EFFECTIVE METHOD OF POLYPHENOL EXTRACTION FROM SESAME CAKE USING MICROBIAL FERMENTATION BY *LACTOBACILLUS BREVIS* NCTH24

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ABSTRACT

Sesame seed is not only nutrient rich food but also of high polyphenol content. The total polyphenol content of *Sesamum indicum's* bran can reach up to 150 mg/g. Among phenolic compounds, sesame lignans and anthocyanin were reported to have bioactive activities against diseases. The research focused on the development of method for sesame cake fermentation by *Lactobacillus brevis* NCTH24 to get high polyphenol content. The fermentation condition includes : 1/10 (w/v) ratio of sesame cake to distilled water, 15 g/l yeast extract, 1g/l glucose and 8 % (v/v) inoculum of *Lactobacillus brevis* NCTH24 equivalent to $8.4 \times 10^{\wedge 7}$ CFU/ml, pH 6.0, fermentation temperature at 30 ^{o}C and fermentation time 12 h. As a result, the total polyphenol, flavonoid contents (calculated per gram dry sesame cake) and antioxidant activity through scavenged DPPH amount obtained from sesame cake fermented supernatant were 21.96 mg, 0.135 mg and 0.38 mg DPPH/30 min, respectively. They were significantly higher than those from sesame cake by extraction using methanol solvent (polyphenol 2.29 mg, flavonoid 0.05 mg and scavenged DPPH amount 0.19 mg/30 min).

Keywords: polyphenol, sesame cake, fermentation.

1. INTRODUCTION

Sesame seed (*Sesamum indicum*) is well-known as a nutrient rich food. It contains oil (48.2–56.3 %), protein (19.1–26.9 %), carbohydrate (10.1–17.9 %), fiber (2.5–3.9 %), ash (2.0–5.6 %), vitamins B1, B2, PP and minerals such as Mg, Cu, Fe, Ca, Na, P [1]. In addition, many bioactive compounds were found in sesame seeds like polyphenol (anthocyanin, sesame lignans), unsaturated fatty acids, phytosterol and lecithin.

In the world, methods were used to obtain sesame lignans from sesame seeds, sesame oil or sesame cake such as extraction using organic solvents, utilization of enzyme or microbial fermentation. Recently, lactic fermentation has become popular. In this study, a method of polyphenol extraction from sesame cake was developed by fermentation using *Lactobacillus brevis* NCTH24.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Materials and microorganism

Black sesame seeds were purchased from the local market. They were defatted to have sesame cake (moisture and fat content: 8.77 ± 0.21 and 8.3 ± 0.52 %, respectively) and stored at 4 ^{o}C.

Strain *Lactobacillus brevis* NCTH24 was obtained from FIRI's collection.

2.1.2. Preparation of sesame cake medium

Sesame cake (SC), after grinding by blender, was added with distilled water. The resultant slurry was sterilized at 121 ^{o}C for 15 min and then stored at 4 ^{o}C until used for fermentation.

2.1.3. Preparation of crude extract of fermented sesame cake

The liquid, obtained from filtration of fermented sesame cake slurry using double layer cheesecloth, was centrifuged at 10000 rpm, 20 ^{o}C for 5 min. The supernatant was stored at -20 ^{o}C until analysis for determination of total phenolic and flavonoid content, antioxidant activity, carbohydrate and dissolved protein amount.

2.2. Methods

2.2.1. Extraction of polyphenols

Polyphenols were extracted using organic solvent as described by Mohdaly AA. et al. [2]. Sesame cake (50 g), after grinding by blender, was mixed with 150 ml of methanol in a conical flask. The mixture was shaken at 200 rpm for 1 h at room temperature and filtered through filter paper. Filtrate was stored at -20 ^{o}C until prior to analysis.

2.2.2. Determination of total polyphenol content

The total polyphenol content was determined by the Folin - Ciocalteu method [3]. The samples were measured with a spectrophotometer at 770 nm. Gallic acid (0–500 mg/l) was used as standard to produce the calibration curve. The results were expressed as mg gallic acid equivalents/g dry sesame cake.

2.2.3. Determination of flavonoid content

The flavonoid content was measured according to the aluminium chloride colorimetric method as described by Arvouet Grand et al. [4**]**. The samples were measured the absorbance at 415 nm. Quercetin (0–25 mg/l) was used as the standard curve. The results were expressed as mg of quercetin equivalents/g of dry SC.

