POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 36(1): 59–78, Y. 2021

THE EFFECTS OF SOAKING TREATMENTS AND FERMENTATION PROCESS ON NUTRITIONAL AND AFLATOXIN CONTENTS OF FERMENTED PEANUT CAKE (*BLACK ONCOM*)

***Azizah Rohimah*[1], *Budi Setiawan*[2], *Katrin Roosita*[3], *Eny Palupi*[4]**

[1] ORCID : 0000-0001-5903-1520
[2] ORCID : 0000-0001-8655-5477
[3] ORCID : 0000-0002-0734-7336
[4] ORCID : 0000-0003-2029-3106
[1–4] Department of Community Nutrition, Faculty of Human Ecology
IPB University, Indonesia

Key words: *black oncom*, processing methods, nutritional value, aflatoxin content.

Abstract

The existing product of fermented peanut cake, namely *black oncom* as a traditional food of West Java contains high aflatoxin and less nutritional contents. This study aimed to evaluate the adaptive processing method using the effect of soaking treatment and fermentation process on nutritional and aflatoxin contents of *black oncom*. Parameters evaluated in this study were the fermentation quality (pH and mold density), nutritional contents (water, ash, protein, lipid, carbohydrate, crude fiber, dietary fiber, and protein digestibility) and aflatoxin (aflatoxin B_1 and total aflatoxin). These parameters were evaluated for peanut cake with unfermented condition and fermented condition such as commercial *black oncom* as a traditional (natural) fermentation and the adaptive processing method of *black oncom* with three soaking treatments of peanut cake, i.e., water (BW), acetic acid 5% (BA) and chitosan 5% w/v (BC) and the use of yeast starter of *Rhizopus oligosporus*. The results showed that growth of mold were spread more homogeneously in BW compared to BC and BA. The adaptive procedure of BW treatment produced *black oncom* with better nutritional value (crude fat 37.46% and protein digestibility 77.91%) and lower aflatoxin content within acceptable level of regulatory standard than the commercial *black oncom* in the market. In conclusion, the adaptive fermentation of *black oncom* processing which used water treatment and the use of yeast starter of *Rhizopus oligosporus* might be applied as a potential solution for *black oncom* production with better nutritional value and acceptable aflatoxin content.

Address: Budi Setiawan, IPB University Campus, Babakan, Dramaga, Bogor, Lingkar Kampus street, West Java 16680, Indonesia, e-mail: bsetiawan@apps.ipb.ac.id

Introduction

Peanut cake is an industrial residue or main by-product of the peanut oil which can be used as a source of nutrients (SADH et al. 2017, 2018). Peanut cake can be improved in quality and nutritional contents with the solid state fermentation technology (SSF) which is defined as the growth of microorganisms (especially fungi or molds) in moist solid materials without free water (BHARGAV et al. 2008, CHI and CHO 2016) and it can improve the functional properties, nutritional characteristics, good sensory characteristics of a product and and the formation of short chain compounds with lower molecular weight (SADH et al. 2018, XIAO et al. 2015). The SSF can be applied in several cereals, nuts and by-products, as well as other food applications (CHI and CHO 2016).

In Indonesia especially West Java, solid state fermentation technology has also been developed in fermented peanut cake (*black oncom*) as a traditional food in West java which contains fungus *Rhizopus oligosporus* (HO 1986, PANDEY 2003). Fermented peanut cake has functional components, such as phenolic compounds, antioxidant, good nutritional and low aflatoxin contents if it is fermented by microorganism starters, such as *Aspergillus oryzae* (SADH et al. 2017), *A. awamori* (DUHAN et al. 2019), *Bacillus natto* bacteria (YANPING et al. 2017), *Neurospora sitophila*, *Rhizopus oligosporus* (KUMBHARE 2017), and *Zygosaccharomyces rouxii* (ZHOU et al. 2017). The production process of *black oncom* still does not use starters, but a natural fermentation process. This is observed from the low nutritional contents (SLAMET and TARWOTJO 1971) and the high aflatoxin content (ROEDJITO et al. 1972, GINTING et al. 2018).

Therefore, it is necessary to modify the production method of *black oncom* with SSF technology to achieve a product with high nutritional contents and low aflatoxin content. Process modification can be performed using soaking treatments, because the fermentation process is begun with a soaking process. Organic acids such as acetic acid, lactic acid as candidates for the soaking treatment were used to reduce the aflatoxin level. HASSAN et al. (2015) showed that 10% acetic acid has the highest inhibitory effect for the growth of aflatoxin-producing *Aspergillus flavus*, and lactic acid can also inhibit the growth of aflatoxin. In addition, a chitosan treatment can reduce the aflatoxin B_1 content in peanuts (HIDAYAH 2015) and increase nutrients in a fermentation process (GANDRA et al. 2018, NWE et al. 2002).

It has been noticed how the nutritional contents can be increased and the toxin can be decreased in an adaptive processing method with soaking treatments and fermentation process using a yeast starter in *black oncom*

production (HASSAN et al. 2006, SITUNGKIR 2005, JANNAH 2005, PURWIJANTININGSIH 2005, RAHARJANTI 2006). Therefore, the aim of this study was to analyze the adaptive processing method with the effect of soaking treatments (water, acetic acid, chitosan) and the fermentation process using the yeast starter of *Rhizopus oligosporus* on nutritional and aflatoxin contents of *black oncom* product compared to unfermented peanut cake and commercial *black oncom* in the market as a traditional fermentation.

Materials and Methods

The Processing Method of Fermented Peanut Cake (*Black Oncom*)

A completely randomized design was applied in this study. Various materials used for the production of *black oncom* were prepared, i.e., peanut cakes collected from the peanut oil factory in Sukasari, Bogor, West Java, Indonesia. *Black oncom* in the market as a traditional fermentation was collected from craftmens in Bantar Kambing, Bogor, West Java, Indonesia. Three types of soaking solution were prepared, i.e., water, acetic acid 5% w/v, and chitosan 5% w/v. The concentration was applied according to DEWI and NUR (2018) that 2.5% of concentration can reduce aflatoxin as much as 70%, and 10% of concentration generates the highest reduction of aflatoxin. The yeast starter of *Rhizopus oligosporus* (Raprima brand) was used for the fermentation process.

The production process of traditional *black oncom* is shown in Figure 1 (*a*). The production used water for soaking treatments and cassava cake 2% for the fermentation process without the addition of *Rhizopus oligosporus* starter, then it was fermented in a bamboo box for 48 h (ROHIMAH et al. 2021). The adaptive processing method is shown in Figure 1 (*b*). For the adaptive fermentation process, the peanut cake materials were soaked in different soaking treatments (water, acetic acid 5%, and chitosan 5%) for 16 h. After soaking, the cake was washed and drained. Then the cake was steamed for one hour. After cooled down, the starter of *Rhizopus oligosporus* was added as much as 0.2% and was spread by mixing it with the cake. Afterwards, it was molded in a square shape. Then, it was fermented at 25–29°C for 48 h in a anaerobic condition in a laboratory. The three different treatment procedures of *black oncom* production were peanut cake was soaked in water and fermented using *Rhizopus oligosporus* (BW), peanut cake was soaked in acetic acid 5% and fermented using *Rhizopus*

oligosporus (BA), peanut cake was soaked in chitosan 5% and fermented using *Rhizopus oligosporus* (BC). This experiment was analyzed with two replicates.

