EFFECTS OF LACTIC FERMENTATION ON TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF GALANGAL (*ALPINIA GALANGA LINN*)

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ABSTRAK. Efek fermentasi laktat pada kandungan total polyfenol dan aktivitas antioksidan Lengkuas (*Alpinia galanga* Linn). Lengkuas merupakan salah satu herbal yang memiliki kandungan senyawa fenolik sehingga banyak digunakan secara luas sebagai bahan obat tradisional selama berabad-abad. Di sisi lain, bakteri asam laktat (BAL) telah terbukti dapat meningkatkan aktivitas antioksidan dari media. Kombinasi kedua bahan tersebut diyakini dapat menghasilkan produk yang sehat. Oleh karena itu, penting untuk mengkaji apakah bakteri asam laktat dapat beradaptasi dengan lengkuas untuk mendapatkan sifat yang diinginkan dari produk fermentasi. Penelitian ini bertujuan untuk mengetahui pengaruh bakteri asam laktat (BAL) terhadap kandungan total polifenol dan aktivitas antioksidan ekstrak lengkuas. Sebelum inokulasi, 150 g lengkuas dicampur dengan 150 ml air steril, kemudian, 90 ml ekstrak tersebut diinokulasi dengan 3 mililiter starter subkultur (L. plantarum dan L. casei) sebelum kemudian diinkubasi pada suhu 37oC selama 24 jam. Setelah itu, ektstrak kemudian dikeringbekukan menggunakan freeze-dryer (48 jam). Total polifenol diukur dengan menggunakan metode Folin-Denis, sedangkan aktivitas antioksidan diukur menggunakan metode DPPH. Hasil penelitian menunjukkan bahwa L. plantarum bisa menaikkan jumlah polifenol lebih tinggi secara signifikan daripada L. casei. Kandungan total polifenol dan aktivitas antioksidan tertinggi dihasilkan oleh ekstrak lengkuas yang difermentasi dengan L. plantarum selama 12 jam, yaitu masing-masing sekitar 53 mg GAE/100 g dan 79%.

Kata kunci: lengkuas, bakteri asam laktat (BAL), fermentasi, kandungan total polifenol, aktivitas antioksidan

ABSTRACT. Galangal is one of herbs that has been widely used as traditional medicines ingredients for centuries due to its phenolic compounds and its antioxidant properties. On the other hand, lactic acid bacteria (LAB) have been proved can increase antioxidant activity of the media. The combination of both functional materials is believed can produce healthful products. Therefore, it is important to analyse whether the lactic acid bacteria are adaptable to the galangal's phenolic compounds characteristics in order to get desirable properties of fermented products. This experiment was aimed to examine the effect of lactic acid bacteria (LAB) fermentation on total polyphenol content and antioxidant activity of ginger extract. Prior to inoculation, 150 g of galangal was blended with 150 ml sterilized water, then, 90 ml of the juice was inoculated by 3 milliliters of subcultured starter (*L. plantarum* and *L. casei*). Then the juice was incubated at 37^{0}C for 24 hours. In order to get powder of fermented samples, freeze-dryer was used (48 hours). The total polyphenol content was measured using Folin-Denis method, while the antioxidant activity was estimated using the DPPH radical-scavenging activity. The result showed that *L. plantarum* could raise the total polyphenol significantly higher than *L. casei*. The highest content of total polyphenol content and antioxidant activity reached by galangal juice which fermented by *L. plantarum* for 12 hours, it was around 53 mg GAE/100 g and 79%, respectively.

Keywords: galangal, lactic acid bacteria (LAB), fermentation, total polyphenol content, antioxidant activity

INTRODUCTION

In Indonesia and some other countries, galangal has been used to flavor various types of foods and beverages both traditional dishes and modern ones. It is also one of many herbs that has been used as traditional medicines ingredients for centuries. It is not only empirically but also scientifically that galangal has been proven can prevent and even cure some illness.

