempedak* are in need to be optimized with the help of starter culture and hygienic fermentation. The rate of growth of *L. casei* in this induced fermentation is almost equal to the growth rate of spontaneous cultures that are predominantly conducted by *L. plantarum* and *Leuconostoc* (Rahmadi *et al.*, 2017; Emmawati, 2015). The sub-optimal fermentation temperature resulted in preferred organoleptic properties of the *mandai cempedak*. General increase of the panelist's acceptance was due to higher pH of the fermented *mandai cempedak*. In derived product, the polyphenol substances of the *mandai cempedak* flour can be maintained when the product was dried at 50°C (Rahmadi *et al.*, 2018).

Fermentation plays a role in the release of phenolic substances from food matrices, while LAB may produce simple hydroxyl (-OH) metabolites, *i.e.* phenyllactic acid, that may be detected as total phenolic (Valerio *et al.*, 2016). Further, the contents of phytochemical compounds are directly related to the antioxidant capacity (Li *et al.*, 2009; Mihai *et al.*, 2011). There is a need of examining phenolic based antioxidant activity to further prove that starter culture in slow and fast fermentation gives better choices of technology that can be commercially applied. This study aims to determine changes in phenolic compounds and DPPH-based antioxidant capacity during spontaneous or induced fermentation with *L. casei* at optimum (37°C) and sub optimum (8°C) temperatures.

MATERIALS AND METHODS

Materials

The raw materials used in this study were mature *cempedak* fruit obtained from traders in the market around Samarinda, East Kalimantan, Indonesia. *Lactobacillus casei* was isolated from Yakult® and cultured in skim milk preparation medium.

Research design

Quantitative study with quasi experimental design approach was utilized, consisting of two parts, namely a comparison between *L. casei* induced and spontaneous fermentations at different temperatures of 37 and 8°C for defined days of observations. Each observation was conducted in two replicates. Parameters observed in this study were total tannins, phenols, flavonoids and antioxidant activity. Retention factors (Rf) for TLC spots were measured after the plates were exposed under 366 and 254 nm of UV lamp.

Mandai cempedak preparation

The inner skin of the *cempedak* fruit was used after being sorted and cleansed. Processing of inner skin of *cempedak* fruit and induction of LAB for fermentation were conducted in accordance with procedure written in patent no. IDS000002032. Spontaneous fermentation was carried out in the same manner without the addition of starter culture of *L. casei*. For the incubation treatment at 37°C, the *mandai cempedak* was observed daily until day 7. For the sub-optimum temperature treatment at 8°C, the *mandai cempedak* was observed every two day up to the 13th day.

Total phenols

Total phenols were measured by modifying methods originally developed by Mu'nisa *et al.* (2012) and Nurhayati *et al.* (2012). As much as 0.3 g of ethanolic extract of *mandai cempedak* was carefully weighed and dissolved in 10 mL of ethanol (Smart-Lab, Indonesia) and distillated water (Soil Laborato-

ry, Mulawarman University) at ratio of 1:1. About 0.2 mL of the extract solution was taken and added with 15.8 mL of distillated water and 1 mL of 50% (v/v) of Folin-Ciocalteu (Sigma-Aldrich, Germany) reagents prepared in aqueous solution. The solution was allowed to stand for ±8 minutes and added with 3 mL of 5% (w/v) of Na_2CO_3 (Sigma-Aldrich, Germany) prepared in aqueous solution. The solution was further allowed to stand for ±2 hours in a dark condition at room temperature (28±2°C). The absorbance was measured at 725 nm of wavelength. The absorbance was plotted against the standard curve of gallic acid prepared in the same manner. Total phenols were expressed in mg equivalent of gallic acid per kg of dry weight.

