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Bacterial Population and Chemical Characteristics of Fermented Mandai Cempedak with Starter Induction

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Traditionally fermented foods can be improved by introducing starter and hygienic production. The study observes the changes in population of lactic acid bacteria (LAB), pH, polyphenolic levels, and antioxidant activity of spontaneous and *Lactobacillus casei* induced *mandai cempedak* fermentation at 37 °C for seven days. The hygienic process included two steps boiling of inner-skin of cempedak at 80-90 °C for 15 minutes. LAB and non-LAB growth were quantified with plate count. Phenolic substances were spectrophotometrically quantified. Gallic acid (GAE), tannic acid (TAE), and catechin (CE) were used as standards. DPPH method was employed to measure antioxidant activity. The LAB dominated bacterial population during the course of fermentation. The LAB grew from 3.3±0.5 to 8.8±0.6 log cfu mL^{-1} for spontaneous fermentation and from 3.3±0.4 to 9.0±0.5 log cfu mL^{-1} for starter induced fermentation. The population of LAB in spontaneous and *L. casei* induced fermentation grew in almost similar pattern and can be approached by linear regression. The degree of acidity increased during the fermentation process and achieving pH 3.5 at the sixth day of fermentation. The fermentation process increased the phenolic contents both in spontaneous and *L. casei* induced fermentation, and resulting in enhancing the antioxidant activity. The phenolic contents, except total tannins, were higher in starter induced fermentation, thus lowering IC_{50} of inhibitions of DPPH reduction. Hence, *L. casei* produced fermented products with better antioxidant activity in comparison to spontaneously fermented products. From these parameters, *L. casei* was successfully used as starter for *mandai cempedak* and optimum fermentation at 37 °C was 6 days.

Key words: antioxidant, bacterial population, induced fermentation, *mandai cempedak*, phytochemicals

Makanan fermentasi tradisional dapat ditingkatkan kualitasnya dengan menggunakan biakan pemula dan produksi yang higienis. Penelitian ini bertujuan untuk mengamati perubahan populasi bakteri asam laktat (LAB), pH, kadar polifenol, dan aktivitas antioksidan dari fermentasi spontan dan biakan pemula *L. casei* dari mandai cempedak pada suhu 37 °C selama tujuh hari. Proses persiapan higienis dilakukan dengan dua tahapan perebusan kulit cempedak bagian dalam pada suhu 80-90 °C selama 15 menit. Pertumbuhan populasi LAB dan non-LAB dikuantifikasi dengan angka lempeng total. Kadar fenolik dikuantifikasi secara spektrofotometri. Asam galat (GAE), tanat (TAE), dan katekin (CE) digunakan sebagai standar. DPPH digunakan untuk mengukur aktivitas antioksidan. Bakteri asam laktat mendominasi populasi bakteri selama waktu fermentasi. Populasi LAB tumbuh dari 3,3±0,5 ke 8,8±0,6 log cfu mL^{-1} untuk fermentasi spontan dan dari 3,3±0,4 ke 9,0±0,5 log cfu mL^{-1} untuk fermentasi dengan biakan pemula. Populasi LAB dalam fermentasi spontan dan yang diinduksi *L. casei* berkembang dengan profil yang serupa dan dapat diprediksi dengan regresi linier. Tingkat keasaman meningkat seiring dengan proses fermentasi dengan pH terendah adalah 3,5 pada hari ke-6. Fermentasi meningkatkan kadar fenolik baik untuk fermentasi spontan maupun yang diinduksi dengan *L. casei*, dan hal ini meningkatkan aktivitas antioksidan. Semua fenolik, kecuali total tanin, terdapat dalam konsentrasi yang lebih tinggi pada fermentasi dengan biakan pemula, sehingga fermentasi cempedak meningkatkan kapasitas pereduksi DPPH menjadi lebih baik. Fermentasi dengan induksi *L. casei* memiliki aktivitas antioksidan yang lebih baik dibandingkan pada fermentasi spontan. Sebagai kesimpulan, *L. casei* dapat digunakan sebagai biakan pemula pada fermentasi mandai dengan waktu optimum fermentasi pada suhu 37 °C adalah enam hari.

Kata kunci: antioksidan, fermentasi yang diinduksi, mandai cempedak, populasi bakteri, *phytochemicals*

In Kalimantan, in addition to be consumed as fresh fruit and seed, the inner-skin of Cempedak or Tiwadak (*Artocarpus champeden*) fruit can be processed into a fermented food called *mandai* or *dami* (Emmawati *et al.* 2015). To produce *mandai cempedak* and other traditional fermented products, spontaneous fermentation is commonly performed at room temperature (Nuraida 2015). *Mandai cempedak* is made from the inner-skin of *cempedak* (or jackfruit) by utilizing salt to promote LAB growth (Nur 2009; Rahmadi *et al.* 2013; Emmawati 2015). *Lactobacillus plantarum* and *Leuconostoc sp.* are predominant bacteria that can be isolated from these products. Stages of processing including peeling the outer skin of the fruit, removing the epidermis, and immersing in

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salt water in order to preserve and soften the texture. The length of fermentation is observed from several hours to one month.

The disadvantage of the traditional fermentation process includes the use of salt in high concentrations (15-25% w/v) that leads to an increase in the body's salt intake (NaCl). High salt diet has health implications (Song *et al.* 2017). The use of high salt content also provides off and bitter taste in the traditionally fermented products as a result of mineral accumulation in the product (Panagou *et al.* 2011). In community, hygienic practice during fermentation is often neglected, resulting in dissimilar quality between batches and potential pathogenic microorganism contamination in the *mandai cempedak* product (Blana *et al.* 2014). There should be an effort to improve the process of traditional food fermentation.