Galangal has been analyzed that it has antimicrobial, antifungal and antioxidant compounds that are very usefull to treat various disease such as allergic, cancer, tumor and even AIDS (HIV)[1,2,3,4]. According to Rusmarilin[1] as an anti-cancer, galangal extracts could inhibit tumor cell growth in mice which transplanted with primary tumor cells. Juntachote and Berghofer[5] stated that galangal extracts showed higher antioxidant activity and galangal extracts exhibited strong superoxide anion scavenging activity, Fe^{2+} chelating activity, and

reducing power in a concentration dependent manner, and furthermore, ethanolic extracts of galangal acted as radical scavenger and also as lipoxygenase inhibitor. Besides that, Juntachote *et al*[6] also mentioned that galangal had higher antioxidant effectiveness than holy basil in ground pork.

It was reported that essential oil of galangal rhizome had shown activity against *S. aureus, Streptococcus suis, Erysipelothrix rhusiopathiac, P. aeruginosa, E. coli, Pasteurella multocida and Arcanobacterium pyogenes* [7]. Before that, Khattak *et al*[8] showed that galangal has only inhibitory effect against *S. aureus*. As well, Prakatthagomol *et al*[9] also showed that inhibition of galangal oil to *S. aureus* was better compared to *E.coli* and *S. typhimurium*.

Lu *et al*[10] showed that among 19 commonly consumed spices in China, galangal exhibited the highest antioxidant capacity and that condition associated with the total phenolic content in galangal. Furthermore, they stated that galangin was identified as the principal phenolic component and the main contributor to the highest antioxidant capacity of galangal. Meanwhile, other study was reported that the main compounds of galangal extract were 1,8-cineole (20.95%), β-bisabolene (13.16%), β-caryophyllene (17.95%) and β-selinene (10.56%)4. Chudiwal *et al* [11] overviewed that many components have been isolated from galangal rhizome such as 1'S'-1'-acetoxychavicol acetate, 1'S'-1'-acetoxyeugenol acetate, 1'S'-1'-hydroxychavicol acetate, trans-p¬-hydroxycinnamaldehyde, trans-p¬-hydroxycinnamyl acetate, and trans-p-coumaryl diacetate. Those components have been reported that they posses many biological activities such as antibacterial, antifungal, antiinflammatory, antitumor/anticancer and antioxidative activity[1,2,3,4,5,6,7,8,9,10].

The utilization of lactic acid bacteria such as *Lactobacillus* sp. and *Bifidobacterium* sp. as probiotics in food have been massively increase since a lot of scientific proofs showed that they can increase antioxidant activity, decrease cholesterol level, and treat diarrhoea, gastroenteritis, and others gastrointestinal infection[12,13,14]. Wohlgemuth *et al* [15] stated that probiotic microbia have considered important for the defense mechanism of gastrointestinal through the modification of the intestinal microbiota composition including the formation of lactic acid as well as the production of antibacterial substances. Meanwhile, Saulnier *et al* [16] stated that the probiotics could enhance the host defense system against pathogen through two mechanisms, they were the production of mucin and the reduction of gut permeability. The both mechanisms able to prevent penetration of pathogens and toxic substances.

Since lactic acid bacteria and galangal are very important for health, the combination of both functional materials is believed can produce healthful products. However, despite of the huge of application in other foods, until now the utilization of galangal as an ingredient of probiotic products is still lacking. It is important to analyse whether the lactic acid bacteria are adaptable to the galangal's compounds and what the effects of lactic acid bacteria on polyphenol content and antioxidant activities of galangal extract. Therefore, this experiment was aimed to examine the effect of lactic acid bacteria (LAB) fermentation on total polyphenol content and antioxidant activity of galangal extract.

MATERIALS AND METHODS

Materials

Galangal rhizomes were purchased from local markets in Pathumthani, Thailand. Meanwhile, lactic acid bacteria (LAB) used in this experiment *Lactobacillus plantarum* and *Lactobacillus casei*, were collected from Thailand Institute of Scientific and Technological Research (TISTR). This experiment also used de Man-Rogosa-Sharpe (MRS) broth as LAB growth medium, Folin-Denis reagent for total polyphenol content measurement, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) for antioxidant activity determination. The measurements of total polyphenol content and antioxidant activity were done using spectrophotometer (Model UV2, UV/Vis spectrometer, UNICAM, UK), while Freeze Dry System (LABCONO) was used as freeze dryer. Besides that it was used Colony counter (tuart Scientific, UK) to count the colony of LAB and pH meter (Model Jenway 3310, Stone, Staffordshire ST15 0SA, UK) to measure pH of the samples.