2.2.4. Determination of antioxidant activity

The antioxidant activity was investigated by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay as described by Gayathri Balakrishnam el al. [5] with modification. 5 ml of the crude extract was extracted with the four times volume of methanol. After centrifugation at 8000 rpm, 10 ^{o}C for 5 min, 1 ml of supernatant was added 2.5 ml of 0.004 % DPPH solution in methanol. The mixture was placed in the dark at room temperature for 30 min and measured the absorbance at 517 nm. The blank sample contained 1 ml of the distilled water-methanol solution (1:4) and 2.5 ml DPPH solution, in the control sample, 2.5 ml of DPPH solution was replaced by methanol. The radical scavenging activity was calculated as follows:

$$\% H = [1 – (\text{sample absorbance} – \text{control absorbance})/\text{blank absorbance})] \times 100$$

The DPPH amount, scavenged by 1 ml of the crude extract from fermented sesame cake in 30 min, was calculated with following equation:

$$Y (\text{mg}/30 \text{ min}) = [(H/100) \times 2.5 \times (0.004/100)]/1000 \times 4$$

where: H is The radical scavenging activity (%),

2.5 - The volume of DPPH solution (ml),

0.004 - The concentration of DPPH solution in methanol (%),

1000 - Conversion factor to mg,

4 - The volume ratio of methanol to the crude extract.

2.2.5. Determination of total carbohydrate

The total carbohydrate amount was estimated by the phenol sulphuric acid method [6].

2.2.6. Determination of dissolved protein concentration

The dissolved protein content was measured by the Lowry method [7].

3. RESULTS AND DISCUSSION

3.1. Effect of fermentation time on total polyphenol and flavonoid production

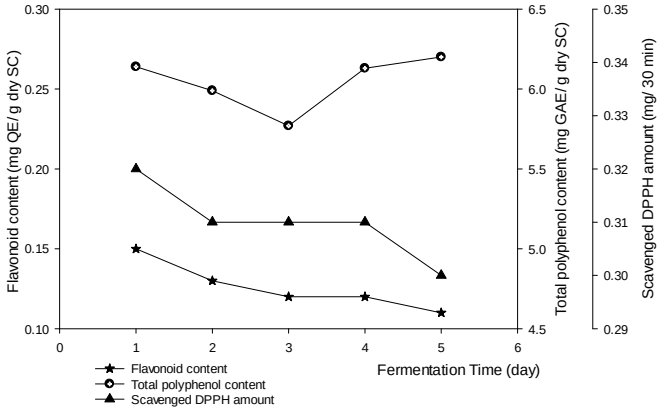

Figure 1. Total phenolic and flavonoid contents during sesame cake fermentation by *Lactobacillus brevis* NCTH24 at 30 ^{o}C.

Sesame cake slurry was fermented by *Lactobacillus brevis* NCTH24 with initial cell density of $1.5 \times 10^{\wedge 7}$ CFU/ml for 5 days at 30 ^{o}C. The crude extract of fermented medium was obtained every 24 h and subsequently used for the analysis. The results were shown in Figure 1.

The flavonoid content and scavenged DPPH amount were the highest at the first 24 h of incubation (respectively, 0.15 mg QE/g dry SC and 0.32 mg DPPH scavenged by 1 ml of crude extract for 30 min.) and decreased slightly in the next days. The total phenolic content of fermented sesame cake at 24 h was 6.14 mg GAE/g dry SC, the same as that at the 5th day of fermentation. Therefore, 24 h was chosen as fermentation time in the following experiments.

3.2. Effect of the volume ratio of sesame cake to water on fermentation

Sesame cake slurries made by adding the different volume of distilled water were incubated with the initial cell density $2.12 \times 10^{\wedge 7}$ CFU/ml of strain NCTH24, for 24 h at 30 ^{o}C. The results (in Table 1) revealed that the pH value, cell population, antioxidant activity as well as flavonoid content of fermented sesame cake slurries were similar.

Table 1. Total phenolic and flavonoid contents of sesame cake fermented supernatant with the different volume ratio of sesame cake to water.