Lactobacillus plantarum is traditionally found in plant-based fermented products. This is due to the versatility of *L. plantarum* enzymes in comparison to other LABs (Siezen *et al.* 2012). *Lactobacillus plantarum* produces amylase, b-glucosidase, decarboxylase, lactate dehydrogenases, peptidases, phenolic acid decarboxylases, phenol reductase, proteinase, and tannase enzymes. On the other hand, regardless more commonly found in dairy-based products, *L. casei* may be employed for plant-based fermentation, as it is recorded to produce amylase, lactate dehydrogenases, peptidases, and proteinase enzymes (Hur *et al.* 2014).

The LAB starter utilized in this research was prepared from *Lactobacillus casei* Shirota strain. This study aims to compare changes in bacterial populations, pH, phenolic (total phenolics, total tannins, and flavonoids) contents, and total antioxidants without addition of salt by spontaneous and starter induced fermentation of *mandai cempedak* at temperature of 37 °C.

MATERIALS AND METHODS

***Mandai Cempedak* Fermentation.** The part of *cempedak* fruit used was the inner-skin that has been sorted and cleaned. The inner-skin of *cempedak* was diced to the size of 3-5 cm^3. The pieces of the inner-skin of cempedak were boiled at temperature of 80-90 °C for 15 minutes to remove the sap and then the water was drained. The inner-skin of cempedak was separated into sealed containers, each was weighted at 100 g. Potable water was poured into the container until the entire dices were submerged. The samples were boiled once more at temperature of 80-90 °C for 15 minutes. Half of the samples were inoculated with *L. casei* at a concentration of $4x10^6$ to $4x10^8$ cfu, or approximately 4 mL of the concentrated *L. casei* inoculum for each container. The samples were further fermented for seven days at 37 °C. Observations on total bacteria, total LAB, pH, phenolic contents, and antioxidant activity potency were performed daily until the seventh day.

Total Plate Count. Total Plate Count (TPC) was performed as described by Fardiaz (1993). Nutrient Agar (NA) medium (Accumedia, USA) was used for total bacteria count, while De Mann Rogosa Sharpe Agar (Himedia, India) medium used for LAB count. The media was sterilized at 121 °C for 15 minutes, then the media was cooled to 60 °C. The warm media was poured aseptically in a sterile Petri dish which was then homogenized to spread evenly across the surface. Petri dishes containing media were allowed to solidify. As many as 0.1 mL of samples were poured into a Petri dish and flattened. The samples were incubated in the incubator in reversed position at 37 °C for 24 hours. The calculation based on the number of colonies ranges from 25 to 250 cfu mL^{-1}, considering dilution factor. The calculation of non-LAB population was performed by subtracting the log of total bacterial population obtained from the NA medium with log of LAB population of the MRSA medium.

pH. The degree of acidity was measured using Sudarmadji *et al.* (2007) method. About ± 50 mL of sample product was placed into a small jar and the pH was measured in duplicate for each product. Prior to analysis, the pH meter had been checked and calibrated in buffer pH 4 and 9.

Total Phenolics. Phenol analysis was performed by spectrophotometric method employing Folin-Ciocalteu (Mu'nisa *et al.* 2012; Nurhayati *et al.* 2012). Gallic acid (Sigma-Aldrich, USA) was used as the standard. The total content of phenolics in the extract was expressed in gallic acid equivalent (GAE). Each sample was weighed as much as 5 mg, then dissolved in 2 mL of 95% ethanol. Further, about 5 mL of aquades and 0.5 mL of Folin-Ciocalteau (Sigma-Aldrich, USA) 50% (v/v) were added. Samples were allowed to stand for 5 minutes and a 5% (w/v) of Na_2CO_3 (Sigma-Aldrich, USA) solution was added to make 10 mL of total volume. The solution was homogenized in dark room for one hour. The solution was measured at a wavelength of 752 nm (Genesys 20, Thermo-Fischer, USA).

Total Tannins. Total tannins were tested according

Table 1 The linear equation of bacterial growth in *mandai cempedak* fermentation with and without starter

Linear equation	Spontaenous Fermentation	*L. casei* induced Fermentation
Total Bacteria	Y = 0.9029*X + 2.687	Y = 0.8717*X + 2.681
Total LAB	Y = 0.9008*X + 2.149	Y = 0.9128*X + 2.081

to Malangngi *et al.* (2012). A total of 0.5 g of the product was extracted with 10 mL of diethyl ether (Merck, USA) for 20 hours, then filtered. The residue obtained was boiled in 100 mL of aquadest (Soil Science Laboratory, Mulawarman University, Indonesia) for two hours, then cooled and filtered. The extract obtained was added with distilled water until the extract volume reached 100 mL. A total of 0.1 mL of the extract was added with 0.1 mL of Folin Ciocalteu reagent (Sigma Aldrich, USA) and was vortexed. About 2 mL of Na_2CO_3 (Sigma Aldrich, USA) was added and the sample was vortexed once more. The absorbance was read at 760 nm of wavelength (Genysis 20, Thermo Fischer, USA) after incubation for 30 min at room temperature (28±2 °C). The results obtained were plotted against the standard curve of tannic acid (Sigma Aldrich, USA) that was prepared in the same procedure. The total content of tannins was expressed as tannic acid equivalent (TAE).