Methods

Experimental Design

This study used a completely randomized design with three replications. This present research consisted of two steps experiments. The first step of this study was fermentation of galangal using *L. casei* and *L. plantarum.* The fermentation process was conducted for 24 hours. During the fermentation process, samples were taken every three hours for the measurement of total plate count and pH. Besides that, samples were also taken at 3, 12 and 24 hours of the fermentation process for the measurement of total polyphenol content and antioxidant activity. Then, on second step of experiment, the sample which gave the best result on total plate count then was dried in freeze dryer. Freeze dried powder samples of the best treatments then were taken for the measurement of

the total polyphenol content and antioxidant activity. All measurements (total plate count, pH, total polyphenol content and antioxidant activity) on all the steps performed in three replications.

Fermentation of spice juice

The experiment followed Chen *et al*[17], 150 g of galangal was blended with distilled water (150 ml) using a commercial blender/chopper and filtered under room temperature. The subcultured starter (*L. plantarum* and *L. casei*) was cultured in MRS broth for 24 h at 37^{0}C. 90 ml of galangal juice then was inoculated by 3 milliliters of starter. Then the juice was incubated at 37^{0}C for 24 hours. In order to get powder of fermented samples, it was use freeze-dryer (48 hours). Each dried sample was milled using a blender machine then sieved (20 mesh; 850 micrometer; 0.0331 inches). The samples were ready for further experiments. pH meter was used to determined pH. Viable bacteria was count by spreading technique on MRS agar plate. Below formula was used for counting :

Cfu/mL = Cfu per plate x (1/mL inoculation sample) x DF (dilution factor)

Determination of TPC (Total Polyphenol Content)

Folin-Denis method was used for measure TPC[18,19]. In this experiment TPC determination was done at *3*, 12 and 24 hours of fermentation time. As the reference standard used gallic acid. Gallic acid solution standard was made in some concentration. Into every 5 ml of gallic acid solution added 0.25 ml of Folin-Denis reagent (5 minutes incubation at room temperature), then added also 1 ml 10% sodium carbonate (Na_2CO_3) solution (10 minutes incubation at room temperature). As a blank for absorbance measurement using UV-VIS spectrometer (760 nm), it was used distilled water and reagent without gallic acid or sample[19]. Data plotting was done to get standard curve and the linear regression from the curve would be used to determined TPC. In order to measure TPC of sample, 1 ml of sample extract was diluted with 4 ml distiled water. Then 0.05 ml of the sample solution was added by 4.95 ml of distilled water to get 5 ml of solution. Those each 5 ml of solution then added by reagent as same as galic acid solution above prior to absorbance measurement. Then TPC was calculated using linear equation from standard curve until it was gotten mg of gallic acid equivalent (GAE) per 100 g sample.

Antioxidant activity determination

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging activity was carried out according to Pianpumepong[20]. In this experiment antioxidant activity determination was done at 3, 12 and 24 hours of fermentation time. The procedure was: 0.025 g of DPPH dissolve in 1 L of methanol, then 5 ml from that solution was added into 0.5 mL sample. Then the mixture was keep minimum 30 minutes in dark room at room. Then the absorbance was measured at wavelength 517 nm. As the blank, it was used methanol instead of DPPH methanol solution while the control contained DPPH methanol solution without sample. The scavenging activity is calculated using the equation below:

DPPH scavenging activity (%) =(1-(absorbance of sample/absorbance of control) x 100%

Freeze drying of fermented spice juice

The process was done using laboratory scale freeze dryer (LABCONO). Freeze drying process was done for 48 hours. The dried sample then was milled using a blender machine then sieved (20 mesh; 850 micrometer; 0.0331 inches). The powder then was tested to measure total polyphenol content and antioxidant activity, using Folin-Denis method and DPPH radical-scavenging activity method, respectively.