Total tannins

A modified method from Malangngi *et al.* (2012) was performed to measure the total tannins. A total of 0.5 g of *mandai cempedak* was macerated with 10 mL of diethyl ether (Merck, Singapore) for 20 hours in a closed tube, then the sample was filtered with Whatman filter paper. The obtained residue was boiled with 100 mL of distillated water for 2 hours, then was cooled and filtered. The extracts obtained were added with distillated water until the extract volume reached 100 mL. A total of 0.1 mL of the extract was added with 0.1 mL of 50% (v/v) of Folin-Ciocalteu reagents prepared in aqueous solution and was vortexed with vortex mixer. The sample was added with 2 mL of 5% (w/v) of Na_2CO_3 prepared in aqueous solution and was vortexed. The absorbance was measured at 760 nm of wavelength after incubation for 30 min at room temperature (28±2°C). The readings were plotted against the standard curve of tannic acid (Sigma-Aldrich, Germany) prepared in the same manner. The total content of tannins was expressed in mg of tannic acid per kg of dried extract.

Total flavonoids

To measure total flavonoids, the method developed by Zou *et al.* (2004) was employed. A total of 1 mg of the *mandai cempedak* extract was weighed and dissolved in 10 mL of 95% ethanol (SmartLab, Indonesia). Distillated water was added to the solution at 0.7 mL. To each solution, 0.1 mL of 5% $NaNO_2$ (Sigma-Aldrich, Germany) prepared in aqueous solution was added. After being stored for 5 minutes at room temperature, the sample was added with 0.1 mL of 10% $AlCl_3$ (Sigma-Aldrich, Germany) prepared in aqueous solution. After 6 minutes, 0.5 mL of 1 M NaOH (Merck, USA) was added. All ingredients were mixed evenly and incubated for 10 minutes at room temperature. The absorbance was measured at a wavelength of 510 nm with 1 mL sample was replaced with 1 mL of 95% ethanol solvent. The results obtained were plotted against the standard catechin curve (Sigma-Aldrich, Germany) prepared in the same way. Total flavonoid was expressed as mg of catechin equivalent per kg of dry weight of extract.

Antioxidant activity

The antioxidant activity test was performed by spectrophotometric method (Farhan *et al.*, 2012) by calculating the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany) reduction. A total of 1 mL of diluted extract in ethanol was added to 1 mL of DPPH (0.15 mM in ethanol) and at the same time, a control consisting of 1 mL DPPH with 1 mL of ethanol was prepared. The solution was well mixed and then incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. Vitamin C was used as a positive control and ethanol was used as a blank. The DPPH capability of the extract was calculated using equation 1:

$$\% \text{ antioxidant activity} = \frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}} X100$$

..... equation (1)

where, the absorbance of the control was the absorption of DPPH + ethanol, the absorbance of the sample was the absorbance of the radical DPPH + sample.

The obtained data was presented with IC_{50} value, and the value was obtained from linear regressing %-DPPH inhibition of sample compound (31.25; 62.5; 125; 250 and 500 ppm). The linear regression equation is calculated as in equation 2 and equation 3:

$$Y = 50 = Ax+B \quad \text{..... equation (2)}$$

$$x = \frac{50-B}{A} \quad \text{..... equation (3)}$$

where, Y is IC_{50} constant value (50), A is The coefficient on the variable x, B is constants and x is IC_{50} value in ppm.

TLC phytochemical profile of *mandai cempedak*

Cempedak powder sample before and after the 13^{th} day of the cold fermentation was prepared in ethanol solution according to method by Hanani (2014). Each sample was then spotted on a 10x10 cm of silica-based TLC plate (Merck, USA). Distance between spots was about 1 to 1.5 cm. The spot was applied with a volume of 5-10 µL. Spot was carefully planted to obtain as small diameter as possible. After the spotting was completed, the plate was left to dry in room temperature. The TLC plate was inserted into a chamber containing eluent in the pre-

pared ratio of: (I) n-hexane (Merck, USA): ethyl acetate (Merck, USA) (1.4: 0.6), (II) n-hexane: ethyl acetate (0.6: 1.4), (III) chloroform (Merck, USA): methanol (Merck, USA) (1.6: 0.4), and (IV) ethyl acetate. Eluent was allowed to reach the limits of the creepage distance of the plate. The plates were removed and dried in the air at room temperature. The resulting spots were observed under ultraviolet lamps at wavelengths of 254 nm and 366 nm.