Total Flavonoids. Total Flavonoids were measured by the method of Zou *et al.* (2004). About 1 mg of extract was dissolved in 10 mL of 95% of ethanol (Kimia Farma, Indonesia) and 0.7 mL of distilled water was added. About 0.1 mL of 5% $NaNO_2$ (Sigma Aldrich, USA) was added into the mixture. After 5 minutes, 0.1 mL of $AlCl_3$ 10% (Sigma Aldrich, USA) was added. After 6 minutes, 0.5 mL of 1 M NaOH (Merck, USA) was added. All ingredients were mixed evenly and the samples were incubated for 10 minutes. The absorbance was measured at a wavelength of 510 nm, after 1 mL of sample was replaced with 1 mL of 95% ethanol solvent. The results obtained were plotted against standard curve of catechin (Sigma Aldrich, USA) that was prepared in the same way. Total flavonoids were expressed as catechin equivalent (CE).

Antioxidant Activity. Total antioxidants were performed by spectrophotometric method using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA) reduction principle (Farhan *et al.* 2012). A total of 1 mL of diluted extract in ethanol (Kimia Farma, Indonesia) was added to 1 mL DPPH (prepared at 0.15 mM in ethanol) and at the same time, a control consisting of 1 mL of DPPH with 1 mL of ethanol was prepared. The reaction mixture was vortexed and then incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 519±2 nm (Genesys 20, Thermo-Fischer, USA). Vitamin C (Sigma Aldrich, USA) was used as a positive control and ethanol was used as the substract. The ability to inhibit DPPH reduction of the extract was calculated by comparing the absorbance of the reduced control of sample divided by the absorbance of the control. The total antioxidant value was then plotted in a linear regression equation: [antioxidant potential] = a [ingredient in ppm] + b to obtain its IC_{50} value.

RESULTS AND DISCUSSION

Total Bacteria and LAB. The growths of microbes in *mandai cempedak* without addition of salt were quantified in seven days of fermentation course with initial microbial concentration of 3,3±0,3 log cfu mL^{-1} for spontaneous fermentation and 3,5±0,5 log cfu mL^{-1} for starter induced fermentation. The dominance of the LAB in *mandai cempedak* fermentation was estimated in linear equations presented in Figure 1 and Table 1. The LAB grew to achieve the density of 8,8±0,6 log cfu mL^{-1} for spontaneous fermentation and 9,0±0,5 log cfu mL^{-1} for starter induced fermentation at the end of the observation period (Fig 2). Until the seventh day, LAB continued to grow and dominate in both spontaneous and starter induced fermentation (Fig 2).

Confirmation of LAB growth was performed with MRSA medium and partial biochemical tests including Gram positive confirmation, microscope observation, the absence of spore, non-motility, and positive catalase. LAB and non-LAB growths were observed from day one to day seven at 37 °C (Fig 1). In the spontaneous *mandai cempedak* fermentation, *L. plantarum* was dominated microbial growth, while in the starter induced fermentation, there were at least two distinct LAB isolates observed under microscope. However, we are yet to determine the species.

This data proved that LAB was able to survive in the heat process applied in the initial processing of *mandai cempedak*. The result is in line with the research of De Angelis *et al.* (2004) and Fiocco *et al.* (2007) which stated that several strains of LAB were able to survive after mild heat processing because they produced proteins that were protective to heat. The inner-skin of *cempedak* contained fibrous materials

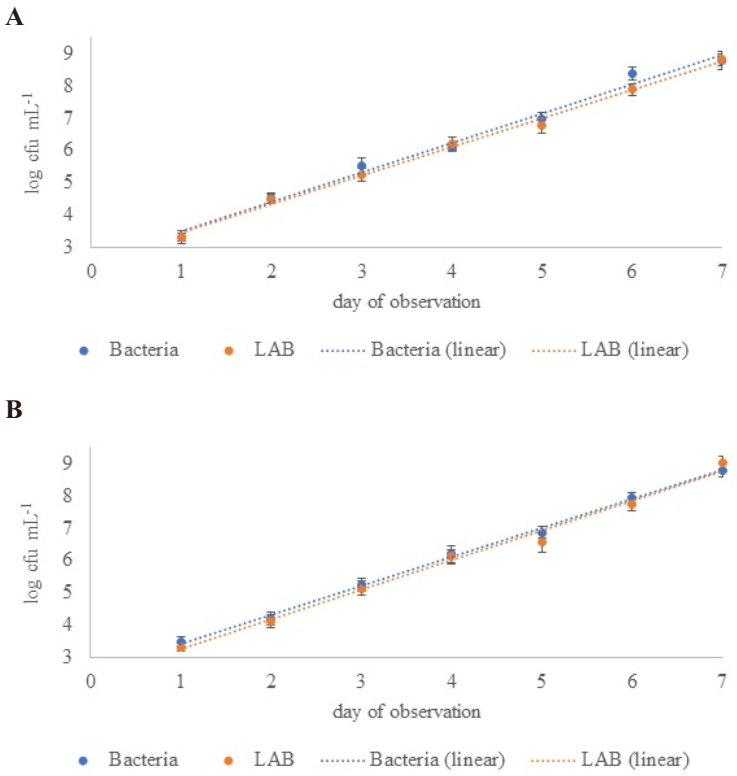

Fig 1 Linear regression of bacterial growth in *mandai cempedak* fermentation with and without starter. A: The linear regression of growth of bacteria and LAB in spontaneous fermentation B: The linear regression of growth of bacteria and LAB in *L. casei* induced fermentation. * Significantly different in the multiple T-test. SEM is symbolized with (┬) located above and below the observation point.