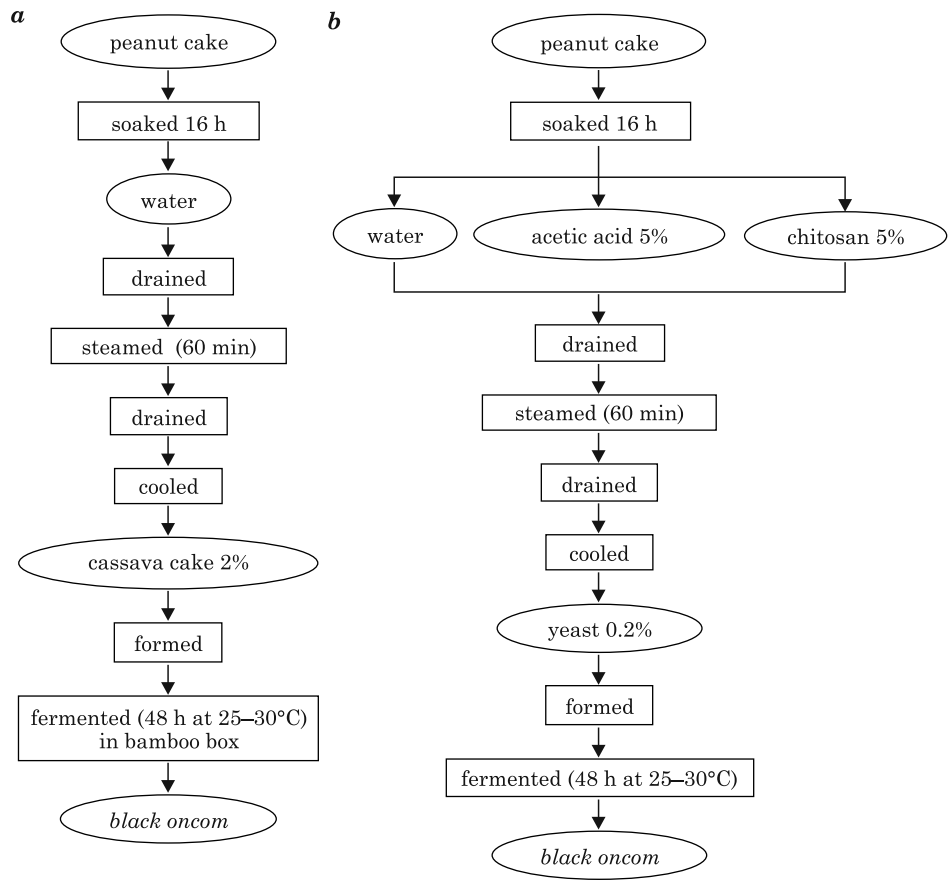

Fig 1. Flow chart of black oncom production processes: *a* – traditional fermentation; *b* – adaptive fermentation

Measurement of Parameters

Determination of Fermentation Quality

Three main parameters were measured in this study, i.e., pH value, mold density, and texture. The level of pH was measured using a OHAUS starter 3100 pH meter. The procedure was as follows: a 10 g sample was prepared, then 10 mL of distilled water was added and stirred until blended. Afterwards, the pH electrode was previously rinsed with distilled

water, then was put in the sample solution. The value of pH was displayed on the device screen. The growth of mold was calculated to determine the mold density that consisted of no mold growth (0); slight mold growth, texture not yet formed (+); sufficient mold growth, the texture is rather compact and soft (++) (SASTRAADMAJA et al. 2002). The spread of mold growth was represented as a percentage. Texture of *black oncom* was examined using a LFRA Stevens texture analyzer with needle-shaped probe, speed of 2.0 mm/s and distance of 10 mm. The data of texture was represented in the equipment as N unit (ISKANDAR et al. 2010).

Determination of Nutritional Content

Nutritional contents determined were the content of water, ash, crude protein, crude fat, carbohydrate, crude fiber, dietary fiber (AOAC 2005), and protein digestibility (SHI et al. 2020). Determination of water content was performed by heating process in an oven at 100 ± 5°C (Eyela, NDO 400, Japan) of 2 g of sample then weighed after being cooled in the desiccator. Water content was determined by the following formula represented as percentage [%].

$$\text{Water content [\%]} = (B/A) \cdot 100$$

where:
A – initial sample weight
B – sample weight after drying.

Ash content was determined by heating of 3 g sample on a hotplate until fume was disappeared. Then, the sample was ashed completely for 5 h at 550°C in a furnace. The ash content was determined as weight of sample after it was ashed compared to weight sample before it was heated and ashed. The ash content was evaluated using the following formula:

$$\text{Ash content [\%]} = (B/A) \cdot 100$$

where:
A – initial sample weight
B – sample weight after ashing

Determination of protein content involved a destruction with 6 mL of H_2SO_4 and 1 g of selenium mix (Foss, DT 208, Denmark). Afterwards, 40 mL of NaOH 40% and 25 mL of aquades were added and the sample was distilled using a KjelDigester instrument (Foss, KT 200, Denmark)

until three times of volume of 4% boric acid. Then, the distillate was titrated using HCl 0.2 N until reached the end point (red colour). The protein content was evaluated using the following formula:

$$\text{Protein content [\%]} = [(V_s - V_b) \cdot N \cdot 1.4007 \cdot F_k\ ]/\ \text{g of sample}$$

where:
V_s – volume of HCl for sample titration
V_b = volume of HCl for blank titration
N = normality of HCl solution
F_k = conversion factor (6.25).

Determination of fat content was performed by hydrolisis method. Two g of sample was added with 30 mL HCl 25 % and 20 mL of aquades, then it was heated at a hotplate for 15 min. The residue was dried at 105°C in an oven for 1 h. Then, the residue was placed in a sample package for fat extraction with hexane using soxhlet equipment that was connected with a boiling flask for 3 h. The boiling flask and residue of fat was dried at 105°C in an oven, and it was weighed after being cooled. The fat content was evaluated using the following formula:

$$\text{Fat content [\%]} = [(C - A)/\ B] \cdot 100$$

where:
A – empty boiling flask weight
B – sample weight
C – boiling flask weight + dried sample.

