



## RESEARCH ARTICLE

# Different drying temperatures modulate chemical and antioxidant properties of mandai cempedak (*Artocarpus integer*) [version 1; peer review: 1 approved, 1 approved with reservations]

Anton Rahmadi , Yuliana Sabarina, Sukmiyati Agustin

Department of Agricultural Products Technology, Mulawarman University, Samarinda, East Kalimantan, 75119, Indonesia

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## Abstract

**Background:** Mandai, the fermented inner skin of cempedak (*Artocarpus integer*), may have further use as industrial ingredient while maintaining its antioxidative capacity. To promote fermentation, *Lactobacillus casei* was induced as the starter culture. This research was carried out (i) to investigate the effect of temperature on yield, chemical properties, and antioxidant activity of starter induced fermented mandai powder, (ii) to find the best drying temperature for the powder, and (iii) to find correlations between phenolic contents and antioxidant activity of the powder.

**Methods:** The drying temperature was used as the variable, and was set at 45, 50, and 55°C at a fixed duration of 18 hours. The control was spontaneously fermented mandai dried at 50°C for 18 hours. Total phenolic content (TPC), hydrolyzed tannic content (HTC), and total flavonoid content (TFC) were spectrophotometrically measured, expressed gallic acid (GAE), tannic acid (TAE), and catechin (CAE) equivalents. Antioxidant capacity was measured by DPPH assay.

**Results:** The best mandai powder had total phenolic content of 348.8±55.6 mg GAE kg<sup>-1</sup>, HTC of 143.8±9.3 mg TAE kg<sup>-1</sup>, TFC of 17.5±1.3 mg CAE kg<sup>-1</sup>, antioxidant activity (IC<sub>50</sub>) of 56.96 ppm, ash content of 4.0±0.7%, pH value of 5.0±0.8, and yield of 9.3±0.8%. There was a strong correlation between TPC, HTC, TFC, and the antioxidant activity.

**Conclusions:** Drying temperature affected all observed parameters but not ash and pH. Temperature of 45°C emerged as the best treatment to produce mandai powder from *L. casei*-inoculated mandai cempedak fermentation. The antioxidant activity of mandai cempedak was contributed by the phenolic components.

## Keywords

Antioxidant activity, *Artocarpus integer*, drying temperature, polyphenols

## Open Peer Review

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**Corresponding author:** Anton Rahmadi ([antonrahmadi@gmail.com](mailto:antonrahmadi@gmail.com))

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## Introduction

In South and East Kalimantan, Indonesia, people historically consume the lactic acid bacteria (LAB)-fermented inner skin of cempedak (*Artocarpus integer*), traditionally termed dami or mandai<sup>1</sup>. The inner skin of cempedak can be used in functional food production, which may partly reduce agricultural waste<sup>2</sup>. Mandai may contain phenolics, flavonoids, tannins, and antioxidant activity.

The unfermented cempedak inner skin (*Artocarpus integer*) contains bioactive components such as phenolics, flavonoids and carotenoids<sup>3</sup>. It has antioxidant activity that is potentially higher than the flesh and seeds of cempedak. Mandai with *L. casei* as the starter had better antimicrobial activity against *S. aureus* and *E. coli* in comparison to spontaneous (*L. plantarum*) mandai<sup>4</sup>. In addition, mandai may function as a probiotic food<sup>5</sup>. While maintaining its antioxidative capacity, fermented mandai may have further use as industrial ingredient primarily as an exotic tropical flavor and a flavor enhancer.

Mandai powder is produced through drying process. To produce good quality mandai powder, the right drying temperature is required. Drying at the correct temperature minimizes antioxidant damage, implicating the ability to reduce free radicals will be higher, as seen in sinom beverage powder<sup>6</sup>, and cumari peppers<sup>7</sup>.

This research aims (i) to measure chemical and antioxidant properties of *L. casei*-fermented mandai at 45, 50, 55°C of drying temperature with constant time of drying at 18 hours, which the results are then compared with spontaneously fermented mandai and dried at 50°C for 18 hours; (ii) to find the best drying temperature on starter induced fermented mandai powder; and (iii) to find correlations between phenolic contents and antioxidant activity on starter induced fermented mandai powder.

