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Effect of ragi tape fermentation on physicochemical properties, phenolic compounds, and antioxidant capacity of turmeric spent

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ABSTRACT

Turmeric spent availability is high due to the increasing production of turmeric herb drinks. However, turmeric spent utilization is low, although it still contains phenolic compounds and antioxidant capacity. This study aims to investigate the effect of fermentation on the physicochemical characteristics of turmeric spent. Ragi tape is used as a starter since it contains various microorganisms. The community of microorganisms in the ragi could inhibit the growth of unwanted microorganisms, thus preventing contamination. Physicochemical characteristics, namely water-holding capacity, swelling capacity, oil-holding capacity, glucose content, crude fiber content, total phenol content, and antioxidant capacity, were observed from non-fermented and fermented turmeric spent. The results show that the duration of fermentation does not affect the physicochemical properties, total phenolic content, antioxidant capacity, and reducing sugars content. Ragi tape contains low concentrations of A. oryzae. Moreover, ragi tape is a consortium of microorganisms consisting of fungi, yeast, and lactic acid bacteria, so further study is needed to investigate the interaction between microorganisms and metabolite production.

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INTRODUCTION

Turmeric is a popular herb due to the curcumin compounds that have antioxidant capacity (Septiana et al. 2018). Turmeric herbal drink is popular since it was believed to improve immunity, especially during the COVID-19 pandemic (Ahmad et al. 2020). The production of turmeric herbal drinks produces waste derived from a filtration process that separates turmeric extract from the insoluble part. Turmeric processing waste named turmeric spent and still contains nutrients that are beneficial to the body, including 45% dietary fiber consisting of 43% water-insoluble dietary fiber and 2% water-soluble dietary fiber (Sowbhagya 2019). Currently, turmeric spent is used in the manufacture of bioplastics with anti-bacterial advantages derived from curcumin compounds (Maniglia and Tapia-Blácido 2019).

Most of the fiber in turmeric spent available as water-insoluble dietary fiber. Several studies have been carried out to modify the structure of water-insoluble dietary fiber to have better functional properties, for example, with physical and chemical modifications in turmeric (Maniglia and Tapia-Blácido 2019), enzymatic modifications (Liu et al. 2021; Wang et al. 2020), modification of fermentation and microwave treatment (Lin et al. 2020). Cellulose and hemicellulose are the main components of water-insoluble dietary fiber, so appropriate treatment is needed to decompose these components into simple carbohydrates. The use of enzymes to extract dietary fiber is quite promising due to its high efficiency, specifications, and high and harmless process conditions (Liu et al. 2021). The enzymes used can be pure enzymes or those produced by microorganisms. The use of enzyme-producing microorganisms has several advantages, namely affordable costs, microorganisms can be propagated many times so that enzyme production can be sustainable.

Wang et al. (2020) reported the use of multiple enzymes improves water holding capacity, oil-holding capacity, and water retention capacity of ginger pulp. Mohamed et al. (2016) reported solid-state fermentation increased phenolic content and enhanced antioxidant capacity. Moreover, fermentation using mixed cultures has several advantages, namely reducing fermentation time, preventing contamination, and interactions between microorganisms can optimize the production of metabolites from microorganisms. Ragi tape is a traditional starter that is widely found in. Hermanto et al. (2021) report that ragi tape contains *A. oryzae*, *Saccharomyces cerevisiae*, and *Lactobacillus* bacteria. *A. Oryzae* is fungi that can produce the enzymes amylase, proteases, and cellulase. However, little information is available on the effect of fermentation on physicochemical properties of turmeric spent.

The aim of the research was to investigate the effect of fermentation using ragi tape on the functional properties, phenolic compounds and antioxidant capacity of the turmeric spent. Fermented turmeric spent is expected to be applied in food products to improve the health benefits of food products.

METHODOLOGY

Turmeric and ragi tape were bought at a local market in Bandung. While chemicals were obtained from Merck (Merck, Darmstadt, Germany).

Preparation of turmeric spent

Turmeric was cut into 1-2 cm then crushed using home blender (HR2115/00, Koninklijke Philips N.V.,Jakarta, Indonesia) followed by heating at 80°C for 30 min. The turmeric spent was separated from turmeric extract by filtering using cheese cloth. The turmeric spent was then dried using hot air oven (XU058, Etuves, Chelles, France) at 50°C until water content reached 30%.