RESULTS AND DISCUSSION

Profile of phenolic substances and antioxidant activity

Bakar *et al.* (2015) mentioned that the potential of total phenolic compounds is 21,000 mg GAE/kg and the potential total flavonoid is 17,000 mg CE/kg in the outer skin of the *cempedak* fruit. The flesh of *cempedak* fruit had potential total phenolic compounds amounted to 4,400 mg GAE/kg and total flavonoids amounted to 820 mg CE/kg. Total phenols of fermented mandai *cempedak* (Table 1) was in close similarity to that reported previously (Bakar *et al.*, 2015). Total flavonoids throughout the fermentation duration were in increasing concentrations, despite the value was not as high as reported by Bakar *et al.* (2015). Of the four combinations of observed fermentation methods, the increase in these three phenolic parameters was consistent with the length of fermentation time (Table 1).

The total phenols increased between 0.5 and 2.5 folds higher in spontaneous and *L. casei* induced fermentation at all observed fermentation temperatures. Total tannins increased up to two folds higher, as well as total flavonoids. This caused the increase of antioxidant activity potential in fermented *mandai cempedak* (Table 1). Dajanta *et al.* (2013) reported that the total phenol content showed a higher increase, giving 217 and 859% in fermented black soybeans and yellow soybeans compared to unfermented ones. Nazarni *et al.* (2016) reported that *jaruk tegarun* extract, LAB fermented *tegarun* (*Crataeva nurvala*, Buch HAM) flower, had higher total tannins compared to the unfermented extract.

Antioxidant activity in fermentation showed an increase, this was indicated by decreasing IC_{50} value of antioxidant activity in *mandai cempedak* (Table 1). The smaller the value of IC_{50}. the higher the antioxidant activity, as lesser concentration of the extract was required to inhibit DPPH reduction. This was consistent with Curiel *et al* (2015) that the inhibition of free radicals from *Myrtus communis* extract fermented with *L. plantarum* was higher than that of the non-fermented ones.

The concentration of phenol, tannin, flavonoid released from the inner skin of *cempedak* was influenced by the use of different fermentation temperatures (Table 1). As a general observation, the total tendency of phenol tannins, and flavonoids at a temperature of 8°C for seven days is approximately 50% of the concentrations of phenolic compounds released at 37°C during the same fermentation time. From this data, it can be concluded that the fermentation temperature affects the release of active compounds from the food matrix.

The temperature and power of hydrogen (pH) may play a role in the release of phenolic substances in the fermentation of *mandai cempedak*. Generally, *Lactobacillus* is a genus of mesophilic bacteria, meaning that the metabolism works optimally at medium temperature, or in this study simulated at 37°C of fermentation temperature. This finding was similar to the previous research of Nur (2009), Rahmadi *et al.* (2013), Emmawati *et al.* (2015), and Nazarni *et al.* (2016) using spontaneous culture and starter *L. plantarum* to ferment *mandai cempedak* and *jaruk tegarun*.

Table 1. Phytochemicals and antioxidants of mandai *cempedak* during fermentation at 37 and 8°C