Based on water, ash, protein and fat contents, carbohydrate content was determined using by difference method. Crude fiber content was determined by firstly removing the fat in the sample that was sieved with 40 mesh of sieve using petroleum ether. Residue of the extraction was refluxed with 100 mL of H_2SO_4 1.25% for 30 min to boil. The filtering process was followed by rinsing using 40 mL hot water for four times. The residue was then rinsed with hot NaOH 1.25% which was collected with an Erlenmeyer. The collected water was refluxed with an upright cooler for 30 min to boil. The solution was then filtered with a filter paper using a vacuum pump. The filter paper containing the residue was rinsed again with 25 mL hot H_2SO_4 1.25%, 25 mL hot water for two times and 25 mL acetone. After rinsing, the filter paper was dried in an oven at 105°C for 2 h. After being heated, the filter paper was cooled and then weighed. If the crude fiber content was above 1%, then the ash content was measured by weighing the heated empty porcelain plate. Paper and residue were char-

red on a hotplate and then was ignited in the furnace (temperature 550°C). After igniting, the plates were cooled in the excicator, then weighed to obtain a fixed weight. Crude fiber content was determined by the gravimetric method.

Determination of dietary fiber was performed by the enzymatic process. A sample of 0.5 g was put into different 50 mL falcon tubes. The sample was extracted with 15 mL of petroleum ether for three times, then was dried in an oven at 100°C. Each sample was transferred to a 500 mL beaker. A total of 40 mL of mes tris buffer was added while stirring until no sample was lumpy. 50 µL *α*-amylase enzyme was added while stirring until homogeneous. The beaker was covered with an aluminum foil and incubated in a waterbath shaker at 100°C for 30 min. The solution was then cooled to 60°C and the walls were rinsed with 10 mL of distilled water. A total of 100 µL of the protease enzyme was added while stirring until there was no clumps of the sample. The solution was closed again with an aluminum foil, then was incubated in a waterbath at 60°C for 30 min. The aluminum was opened and 0.5 M HCl was added. The pH of the sample was adjusted in the range 4.1–4.6 with 1 M HCl or 1 M NaOH. The solution was added with 200 µL of amyloglucosidase enzyme and was incubated again at 60°C for 30 min. 225 ml of 95% ethanol at 60°C was added and stirred until homogeneous. The solution was left to stand for 1 h, then was filtered with a known weight filter paper and rinsed with 15 mL 78% ethanol for two times, 15 mL 95% ethanol for two times and 15 mL acetone for two times. The filter paper was dried in an oven at 103°C overnight. The filter paper containing the residue was weighed, then the weight was determined for ash analysis and the weight for protein analysis. Calculation of dietary fiber content was determined by corrected ash and protein contents of the residue.

Protein digestibility [%] was assessed using enzymatic method (SHI et al. 2020). Protein digestibility was determined based on comparison of the protein content that was digested by enzyme and the protein content in the original sample.

Determination of Aflatoxin

Aflatoxin is one of parameters that can be used for food safety. Aflatoxin B_1 and total aflatoxin was evaluated in the peanut cake and their fermentation. The aflatoxin measurements were performed by using a liquid chromatography/mass spectrophotometry (LC/MS). The column used was Acquity UPLC BEH C18 2.1 · 100 mm; 1.7 µm, mobile phase A of formic acid 0.1% in aquabidest, mobile phase B of formic acid 0.1% in acetonitrile, with

injection volume of 5 μL and column temperature of 30°C. Aflatoxin content in ppb unit was determined using a calibration curve with a line equation (YOUNG et al. 2015, JETTANAJIT and NHUJAK 2016).

Statistical Analysis

The results were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS 16. The assessment of the significance of differences between the means was performed using Duncan test ($p < 0.05$).

Results and Discussions

Fermentation Quality

The quality of fermentation process can be assessed from pH value and mold density (both qualitative and quantitative). Based on the pH indicator, BW treatment had significantly higher pH level compared to other treatments ($p < 0.05$) – Table 1, which mean that the treatment can produce *black oncom* when the pH value was high (pH > 5).

Table 1

The physical characteristics of fermented peanut cakes

Treatment		pH value	Texture (N)	Mold density	
				qualitative (visual)	quantitative [%]
Traditional fermentation	*black oncom* in the market	5.01 ± 0.11^b	n/a	++	100^c
Adaptive fermentation	BW	5.823 ± 0.061^c	64.33–76.67	++	100^c
	BA	3.865 ± 0.022^a	n/a	0	0^a
	BC	5.203 ± 0.30^b	n/a	+	30.83^b

Explanation: data are presented as mean ± sd; in one column; the different superscript (^{a,b,c}) letters mean significantly different ($p < 0.05$); n/a – data can not be measured; 0 – no mold growth; + slight mold growth, texture not yet formed; ++ sufficient mold growth, the texture is rather compact and soft

Mold density of *black oncom* can be observed when the growth of mold produced spread after 48 h fermentation (Fig. 2). Therefore, the treatment that can be measured for the texture was only BW treatment because in other treatments the mold has not been formed. Furthermore, there was a significant correlation between pH and mold density ($p < 0.05$), It means that the higher the pH, the more mold growth. pH has been one of factors which can influence the growth of mold.

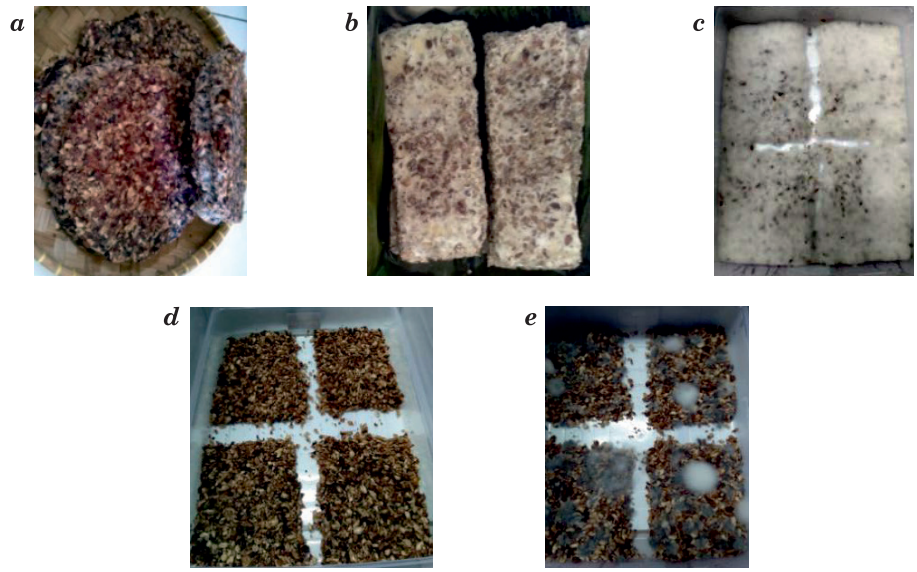

Fig. 2. Appearance of fermented peanut cakes: *a* – peanut cake; *b* – *black oncom* in the market; *c* – *black oncom* produced using adaptive processing methods BW; *d* – BA treatment; *e* – BC treatment

This study showed that peanut cake that can be appropriately fermented was BW in the fermentation process. This was supported by the environment of the fermentation process, i.e., temperature (25–30°C), humidity (70–90%) and pH (KALSUM and SJOFJAN 2008). The water treatment environment was good for the growth of mold, which was shown in the highest pH value. The high pH indicated the activity of extracellular enzymes especially proteolytic enzymes produced by mold, which breaks down proteins into amino acids. Furthermore, the degradation of amino acids produces ammonia so that the pH is high (ISKANDAR et al. 2010).

