Characteristic of Chocolate Candy Produced from Fermented Cocoa Bean with *Lactobacillus plantarum* HL-15 Starter Culture for Inhibition Growth of Mycotoxin-Producing Fungi

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Abstract: Chocolate candy is a snack product that is liked by many people. The presence of mycotoxin producing fungi is a problem in chocolate product. The objective of this research was to observe the characteristic of chocolate candy which is produced from fermented cocoa bean with *Lactobacillus plantarum* HL-15 starter culture. The cocoa bean (47.5 kg) is fermented by adding *L. plantarum* HL-15 culture about 500 mL (10^{10} CFU/mL) in the new and old fermentation box and in another new and old fermentation box without adding culture. Chocolate candy processing is done based on standard processing of chocolate candy. The results showed that the addition of *L. plantarum* HL-15 culture in cocoa bean fermentation and use of the new fermentation box give lower fungi concentration on chocolate candy, reducing 1 log cycle from 1.7×10^3 to $< 10^2$ colony/g, while the addition of *L. plantarum* HL-15 culture in the old fermentation box is not effect. The average a_w value of chocolate candy is 0.64 and pH value is 6.7. Fat content of chocolate candy is about 44.9%-46.2%.

Key words: Chocolate candy, *Lactobacillus plantarum* HL-15 culture, fungi contamination.

1. Introduction

Cocoa (*Theobroma cacao* L.) is one of the leading and important commodities in Indonesia. As the third largest cocoa producer in the world, Indonesia's cocoa production reached 760,429 tons in 2016 and in 2012-2016, cocoa production growth increased by 1.63% and is predicted to increase to 2.78% in the range of 2016-2020 [1]. This condition has a positive impact on the economics of Indonesia, because the cocoa was able to donate the state foreign exchange of \$1.014 billion in 2015.

The final quality of chocolate candy such as flavor and its resistance to fungi can be influenced by the

quality of cocoa pods, the production of dried fermented cocoa beans and its processing [2-5].

The dry fermented cocoa beans from small plantations still face post-harvest problems. The quality problems of Indonesian cocoa are the high level of acidity of beans followed by the weak flavor, the lack of the quality consistency and unfermented beans. According to Haryadi and Supriyanto [6], the cocoa beans of Indonesian farmers, especially those produced by small plantations are the lowest valued in the international market due to the low quality of the beans and dominated by unfermented beans (slaty).

Another problem is the presence of fungus in cocoa beans. Asrul [7] reported the contamination of *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp., *Rhizopus* sp.,

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Mucor sp., and *Verticillium* sp. with a population of 1.4×10^9 CFU/mL in cocoa beans. Nugroho *et al.* [8] also reported that dried cocoa beans from Kulon Progo, Yogyakarta were contaminated with black *Aspergilli* fungi. This fungi produces Ochratoxin A (OTA). According to Samson *et al.* [9] and Frisvad *et al.* [10], many agriculture products are contamination of fungus-produced aflatoxin and OTA which endangers. OTA is produced by some species of *Aspergillus* and *Penicillium* such as *A. niger* and *P. citrinum* [8, 11]. OTA can contaminate chocolate products and is carcinogenic [12, 13]. The fungi-produced OTA can also be transferred to the chocolate product [14].

Most of the mycotoxinogenic fungi, particularly those producing aflatoxin and OTA are soil born fungi and their conidia could be present in dust, people etc. The contamination of the mycotoxin-producing fungus can come from anywhere, one of which is presumably derived from a recycled wooden fermentation box used as well as from the cacao drying process [15, 16]. The use of fermentation boxes repeatedly without sanitation, affects the fermented cocoa beans quality.

A good handling in fermentation process can ensure a strong cocoa flavor and inhibit the growth of mycotoxin-producing fungi. The traditional fermentation process occurs spontaneously, but can be controlled, for example by the addition of a starter culture. In the previous study Marwati *et al.* [17] have isolated the bacteria from the fermentation process of cocoa beans in Gunung Kidul Regency, Yogyakarta. Isolate *Lactobacillus plantarum* HL-15 was identified as the most effective in inhibiting fungal growth. Lactic acid bacteria cultures about 10^8 - 10^{10} CFU/mL can be added as starters in the fermentation process. Addition of *L. plantarum* HL-15 as a starter could inhibit fungal contamination both by competition and production of antifungal metabolites [18]. Therefore, this study begins with fermentation of cocoa beans using *L. plantarum* HL-15 culture as a starter and then produces chocolate candy. This study aimed to determine the effect of variations on old and new

fermentation boxes and the addition of *L. plantarum* HL-15 starter which has antifungal activity on the quality of chocolate candy.

2. Materials and Methods

2.1 Materials

Cocoa beans were obtained from Gunung Kidul Regency, Yogyakarta. *L. plantarum* HL-15 culture was obtained from Food Nutrition Culture Collection, Food and Nutrition Study Center, Gadjah Mada University, Yogyakarta. The additional ingredients for processing chocolate candies such as sugar, lecithin, milk powder were obtained from the local market.

