RESEARCH ARTICLE

REVISED Different drying temperatures modulate chemical and antioxidant properties of mandai cempedak (*Artocarpus integer*) [version 2; peer review: 2 approved]

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Abstract

Background: Mandai, the fermented inner skin of cempedak (*Artocarpus integer*), may have further use as an industrial ingredient while maintaining its antioxidative capacity. The starter culture of *Lactobacillus casei* may induce the Mandai fermentation. 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. The DPPH assay measured antioxidant capacity.

Results: The best mandai powder had total phenolic content of 348.8 ± 55.6 mg GAE kg⁻¹, HTC of 143.8 ± 9.3 mg TAE kg⁻¹, TFC of 17.5 ± 1.3 mg CAE kg⁻¹, antioxidant activity (IC₅₀) of 56.96 ppm, ash content of $4.0 \pm 0.7\%$, pH value of 5.0 ± 0.8 , and yield of $9.3 \pm 0.8\%$. There was a strong correlation between TPC, HTC, TFC, and antioxidant activity.

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

Keywords

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

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REVISED Amendments from Version 1

We agree with some of the reviewers suggestion, i.e. (1) the yields of mandai powder at drying temperatures of 45, 50, and 55°C did not differ significantly, (2) Lowering the drying temperature may additionally preserve the content of polyphenols, therefore, may further increase the antioxidant capacity. In the reviewer's comment no.8, we made clear that In the concept of IC_{50} value, the lower the IC_{50} value means the substance is more potent or showing a better antioxidant capacity. We also add some explanatory notes on the method section, i.e., (1) The drying time of the mandai powder and the liquid extract were opted from the result of the preliminary optimization research, subjected to our dryer capability. A supplementary file including materials used was also added.

Any further responses from the reviewers can be found at the end of the article

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 mandai¹. The use of the inner skin of cempedak in functional food production may partly reduce agricultural waste². Mandai may contain phenolics, flavonoids, tannins, and antioxidant activity.

The unfermented cempedak inner skin (*Artocarpus integer*) contains bioactive components such as phenolics, flavonoids, and carotenoids³. 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*) mandai⁴. Besides, mandai may function as a probiotic food⁵. While maintaining its antioxidative capacity, fermented mandai may have further use as an industrial ingredient primarily as an exotic tropical flavor and a flavor enhancer.

Mandai powder is produced through a drying process. The right drying temperature is required to produce good quality mandai powder. Drying at the correct temperature minimizes antioxidant damage, implicating the ability to reduce free radicals will be higher, as seen in sinom beverage powder⁶, and cumari peppers⁷.

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 was 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 two weeks at a temperature of $8 \pm 2^\circ\text{C}$ 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 45, 50, and 55°C, then ground and screened with an 80-mesh sieve. The 18 hours drying time opted from the result of the preliminary research. All reagents and corresponding suppliers are listed in [Supplementary File](#).

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 (Whatman no. 4), and the liquid extract was dried at 50°C for 16 hours. The duration of liquid drying opted from the preliminary optimization research.

Yield, ash, and pH

The yield was measured as the ratio of mandai powder to initial mandai cempedak (w/w), while ash contents were measured as described⁸. About 2 g of the 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^{9,10}. HTC was estimated by the Folin-Ciocalteu assay and expressed in mg kg^{-1} tannic acid equivalent (TAE), as described¹¹. TFC was estimated using the aluminum chloride (AlCl_3) method and expressed in mg kg^{-1} catechin equivalent (CAE), as described¹². The antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, as described¹³. All standards were purchased from Sigma-Aldrich.

Statistical analysis

Data in [Table 1](#) except IC_{50} of antioxidant activity were subjected to analysis of variance (ANOVA), and Fisher's least significant difference test determined the significance of the difference between the averages (at $\alpha = 5\%$, analyzed with GraphPad 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 the 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 yields of mandai powder at drying temperatures of 45, 50, and 55°C did not differ significantly ([Table 1](#)). The yield of each

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 ₅₀ in mg/kg)	
							Average	R-square
45	9.3±0.8a	4.0±0.7	5.0±0.8	358.8±55.6 ^a	143.8±9.3 ^a	17.5±1.3 ^a	56.96	0.93
50	9.1±0.8a	3.5±0.8	4.8±0.8	236.3±12.4 ^c	96.5±8.7 ^c	5.1±1.8 ^{cb}	66.76	0.96
55	9.1±0.6a	3.0±0.7	4.5±0.5	199.2±13.4 ^c	67.3±6.6 ^d	1.6±1.0 ^d	84.74	0.96
control	9.3±0.6a	3.2±0.9	5.3±0.2	275.1±14.2 ^b	115.3±8.2 ^b	7.0±1.3 ^b	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 $\alpha=5\%$

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±0.5 to 5.3±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, the 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. The 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 (IC₅₀) value of DPPH for each treatment was obtained through one-phase association equation. In the concept of IC₅₀ value, the lower the IC₅₀ value means the substance is more potent or showing a better antioxidant capacity. The drying temperature significantly affected the antioxidant activity of mandai powder. Control mandai powder extract had the highest IC₅₀ value, while mandai powder extract dried at 45°C had the lowest IC₅₀ 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 the antioxidant activity in mandai powder (Table 3). Lowering the drying temperature may additionally preserve the content of polyphenols, therefore, may further increase the antioxidant capacity.

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

Table 2. IC₅₀ 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

Y₀, (0% inhibition / without extract or Vitamin C); Plateau, maximum peak value; K, inhibition rate constant depended on concentration of Vitamin C or extract; IC₅₀, 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.

strongest correlation occurred between total tannin content ($r = 0.910$) and antioxidant activity in mandai powder.