In the BA adaptive processing treatment, fungi did not grow at all because the concentration of the soaking solution is not optimal, in which the media containing acetic acid more than 7 g/L can stop *Rhizopus* mold growth (ASADOLLAHZADEH et al. 2018). Acetic acid concentration in BA treatment was still too strong in inhibiting mold growth because acetic acid has an inhibition of mold growth (PUNDIR and JAIN 2010, ROGAWANSAMY et al. 2015). Hence, the concentration of acetic acid have to be optimized to support *Rhizopus oligosporus* growth in *black oncom* production.

Meanwhile, the pH value in BC treatment of 5.20, was not significantly different from commercial black oncom. This pH value still supported mold growth, but mold growth was only at certain points with a spread in uneven position. This treatment is still needed to optimize in the

appropriate concentration of treatment so that it can produce *black oncom* with good mold growth. Therefore, the chitosan concentration still have strong activity as an antifungal growth as described by DUTTA et al. (2009), but another study showed the higher biomass production of *Rhizopus oryzae* generates higher chitosan content (TAI et al. 2010).

Nutritional Contents

The nutritional contents are shown in Table 2. The fermentation process experienced a decrease in carbohydrate, total fat, ash, crude protein, and energy in wet basis. Meanwhile, in dry basis, total fat, crude protein, protein digestibility and crude fiber were increased. The differences in the content occurred because a high change of water content in the fermentation product. The highest increase in water and carbohydrate contents was in the commercial *black oncom* where the values between treatments were significantly different ($p < 0.05$). This is because *black oncom* from the market has been added with cassava cake or tapioca. Microbial activity requires energy and nutrients during fermentation so that the carbohydrate content decreased which acts as the main source of energy. This can be confirmed also in the total energy which was decreased after the fermentation compared to raw materials (peanut cake) (YANG et al. 2016).

The adaptive procedure of peanut cake fermentation can significantly increase fat content ($p < 0.05$) than peanut cake and the traditional fermentation. BW treatment had the highest content and the contents were significantly different between treatments ($p < 0.05$). Fat increase occurs due to the activity of fat decomposition by the lipase enzyme produced by mold (WANG et al. 1975). This is supported by a study related to the fermentation process that affects fat distribution and fatty acid composition (ZOU et al. (2018). This study showed in general fungi can synthesis lipids which its lipid changes and composition in solid state fermentation of rice bran. The increasing of fat content is also the result of lipid accumulation from carbon source, such as glucose (ATHENAKI et al. 2018).

Fermentation of peanut cake in the traditional fermentation and adaptive fermentation enhanced the protein content, but the increase of protein content after fermentation in the treatment group tended to be higher than commercial *black oncom* in the market. Protein content of commercial *black oncom* was not significantly different compared to the peanut cake. The increasing of protein content in BW treatment was significantly different compared to peanut cake and commercial *black oncom* in the market.

Table 2

Nutritional contents of fermented peanut cakes

Parameter	Unit	Non-fermentation		Traditional fermentation		Adaptive fermentation					
		peanut cake		commercial *black oncom* in the market		BW		BA		BC	
		wb	db	wb	db	wb	db	wb	db	wb	db
Carbohydrate	%	34.96 ± 3.71^{b}	38.50 ± 4.07^{c}	9.89 ± 0.1^{a}	33.53 ± 0.01^{bc}	8.57 ± 0.15^{a}	22.64 ± 0.38^{a}	10.073 ± 0.08^{a}	25.56 ± 0.23^{ab}	9.21 ± 0.014^{a}	22.67 ± 0.18^{ab}
Total fat	%	27.17 ± 0.31^{c}	29.92 ± 0.35^{a}	9.17 ± 0.02^{a}	31.07 ± 0.01^{a}	14.20 ± 0.32^{b}	37.46 ± 0.37^{c}	13.72 ± 0.26^{b}	34.82 ± 0.63^{b}	15.003 ± 0.80^{b}	36.96 ± 2.22^{b}
Ash content	%	4.89 ± 0.48^{c}	5.37 ± 0.56^{b}	1.93 ± 0.02^{b}	6.53 ± 0.004^{c}	1.47 ± 0.25^{ab}	3.87 ± 0.61^{a}	1.21 ± 0.042^{a}	3.07 ± 0.11^{a}	1.34 ± 0.1^{ab}	3.30 ± 0.22^{a}
Crude protein	%	23.58 ± 2.92^{b}	25.96 ± 3.22^{a}	8.52 ± 0.09^{a}	28.87 ± 0.09^{ab}	14.94 ± 0.39^{a}	36.02 ± 0.95^{bc}	14.39 ± 0.10^{a}	36.54 ± 0.29^{bc}	15.04 ± 0.99^{a}	37.05 ± 2.19^{c}
Protein digestibility	%	39.28 ± 1.47^{a}		57.52 ± 4.05^{b}		77.91 ± 0.80^{c}		44.06 ± 3.08^{a}		55.50 ± 0.40^{b}	
Water content	%	8.76 ± 0.71^{a}	9.61 ± 0.84^{a}	70.51 ± 0.30^{c}	237.31 ± 1.02^{e}	62.117 ± 0.75^{b}	164.06 ± 5.29^{d}	60.6 ± 0.035^{b}	153.82 ± 0.23^{b}	59.42 ± 0.27^{b}	146.38 ± 1.68^{c}
Crude fiber	%	2.99 ± 0.01^{b}	3.33 ± 0.013^{a}	6.81 ± 1.71^{ab}	7.88 ± 0.06^{b}	1.39 ± 0.60^{a}	3.67 ± 1.57^{a}	2.18 ± 0.48^{ab}	5.55 ± 1.22^{ab}	1.06 ± 0.04^{a}	3.16 ± 0.71^{a}
Dietary fiber	%	23.53 ± 1.007^{c}	26.17 ± 1.13^{a}	14.13 ± 2.79^{b}	47.87 ± 8.96^{b}	9.34 ± 2.09^{a}	27.16 ± 4.33^{a}	8.94 ± 0.24^{a}	22.67 ± 0.59^{a}	8.03 ± 1.38^{a}	22.12 ± 0.07^{a}
Energy from fat	kcal/100 g	244.60 ± 2.79^{c}		82.5 ± 0.09^{a}		127.74 ± 2.84^{b}		123.48 ± 2.35^{b}		135.02 ± 7.22^{b}	
Total energy	kcal/100 g	478.81 ± 0.41^{c}		156.11 ± 1.6^{a}		216.62 ± 3.43^{b}		221.36 ± 1.62		232.01 ± 3.30^{b}	

Explanation: data are presented as mean ± sd; in one row; the different superscript (^{a,b,c}) letters mean significantly different ($p < 0.05$); db – dry basis; wb – wet basis

This showed that BW treatment can penetrate and synthesize protein better than traditional fermentation and support extracellular enzyme for protein synthesis process (MANPREET et al. 2005). The protein content of BA treatment was also significantly increased and different compared to peanut cake. However, the increasing protein in BC treatment was significantly different compared to other treatments. The increasing of protein in BC treatment is in agreement with a research by GANDRA et al. (2018) that the addition of chitosan of 5 g/kg sample can significantly increase the protein content.