Statistical analysis

Analysis of variance (ANOVA) and Duncan's test for post hoc test, using SPSS 15.0 (p 0.05) were used for analysing of the results of experiment statistically.

RESULTS AND DISCUSSION

Lactic acid bacteria fermentation

Prior to fermentation, galangal chopped and blended with water (1:1) using commercial food processor (blender), filtered and subsequently inoculated by *Lactobacillus plantarum* or *Lactobacillus casei*. This experiment proved Rodriguez *et al*[21] and also Usmiati and Utami[22] reports which stated that starter culture, in this case was lactic acid bacteria (LAB), led a rapid acidification on fermentation medium. As can be seen in Figures 1, pH changed rapidly in first 3 hours of fermentation from 5.39 to 4.80. from the Figure also can be seen that *L. plantarum* (LP) was better because it could decrease the pH lower than what *L. casei* (LC) did. However, the pattern was almost same that in the first 3 hours of fermentation, pH value decreased rapidly. That fast decreasing moment

happened because there was acids formation by LAB as products of the digestive process of energy source (carbohydrate/glucose found in the media), especially lactic acid but might be also acetic acid and propionic acid[22,23].

Related to bacterial growth, as can be seen in Figure 2, the both LAB used in this experiment reached the exponential phase around 3 hours. After that, there was a continuous growth decrease. Besides that, it seem that *L.plantarum* could growth better than *L.casei*. Although the both LAB reached the end of exponential phase at same time in galangal juice, population of *L. plantarum* was more high than *L. casei*.

Therefore, this result also support the result of the pH test. Because the population of *L. plantarum* was higher than *L. casei* so the production rate of acids by *L. plantarum* should be also higher than acid production by *L.casei*, so that *L. plantarum* could decrease the pH lower than what *L. casei* did. Meanwhile, the fast decreasing of pH happened in first three hours of fermentation process occured simultaneously with the process of the phase change of the bacteria growth which striking to the peak of the curve (3 hours).

Figure 2 also shows that the growth rate of bacteria was relatively low, so it is hard to separate the growth phases clearly. Since the juice was not added by any nutrition sources and only relied on the nutrients contained by the juice itself, so the low growth process of the LABs could be happen because of the lack of nutrition in the medium (galangal juice). Table 1 shows that after 12 hours of fermentation process around 50% total soluble solid in the juice was exhausted. It indicated that the amount of glucose and other energy sources would be decrease up to 50% after 12 hours fermentation process and the bacteria would be suffer and hard to growth well. In line with that, stressfull of bacterial cell which caused by the phenolic content of galangal might also be the reason why the growth rate was low. As other study has been reported that antibacterial contents of the spices especially phenolic compounds were believed inhibited the growth of LAB[24].

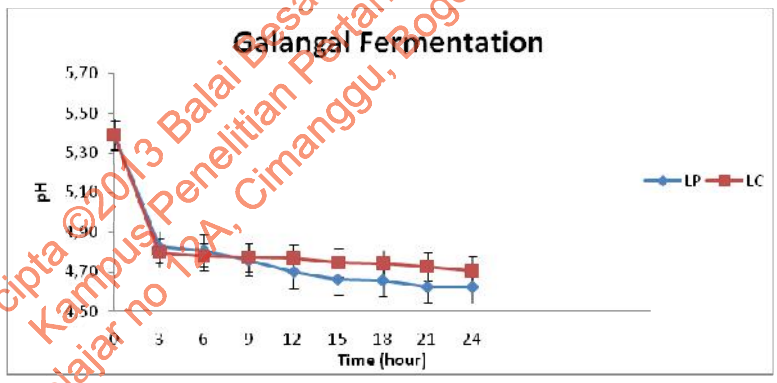

Figure 1. Changes in pH during galangal fermentation
Gambar 1. Perubahan pH selama proses fermentasi