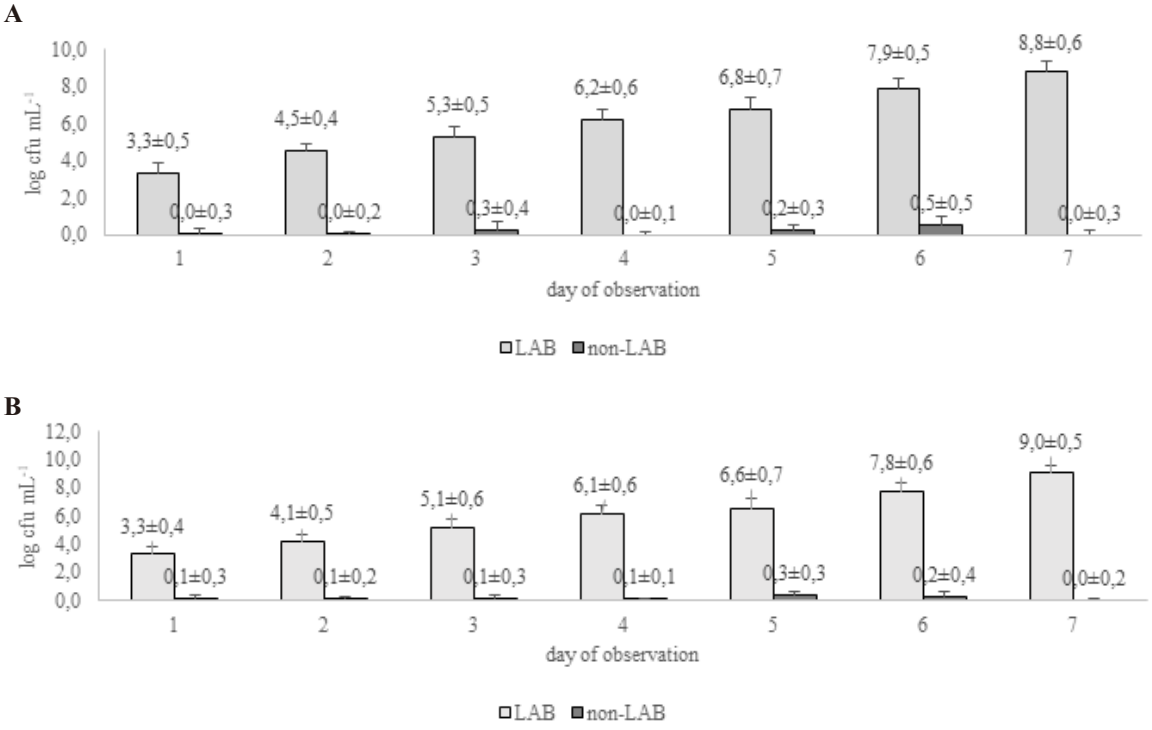

Fig 2 The LAB and non-LAB population in *mandai cempedak* fermentation with and without starter. A: The LAB and non-LAB population in spontaneous fermentation B: The LAB and non-LAB population in *L. casei* induced fermentation. * Significantly different in the multiple T-test. SEM is symbolized with (┬) located above and below the observation point.

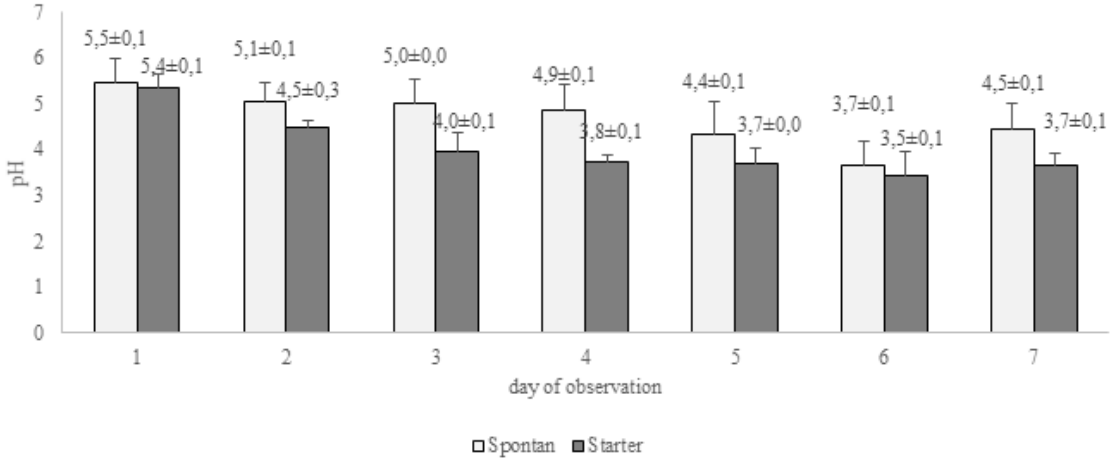

Fig 3 pH of *mandai cempedak* fermentation with and without starter. * Significantly different in the multiple T-test. SEM is symbolized with (┬) located above and below the observation point.

that provide an extent of supports for cell immobilization, giving protection to the viability of lactobacilli (Lye *et al.* 2012). This also explained the survivability of the LAB during mild heat treatment.