Fermentation process can decrease the ash content in the traditional fermentation and adaptive fermentation. However, the traditional fermentation had the highest ash content compared to adaptive fermentation. This was due to the addition of cassava cake in black oncom production using traditional fermentation that might be contributed to the total ash content. Decreasing ash content during the fermentation is due to dissolution of water-soluble minerals or the metabolic activity of microorganisms (NNAM 2001) or leaching process which affected the total of ash content (ADEGBEHINGBE et al. 2014, SIMWAKA et al. 2017). The highest ash content was found in the commercial *black oncom,* and the ash contents were significantly different between treatments ($p < 0.05$).

The results in this study is in agreement with STEINKRAUS (2002) that the fermentation process improves nutritional contents. The nutritional contents of *black oncom* production in this study was higher than that of (SLAMET et al. 2002) and similar to SOFYAN (2003) and GINTING et al. (2018), especially in the proximate components such as carbohydrate, water, ash, protein and fat contents. However, the nutritional contents showed a lower value compared to KUMBHARE (2017).

The adaptive fermentation can also increase the protein digestibility especially in BW treatment that achieved the highest protein digestibility ($p < 0.05$) compared to the commercial *black oncom* in the market and other treatments. Commercial *black oncom* as the traditional fermentation process can not effectively increase protein digestibility. Meanwhile, the protein digestibility in BA treatment was lower than BA treatment because there was no growth of *Rhizopus oligosporus* that modified protein to amino acids. The BC treatment had the highest protein, but the protein digestibility was lower than BW treatment. This was due to the addition of chitosan which can add the amine group that is difficult to digest.

This study is in agreement with YANG et al. (2016) that controlled peanut cake fermentation can increase the crude protein and in vitro protein digestibility in plant protein compared to the traditional fermentation

(ALKA et al. 2012, PRANOTO et al. 2013). The process of increasing protein occurs because crude protein can increase in 36 h of fermentation (SIMWAKA et al. 2017). There are also microorganisms activities that use carbohydrates for energy (ONYANGO et al. 2005) and the process of proteolysis during fermentation produces peptides and amino acids, thus producing more soluble proteins (PRANOTO et al. 2013, EL KHALIFA et al. 2005).

Fermentation can be carried out using a starter culture or a natural process. Natural or traditional fermentation is less effective and unpredictable. This study showed that naturally fermented *black oncom* had lower protein digestibility than BW treatment. A previous study has shown that the digestibility of sorghum flour protein fermented using *Lactobacillus plantarum* achieved 92%, whereas the digestibility of the natural fermentation only reached 47% (PRANOTO et al. 2013). The increase in digestibility occurs due to the process of breaking down the food matrix into free nutrients (NKHATA et al. 2018). In BA and BC treatments, the digestibility was not increased because of the soaking treatment factors, i.e., the concentration, and there was no growth of *Rhizopus oligosporus* to perform the fermentation process in order to increase the nutritional contents especially the total fat and protein contents, and the protein digestibility.

Fermentation process significantly increased the crude fiber in the commercial *black oncom*, BW and BA, but there was no increase of crude fiber in the BC treatment. The increase of crude fiber occurred because mold cannot use carbohydrate that is difficult to digest, so it increases the crude fiber content. In addition, dietary fiber was increased in the commercial *black oncom* and BW treatment, but the commercial *black oncom* had the highest dietary fiber than BW treatment. This is because in the commercial *black oncom* production there was an addition of cassava cake as a carbohydrate source for the fungi. This study is in agreement with ZHAO et al. (2017) that solid state fermentation of wheat bran using lactic acid bacteria increases total dietary fiber. Meanwhile, total dietary fiber was decreased in BA and BC treatments. The BA and BC treatments might contain different microorganisms. According to TENG et al. (2017), the use of *Saccharomyces cerevisiae* can increase total dietary fiber, but the use of *Bacillus amyloquefaciens* can decrease total dietary fiber. Based on the nutritional contents, BW treatment was the best adaptive fermentation that could be increased increase nutritional contents especially in content of fat, protein and protein digistibility, so it can be used as food ingredient for product development such as biscuits (SETIAWAN et al. 2020).

Aflatoxin Content

Food safety of *black oncom* can be determined based on the aflatoxin content because the peanut product had a risk of aflatoxin which is produced by *Aspergillus* sp. The adaptive fermentation significantly decreased aflatoxins contents ($p < 0.05$) such as aflatoxin B_1 and total aflatoxin compared to the peanut cake. The aflatoxin content in BC treatment was the lowest with aflatoxin B_1 and total aflatoxin were significantly different within treatments ($p < 0.05$). This decrease occurred because the production process of *black oncom* especially in the soaking and fermentation processes.

Table 3

Aflatoxin content of fermented peanut cakes

Parameter	Non-fermentation	Traditional fermentation	Adaptive fermentation			Standard		
	peanut cake	commercial *black oncom*	BW	BA	BC	ID*	EU**	codex***
Aflatoxin B_1 [µg/kg]	287.27 ± 254.28^b	55.05 ± 69.81^{ab}	3.80 ± 1.002^a	1.47 ± 0.28^a	0.07 ± 0.056^a	15	2	15
Total aflatoxin [µg/kg]	370.79 ± 332.97^b	73.37 ± 93.31^{ab}	6.13 ± 1.55^a	2.35 ± 0.40^a	0.15 ± 0.035^a	20	4	20

Explanation: data are presented as mean ± sd; in one row; the different superscript (^{a,b,c}) letters mean significantly different ($p < 0.05$); *BPOM 2018; **EU (European Commission 2010); ***Codex 2017

The aflatoxin content decreased in the production process of *black oncom* (Table 3). The production process of *black oncom* with adaptive fermentation treatments in this research showed a higher reduction in aflatoxin compared to commercial *black oncom* in the market. The higest reduction of aflatoxins were BC, BA and BW treatment, respectively. The traditional fermentation cannot decrease aflatoxin contents effectively compared to the adaptive fermentation. The decreasing process of aflatoxin can be influenced by the fermentation process including soaking treatments and fermentation by the fungus (*Rhizopus oligosporus*). The aflatoxin content of adaptive fermentation in this study is smaller than other studies (ROEDJITO et al. 1972, JANNAH 2005, GINTING et al. 2018) and is classified as low aflatoxin according to the regulatory standards. The aflatoxin upper limit in Indonesia and codex is 20 ppb for total aflatoxin and 15 ppb for aflatoxin B_1 in processed peanut and corn products (BPOM 2018, CODEX 2017), but the upper limit in EU was lower than other standards (European Commission 2010).