Volume ratio of sesame cake to water (w/v)	Flavonoid content (mg QE/g dry SC)	Total polyphenol content (mg GAE/g dry SC)	Scavenged DPPH amount (mg/30min)	Viable cells, CFU/ml	pH value at the end of fermentation
1/8	0.09 ± 0.01	5.79 ± 0.03	0.30 ± 0.01	$2.31 \pm 0.05 \times 10^{\wedge 8}$	5.82
1/9	0.09 ± 0.01	6.13 ± 0.01	0.30 ± 0.01	$2.36 \pm 0.02 \times 10^{\wedge 8}$	5.83
1/10	**0.10 ± 0.00**	**6.48 ± 0.01**	0.30 ± 0.01	**$2.39 \pm 0.01 \times 10^{\wedge 8}$**	**5.79**
1/11	0.09 ± 0.00	6.68 ± 0.01	0.29 ± 0.01	$2.39 \pm 0.02 \times 10^{\wedge 8}$	5.73

There were no significant differences in polyphenol content among the 1/10 and 1/11 ratio of material to distilled water after fermentation, respectively 6.48 mg GAE/g dry SC and 6.68 mg GAE/g dry SC. However, they were higher than those of fermented sesame cake added by 8 and 9 fold of water (w/v). Hence, the 1/10 ratio of sesame cake to distilled water was used in the subsequent experiments.

3.3. Effect of nitrogen supplement on total polyphenol and flavonoid production

The total carbohydrate and dissolved protein concentration of sesame cake slurry are low (5.16 g/l and 4.12 g/l, respectively) and may be not enough for bacteria growth. Therefore, nutrient supplementing in sesame cake slurry was surveyed. In this experiment, yeast extract (YE) was used as nitrogen source, added to fermentation medium from 0 to 17.5 g/l. After incubation for 24 h, the total polyphenol and flavonoid contents were analyzed and shown in Figure 2.

The results showed that the supplementation of yeast extract enhanced significantly the production of total phenolic and flavonoid. In the survey range, the highest content of polyphenol was 19.75 mg GAE/g dry SC in fermented sesame slurry with addition of 17.5 g/l yeast extract, higher 3 times than that in this medium without adding nitrogen source (6.45 mg

GAE/g dry SC). While, fermented sesame medium with supplementation of 15 g/l YE exhibited the lower level of that (18.04 mg GAE/g dry SC) and the highest flavonoid content (0.24 mg QE/g dry SC) and antioxidant activity through DPPH amount scavenged by 1 ml of crude extract for 30 min (data not shown). Moreover, this medium had the residual protein concentration at the end of fermentation less than that added 17.5 g/l YE (8.35 g/l and 10.07 g/l residual protein, respectively). It was easier to extract phenolic components like sesaminol glucosides [8]. Therefore, the 15 g/l of yeast extract was used as a nitrogen supplement in the next experiments.

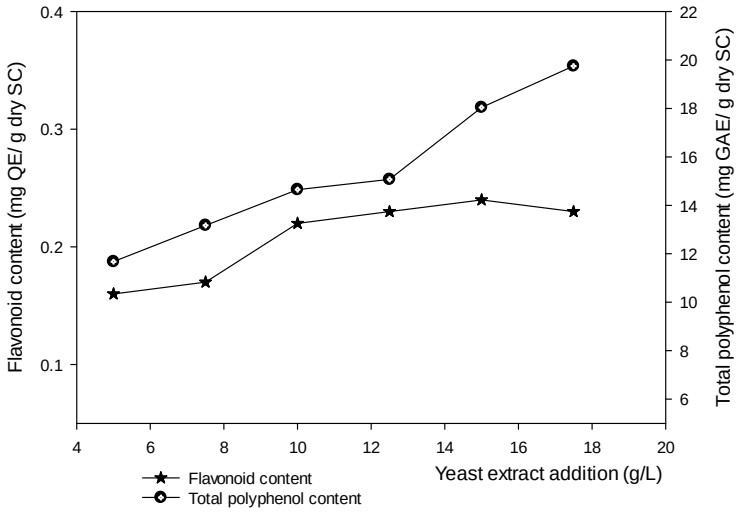

Figure 2. Effect of yeast extract supplement on polyphenol and flavonoid production.

3.4. Effect of glucose supplement on total polyphenol and flavonoid production

The fermentation of sesame cake slurries with addition of different glucose concentration (0 to 5 g/l) was carried out. The total phenolic, flavonoid contents and scavenged DPPH amount of fermented sesame cakes were shown in Figure 3.