It is noted that *cempedak* medium promoted LAB growth and domination. Heat treatment of *mandai cempedak* may resulted in the release of nutritious compounds such as free amino, nitrogen, and sugars into the fermentation media, leading to their increased availability to be utilized by LAB. Teh *et al.* (2010) postulated that the sugars from the skin of durian (*Durio zibethinus*), cempedak, and mangosteen were assimilated by lactobacilli and utilized for growth.

Acidity. LAB is known to produce lactic acid in an amount that is sufficient to increase the acidity of the fermented product. The increase of acidity of *mandai cempedak* started from the second day of the fermentation course to the last observed day. The lowest pH (3.5) was observed on the sixth day of fermentation. Overall, there was no significant difference of the lowest pH of spontaneous and starter induced *mandai cempedak*, although slight variations of pH on day to day observation were measured (Fig 3). From the lowest achieved pH, both optimum fermentations at 37 °C were 6 days. The pH is used as general indication of optimum LAB fermentation as stated by Mousavi *et al.* (2010). Rhee *et al.* (2011) reported a decrease in pH as a result of lactic acid production, which was also an indicator of the success of fermentation of traditional food products by LAB.

The *L. plantarum* and *L. casei* are regarded to be facultatively heterofermentative bacteria (Ashraf *et al.* 2011; Zago *et al.* 2011), therefore produces a variety of organic acids to reduce the pH. Cueva *et al.* (2013) stated that during the in vitro fermentation of up to 48 h of grape seeds by *Lactobacilli*, organic acids such as 4-hydroxyphenylacetic acid, phenylpropionic acid, 3-hydroxyphenylacetic acid, phenylacetic acid, 3-(4-hydroxyphenyl)-propionic acid, and 4-hydroxy-5-(phenyl)-valeric acid were significantly increased by the fermentation process. These compounds, along with lactic acid, caused the increasing acidity of the fermented *mandai cempedak.*

Phytochemistry and Antioxidant Activity. Fermentation is proven to increase the phenolic contents in certain food products, and as resulting in enhancing the antioxidant activity. These phenomena were highlighted in *mandai cempedak* fermentation (Fig 4). The difference of total phenolic and flavonoid contents between spontaneous and starter induced fermentation products was observed, in which the use of *L. casei* induced fermentation resulting in higher concentrations of total phenolic and flavonoid contents. In addition, there was no difference of total tannins for both products in any day of observation (Fig 4). Hur *et al.* (2014) stated that antioxidative activity may be increased in fermented plant-based food products as a result of microbial enzymatic hydrolysis of phenolic compounds.

Lactobacillus casei and *L. plantarum* are known to have -glucosidase and -galactosidase activities that increase flavonoids in fermented food products, i.e. in soybean fermentation (Marazza *et al.* 2009). In this regard, Hur *et al.* (2014) proposed that LAB fermentation causes formation of organic acids that influences pH, Maillard reaction, and pentose phosphate pathway which subsequently attenuate redox balance and radical scavenging activity. The