Fermentation of turmeric spent

Dried turmeric spent (water content of 30%) was mixed with 10% w/w ragi tape solution. The ratio of dried turmeric spent and ragi tape was 5: 1. Dried turmeric spent was mixed thoroughly then incubated for 6 days at 28-30°C using solid state fermentation method. Sample was taken at day 0, 3 and 6 to investigate the changes of reducing sugar, (poly)phenolic compounds, and antioxidant capacity.

Evaluation

Water holding capacity

The water holding capacity of non-fermented and fermented turmeric spent was analyzed based on method proposed by Subagio et al. (2003). One gram of turmeric spent was added to 30 ml distilled water and mixed using vortex mixer (IKA Genius 3, IKA Werke GmbH & Co, Staufen im Breisgau, Germany) every 5 minutes for 30

minutes. The mixture was then centrifuged using Gyrozen centrifuge at 2000*g* for 15 min. The supernatant was separated, and the filtrate was weighted and calculated.

Oil holding capacity

Oil holding capacity of non-fermented and fermented turmeric spent was analyzed based on method proposed by Subagio et al. (2003). One gram of turmeric spent was added to 30 ml vegetable oil (Fortune, Wilmar International Ltd., Gresik, East Java, Indonesia) and mixed using vortex mixer (IKA Genius 3, IKA Werke GmbH & Co, Staufen im Breisgau, Germany) every 5 min for 30 min. The mixture was then centrifuged using Gyrozen centrifuge at 2000 *g* for 15 min. The supernatant was separated, and the filtrate was weighted and calculated.

Swelling Power

Analyses of swelling power in turmeric spent was conducted following method proposed by Kaur et al. (2011). The sample of 0.1 g was added to 10 ml of distilled water. The mixture was then heated at 60°C for 30 min. The mixture was then cooled down until reached room temperature then centrifuged at 1500 *g* for 15 min. The swelling power was calculated by dividing the weight of paste and the weight of dried sample.

Color

The effect of fermentation on turmeric spent color was analyzed using a colorimeter (JZ-300 Universal, Shenzhen Kingwell Instrument, Guangdong, China) based on method proposed by Li et al. (2014). The result was presented as L*, a* and b* values.

Extraction of turmeric spent.

Extraction of phenolic compounds from turmeric spent was conducted based on Cakrawati et al. (2021). One gram of dried non-turmeric spent, and fermented turmeric spent was mixed with 10 mL of distilled water. The mixture was mixed using vortex mixer for 30 min followed by centrifugation (Gyrozen 406, Seoul, South Korea) at 1500 *g* for 15 min. The supernatant was separated from the filtrate using Whatman filter paper (Whatman filter paper no 1, Whatman International Ltd., Maidstone, England).

Reducing sugar

The amount of reducing sugar in turmeric spent during the incubation time was determined following the method proposed by Saqib and Whitney (2011). A DNS reagent was made by mixing 10 g of DNS, 300 g of sodium potassium tartrate and 20 g of sodium hydroxide and adjusted until the volume was 1l. A sample of 0.5 ml was added to 1.5 ml distilled water and 1.5 ml of DNS reagent. The mixture was heated for 5 min and cooled down at room temperature before measuring the absorbance at 540 nm. The result was reported as mg glucose/g sample.

Total Phenolic compound

Total phenolic compounds was determined using the method proposed by Dewanto et al. (2002). The sample of 0.1 ml was mixed with 0.1 ml of Folin-Ciocalteau reagent. The mixture was set for 6 minutes at room temperature to allow reaction then added with 1 ml $NaCO_3$ 7% and 1 ml of distilled water and held for 90 min incubation. The absorbance was measured at 760 nm using a Mapada UV-1 100 spectrophotometer (Mapada UV-1 100, Shanghai Mapada Instrument Co.,Ltd., Shanghai, China) and expressed as mg GAE/ 100 g sample.