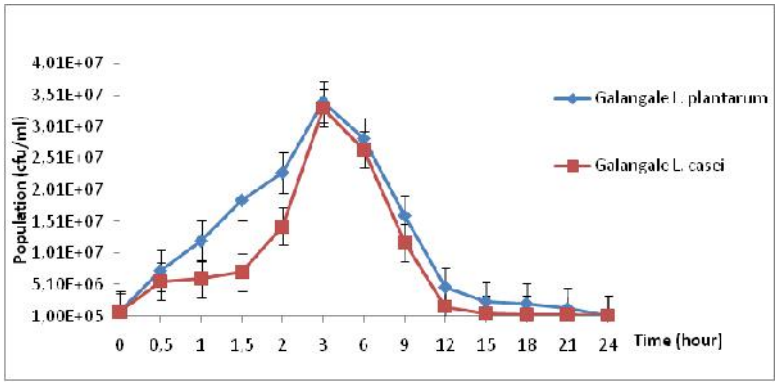

Figure 2. Bacterial growth curve in galangal juice
Gambar 2. Kurva pertumbuhan bakteri di dalam media ektsrak lengkuas

Table 1. Total Soluble solid of galangal juice before and after 12 hours fermentation

Tabel 1. Kandungan total padatan terlarut ekstrak lengkuas sebelum dan setelah 12 jam proses fermentasi

Sample of Galangal Juice / *Sampel ekstrak lengkuas*	Total soluble solid (^{0}brix)/ *Total padatan terlarut (^{0}brix)*
Unfermented (initial) / *Tidak difermentasi (kondisi awal)*	2.0
After 12 hours fermentation /*Setelah 12 jam fermentasi*	1.1

Total polyphenol content and antioxidant activity

Related to bioactive content, it is important to know the phenolic compounds and the effect of fermentation on its amount. Therefore, in order to know the effect of lactic acid bacteria fermentation, this study was also aimed to measure total polyphenol content in galangal extract before (unfermented galangal extract) and after fermentation (3, 12, and 24 hours fermentation process).

As can be seen in Figure 3, in general and compared to unfermented galangal extract, the fermentation process tended to raise the amount of polyphenol in fermented galangal media using *L. plantarum* as starter. The polyphenol content of 3 hours fermented galangal extract was same as unfermented galangal extract. And then, the polyphenol of the galangal was going to raise and reach the higher point at the 12 hours fermentation process. After that, the polyphenol content tended to decrease even it was lower then the content of unfermented galangal extract. In other words, the polyphenol content of galangal extract which fermented using *L. plantarum* reached the highest point not at the beginning (and also the peak) of the exponential phase of the bacteria growth curve (3 hours) but it was at at the end of the exponential phase of bacteria growth curve (12 hours). And, all the each treatments using *L. plantarum* as starter gave significant difference effects ($p< 0.05$) compared to unfermented galangal extract which showed by the different notation (a, b, c, etc) in the graph.

In contrast, the polyphenol content of galangal extract tended to decrease in fermented galangal extract using *L. casei* as starter compared to unfermented media. The polyphenol content of unfermented galangal extract was higher then all fermentation treatments using *L. casei*. The polyphenol content of fermented galangal extract was gradually decrease along with the fermentation process time. Polyphenol content of 3 hours fermented galangal extract was higher than polyphenol content of 12 hours fermented galalangal extract and so on polyphenol content of 12 hours fermented galangal extract was higher than 24 hours fermented galangal extract. In other words, the addition of *L. casei* gave the negative effects to the polyhenol content of galangal extract. And, all the treatments gave the significant difference effect ($p< 0.05$) which showed by the different notation (a, b, c, etc) in the graph.