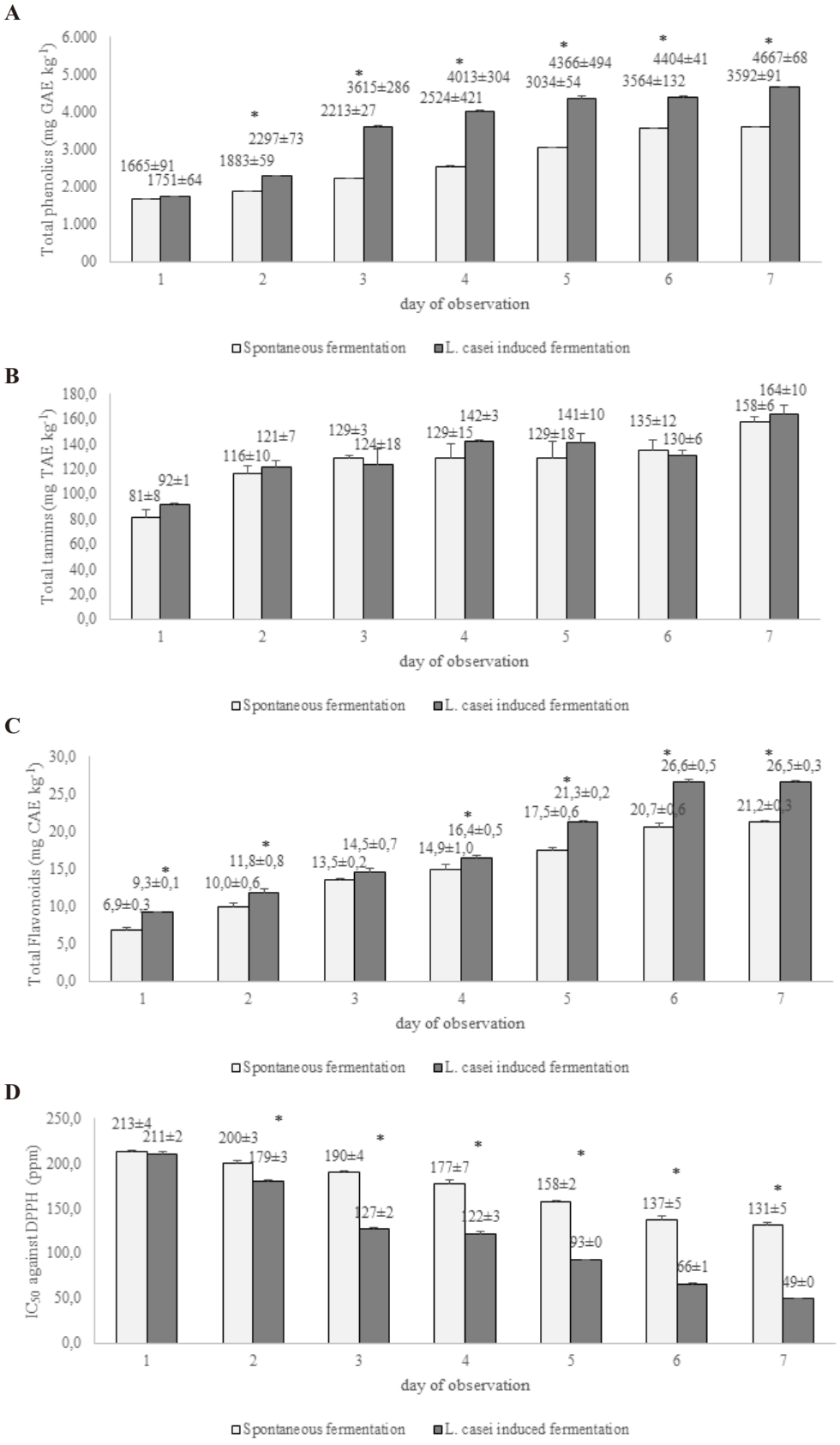

Fig 4 The total phenolic, tannins, flavonoid contents and antioxidant activity in fermentation of *mandai cempedak* with and without starter. A: The total phenolic contents in spontaneous and *L. casei* induced fermentation. B: The total tannin contents in spontaneous and *L. casei* induced fermentation. C: The total flavonoid contents in spontaneous and *L. casei* induced fermentation. D: The IC_{50} of inhibition of DPPH reduction in spontaneous and *L. casei* induced fermentation. * Significantly different in the multiple T-test. SEM is symbolized with (┬) located above and below the observation point.

fermentation also causes activations of -glucosidase, -galactosidase, tannase, and phosphoketolase that assist hydrolysis and de-polymerization of phenolics. The downstream processes have an implication of liberation of phenolic substances which in turn provides hydrogen or electron donor and positively modulates metal ion chelation activity. As a result, radical scavenging activity may increase. Quercetin and gallic acid were significantly higher during LAB fermentation of *Graptopetalum paraguayense* in any stage of fruit maturity (Wu *et al.* 2011). Hole *et al.* (2012) concluded that fermentation of cereal products with specific probiotics exhibited significant increase of free phenolic acids, i.e. caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, 5,5-diferulic acid, 8-o-4-diferulic acid, and 8,5-diferulic acid. Selected LABs were able to increase free phenolic acids due to high feruloyl esterase activity (FAE).

In solid state fermentation, the increase of antioxidant activity with regard to modulation of polyphenolic content is also proven. Lee *et al.* (2008) reported that an increase of antioxidant activity is observed when employing different starter to ferment koji bean, which was attributed to the increase of phenol and anthocyanin contents. Further, the increase in phenolic substances were measured during fermentation of cooked grass pea seed, wheat grains, and soybean products (Starzynska-Janiszewska *et al.* 2008; Bhanja *et al.* 2009; Singh *et al.* 2010; Dajanta *et al.* 2013).

However, the changes of phytochemical contents are medium and strain specific (Martins *et al.* 2011). In other condition, i.e. tea fermentation, it is stated that the monomeric flavonoids were transformed to polymeric derivatives as the tea leaves were further fermented (Kim *et al.* 2011). As a result, polyphenolic contents were lessened as the higher degree of fermentation occurred. Further, external factors affecting the changes of phytochemical contents are duration of fermentation course, temperature, pH, inhibitors, stimulators, and the composition of atmosphere in the fermentation chamber (Hur *et al.* 2014).

The change in IC_{50} values of DPPH reduction inhibition is limitedly influenced by pH if the difference of the pH is less than 1. However, if the difference of pH at range of greater than 2, the IC_{50} value of DPPH reduction inhibition between products will differ significantly (Pekal and Pyrzynska 2015). In this study, the comparison of IC_{50} inhibition of DPPH reduction was performed only between the same day fermented of *mandai cempedak* products, where the difference of pH was less than 1 between spontaneous and starter induced fermented products. It was concluded that IC_{50} inhibitions of DPPH reduction of starter induced fermentation were lower than the values produced from spontaneous fermentation. *Lactobacillus casei* produced fermented products with better antioxidant activity in comparison to *L. plantarum* fermented products.

CONCLUSION

The growths of bacteria in *mandai cempedak* initially started from 3,3±0,3 log cfu mL^{-1} and 3,5±0,5 log cfu mL^{-1} for spontaneous fermentation and starter induced fermentation, respectively. The growth curves of the LAB in spontaneous and *L. casei* induced *mandai cempedak* fermentation in the seven day of fermentation at 37 °C were in log-linear pattern. The LAB grew to achieve the density of 10^9 cfu mL^{-1} for both types of fermentation at the end of the observation period. There was no significant difference of the lowest pH of spontaneous and starter induced *mandai cempedak*, although slight variations of pH on day to day observation were measured. The starter induced fermentation products contained higher total phenolic and flavonoid in comparison to spontaneous fermentation products, while there was no difference of total tannins for both products in any day of observation. *Lactobacillus casei* produced fermented products with better antioxidant activity in comparison to *L. plantarum* fermented products, indicated by IC_{50} inhibitions of DPPH reduction. From these parameters, *L. casei* was successfully used as starter for *mandai cempedak* and optimum fermentation at 37 °C was 6 days.