The aflatoxin content in BC treatment was the lowest because chitosan inhibited the growth of pathogenic microorganisms (DUTTA et al. 2009) such as *Aspergillus flavus*. According to (SIMPSON et al. 1997), the interaction between chitosan and pathogenic molds shows that chitosan can resist the mold growth by binding the hosting DNA and damaging its biological membrane. According to KUMAR (2000), chitosan can provide a negative charge on carboxylic group and a positive charge on NH group which will be interact each other which causes expansion of cell surface and changes the cell wall permeability. This will cause the loss of several cell constituents and chitosan will inhibit the metabolisms of pathogenic microorganism (KURNIASIH and KARTIKA 2009). According to HIDAYAH (2015), The level of peanut chemical composition was decreased after 30 days of storage and the addition of 1% chitosan.

BA treatment of *black oncom* production also contributed to the decrease of aflatoxin contents because a concentration of 10 % acetic acid has the highest inhibitory effect on *Aspergillus flavus* of 45.21 (HASSAN et al. 2015). Acetic acid is more effective than lactic acid and has the best inhibition of *Aspergillus flavus* growth (KURNIASIH and KARTIKA 2009, DALIÉ et al. 2010). The mechanism of aflatoxin inhibition by organic acids, such as acetic acid by lowering the media pH is known as the hydrophobic feature which allows free protonized diffusion through cell membranes, and then the cell allocates a part of its energy content to remove the newly formed proton. Therefore, this slows down the growth of the fungus (LEÓN PELÁEZ et al. 2012). In addition, the decrease of aflatoxin contents occurs because a degradation of aflatoxin that formed β-keto acid structure that was catalyzed by acid medium. Lactone ring structure experiences hydrolysis that changes aflatoxin to non toxic components (ALBORES et al. 2005).

Fermentation process, especially in the inoculation process also contributed to the decrease of aflatoxin contents. The degradation mechanism of aflatoxin by *Rhizopus oligosporus* is apparently to occur enzymatically by reducing cyclopentanone bonds, degrading lactone rings, and opening difuran rings (SARDJONO et al. 2004, WU et al. 2009). JANSSEN et al. (1997) stated that the degradation of AFB_1 occurs resulting in aflatoxicol A formation which is 10 times lower in toxicity compared to AFB_1. CHEN et al. (2015) mentioned that there is a biotransformation of aflatoxin to non toxic compounds in the fermented peanut cake. BW treatment also decreased aflatoxins level and the content have no significantly different with BC and BA treatment ($p > 0.05$). Based on this, the adaptive fermentation could be used as alternatife procedure to produce *black oncom* that have low aflatoxin.

Conclusions

The adaptive fermentation method of peanut cake using soaking treatments (water, acetic acid 5%, chitosan 5%) and fermentation process using the yeast starter of *Rhizopus oligosporus* can increase nutritional contents and decrease the aflatoxins content. The adaptive fermentation that produced the best *black oncom* was the BW treatment compared to the commercial *black oncom* in the market, BA, and BC treatments. This was revealed from the pH value, mold density, and the protein digestibility. For the BC treatment, mold growth was only performed at certain points; while for the BA treatment, there was no mold growth. Generally, natural fermentation and adaptive fermentation processes can decrease carbohydrate, energy, and ash contents and can increase water content, total fat, crude protein, protein digestibility. Meanwhile, the crude fiber and dietary fiber showed a different result between natural and adaptive fermentation that might be affected by substrate additions and miroorganisms in the fermentation process. Furthermore, the adaptive fermentation process can decrease the aflatoxin contents below the standard limit set by the Indonesian and Codex standard compared to the commercial *black oncom* in the market. Meanwhile, the aflatoxin content of BC treatment was below the standard limit set by EU. Finally, the adaptive processing BW treatment is potential to produce *black oncom* with high mold growth, fat, protein, protein digestibility and low aflatoxin content compared to the traditional fermentation. BA and BC treatments produced a low aflatoxin profile, but these treatments need a further research especially in optimization of the soaking solution concentration to achieve better nutritional contents.

Acknowledgment

The authors are grateful to the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia that funded this research (2018–2019) and Directorate of General Higher Education, Ministry of Education and Culture of the Republic of Indonesia that provided the education scholarship (Batch 3 PMDSU Scholarship) to the researcher.

Accepted for print 18.12.2020

References

ADEGBEHINGBE K.T., ADETUYI F.C., AKINYOSOYE F.A. 2014. *Effect of fermentation on nutrient and anti- nutrient contents of ground-cooked Lima Bean (Phaseolus lunatus) seeds using Bacillus subtilis and Bacillus pumilus*. Br. Microbiol. Res. J., 4(11): 1285–1298.

ALKA S., NEELAM Y., SHRUTI S. 2012. *Effect of fermentation on physicochemical properties & in vitro starch and protein digestibility of selected cereals*. Int. J. Agr. Food. Sci., 2(3): 66–70.

AOAC Association of Official Analitycal of Chemist. 2005. *Official Methods of Analysis of AOAC International 18th Edition, 2005*. Ed. W.H. Hotwiz. The Association of Official Analitycal Chemist, Washington D.C.

ASADOLLAHZADEH M., GHASEMIAN A., SARAEIAN A., RESALATI H., TAHERZADEH M. 2019. *Effect of acetic acid on growth and ethanol fermentation of filamentous fungi Rhizopus oryzae, Mucor indicus, Neurospora intermedia and Aspergilus oryzae*. J. Exp. Anim. Sci., 7(3): 119–130.

ATHENAKI M., GARDELI C., DIAMANTOPOULOU P., TCHAKOUTEU S.S., SARRIS D., PHILIPPOUSSIS A., PAPANIKOLAOU S. 2017. *Lipids from yeasts and fungi: physiology, production and analytical considerations*. J. Appl. Microbiol., 124(2): 336–367.

BHARGAV S., PANDA B., ALI M., JAVED S. 2008. *Solid-state fermentation systems – An overview*. Chem. Biochem. Eng. Q., 22(1): 1–30.

[BPOM] Food and Drug Administration of Indonesia. *Peraturan BPOM NO 8 Tahun 2018 Tentang Batas Maksimum Cemaran Kimia dalam Pangan Olahan*. Regulatory of BPOM No. 8, Jakarta, Indonesia (in Indonesian).

CHEN Y., KONG Q., CHI C., SHAN S., GUAN B. 2015. *Biotransformation of aflatoxin B_1 and aflatoxin G1 in peanut meal by anaerobic solid fermentation of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus*. Int. J. Food Microbiol., 211(2015): 1–5.

CHI C.H., CHO S.J. 2016. *Improvement of bioactivity of soybean meal by solid-state fermentation with Bacillus amyloliquefaciens versus Lactobacillus* spp. *and Saccharomyces cerevisiae*. LWT – Food Sci. Technol., 68(2016): 619–625.