The chemical used for analysis such as HCl was obtained from Mallinckrodt Baker, Inc. Petroleum benzine and AgNO₃ were obtained from Merck KGaA, Germany. The materials for fungi analysis were Dichloran-Glycerol (DG18) Agar Base CM0729 from Oxoid LTD, England, glycerol from Merck KGaA, Germany and bacteriological peptone LP0037 from Oxoid LTD, England.

2.2 Methods

2.2.1 Cocoa Beans Fermentation

The equipment needed in the cocoa beans fermentation process are fermentation box, size $p \times l \times t = 40 \text{ cm} \times 40 \text{ cm} \times 40 \text{ cm}$ with new condition and fermentation box which is often used by farmer in Processing Unit of Ngudi Raharjo II Gunungkidul Regency, Yogyakarta. Other equipment used is bucket for washing, bamboo wicker board for drying cocoa.

The fermentation process begins with the breakdown of fresh cocoa fruits to obtain cocoa pods (still coated in pulp). Forty-seven point five kilograms (47.5 kg) cocoa pod was put into the fermentation box. Fermentation of cocoa beans was conducted with four treatments as follows: (1) old box without *L. plantarum* HL-15 starter (OB); (2) new box without *L. plantarum* HL-15 starter (NB); (3) old box with *L. plantarum* HL-15 culture (OBS); (4) new box with *L. plantarum* HL-15 culture (NBS). The *L.*

plantarum HL-15 culture was added as a starter of 500 mL (10^{10} CFU/mL). Fermentation is done at room temperature for 5 d. After that, the fermented cocoa beans are soaked in water for 15 min then washed thoroughly from the pulp. Wet cocoa beans fermented were dried with sunlight for 5 d. Furthermore, the dry cocoa beans fermented were packed in a vacuum plastic bag to further process into chocolate candy.

2.2.2 Chocolate Candy Processing

Chocolate candy processing was using the equipment available in Taman Teknologi Pertanian (TTP)/Agricultural Techno Park (ATP) Nglanggeran, Patuk, Gunungkidul Regency, Yogyakarta namely steamer, roasting machine, deshelling, grinder machine, ballmill machine for mixing and refining, conching machine, aluminum basin, thermometer, candy mold and cooling machine.

A total of 2.5 kg of dried cocoa beans fermented were steamed for 20 min. After that, roasted process was conducted at 100-110 °C for 15-19 min. Furthermore, stripping of the epidermis of cocoa beans was done using desheller which has a blower (winnow) to produce nib. Nib is subsequently crushed into a paste (cocoa liquor) with a grinder machine. Chocolate paste was mixed with cocoa butter, sugar and milk powder in a ball mill, into a ball mill put balls to help mixing and refining. The mixing in a ball mill is carried out at 60 °C for 10-12 h, reversing the dough was conducted two times at the 2nd and 4th hours. Then, conching at a temperature of 60-70 °C for 2 h to produce a chocolate dough. Furthermore, the mixture is lowered to 30 °C and candy molding is immediately carried out. After that, candy is put into the refrigerator.

2.2.3 Analysis of Chocolate Candy Quality

Analysis of fat content of chocolate candy used Soxhlet by Association of Official Analytical Chemists (AOAC) 2016 [19]. Fat content analysis was using equipment, such as beaker glasses, measuring cup, boiling rock, 10 mL measuring pipette, filter

paper free fat, funnel, drop pipette, oven (temperature 100 ± 2 °C), Soxhlet extraction tool and the calibrated analytical scales. The principle of extraction using the Soxhlet method, which is chocolate candy, is inserted into the thimble and inserted into the Soxhlet extraction tool. The fat pumpkin is then filled with fat solvents. Fat pumpkin is heated and extracted for 4-6 h. Then the extract was poured in a weighing bottle and the solvent was evaporated. Then dried in an oven until the constant weight is obtained at 100 °C. The residual weight in the bottle is expressed as fat weight.

To analyze the water activity of chocolate candy, water activity meter was used [19]. Chocolate candy is put in a round to half height container. After that, the sample container is attached to the meter tool. The sample is waited for about 5 min, then the temperature and water activity will be read. Temperature will be used as a correction factor.

The pH value of chocolate candy was analyzed using pH meter HI2210 by AOAC 2016 [19], standardization electrode and potentiometer with buffer solution pH 4.00.

Total fungi of chocolate candy were analyzed using dilution and plating method [20]. Fungi analysis was using laminar air flow, vortex-mixer, autoclave, analytical scales, knife, plastic bags, Petri dish, micropipette (Soccorex) 0.1 mL and 1 mL, tip pipette 0.1 mL and 1 mL, cool box sample, Eppendorf and tissue.

2.2.4 Experimental Design

This research was conducted using a complete randomized design with three replications. The data presented in the research are the average of the repetition.

3. Results and Discussion

3.1 Fat Content of Chocolate Candy

The fat content of chocolate candy is presented in Table 1. The fat content of chocolate candy is ranged from 44.9% to 46.2% and there is no significant difference for each treatment ($p = 0.05$). This shows

Table 1 Fat content of chocolate candy.