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REFERENCES

Ashraf R, Shah NP. 2011. Selective and differential enumerations of *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium spp.* in yoghurt — A review, International Journal of Food Microbiology 149(3): 194-208. doi:10.1016/j.ijfoodmicro.2011.07.008.

Bhanja T, Kumari A, Banerjee R. 2009. Enrichment of phenolics and free radical scavenging property of wheat koji prepared with two filamentous fungi. Bioresour. Technol. 100:2861–6. doi: 10.1016/j.biortech.2008.12.055.

Blana VA, Grounta A, Tassou CC, Nychas GJE, Panagou EZ. 2014. Inoculated fermentation of green olives with potential probiotic *Lactobacillus pentosus* and *Lactobacillus plantarum* starter cultures isolated from industrially fermented olives, Food Microbiology 38: 208-218. doi:10.1016/ j.fm.2013.09.007.

Cueva C, Sanchez-Pata F, Monangas M, Walton GE, Gibson GR, Martin-Alvarez PJ, Bartolome B, Moreno-Arribas V. 2013. In vitro fermentation of grape seed flavan-3-ol fractions by human faecal microbiota: changes in microbial groups and phenolic metabolites. FEMS Microbiol Ecol 83: 792–805. doi:10.1111/1574-6941.12037.

Dajanta K, Janpum P, Leksing W. 2013. Antioxidant capacities, total phenolics and flavonoids in black and yellow soybeans fermented by *Bacillus subtilis*: A comparative study of Thai fermented soybeans (*thua nao*). International Food Research Journal. 20(6): 3125-3132.

De Angelis M, Di Cagno R, Huet C, Crecchio C, Fox PF, Gobbetti M. 2004. Heat Shock Response in *Lactobacillus plantarum*. Applied and Environmental Microbiology. Vol 70(3): 1336-1346. doi: 10.1128/AEM.70.3.1336-1346.2004.

Emmawati A, Jenie BSL, Nuraida L, Syah D. 2015. Karakterisasi Isolat Bakteri Asam Laktat dari Mandai yang Berpotensi Sebagai Probiotik. Agritech 35(2): 146-155. doi: 10.22146/agritech.9400.

Fardiaz S. 1993. Analisis Mikrobiologi Pangan [Food Microbiology Analysis]. Raja Grafindo Persada. Jakarta.

Farhan H, Rammal H, Hijazi A, Hamad H, Daher A, Reda M, Badran B. 2012. Invitro Antioxidant Activity of Ethanolic and Aqueous Extracts from Crude *Malva parviflora* L. Grown in Lebanon. *Asian Journal of Pharmaceutical and Clinical Research* 5(3): 234-238.

Fiocco D, Capozzi V, Goffin P, Hols P. 2007. Improved Adaption to Heat, Cold and Solvent Tolerance in *Lactobacilus plantarum*. Applied Genetics and Molecular Biotechnology 77: 909-915. doi: 10.1007/s00253-007-1228-x.

Hole AS, Rud I, Grimmer S, Sigl S, Narvhus J, Sahlstrøm, S. 2012. Improved Bioavailability of Dietary Phenolic Acids in Whole Grain Barley and Oat Groat following Fermentation with Probiotic *Lactobacillus acidophilus, Lactobacillus johnsonii,* and *Lactobacillus reuteri*. J. Agric. Food Chem. 60: 6369−6375. doi:10.1021/jf300410h.

Hur SJ, Lee SY, Kim YC, Choi I, Kim GB. 2014. Effect of fermentation on the antioxidant activity in plant-based foods. Food Chemistry 160: 346–356. doi:10.1016/j.foodchem.2014.03.112.

Kim Y, Goodner KL, Park JD, Choi J, Talcott ST. 2011. Changes in antioxidant phytochemicals and volatile composition of *Camellia sinensis* by oxidation during tea fermentation. Food Chemistry 129: 1331–1342. doi:10.1016/j.foodchem.2011.05.012.

Lee IH, Hung YH, Chou CC. 2008. Solid-state fermentation with fungi to enhance the antioxidative activity, total phenolic and anthocyanin contents of black bean. Int J Food Microbiol 121:150–6. doi: 10.1016/j.ijfoodmicro.2007.09.008.

Lye HS, Teh SS, Lim TJ, Bhat R, Ahmad R, Wan-Abdullah W, Liong MT. 2012. Bioactive property of soymilk fermented by agrowastes-immobilized *lactobacilli*. British Food Journal 114 (9): 1339 – 1353 doi:10.1108/00070701211258862.

Malangngi LP, Sangi MS, Paedong JJE. 2012. Penentuan Kandungan Tanin dan Uji Aktivitas Antioksidan Ekstrak Biji Buah Alpukat (*Persea americana* Mill.) [Determination of Tanin Contents and Antioxidant Activity of Avocado Fruit Extract (*Persea americana* Mill.)]. *Jurnal Mipa Unsrat Online* 1(1): 5-10.

Marazza JA, Garro MS, de Giori SG. 2009. Aglycone production by *Lactobacillus rhamnosus* CRL981 during soymilk fermentation. Food Microbiology, 26(3), 333–339. doi: 10.1016/j.fm.2008.11.004.