Day	Total Phenol (mg GAE/kg)		Total Tannin (mg TAE/kg)		Total Flavonoid (mg CE/kg)		Antioxidant activity (IC_{50} ppm)	
	Spontaneous	*L. casei* Induced	Spontaneous	*L. casei* Induced	Spontaneous	*L. casei* Induced	Spontaneous	*L. casei* Induced
Temperature of Fermentation: 37°C								
1	1665±91a	1751±64a	32.51±3.34a	36.69±0.51a	6.886±0.30a	9.252±0.817a	212.6±3.5a	210.6±2.5a
2	1883±59a	2297±73ab	46.33±3.86b	48.51±2.82b	10.0±0.64b	11.77±0.76b	200.4±3.1b	179.4±2.5b
3	2213±27b	3615±286bc	51.42±1.29b	49.42±7.20b	13.50±0.17c	14.52±0.68c	189.5±3.7c	127.4±2.2c
4	2524±421bc	4013±304c	51.60±6.17b	56.69±1.03b	14.91±0.97c	16.38±0.51d	176.8±7.2d	122.0±3.4d
5	3034±54cd	4366±494d	51.60±7.20b	56.33±4.11b	17.46±0.59d	21.29±0.17e	157.5±1.9e	93.19±0.17e
6	3564±132cd	4404±41e	53.97±4.89b	52.15±2.31bc	20.66±0.64e	26.56±0.51f	137.0±5.3f	65.88±0.73f
7	3592±91d	4667±68e	63.05±2.31c	65.60±3.86c	21.20±0.30e	26.53±0.30f	130.9±4.6f	49.32±0.45g
Temperature of Fermentation: 8°C								
1	1443±154a	1607±14a	39.42±1.29a	41.42±5.14a	6.856±0.17a	7.006±0.13a	205.0±0.16a	217.8±16a
3	1463±109a	1787±95ab	39.42±0.26a	29.05±1.54b	7.545±0.21b	7.695±0.50b	180.3±0.47b	164.8±0.37b
5	1636±36a	2040±9.07bc	43.24±1.03a	37.78±3.09a	8.623±0.21c	8.802±0.13bc	170.3±3.21bc	133.5±1.88c
7	1687±45b	2204±59cd	37.24±3.34a	41.42±0.51a	9.192±0.34cd	9.731±0.34cd	132.8±12d	111.9±5.30d
9	1745±45b	2524±422d	40.69±0.51a	40.33±1.54a	9.521±0.13d	11.02±0.47d	112.4±0.39e	95.07±1.29e
11	1828±18b	3034±54e	40.87±3.86a	45.60±1.29ac	9.820±0.04de	13.68±0.93e	88.62±2.71(f)	94.29±1.45e
13	1860±109b	3592±91f	52.15±1.29b	43.42±2.82c	11.44±0.55e	13.59±0.64e	63.51±3.60(g)	87.24±1.66e

Note: The number followed by the same letter signifies no significant difference at α 5%. The stated value is expressed as Me an ± standard deviation

The metabolism of *L. casei* and *L. plantarum* in *mandai cempedak* tended to slow down at fermentation temperature of 8°C, with the final value of pH ranging from 4.5 to 5.0 after 13 days, while at 37°C produced pH of 3.5 after 7 days (Rahmadi *et al.*, 2017).

The use of specific species and strain of *Lactobacillus* may result in different concentration of metabolites, leading to dissimilar antioxidant activity (Park *et al.*, 2015). The induction of *L. casei* in *mandai cempedak* fermentation consistently increased the total concentration of phenol, tannin, flavonoid at two different temperatures. However, the induction of *L. casei* was more effective in increasing antioxidant activity when the *mandai cempedak* was fermented at optimum temperature of 37°C. Spontaneous fermentation at cold temperatures produced compounds capable of better inhibiting DPPH reduction after 11 days (Table 1). Rye bran that was fermented with lactic acid bacteria (*L. lactis ssp. Lactis, L. plantarum, L. brevis*, and *L. helveticus*) had higher antioxidant activity than spontaneous fermented buckwheat dough (Banu *et al.*, 2010).

Correlation of antioxidant activity and phenolic substance concentrations

There is a relationship of the higher total phenols, total tannins, and flavonoids with the higher the antioxidant activity for both spontaneous and *L. casei* induced fermentation process (Table 2). Based on the table, it can be concluded that higher total content of phenols, tannins, and flavonoids during fermentation of *mandai cempedak* resulted in the higher the antioxidant activity indicated by the lower value of IC_{50}.