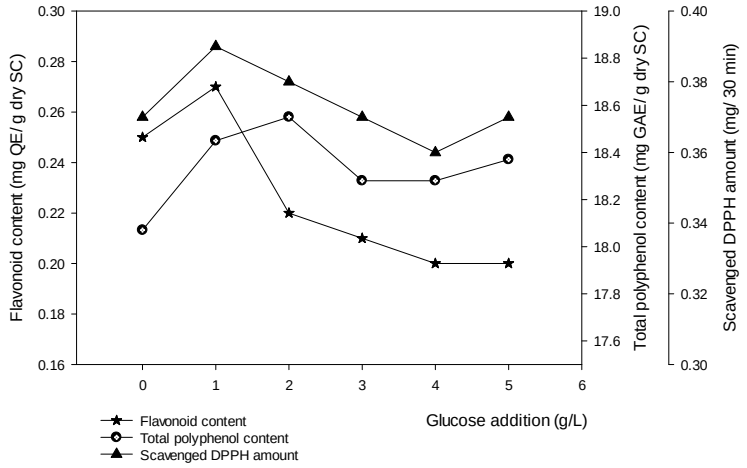

Figure 3. Effect of glucose addition on polyphenol and flavonoid production.

The results demonstrated that, addition of 1 g/l glucose in fermentation medium increased polyphenol and flavonoid production more than that without supplementation of glucose. However, the higher concentration of glucose was added (from 2 to 5 g/l), the lower content of obtained polyphenol and flavonoid became at the end of fermentation. It was considered that related to β-glucosidase activity of lactic acid bacteria [9]. During fermentation of sesame cake without addition of sugar, bacteria used fermentable sugars from sesame cake. When the fermentable carbohydrates were limited, they produced β-glucosidase to hydrolyze sesaminol triglucoside to sesaminol aglycone and glucose. The sesaminol aglycone contains more reactive phenolic compound results in higher total phenolic content and antioxidant activity.

Ulyatu Fitrotin et al. [9] have reported that the total phenolic content of sesame milk fermented by *L. plantarum* Dad 13 declined from 8 mg GAE to 7 mg GAE/g dry sesame seed in the case of 2 % and 4 % sucrose supplementation and the β-glucosidase activity diminished from 70.3 mU/ml in fermented sesame milk without addition of sucrose to 30.83 mU/ml and 29.4 mU/ml in the medium added with 2 % and 4 % sucrose, respectively.

The results also exhibited that the antioxidant activity of fermented sesame cake slurries was the highest in case of adding 1 g/l glucose. Moreover, due to low of fermentable carbohydrate in raw material so 1 g/l glucose was essential for the initial growth of strain NCTH24 and used in the next experiments.

3.5. Effect of initial fermentation pH on total polyphenol and flavonoid production

The initial pH of sesame cake slurry could influence directly to the cell growth and so it might affect to polyphenol and flavonoid production during fermentation. Hence, the variation of the initial medium pH was surveyed. It was adjusted in pH range (pH 5.5 to pH 7.0) and then fermented by strain NCTH24 with the initial cell density of $2.12 \times 10^{\wedge 7}$ CFU/ml, for 24 h at 30 ^{o}C.

Table 2. Effect of the initial fermentation pH on sesame cake fermentation.

Initial pH value	Flavonoid content (mg QE/g dry SC)	Total polyphenol content (mg GAE/g dry SC)	Scavenged DPPH amount (mg/30 min)	Viable cells, CFU/ml	pH value at the end of fermentation
pH 5.5	0.17 ± 0.01	21.67 ± 0.01	0.38 ± 0.00	$1.85 \pm 0.02 \times 10^{\wedge 8}$	5.71
pH 6.0	**0.17 ± 0.01**	**22.58 ± 0.02**	0.38 ± 0.01	**$2.28 \pm 0.02 \times 10^{\wedge 8}$**	**5.69**
pH 6.5	0.18 ± 0.01	21.59 ± 0.01	0.38 ± 0.02	$1.73 \pm 0.01 \times 10^{\wedge 8}$	5.81
pH 7.0	0.2 ± 0.01	20.75 ± 0.03	0.38 ± 0.00	$1.5 \pm 0.03 \times 10^{\wedge 8}$	5.97

The highest total phenolic content and viable cells of fermented sesame slurry were 22.58 mg GAE/g dry SC and $2.28 \times 10^{\wedge 8}$ CFU/ml, respectively, showing that the initial pH 6.0 was optimum for the cell growth and total polyphenol production.