Codex Alimentarius Commision. 2017. *Request for comments at step 3 on the proposed draft maximum level for total aflatoxins in ready-to-eat peanuts*. Joint FAO/WHO Food Standards Programme No. 11, Rio De Janeiro, Brazil.

DALIÉ D.K.D., DESCHAMPS A.M., RICHARD-FORGET F. 2010. *Lactic acid bacteria. Potential for control of mould growth and mycotoxins: A review*. Food Control., 21(4): 370–380.

DEWI R., NUR R.M. 2018. *Antifungal Activity of chitosan on Aspergillus* spp. IJBB., 2(4): 24–30.

DUHAN J.S., SAHARAN P., SADH P.K. 2019. *Effect of production parameters on release of phenolic content of peanut press cake fermented w*ith *A. oryzae and A. awamori*. Int. J Pharmacy Biol. Sci., 9(2): 434–444.

DUTTA P.K., TRIPATHI S., MEHROTRA G.K., DUTTA J. 2009. P*erspectives for chitosan based antimicrobial films in food applications*. Food Chem., 114(4): 1173–1182.

ELKHALIFA A.E.O., SCHIFFLER B., BERNHARDT R. 2005. *Effect of fermentation on the functional properties of sorghum flour*. Food Chem., 92(1): 1–5.

EUROPEAN COMMISSION. 2010. *Commission regulation (EU) no 165/2010 of 26 February 2010,* A*mending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins*. Official Journal of the European Union, No. 165, Brussel, Belgium.

GANDRA J.R., TAKIYA C.S., DEL VALLE T.A., OLIVEIRA E.R., DE GOES R.H.T.B., GANDRA E.R.S., BATISTA J.D.O., ARAKI H.M.C. 2018. *Soybean whole-plant ensiled with chitosan and lactic acid bacteria: Microorganism counts, fermentative profile, and total losses*. J. Dairy Sci., 101(9): 787–7880.

GINTING E., RAHMIANNA A.A., YUSNAWAN E. 2018. *Aflatoxin and nutrient contents of peanut collected from local market and their processed foods*. IOP Conference Series: Earth and Environmental Science, 102: 1–8.

HASSAN A., MA., NM O., ELTAYEB M., GA O., EE B. 2006. *Effect of processing treatments followed by fermentation on protein content and digestability of pearl millet (Pennisetum typhoideum) cultivars*. Pak J Nutr., 5(1): 86–89.

HASSAN R., EL-KADI S., SAND M. 2015. *Effect of some organic acids on some fungal*. Ijab., 2(1): 1–11.

HIDAYAH N. 2015. *The Application of chitosan to prevent aflatoxin B_1 contaminant on the peanut (Arachis hypogaea L.) during storage at room temperature*. IPB University Repository, pp. 1–41.

HO C.C. 1986. *Identity and characteristics of Neurospora intermedia responsible for oncom fermentation in Indonesia*. Food Microbiol., 3(2): 115–132.

ISKANDAR Y.M., PRIATNI S., KARDONO L.B.S. 2010. *Influence of tapioca flour concentration and legumes varieties on characteristic of black oncom with Mucor sp. as inoculum*. Teknol. Indonesia., 33(1): 38–44.

JANNAH S. 2005.*Inhibition of growth of Aspergillus flavus and reduction of aflatoxin by Lactobacillus plantarum pi28a during the process of making black oncom*. IPB University Repository, pp 1–82.

JANSSEN M., PUT H., NOUT M. 1997. Natural Toxin. In: textbook of *Food Safety and Toxicity*. Ed. J. De Vries. CRC Press, Boca Raton, pp. 16–46.

JETTANAJIT A., NHUJAK T. 2016. *Determination of Mycotoxins in Brown Rice Using QuEChERS Sample Preparation and UHPLC-MS-MS*. J. Chromatogr Sci., 54(5): 720–729.

KALSUM U., SJOFJAN O. 2008. *Effects of incubation time of tofu by-product and tapioca waste mixture fermented by Neurospora sitophila on nutrient contents*. Seminar Nasional Teknologi Peternakan dan Veteriner, pp. 226–232.

KUMAR M. 2000. *A review of chitin and chitosan applications*. React. Funct. Polym., 46(1): 1–27.

KUMBHARE P.H. 2017. *Evaluation of nutritive value of laboratory scale fermented peanut Press Cake*. IJRBAT, 5(2): 1–4.

KURNIASIH M., KARTIKA D. 2009. *Antibacterial activity of chitosan against S. Aureus bacteria*. Molekul., 4(1): 1–5.

LEÓN PELÁEZ A.M., SERNA CATAÑO C.A., QUINTERO YEPES E.A., GAMBA VILLARROEL R.R., DE ANTONI G.L., GIANNUZZI L. 2012. *Inhibitory activity of lactic and acetic acid on Aspergillus flavus growth for food preservation*. Food Control., 24(1–2): 177–183.

MANPREET S., SAWRAJ S., SACHIN D., PANKAJ S., BANERJEE U.C. 2005. *Influence of process parameters on the production of metabolites in solid-state fermentation*. Malays. J. Microbiol., 1(2): 1–9.

MÉNDEZ-ALBORES A., ARÁMBULLA-VILLA G., LOARCA-PIÑA M.G.F.L., TOSTADO E.C., MORENO--MARTÍNEZ E. 2005. *Safety and efficacy evaluation of aqueous citric acid to degrade B-aflatoxins in maize*. Food. Chem.Toxicol., 43(2): 233–238.

NKHATA S.G., AYUA E., KAMAU E.H., SHINGIRO J.B. 2018. *Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes*. Food Sci. Nutr., 6(8): 2446–2458.

NNAM N.M. 2001. *Chemical, sensory and rheological properties of porridges from processed sorghum (Sorghum bicolor), bambara groundnut (Vigna subterranea* L. *Verdc) and sweet potato (Ipomoea batatas) flours*. Plant Foods Hum. Nutr., 56(3): 251–264.

NWE N., CHANDRKRACHANG S., STEVENS W.F., MAW T., TAN T.K., KHOR E., WONG S.M. 2002. *Production of fungal chitosan by solid state and submerged fermentation*. Carbohydr. Polymer., 49(2): 235–237.

ONYANGO C., NOETZOLD H., ZIEMS A., HOFMANN T., BLEY T., HENLE T. 2005. *Digestibility and antinutrient properties of acidified and extruded maize-finger millet blend in the production of uji*. LWT – Food Sci. Technol., 38(7): 697–707.

PANDEY A. 2003. *Solid state fermentation*. Biochem. Eng. J., 13(2–3): 81–84.

PRANOTO Y., ANGGRAHINI S., EFENDI Z. 2013. *Effect of natural and Lactobacillus plantarum fermentation on in-vitro protein and starch digestibilities of sorghum flour*. Food Biosci., 2: 46–52.