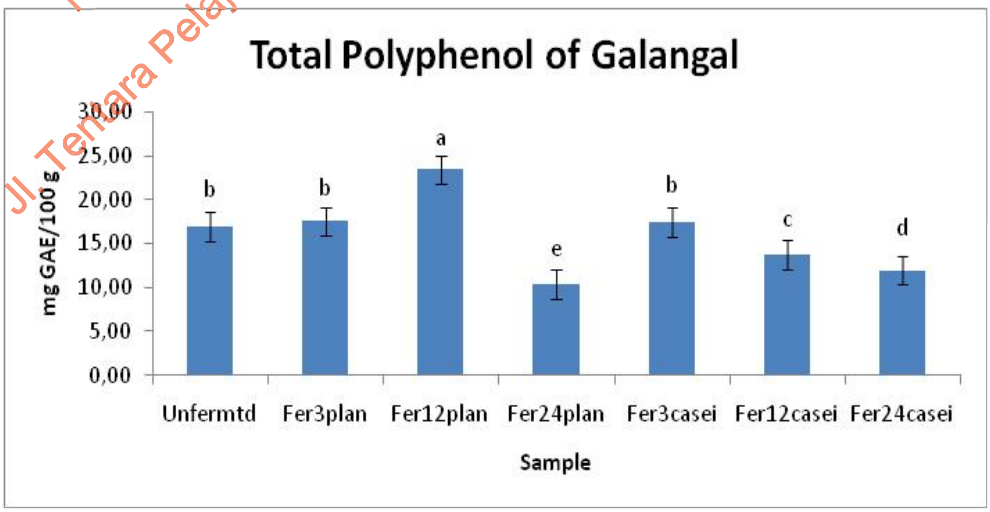

Figure 3. Total polyphenol (gallic acid equivalent(GAE)) content of galangal juice (Fer = fermentation; 3/12/24= hour; plan = L. plantarum; casei = L. casei)

Gambar 3. Kandungan total polyphenol ektsrak lengkuas (Fer = fermentasi; 3/12/24 = jam; plan = L. plantarum; casei = L. casei)

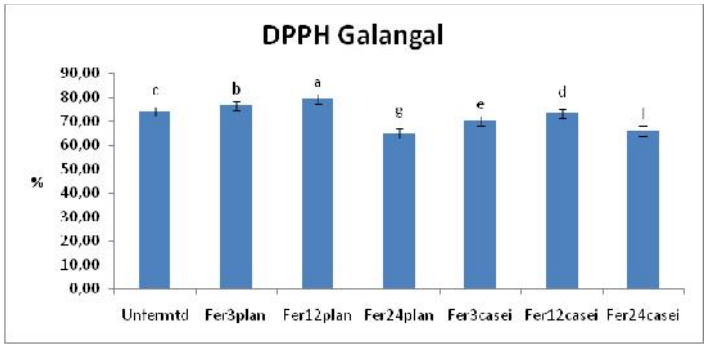

Figure 4. Antioxidant activity of galangal juice
(Fer = fermentation; 3/12/24= hour; plan = L. plantarum; casei = L. casei)
Gambar 4. Aktivitas antioksidan ekstrak lengkuas
(Fer = fermentasi; 3/12/24 = jam; plan = L. plantarum; casei = L. casei)

Furthermore, Figure 3 also shows that *L. plantarum* was better than *L. casei* because it could raise the total polyphenol significantly higher than *L. casei*. In this case, the highest result gave by *L. plantarum* at 12 hours fermentation. The highest content of total polyphenol was around 23 mg GAE/100 g. Lu *et al*[10] stated that phenol content of galangal extract was around 58 mg GAE/100 g. In this experiment, the polyphenol content was lower than what they have reported because this experiment used fresh spiced not dried spices. Besides that, this experiments used water as solvent, while they used ethanol as solvent. Meanwhile, according to Hernani *et al*[25] the best solvent to extract galangal was 80 % hexane.

Beside total polyphenol content, this experiment also was aimed to measure antioxidant activity in order to know the effect of lactic acid bacteria fermentation on antioxidant activity of galangal extract. As can be seen in Figure 4, the antioxidant activity of fermented galangal extract sometimes was higher and sometimes was lower compared to unfermented galangal juice. It also shows that 12 hours fermentation using *L. plantarum* gave higher antioxidant activity compared to unfermented samples. In contrast, fermentation using *L. casei* could not raise antioxidant activity more than unfermented samples. The highest value was reached by 12 hours *L. plantarum* fermented galangal, was 79% compared to 74% of unfermented galangal extract.