According to Idayu Muhamad et al. [10], the production of β-glucosidase is associated to the cell growth, so the higher cell population during fermentation the higher produced total phenolic content. In addition, the maximum β-glucosidase activity is exhibited at pH 6.0.

3.6. Effect of fermentation temperature on total polyphenol and flavonoid production

The fermentation temperature had great effect on phenolic and flavonoid production as shown in Figure 4. The highest obtained polyphenol, flavonoid contents and scavenged DPPH amount were 22.30 mg GAE/g dry SC, 0.18 mg QE/g dry SC and 0.39 mg/30 min, respectively at 30 ^{o}C. They declined in case of the fermentation temperature increase (35 ^{o}C and 37 ^{o}C). Furthermore, at 30 ^{o}C, the cell population of strain NCTH24 was the highest (data not shown). Therefore, it was chosen as an optimum temperature for strain NCTH24 to ferment sesame cake.

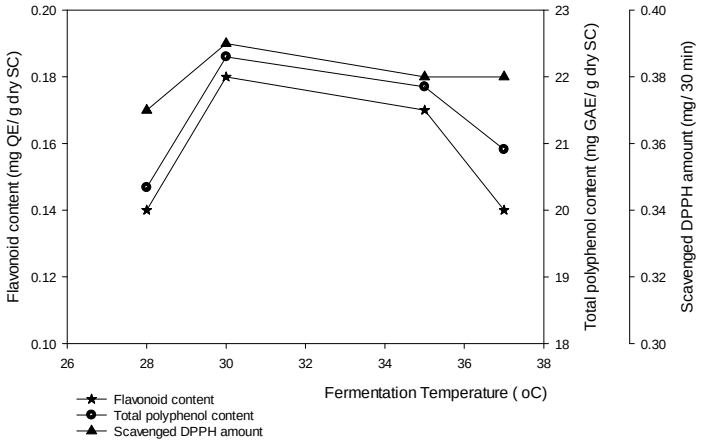

Figure 4. Effect of fermentation temperature on polyphenol and flavonoid production.

3.7. Effect of initial cell density on total polyphenol and flavonoid production

Different inoculum size affected the initial population of bacteria in the fermentation medium. Initial cell densities of strain NCTH24 in sesame cake medium with inoculum size of 2, 4, 6, 8 and 10 % (v/v) were 2.2 × 10^7 CFU/ml, 4.4 × 10^7 CFU/ml, 6.4 × 10^7 CFU/ml, 8.4 × 10^7 CFU/ml and 1.03 × 10^8 CFU/ml, respectively.

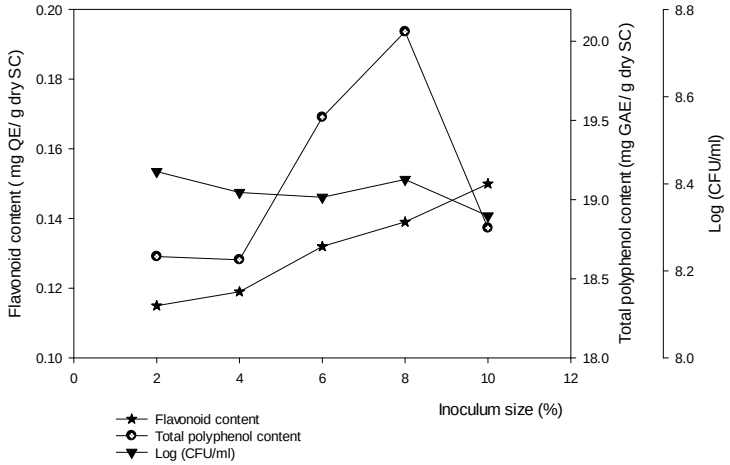

Figure 5. Effect of initial cell density of strain NCTH24 on polyphenol and flavonoid production.

It can be seen from Figure 5 that total polyphenol and flavonoid content of were lowest in the fermentation medium with 2 % inoculum and rose significantly with the increase of

inoculum size (from 4 % to 8 %). However, the total polyphenol content fell remarkably in the case of 10 % inoculum. The polyphenol and flavonoid content obtained from sesame cake medium added 8 % inoculum were 20.06 mg GAE/g dry SC and 0.139 mg QE/g dry SC, respectively.