Analysis of antioxidant capacity

The electron transfer mechanism by TEAC assay was used to measure antioxidant capacity. The TEAC assay was conducted following the method proposed by Re et al. (1999). The ABTS•+ was made by adding 5 mL of 7 mM ABTS to 88 µL of 140 mM $K_2S_2O_8$ in the dark and held for 24 h at room temperature before use. The $ABTS^{\bullet+}$ stock solution was diluted in phosphate buffer saline (PBS). to get an absorbance of 0.700 ± 0.020 at 734 nm. Antioxidant capacity was measured by mixing 5 µl of a diluted sample with 1 ml of $ABTS^{\bullet+}$ radical solution. The absorbance at 734 nm was recorded using a UV-100 Mapada spectrophotometer by analysing the decolorisation of the $ABTS^{\bullet+}$ at 734 nm after 4 min at 30°C using PBS as a control. The standard curve was made using Trolox at 0.5- 5 mM.

Statistical analysis

The research was conducted by experimental design by completely randomized design. All data obtained from two separate replications. Data were analyzed using analysis of variance (ANOVA) and means were compared using Duncan's Multiple Range Test at significant level of 0.05 using Microsoft Excell.

RESULTS AND DISCUSSION

Physicochemical properties observed in turmeric spent namely water holding capacity, oil

holding capacity, and swelling capacity (Table 1). Although fermentation was carried out using ragi tape that contained mold and yeast, incubation for 6 days did not affect the physicochemical properties of turmeric pulp. The ability of turmeric spent to absorb water (WHC) is related to the fiber content and carboxyl group in the protein. While the ability of turmeric spent to absorb oil is related to the lipophilic group of the protein. The duration of fermentation does not affect the value of WHC and OHC. This was in agreement with Rohmawati et al. (2015) which shows the duration of fermentation has no effect on crude fiber and protein levels of soy by-products.

The value of WHC is related to the value of swelling power and the fiber content in turmeric spent. Table 1 shows the WHC and swelling power values decreased after fermentation. It might be due to the hydrolysis of fiber into simple sugar by fungi. The absorbency of oil is influenced by the amount of hydrophobic protein in turmeric spent (Lin et al. 1974).

Curcumin is a substance that gives a yellow color to turmeric, it also has bioactive compounds that act as antimicrobials (Purwani et al. 2012). The L*a*b*color analysis of non-fermented and fermented turmeric spent by ragi tape for 6 days are presented in Table 2. The results showed that fermentation using ragi tape decreased the lightness of 6-days fermented turmeric spent ($P<0.05$). However, fermentation did not affect the red/green value and blue/yellow value of turmeric spent ($P \geq 0.05$). Fermentation might lead to oxidation of curcumin therefore decrease the lightness of fermented turmeric spent.

Figure 1 shows the effect of incubation time on the total phenolic compounds and antioxidant capacity. Incubation time did not affect ($P \geq 0.05$) the total phenolic compounds, and antioxidant capacity. On the contrary, Đorđević et al. (2010) reported the use of yeast and BAL can increase the phenolic content in cereal fermentation. Moreover, Cai et al. (2012) added *A. oryzae* and *A. niger* were able to increase the phenolic content in oat fermentation. *A. oryzae* and *A. niger* produces enzymes namely glycoside hydrolase, cellulase/xylanase, and esterase which work by breaking the cell wall bonds and releasing nutrients and bound phenol groups (Cai et al. 2012). Ragi tape contains *a* low concentration of *A. oryzae* also a consortium of microorganisms of yeast and lactic acid bacteria. Therefore, further investigation is needed to understand the interaction between microorganisms.

Figure 2 shows the effect of incubation time on the reducing sugar content. The result shows that incubation time did not affect reducing sugar content. Villanueva-Suárez et al. (2013) reported most sugar available in the form of oligosaccharide after fermentation of okara using *Bifidobacterium bifidum*Turmeric spent dominantly consist of dietary fiber, while protein and glucose as nitrogen and carbon source were limited. This might lead to the limited metabolites production of microorganisms. Moreover, ragi tape as traditional starter contains various microorganisms that interaction among microorganisms need to be studied further.