## Methods

### Producing mandai powder from the inner skin of cempedak

Cempedak was peeled and separated from its husk and flesh, then washed and cut into pieces. The pieces of cempedak inner skin were boiled at 100°C for 5 minutes to remove the sap. The sample was drained and then boiled once more in a sealed

container at 100°C for 5 minutes to soften the texture. The sample was then cooled until the temperature was less than 40°C. *L. casei* strain Shirota isolated from Yakult® as starter culture were inoculated at the concentration of 2% (v/v). For spontaneous fermentation, the mandai was directly stored without inoculation. The spontaneous and inoculated mandai were stored for 2 weeks at temperature of 8±2°C to allow slow fermentation to occur. After incubation, mandai was drained and blended. The puree of mandai then was dried for 18 hours at the appropriate temperature treatment, then ground and screened with an 80-mesh sieve. All reagents and corresponding suppliers are listed in [Supplementary File 1](#).

### Extraction of mandai powder

For the analysis of TPC, HTC, TFC, and antioxidant activity, 20 g mandai powder was dissolved in 60 ml 95% ethanol (SmartLab cat no. A1035, Indonesia) and macerated for 24 hours. Mandai was filtered through filter paper (Whatmann no. 4) and the liquid extract was dried at 50°C for 16 hours.

### Yield, ash, and pH

Yield was measured as the ratio of mandai powder to initial mandai cempedak (w/w), while ash contents were measured as described<sup>8</sup>. About 2 g of sample was diluted with distilled water to a volume of 20 ml. This mixture was homogenized and allowed to soak for 15 minutes before the pH was measured.

### Phenolic content and antioxidant activity measurement

TPC was estimated by the Folin-Ciocalteu assay and expressed as gallic acid equivalents (GAE), as described previously<sup>9,10</sup>. HTC was estimated by the Folin-Ciocalteu assay and expressed in mg kg<sup>-1</sup> tannic acid equivalent (TAE), as described<sup>11</sup>. TFC was estimated using the aluminum chloride (AlCl<sub>3</sub>) method and expressed in mg kg<sup>-1</sup> catechin equivalent (CAE), as described<sup>12</sup>. The antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, as described<sup>13</sup>. All standards were purchased from Sigma-Aldrich.

### Statistical analysis

Data in [Table 1](#) except IC<sub>50</sub> of antioxidant activity were subjected to analysis of variance (ANOVA) and the significance of the difference between the averages was determined by Fisher's least significant difference (at  $\alpha$  =5%, analyzed with GraphPad

**Table 1.** Yield, ash, pH phenolic contents and antioxidant activity of powdered mandai cempedak.

Temperature (°C)	Yield (%)	Ash (%)	pH	TPC (GAE mg/kg)	HTC (TAE mg/kg)	TFC (CAE mg/kg)	Antioxidant Activity (IC <sub>50</sub> in mg/kg)	
							Average	R-square
45	9.3±0.8 <sup>ac</sup>	4.0±0.7	5.0±0.8	358.8±55.6 <sup>a</sup>	143.8±9.3 <sup>a</sup>	17.5±1.3 <sup>a</sup>	56.96	0.93
50	9.1±0.8 <sup>ac</sup>	3.5±0.8	4.8±0.8	236.3±12.4 <sup>c</sup>	96.5±8.7 <sup>c</sup>	5.1±1.8 <sup>cb</sup>	66.76	0.96
55	9.1±0.6 <sup>bc</sup>	3.0±0.7	4.5±0.5	199.2±13.4 <sup>c</sup>	67.3±6.6 <sup>d</sup>	1.6±1.0 <sup>d</sup>	84.74	0.96
control	9.3±0.6 <sup>c</sup>	3.2±0.9	5.3±0.2	275.1±14.2 <sup>b</sup>	115.3±8.2 <sup>b</sup>	7.0±1.3 <sup>b</sup>	136.78	0.97

Yield, ash, pH, total phenolic content (TPC), hydrolyzed tannin content (HTC), total flavonoid content (TFC) are presented in average ± standard deviation.