The relationship between the total phenolic content and antioxidant activity is recognized in strong correlation. Li *et al.* (2009) also reported the antioxidant activities and phenolic contents of danggui (*Angelicae sinensis*) root had correlation coefficients (R) from 0.642 to 0.941, with the average value of R at 0.839. Mihai *et al.* (2011) reported that the relationship between DPPH radical scavenging activity of propolis sample from Transylvania and the concentration of total polyphenols had a positive correlation with coefficient of multiple correlation (R^2) at 0.8387. Kusumowati *et al.* (2012) reported an association between antiradical and total phenolic content from the extracts of betel leaf (*Piper betle*), teak leaf (*Tectona grandis*) and *katuk* leaf (*Sauropus androgynous*). The positive correlation resulted in greater antiradical activity. Other study also examined strong relationship between inhibitory concentration (IC_{50}) of DPPH reduction and total phenolic and total flavonoid contents in *tanjung* fruit extract (*Mimusops elengi*), resulted in correlation coefficient valued (R) at 97 and 99%, respectively (Perwiratami *et al.*, 2014).

TLC phytochemical profile of *mandai cempedak*

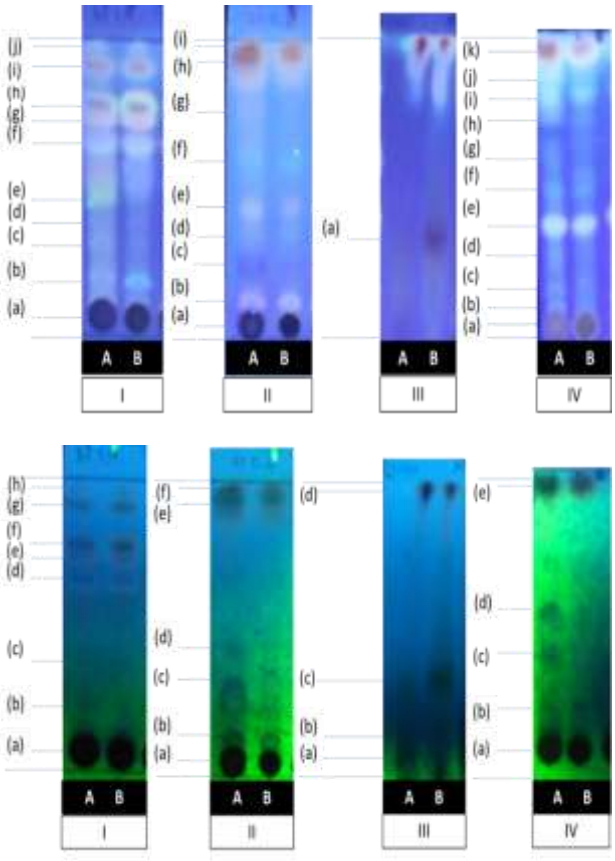
There were different stain patterns for the separated phyto-compounds on each solvent used (Table 3). In the n-hexane:ethyl acetate (1.4:0.6) solvent, the fermented inner skin of *cempedak* contained compounds that were not present in the unfermented *cempedak*. In the eluent of n-hexane:ethyl acetate (0.6:1.4), there were compounds in the unfermented inner skin of *cempedak* that degraded after fermentation. The better separation when n-hexane mixes were employed indicated the tendency of higher hydrophobicity in some compounds. The better separation phenomenon was observed in ethyl acetate solvent as well. In chloroform:methanol (1.6:0.4) eluent, there were more concentrated compounds observed after the inner skin of *cempedak* was fermented.

Table 2. Correlation Analysis of Phenolic substance vs IC_{50} of inhibition of DPPH reduction

Spontaneous Fermentation at 37°C			r value
Total Phenol (mg GAE/Kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.998
Total Tannin (mg TAE/kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.853
Total Flavonoid (mg CE/kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.986
L. casei induced Fermentation at 37°C			r value
Total Phenol (mg GAE/Kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.969
Total Tannin (mg TAE/kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.861
Total Flavonoid (mg CE/kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.969
Spontaneous Fermentation at 8°C			r value
Total Phenol (mg GAE/Kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.964
Total Tannin (mg TAE/kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.585
Total Flavonoid (mg CE/kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.967
L. casei induced Fermentation at 8°C			r value
Total Phenol (mg GAE/Kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.824
Total Tannin (mg TAE/kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.424
Total Flavonoid (mg CE/kg)	vs	IC_{50} of inhibition of DPPH reduction (ppm)	-0.868