Table 1 Water holding capacity, oil holding capacity dan swelling power of non-fermented and a 6-days fermented turmeric spent [ns]

	non-fermented turmeric spent	**6-days fermented turmeric spent**
Water holding capacity (%)	925±190,92	595±28,28
Oil holding capacity	352±31,82	357,5±74,25
Swelling capacity	21,5±7,78	17,5±5,66

Table 2 Color of non-fermented and a 6-days fermented turmeric spent

Sampel	**Result**		
	L*	**a***	**b***
non-fermented turmeric spent	60,60±0,75*	24,95±2,29	33,25±2,80
6-days fermented turmeric spent	52,52±1,2	26,08±8,04	61,07±23,87

Value with (*) on the same column was significantly different ($P< 0.05$)

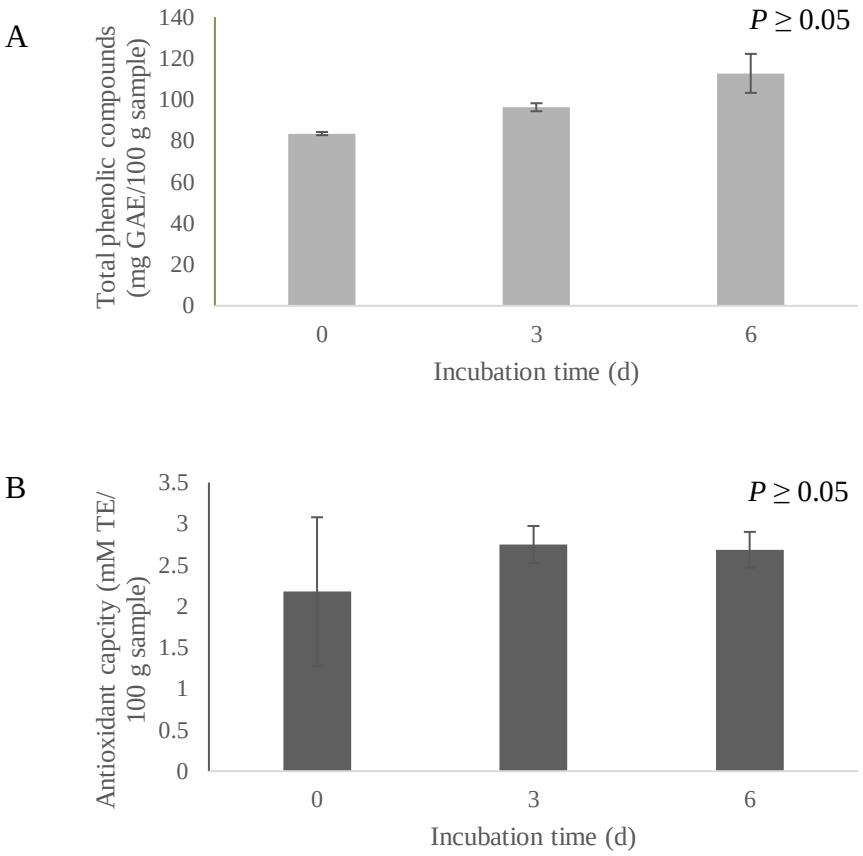

Figure 1 Effect of incubation time on (A) total phenolic compounds; dan (B) antioxidant capacity of fermented turmeric spent

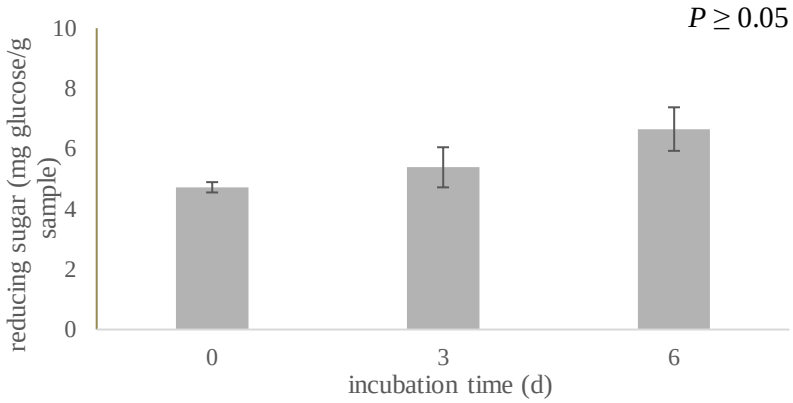

Figure 2 Effect of incubation time on reducing sugar

CONCLUSION

Fermentation of turmeric spent using ragi tape did not affect physicochemical properties, phenolic compounds, antioxidant capacity, reducing sugar and crude fiber. This research provides an understanding that the numbers of microorganisms in the starter is important to produce enzymes needed to improve physicochemical properties and phenolic content if turmeric spent.

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