3.8. Kinetic analysis of sesame cake fermentation

After selection of optimum fermentation conditions, kinetic analysis of the sesame cake fermentation was carried out. The sesame cake slurry had the 1/10 ratio of material to distilled water (w/v), added 15 g/l yeast extract, 1g/l glucose and adjusted to pH 6.0, was fermented by 8 % (v/v) inoculum size of strain NCTH24 (equivalent to $8.1 \times 10^{\wedge 7}$ CFU/ml) for 48 h at 30 ^{o}C. The number of cells, total polyphenol and flavonoid content, DPPH amount scavenged by 1 ml of the crude extract from fermented medium and the residual total carbohydrate and dissolved protein concentration, were analyzed every 6 h and shown in figure 6.

The raw sesame cake was extracted by using methanol solvent and used for comparison. The obtained polyphenol and flavonoid contents were 2.29 mg GAE and 0.05 mg QE per gram of dry sesame cake. The DPPH amount scavenged by 1 ml of methanol extract (that was calculated to be equavalent to 1 ml of fermented sesame cake slurry) was 0.19 mg/30 min.

From Figure 6 it can be seen that the first 6 h of fermentation was the exponential phase of strain NCTH24. The cell population reached a peak of $4.10 \times 10^{\wedge 8}$ CFU/ml at 6th h, following by the stationary stage. From 30th h to 48th h, it declined slightly to $2.92 \times 10^{\wedge 8}$ CFU/ml at the end of fermentation.

The level of flavonoid content and scavenged DPPH amount of sesame cake fermented supernatant also rose with the increase of cell number. At the 6th h of incubation, they were 0.135 mg QE/g dry SC and 0.38 mg DPPH/ 30 min, respectively and then grew no significantly in the next 42 h.

The polyphenol content escalated more three times during the first 12 h of process, from 7.05 mg GAE/g dry SC to 21.96 mg GAE/g dry SC, then increased slowly to 22.83 mg GAE/g dry SC at the 48th of fermentation.

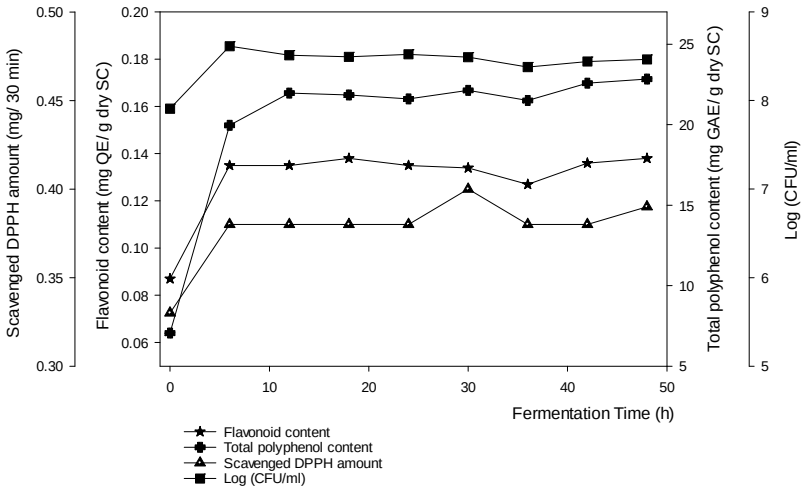

Figure 6. The change of the polyphenol and flavonoid contents, scavenged DPPH amount and cell population during sesame cake fermentation.

As can be seen from Figure 6, fermentation time extending (from 12 h to 48 h) did not significantly increase flavonoid content as well as total polyphenol concentration. Therefore, the optimum sesame cake fermentation time was 12 h.

The results exhibited that the polyphenol and flavonoid contents, scavenged DPPH amount obtained from sesame cake by the fermentation method were significantly higher than those by extraction using methanol solvent.

4. CONCLUSION

The fermentation method by *Lactobacillus brevis* NCTH24 increased remarkably the polyphenol content extract of sesame cake. The results showed that the total polyphenol, flavonoid contents and antioxidant activity through scavenged DPPH amount of sesame cake fermented supernatant reached to 21.96 mg GAE/g dry SC, 0.135 mg QE/g dry SC and 0.38 mg DPPH/30 min, respectively. The conditions for sesame cake fermentation by *Lactobacillus brevis* NCTH24 included: the ratio of material to distilled water 1/10 (w/v); nutrient supplementation 15 g/l yeast extract and 1g/l glucose; the initial medium pH 6.0; the inoculum size 8 % (v/v) equivalent to $8.4 \times 10^{\wedge 7}$ CFU/ml; temperature 30 ^{o}C and fermentation time 12 h.

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