PUNDIR R.K., JAIN P. 2010. *Screening for antifungal activity of commercially available chemical food preservatives Lactic acid Benzoic acid Citric acid*. Int. J. Pharm. Sci. Rev. Res., 5(2): 25–27.

PURWIJANTININGSIH E. 2005. *Penghambatan Pertumbuhan dan Produksi Aflatoksin dari Aspergillus flavus oleh Kapang dan Khamir Ragi Tempe*. IPB University Repository, pp. 1–65 (in Indonesian).

RAHARJANTI D.S. 2006. *Inhibition of growth of Aspergillus parasiticus and reduction of aflatoxins by mold and yeast yeast tape*. IPB University Repository, pp. 1–95.

ROEDJITO D., MUHILAL, WIROHUSODO H.S.P., KARYADI D. 1972. *Aflotoxin in peanuts, oil, cake and Oncom*. The Journal of Nutrition and Food, 2: 80–87.

ROGAWANSAMY S., GASKIN S., TAYLOR M. 2015. *An evaluation of antifungal agents for the treatment of fungal contamination in indoor air environments.* Int. J. Environ. Res. Public Health, 12: 6319–6332.

ROHIMAH A., SETIAWAN B., ROOSITA K., PALUPI E. 2021. THE AMINO ACID AND MINERAL CONTENT OF BLACK ONCOM PROCESSED WITH FERMENTATION MODIFICATIONS. J. GIZI PANGAN., 16(SUPPL.1): 115–120.

SADH P.K., CHAWLA P., BHANDARI L., DUHAN J.S. 2017. *Bio-enrichment of functional properties of peanut oil cakes by solid state fermentation using Aspergillus oryzae.* J. Food Meas. Charact., 12(1): 622–633.

SADH P.K., CHAWLA P., DUHAN J.S. 2018. *Fermentation approach on phenolic, antioxidants and functional properties of peanut press cake.* Food Bioscience, 22: 113–120.

SARDJONO, RAHARJO S., RAHAYU E.S., KUSWANTO K.R. 2004. *The Role of Extracellular Enzyme Produced by Aspergillus oryzae KKB4 in Biodegradation of Aflatoxin B1.* IFNP., 11(2): 65–71.

SASTRAATMADJA D.D., TOMITA F., KASAI T. 2002. *Production of high quality oncom, a traditional indonesian fermented food, by the inoculation with selected mold strains in the form of pure culture and solid inoculum*. J. Grad. Sch. Agr. Hok. 70(2): 111–127.

SETIAWAN B., ROHIMAH A., PALUPI E., SULAEMAN A., HANDHARYANI E. 2020. *Physical-sensory characteristics and nutritional contents of black oncom and peanut ingredients-based biscuits as an elderly supplementary food*. AIMS Agric. Food., 5(4): 868–881.

SHI H., SU B., CHEN X., PIAN R. 2020. *Solid state fermentation of Moringa oleifera leaf meal by mixed strains for the protein enrichment and the improvement of nutritional value.* Peer J., 8(e10358): 1–22.

SIMPSON B.K., GAGNÉ N., ASHIE I.N.A., NOROOZI E. 1997. *Utilization of chitosan for preservation of raw shrimp (Pandalus borealis).* Food Biotechnol., 11(1): 25–44.

SIMWAKA J.E., CHAMBA M.V.M., HUIMING Z., MASAMBA K.G., LUO Y. 2017. E*ffect of fermentation on physicochemical and antinutritional factors of complementary foods from millet, sorghum, pumpkin and amaranth seed flours*. Int. Food Res. J., 24(5): 1869–1879.

SITUNGKIR R.U. 2005. *Application of lactic acid bacteria culture with salt to reduce aspergillus flavus and aflatoxin in the processing of salted peanuts*. IPB University repository, pp. 1–93.

SLAMET D.S., TARWOTJO I. 1971. *The nutrient content of "ontjom"*. The Journal of Nutrition and Food Research, 1: 49–52.

SOFYAN H. 2003. *The effect of incubation temperature and Rhizopus oligosporus inoculum concentration on the quality of peanut cake oncom*. J. Infomatek., 5(2): 74–86.

STEINKRAUS K.H. 2002. *Fermentations in world food processing*. Compr. Rev. Food Sci. Food Saf., 1(1): 23–32.

TAI C., LI S., XU Q., YING H., HUANG H., OUYANG P. 2010. *Chitosan production from hemicellulose hydrolysate of corn straw. Impact of degradation products on Rhizopus Oryzae growth and chitosan fermentation*. Lett. Appl. Microbiol., 51(2010): 278–284.

TENG P.Y., CHANG C.L., HUANG C.M., CHANG S.C., LEE T.T. 2017. *Effects of solid-state fermented wheat bran by Bacillus amyloliquefaciens and Saccharomyces cerevisiae on growth performance and intestinal microbiota in broiler chickens*. Ital. J. Anim Sci., 16(4): 552–562.

WANG H., SWAIN E.W., WALLEN L., CW H. 1975. *Free fatty acids identified as antitryptic factor in soybeans fermented by Rhizopus oligosporus*. J. Nutr., 105(10): 1351–1355.

WU Q., JEZKOVA A., YUAN Z., PAVILKOVE L., DOHNAL V. 2009. *Biological degradation of aflatoxins*. Drug Metab. Rev., 41(1): 1–7.

XIAO Y., XING G., RUI X., LI W., CHEN X., JIANG M., DONG M. 2015. *Effect of solid-state fermentation with Cordyceps militaris SN-18 on physicochemical and functional properties of chickpea (Cicer arietinum* L.*) flour*. LWT–Food Sci.Technol., 63(2): 1317–1324.

YANG X., TENG D., WANG X., GUAN Q., MAO R., HAO Y., WANG J. 2016. *Enhancement of nutritional and antioxidant properties of peanut meal by bio-modification with Bacillus licheniformis*. Appl. Biochem. Biotechnol., 180(6): 1227–1242.

YANPING W., YINGFEN H., YUPING M., YUANJIE Z., QING, L. 2017. *Study on antioxidant activity of peanut meal using bacillus natto by solid state Fermentation*. Int. J. Sci., 6(1): 44–51.

YOUNG M.S., TRAN K., SHAH D., CORPORATION W. 2015. *Evaluation of a modified quechers method for uplc-ms/ ms determination of multiresidue mycotoxins in Grains*. Waters the science of what's possible, pp. 1

ZHAO H.M, GUO X.N, ZHU K.X. 2017. *Impact of solid state fermentation on nutritional, physical and flavor properties of wheat bran*. Food Chem., 217: 28–36

ZHOU G., CHEN Y., KONG Q., MA Y., LIU Y. 2017. *Detoxification of Aflatoxin* B_1 *by Zygosaccharomyces rouxii with solid state fermentation in peanut meal*. Toxins., 9(42): 1–9.

ZOU M., ZHU X., LI X., ZENG X. 2018. *Changes in lipids distribution and fatty acid composition during soy sauce production*. Food Sci. Nutr., 7: 764–772.