The pattern of the graph of the antioxidant activity was almost same with the pattern of total polyphenol content graph (Figure 3). In other words, the 12 hours fermentation process using *L. plantarum* gave the highest antioxidant activity related to the polyphenol content in which the treatment also gave the highest value of total polyphenol content in galangal juice. Meanwhile, the antioxidant activities of fermented galangal extracts using *L. casei* were never higher than the unfermented galangal extract also related to the polyphenol content of them in which lower than polyphenol content of unfermented galangal extract. In this case, Mayachiew and Devahastin [4] stated that phenolic compounds contribute to the overall antioxidant activities of herbs and spices where generally, the mechanisms of phenolic compounds for antioxidant activity are inactivating lipid free radicals and preventing decomposition of hydroperoxides into free radicals.

Therefore, based on above results, it was known that L. plantarum was better to be starter of fermentation with the best long fermentation time was 12 hours. This result then used in the next step of experiment. The sample then treat by freeze dryer prior to be powder. The freeze drying process for the samples was 2 days (48 hours), and immediately, after the samples was taken out from freeze dryer then the dried samples were blended/chopped by blender machine then sieved (20 mesh; 850 micrometer; 0.0331 inches). The samples then were placed in container covered by aluminium foil and keeped in desiccator to maintain moisture content level and ready for further experiments.

The characteristic of freeze dried powder samples was different each other. The colour of unfermented galangal powder was white just as white as the galangal fresh flesh and also same with the color of the galangal juice. Meanwhile, the fermented galangal powder was light red yellow.The unfermented galangal powder had same colour with the fresh galangal juice because it was dried in the freeze dryer immediately after the juice was made, while the fermented powder had red yellow colour because it had to waited until 12 hours ferementation process prior to freeze dryer. The unfermented galangal powder did not affect by browning reaction while the fermented one affected by browning

reaction. Besides that, the light brown colour of the *L. plantarum* in MRS media also played role in the change of the colour.

Related to total polyphenol and antioxidant activity after freeze drying process, there was difference result in the both parameters. For total polyphenol, there was a significant increasing of the value in the powder compared to fresh fermented ginger juice. In contrast, for antioxidant, there was a slightly decreasing value in the powder compared to fresh fermented ginger juice. The initial highest content of total polyphenol was around 23 mg GAE/100 g in fermented galangal juice, then it increased to about 53 mg GAE/100 g in fermented galangal powder. That so, the result was almost same with what Lu et al10 had reported that phenol content of dried galangal was around 58 mg GAE/100 g. The increasing value of the polyphenol content could be happen related to the decreasing value of moisture content as result of freeze drying process. Fresh galangal was used in this experiment had moisture content about 78.90%, while after freezed drying process, the moisture content was decreased became 13.10%. Besides that, hydration and diffusion process of dried sample could improve extraction process of polyphenol so that its content became higher[26].

Meanwhile, as mentioned before, antioxidant activity of fermented galangal powder were decreased compared to the fermented galangal juice. The highest antioxidant activity of the fermented galangal powder was about 67.4%, while the highest activity for fermented galangal juice was 79% and the antioxidant activity of the unfermented galangal juice was 74%. The decreasing value might be happen because of the drying process. Although the drying process carried out in freeze dryer, the process still would involved heat transfer which affect the antioxidant activity. Besides that, contact with oxigen and light also might be happen during the drying process. However this result same with what Chen *et al*[17] have been reported that freeze drying exhibited a decreased of 15-20% of antioxidant activity.

CONCLUSION

The result of the experiments showed that *L. plantarum* was better because it could growth well in galangal juice and so it could decrease the pH lower than what *L. casei* did. As well, *L. plantarum* could raise the total polyphenol significantly higher than *L. casei*. The highest content of total polyphenol reached by 12 hours fermentation, it was around 23 mg GAE/100 g. Those values then increased more than two times in freeze dried fermented galangal powder. The value was 53 mg GAE/100 g. Meanwhile, the highest value of antioxidant activity was 79% in fermented galangal juice and after drying, the value was decreased compared to the fermented juice, it was about 67.4%.

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