Martins S, Mussatto SI, Martinez-Avila G, Montanez-Saenz J, Aguilar CN, Teixeira JA. 2011. Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. Biotechnology Advances 29 (2011) 365–373. doi:10.1016/j.biotechadv.2011.01.008.

Mousavi ZE, Mousavi SM, Razavi SH, Emam-Djomeh Z, Kiani H. 2010. Fermentation of pomegranate juice by probiotic lactic acid bacteria. World Journal of Microbiology and Biotechnology 27(1): 123-128. doi:10.1007/s11274-010-0436-1.

Mu'nisa A, Wresdiyati T, Kusumorini N, Manalu W. 2012. Aktivitas Antioksidan Ekstrak Daun Cengkeh [Antioxidant Activity of Clove Leaf Extract]. *Jurnal Veteriner* 13(3): 272-277.

Nur HS. 2009. Suksesi Mikroba dan Aspek Biokimiawi Fermentasi Mandai dengan Kadar Garam Rendah [Succession of Microbes and Biochemistry Aspects of

Mandai Fermentation with Low Salt]. Makara Sains 13(1): 13-16.

Nuraida L. 2015. A Review: Health Promoting Lactic Acid Bacteria in Traditional Indonesian Fermented Foods. Food Science and Human Wellness 4(2): 47-55.

Panagou EZ, Hondrodimou O, Mallouchos A, Nychas GJE. 2011. A study on the implications of NaCl reduction in the fermentation profile of *Conservolea* natural black olives. Food Microbiology 28(7): 1301-1307. doi:10.1016/j.fm.2011.05.008.

Park E, Kim H, Eom SJ, Paik H. 2015. Antioxidative and Anticanceric Activities of Magnolia (*Magnolia denudata*) Flower Petal Extract Fermented by *Pediococcus acidilactici* KCCM 11614. Molecules 20: 12154-12165. doi: 10.3390/molecules200712154.

Pekal A, Pyrzynsk K. 2015. Effect of pH and metal ions on DPPH radical scavenging activity of tea. Int J Food Sci Nutr 66(1): 58-62. doi:10.3109/09637486.2014.959899.

Rahmadi A, Abdiah I, Sukarno MD, Purnaningsih T. 2013. Karakteristik Fisikokimia dan Antibakteri Virgin Coconut Oil Hasil Fermentasi Bakteri Asam Laktat [Physicochemical and Antibacterial Characteristics of Virgin Coconut Oil fermented with Lactic Acid Bacteria]. J. Teknol. Industri Pangan. 24(2):178-183. doi: 10.6066/jtip.2013.24.2.178.

Rhee SJ, Lee JE, Lee CH. 2011. Importance of Lactic Acid Bacteria in Asian Fermented Foods. Microbial Cell Factories 10(1): 1-13. doi:10.1186/1475-2859-10-S1-S5.

Siezen RJ, Francke C, Renckens B, Boekhorst J, Wels M, Kleerebezem M. 2012. Complete Resequencing and Reannotation of the *Lactobacillus plantarum* WCFS1 Genome. J. Bacteriol. 194(1): 195-196. doi: 10.1128/JB.06275-11.

Singh HB, Singh BN, Singh SP, Nautiyal CS. 2010. Solid-state cultivation of *Trichoderma harzianum* NBRI-1055 for modulating natural antioxidants in soybean seed matrix. Bioresour Technol 101:6444–53. doi: 10.1016/j.biortech.2010.03.057.

Song HJ, Park SJ, Jang DJ, Kwon DY. 2017. High consumption of salt-fermented vegetables and hypertension risk in adults: a 12-year follow-up study. Asia Pac J Clin Nutr 26(4):698-707. doi: 10.6133/apjcn.042016.13.

Starzynska-Janiszewska A, Stodolak B, Jamróz M. 2008. Antioxidant properties of extracts from fermented and cooked seeds of Polish cultivars of *Lathyrus sativus*. Food Chem 109: 285–92. doi: 10.1016/j.foodchem. 2007.12.028.

Sudarmadji S, Haryono B, Suhardi. 2007. Prosedur Analisis Untuk Bahan Makanan dan Pertanian [Analytical Procedures for Food Ingredients and Agriculture]. Liberty. Yogyakarta.

The SS, Ahmad R, Wan-Abdullah W, Liong MT. 2010. Enhanced Growth of *Lactobacilli* in Soymilk upon Immobilization on Agrowastes. J. Food. Sci. 75(3): M155-M164. doi:10.1111/j.1750-3841.2010.01538.x.

Wu SC, Su YS, Cheng HY. 2011. Antioxidant properties of *Lactobacillus*-fermented and non-fermented *Graptopetalum paraguayense* E Walther at different stages of maturity. Food Chemistry 129 (2011) 804–80. doi: 10.1016/j.foodchem.2011.05.025.

Zago M, Fornasari ME, Carminati D, Burns P, Suarez V, Vinderola G, Reinheimer J, Giraffa G. 2011. Characterization and Probiotic Potential of *Lactobacillus plantarum* Strains Isolated from Cheeses. Food Microbiology 28(2011): 1033-1040. doi: 10.1016/j.fm.2011.02.009.

Zou Y, Lu Y, Wei D. 2004. Antioxidant Activity of Flavonoid Rich Extract of *Hypericum pertoratum* L. in Vitro. Journal Agriculture and Food Chemistry 52(16): 5032-5039. doi: 10.1021/jf049571r.