Letters after the numbers indicate the least significant difference at  $\alpha$ =5%

Prism version 6.0. Values were expressed in average  $\pm$  standard deviation (SD).  $IC_{50}$  of antioxidant activity was produced by employing the non-linear fit of one-phase association method with GraphPad Prism version 6.0. Pearson correlation analysis was performed with Microsoft Excel 2016.

## Results

### Yield, ash, and pH

The yield of mandai powder at drying temperatures of 45 and 50°C did not differ significantly. However, the yield of mandai powder dried at a temperature of 55°C was significantly different to the yield resulting from treatment temperatures of 45 and 50°C (Table 1). The yield of each treatment did not differ significantly with the yield of mandai powder with spontaneous fermented that dried at 50°C (control). A comparison between the ash content of each treatment with control showed no significant difference (Table 1). The pH values of dissolved mandai powder ranged from 4.5 $\pm$ 0.5 to 5.3 $\pm$ 0.2. The drying temperature did not affect the pH value of the dissolved mandai powder.

### TPC, HTC and TFC

The drying temperature significantly affected the TPC of mandai powder. The highest average value of TPC in mandai powder dried at 45°C. In comparison to control, TPC of each treatment was significantly different. The drying temperatures in each treatment resulted in significantly different HTC in comparison to that of control mandai. Drying temperature of 45°C produced higher HTC than that of mandai powder dried at 50°C and 55°C. The drying temperature significantly affected the TFC of mandai powder. The TFC of mandai powder which dried at 45 and 55°C were significantly different from the control, but that of dried at 50°C was not significantly different from control.

### Antioxidant activity

Half-maximal inhibitory concentration ( $IC_{50}$ ) value of DPPH for each treatment was obtained through one phase association equation. The drying temperature significantly affected the antioxidant activity of mandai powder. Control mandai powder extract had the highest  $IC_{50}$  value, while mandai powder extract dried at 45°C had the lowest  $IC_{50}$  value of (Table 2). The TPC, HTC, and TFC have a strong correlation with antioxidant activity. The higher TPC, HTC, and TFC value, the higher antioxidant activity in mandai powder (Table 3).

### Correlation of TPC, HTC, TFC and antioxidant activity

Based on Pearson correlation analysis, there was a strong correlation between total phenolic content ( $r = 0.796$ ) and total flavonoid content ( $r = 0.783$ ) with antioxidant activity. The strongest correlation occurred between total tannin content ( $r = 0.910$ ) and antioxidant activity in mandai powder.

#### Dataset 1. All raw data obtained in the present study

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Data include yield, ash weight, pH, and phenolic, tannin and flavonoid contents.

**Table 2.  $IC_{50}$  of antioxidant activity of powdered mandai cempedak.**

Treatment	$Y_0$	Plateau	K	$IC_{50}$ (Interpolated)
Vitamin C	0	103.40	0.003	209.11
45°C	0	89.99	0.014	56.96
50°C	0	91.12	0.011	66.76
55°C	0	91.44	0.009	84.74
Control	0	87.39	0.006	136.78

$Y_0$ , (0% inhibition / without extract or Vitamin C); Plateau, maximum peak value; K, inhibition rate constant depended on concentration of Vitamin C or extract;  $IC_{50}$ , concentration of Vitamin C or extracts inhibiting DPPH reduction by 50%.

**Table 3. Pearson correlation between total phenolic, tannin, flavonoid and antioxidant activity of mandai powder.**

Variable	R-value		
	TPC	HTC	TFC
Antioxidant activity	0,796	0,910	0,783
TPC		0,973	0,999
HTC			0,967

0,01-0,19 very weak correlation; 0,20-0,39 weak correlation; 0,40-0,59 moderate correlation; 0,60-0,79 strong correlation; 0,80-1,00 very strong correlation.