Discussion

Yield, ash, and pH

More water in mandai evaporated at higher temperatures. The evaporated water caused the yield of mandai powder to reduce with increasing drying temperature; however, the water content

of the final product is also lesser. A previous report documents that the yield may decrease with increased drying temperatures¹⁴. The drying temperature did not affect the ash content of mandai powder. Microbes equally used the mineral resources in the spontaneous and *L. casei*-fermented mandai, so it did not have a different effect on ash content.

During mandai fermentation, the population of LAB increased until day 14, thus lowering pH¹⁵. Inferring from previous research, organic acids such as lactic acid and acetic acid were produced to lower the pH and caused a more acidic environment on day 12 of fermentation⁵. 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 dough¹⁵. However, cucumber pickle fermented with LAB produced organic acids that were higher than that of spontaneous fermentation¹⁶.

TPC, HTC, and TFC

TPC of the inner skin of cempedak was at 21.29 mg GAE kg⁻¹. TPC of mandai powder ranged from 199.2±13.4 to 348.8±55.6 mg GAE kg⁻¹, higher than that of unfermented cempedak. Phenolic compounds were sensitive to heat treatment so that the drying process reduced the TPC¹⁷. 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 temperatures (i.e., 55°C), combined with the long drying time duration (i.e., 18 hours), resulted in loss of antioxidant activity¹⁸.

In dry conditions, components in the cell, such as membranes and organelles, clump together, resulting in fewer extracted phenolic compounds¹⁴. Drying at 50°C quickly disabled the oxidation of polyphenols. However, the initial oxidation of polyphenols might have occurred before drying and led to polyphenol degradation. Phenolic compounds are sensitive, unstable, and susceptible to degradation by oxygen and light¹⁹. The enzymatic oxidation of polyphenols components is mostly caused by polyphenol oxidase⁷. Injury to the cell membrane liberates and therefore activates these enzymes, which convert phenolic compounds to quinones.

Phenolic contents may vary on the types of heat processing, such as drying, boiling, and steaming¹⁹. Two consecutive boilings at 100°C for 5 minutes assisted removal of sap in the inner skin of cempedak and texture softening. This two-step boilings can be replaced by one-step boiling at a longer time. However, the use of two consecutive boilings is necessary to reduce the length of heat exposure; therefore, this was included as an essential preparation step in our patent application on mandai fermentation.

Environmental factors affecting phenolic concentrations include weather conditions, seasons, and post-harvest conditions²⁰. Phenolic contents are also related to varieties of different fruits, diversity of extraction methods²¹, 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^{22–24}.

L. casei may modify the phenolic component, causing a significant difference between TPC of each treatment and control^{25,26}. The fermentation and metabolic activity of LAB may play a role on levels of total phenolic in rye dough and bread¹⁵ and *Moringa oleifera* leaf powder²⁷. HTC was inversely correlated with drying temperature. Also, the duration of drying contributes to the loss of tannins^{28,29}, consistent with the results in the drying of yacon (*Polymnia sonchifolia*) and coffee leaf tea^{30,31}. Degradation of flavonoid structures is linked to the degree of heat exposure^{30,32}.

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 the antioxidant activity of the powder (Table 2). The temperature had a significant effect on the inhibition of free radicals of DPPH in grass jelly (*Premna serratifolia*)³³. 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^{34,35}. The 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 molecules³⁶.

Conclusions

Drying temperature affected total phenolic, tannin and flavonoid contents, and antioxidant activity, but did not affect ash content, yield, 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⁻¹ dry sample, HTC of 143.8±9.3 mg TAE kg⁻¹ dry sample, TFC of 17.5±1.3 mg CAE kg⁻¹ dry sample, antioxidant activity (IC₅₀) 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 IC₅₀ of antioxidant activity on starter induced fermented mandai powder. The phenolic components contributed to the antioxidant activity of mandai cempedak.

Data availability

Underlying data

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/10.17605/OSF.IO/Q76SH>³⁷.

Extended data

Supplementary File 1. List of all reagents used in the current study, with suppliers³⁷.

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No further comments to make.

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.

Version 1

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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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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.

Author Response 14 Feb 2020

anton rahmadi, Mulawarman University, Samarinda, Indonesia

Addressing Comment (1)

Abstract structure is assigned by the journal already.

Addressing Comment (2)

Two consecutive boilings at 100°C for 5 minutes assisted removal of sap in the inner skin of cempedak and texture softening. This two-step boilings can be replaced by one-step boiling at a longer time. However, the use of two consecutive boilings is necessary to reduce the length of heat exposure; therefore, this was included as an essential preparation step in our patent application on mandai fermentation.

Addressing Comment (3, 4, and 5)

The changes are incorporated in the second version of the article. The drying time of the mandai powder and the liquid extract were opted from the result of the preliminary optimization research, subjected to our dryer capability.

Addressing Comment (6 and 7)

We agree with the reviewers suggestion, i.e. (1) the yields of mandai powder at drying temperatures of 45, 50, and 55 °C did not differ significantly, (2) Lowering the drying temperature may additionally preserve the content of polyphenols, therefore, may further increase the antioxidant capacity.

Addressing Comment (8)

In response to the reviewer's comment no.8, we made clear that In the concept of IC₅₀ value, the lower the IC₅₀ value means the substance is more potent or showing a better antioxidant capacity.

Competing Interests: No competing interests were disclosed.

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