Table 3. Rf values of compounds in fermented and unfermented *cempedak* at 366 and 254 nm

Spot No.	I. n-Hexane:Ethyl Acetate (1.4:0.6)			II. n-Hexane:Ethyl Acetate (0.6:1.4)			III. Chloroform:Methanol (1.6:0.4)			IV. Ethyl Acetate		
	Rf	Spot Visibility		Rf	Spot Visibility		Rf	Spot Visibility		Rf	Spot Visibility	
		Before Fermentation (A)	After Fermentation (B)		Before Fermentation (A)	After Fermentation (B)		Before Fermentation (A)	After Fermentation (B)		Before Fermentation (A)	After Fermentation (B)
UV lamp λ=366 nm												
(a)	0.06	+	+	0.04	+	+	0.34	-	+	0.04	+	+
(b)	0.19	-	+	0.13	+	+	0.79	-	+	0.06	+	+
(c)	0.30	+	-	0.25	+	-	0.87	-	+	0.09	+	-
(d)	0.39	-	+	0.34	+	+	1.00	+	+	0.16	+	+
(e)	0.46	+	-	0.44	+	+				0.27	+	-
(f)	0.65	+	+	0.59	+	-				0.34	+	+
(g)	0.72	+	+	0.72	+	-				0.50	+	+
(h)	0.77	+	+	0.87	+	-				0.58	+	-
(i)	0.90	+	+	0.94	+	+				0.72	+	-
(j)	0.97	+	+							0.79	+	+
(k)										0.95	+	+
UV lamp λ=254 nm												
(a)	0.06	+	+	0.05	+	+	0.06	-	+	0.09	+	+
(b)	0.20	-	+	0.13	+	+	0.13	-	+	0.16	+	+
(c)	0.37	+	-	0.32	+	-	0.32	-	+	0.42	+	-
(d)	0.66	-	+	0.43	+	+	0.97	+	+	0.56	+	+
(e)	0.71	+	-	0.92	+	+				0.97	+	-
(f)	0.77	+	+	0.98	+	-						
(g)	0.77	+	+									
(h)	0.96	+	+									



Note: I = n-hexane: ethyl acetate (1.4: 0.6); II = n-hexane: ethyl acetate (0.6: 1.4); III = chloroform: methanol (1.6: 0.4); IV = ethyl acetate. A = *Cempedak* samples before fermentation; B = *Cempedak* samples after fermentation

Figure 4. Thin Layer Cromatography of Mandai *Cempedak* (top) at λ 366 nm (bottom) at λ 254 nm

In general, active compounds in plants are obtained by extraction using a solvent. The isomers of active polyphenol compound have a wide spectrum of solubility in different solvents. This is due to the hydroxyl group possessed by the compound differing in number and position. Extraction using a variety of solvents will produce different polyphenolic components. Polyphenol compounds presented in plants are normally extracted with methanol and ethanol. Several studies related to polyphenol compounds and antioxidant activity utilized aqua and dichloromethane, ethyl acetate, chloroform, methanol solvent mixtures, a combination of acetic acid mixtures, hexane and ether (Liu *et al.*, 2017). The optimal use of various solvents was due to better separation of the intended compounds. This TLC phytochemical information will be used as a lead for further isolating the specific polyphenolic compounds from *mandai cempedak*.

CONCLUSION

Unsalted spontaneous and induced fermentation of *mandai cempedak* was carried out successfully. Fermentation increased the release of total phenolic, tannins, and flavonoids from *mandai cempedak*, which in turn positively modulated antioxidant activity. The use of starter culture resulted in higher overall phenolic substances and antioxidant activity in optimum fermentation temperature, while spontaneous LAB culture performed better to ferment *mandai cempedak* at sub-optimum temperature. In general, the rate of release of phenolic substances from the inner skin of fruit was halved when the fermentation was carried out at sub-optimal temperature of 8°C. Ethyl acetate alone or in combination with n-hexane produced better separation of the phytochemical compounds from fermented and unfermented *mandai cempedak*.

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