## Discussion

### Yield, ash and pH

More water in mandai evaporated at higher temperatures. This caused the yield of mandai powder to reduce with increasing drying temperature. A previous report documents that the yield may decrease with increased drying temperatures<sup>14</sup>. The drying temperature did not affect the ash content of mandai powder. The mineral resources in the spontaneous and *L. casei*-fermented mandai were equally used by microbes, so it did not have a different effect to ash content.

During mandai fermentation, population of LAB increased until day 14, thus lowering pH<sup>15</sup>. Inferring from previous research, it was deduced that organic acids such as lactic acid and acetic acid were produced to lower the pH and caused more acidic environment on day 12 of fermentation<sup>5</sup>. The fermentation medium, duration of fermentation, and the use of starter cultures may play a role in the final pH and organic acid contents of LAB-fermented products. The previous research stated that the pH of spontaneously fermented rye dough was higher than the pH of starter-fermented rye dough<sup>15</sup>. However, cucumber pickle fermented with LAB produced organic acids that were higher than that of spontaneous fermentation<sup>16</sup>.

### TPC, HTC and TFC

TPC of the inner skin of cempedak was at 21.29 mg GAE kg<sup>-1</sup>. TPC of mandai powder ranged from 199.2±13.4 to 348.8±55.6 mg GAE kg<sup>-1</sup>, higher than that of unfermented cempedak. Phenolic compounds were sensitive to heat treatment, so that the drying process reduced the TPC<sup>17</sup>. The TPC of mandai powder dried at 55°C was the lowest value observed when compared to other treatments. The drying process, especially at higher temperature (i.e. 55°C), combined with the long drying time duration (i.e. 18 hours) resulted in loss of antioxidant activity<sup>18</sup>.

In dry conditions, components in the cell, such as membranes and organelles, clump together, resulting in fewer extracted phenolic compounds<sup>14</sup>. Drying at 50°C quickly disabled the oxidation of polyphenols. However, the initial oxidation of polyphenols might have occurred prior to drying and led to polyphenol degradation. Phenolic compounds are sensitive, unstable and susceptible to degradation by oxygen and light<sup>19</sup>. The enzymatic oxidation of polyphenols components is mostly caused by polyphenol oxidase<sup>7</sup>. Injury to the cell membrane liberates and therefore activates these enzymes, which convert phenolic compounds to quinones.

Phenolic contents are often subjected to heat processing, such as drying, boiling, and steaming<sup>19</sup>. Environmental factors affecting phenolic concentrations include weather conditions, seasons, and post-harvest conditions<sup>20</sup>. Phenolic contents are also related to varieties of different fruits, diversity of extraction methods<sup>21</sup>, and the type of phenolic components in the plant and its location in the cell, as well as the type of solvent and method of extraction<sup>22–24</sup>.

*L. casei* may modify the phenolic component, causing a significant difference between TPC of each treatment and control<sup>25,26</sup>. The type of fermentation and metabolic activity of LAB may play a role on levels of total phenolic in rye dough and bread<sup>15</sup> and *Moringa oleifera* leaf powder<sup>27</sup>. HTC was inversely correlated with drying temperature. In addition, duration of drying contributes to the loss of tannins<sup>28,29</sup>. This is consistent with the results in yacon (*Polymnia sonchifolia*) and coffee leaf tea<sup>30,31</sup>. Degradation of flavonoid structures is linked to the degree of heat exposure<sup>30,32</sup>.

### Correlation of TPC, HTC, TFC and antioxidant activity

The antioxidant activity of mandai powder was low when compared to the fresh form. Temperature plays a role in retaining

antioxidant activity of the powder (Table 2). Temperature had a significant effect on the inhibition of free radicals of DPPH in grass jelly (*Premna serratifolia*)<sup>33</sup>. A strong correlation was observed between antioxidant activity and the polyphenol contents of mandai powder. Previous studies have reported strong correlations between TPC, HTC, TFC and antioxidant activity<sup>34,35</sup>. Phenolic chemical structure has a role in the inhibition of free radicals, largely depending on the number and position of the hydrogen donation from the hydroxyl group to the aromatic ring of the phenol molecules<sup>36</sup>.

### Conclusions

Drying temperature affected total phenolic, tannin and flavonoid contents, antioxidant activity, and yield but did not affect ash content and pH of starter induced fermented mandai powder. Mandai powder dried at 45°C for 18 hours emerged as the best treatment, with a TPC of 358.8±55.6 mg GAE kg<sup>-1</sup> dry sample, HTC of 143.8±9.3 mg TAE kg<sup>-1</sup> dry sample, TFC of 17.5±1.3 mg CAE kg<sup>-1</sup> dry sample, antioxidant activity (IC<sub>50</sub>) of 56.96 ppm, ash content of 4.0±0.7%, pH value of 5.0±0.8, and yield of 9.3±0.8%. The strongest correlation was shown between HTC and IC<sub>50</sub> of antioxidant activity on starter induced fermented mandai powder. The antioxidant activity of mandai cempedak was contributed by the phenolic components.

### Data availability

**Dataset 1. All raw data obtained in the present study.** Data include yield, ash weight, pH, and phenolic, tannin and flavonoid contents. DOI: <https://doi.org/10.5256/f1000research.16617.d221911><sup>37</sup>.

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### Supplementary material

**Supplementary File 1. List of all reagents used in the current study, with suppliers.**

[Click here to access the data](#)



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**Francisc Vasile Dulf** 

Faculty of Agriculture, Department of Environmental and Plant Protection, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania

This paper aims: to measure chemical and antioxidant properties of *L. casei*-fermented mandai at different drying temperatures with constant time of drying at 18 hours. The results are then compared with spontaneously fermented mandai and dried at 50°C for 18 hours. The paper proposed also to find the best drying temperature on starter induced fermented mandai powder; and to find correlations between phenolic contents and antioxidant activity on starter induced fermented mandai powder.

The article is well written. Data is interpreted statistically. They are compared to literature data.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** biochemistry, fermentation

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 13 December 2018

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**Syed Ziaur Rahman**

<sup>1</sup> Department of Pharmacology & Deputy Medical Superintendent, Jawaharlal Nehru Medical College Hospital, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

<sup>2</sup> Ibn Sina Academy of Medieval Medicine & Science, Aligarh, India

**Mohd Ashraf Alam**

Department of Pharmacology, Jawaharlal Nehru Medical College Hospital, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

It is very interesting research which has tried to explore the use of Mandai as industrial ingredient while maintaining its antioxidant activity. However, there are some suggestions and queries, which needs to be corrected along with grammatical errors.

1. Abstract: Better, if is structured like introduction, aims and objectives, material and methods, results and conclusion.
2. Methods: On page 3, it has been mentioned that cempedak was boiled two times at 100°C for 5 minutes to remove sap and to soften its texture, then there might be a possibility that at this temperature of 100°C, flavonoid and retinoid content be lost or decreased.
3. The mandai storage temp, and the drying period of 18 hours, are selected but without proper referencing. Please add reference if any
4. Extraction process: Here the liquid extract was dried at 50°C for 16 hours. Please add reference if any.
5. Methods: There should be a mentioning of temperatures. Nowhere, temperature of 45, 50 and 55°C is mentioned, though the same is provided in the table.
6. Results: The values mentioned on page no. 4 and in table 1 are not matching. The yield mentioned in table at 50 and 55°C are approximately same, but in the text it is mentioned that there was a significant difference.
7. There should be proper mentioning in text under result section whatever is shown in table 1. With increase of drying temperature, there is decrease in TPC, HTC and TFC. There might be a chance to get more flavonoid and retinoid content in further lowering temperatures.
8. Antioxidant activity results/values shown on page 4 and in table 1 are not matching. In the text it is mentioned that higher TPC, HTC, TFC value means higher the antioxidant activity, but in table, as the temperature is increasing the values for TPC, HTC and TFC are decreasing, and antioxidant activity increasing.



**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Pharmacology, Nutraceuticals

**We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.**

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