Candy type (treatment)	Fat content (%)
Old box without <i>Lactobacillus plantarum</i> HL-15 starter (OB)	46.0 ± 0.50
New box without <i>L. plantarum</i> HL-15 starter (NB)	44.9 ± 0.34
Old box with <i>L. plantarum</i> HL-15 starter (OBS)	46.2 ± 0.34
New box with <i>L. plantarum</i> HL-15 starter (NBS)	45.5 ± 0.81

that the fat content is not influenced by the culture starter addition or fermentation box variation. The fat content of chocolate candy has fulfilled the Indonesian National Standard (SNI) 7934: 2014 on Chocolate and Chocolate Products. The SNI stated that the requirement of quality of chocolate candy is to have total fat content $\geq 31\%$ [21].

Fat on chocolate candy is closely related to the texture of chocolate candy. According to Beckett [22], the dominant fatty acid combination of oleic (C18:1), stearate (C18:0) and palmitate (C16:0) makes cocoa melt rapidly in the range of small temperature changes that are solid at room temperature and melt at body temperature.

3.2 Water Activity of Chocolate Candy

Water is the main requirement for the growth process of microorganisms [20]. In food, water for the growth of microorganisms is available in the form of water activity (a_w). Therefore, a_w measurement on chocolate products is important to determine the possibility of microbial contamination in chocolate. The water activity (a_w) value of chocolate candy is 0.64 which is low and could inhibit the destructive microbial growth of food. However, according to Rahayu *et al.* [20], at a_w 0.60 *Aspergillus* sp. and *Penicillium* sp. spores survive for several years. An important factor that affects the growth of mold and the formation of OTA is water activity (a_w). *A. niger* has ability to grow and produce OTA rapidly at a_w 0.92-0.99 [23].

3.3 The pH Value of Chocolate Candy

The pH value is one of the important attributes in

food especially chocolate. Acidity and pH are among the intrinsic factors of pollutants microbial growth. In good nutritional conditions, the fungus can grow in a very wide pH range of 2.0-8.5 [20]. The pH value of chocolate candy ranges from 6.7 to 6.8, and is presented in Table 2.

Sandhya *et al.* [24] reported that an increase in pH of cocoa beans accelerated fermentation process due to the addition of *Saccharomyces cerevisiae*, *L. plantarum* and *Acetobacter aceti* by 10%-60% as culture starter. The increase in pH occurs due to the consumption of sugar by yeast and the use of citric acid by lactic acid bacteria.

The results showed no significant difference between samples at various treatments ($p \geq 0.05$). This dissimilarity is thought to be due to alkalization and mixing with other ingredients in chocolate. To prove this, a pH test with cocoa paste has not been mixed with other ingredients and has not been alkalized (Table 3). In Table 3, it can be seen that the pH value of cocoa paste is lower than the chocolate candy and is not significantly different between the samples at various treatments ($p \geq 0.05$).

3.4 Microbiology Quality of Chocolate Candy

Fungi contamination detected on chocolate candy is presented in Table 4. Fungi contamination on chocolate candy from cocoa beans fermentation using old box is greater (2.6×10^3 CFU/g) than the other treatment while the fermentation using new box with addition of the *L. plantarum* HL-15 culture as starter can suppress the fungus growth ($< 10^2$ CFU/g). Contaminations of fungi on chocolate candy produced from fermentation using old box without *L. plantarum*

Table 2 The pH value of chocolate candy.

Candy type (treatment)	pH value
Old box without <i>L. plantarum</i> HL-15 starter (OB)	6.7
New box without <i>L. plantarum</i> HL-15 starter (NB)	6.7
Old box with <i>L. plantarum</i> HL-15 starter (OBS)	6.8
New box with <i>L. plantarum</i> HL-15 starter (NBS)	6.7

Table 3 The pH value of chocolate pasta.

Pasta type (treatment)	pH value
Old box without <i>L. plantarum</i> HL-15 starter (OB)	5.2
New box without <i>L. plantarum</i> HL-15 starter (NB)	5.2
Old box with <i>L. plantarum</i> HL-15 starter (OBS)	5.2
New box with <i>L. plantarum</i> HL-15 starter (NBS)	5.2

Table 4 Fungi contamination in chocolate candy.

Candy type (treatment)	Colony per g sample		
	Chocolate candy	Dried fermented cocoa beans [25]	Chocolate powder [26]
Old box without <i>L. plantarum</i> HL-15 starter (OB)	2.6×10^3	1.6×10^3	7×10^2
New box without <i>L. plantarum</i> HL-15 starter (NB)	1.7×10^3	7×10^2	5.5×10^2
Old box with <i>L. plantarum</i> HL-15 starter (OBS)	1.3×10^3	6×10^2	1.5×10^2
New box with <i>L. plantarum</i> HL-15 starter (NBS)	$< 10^2$	5×10^2	1.5×10^2

HL-15 (OB), new box without *L. plantarum* HL-15 (NB) and old box with *L. plantarum* HL-15 starter (OBS) are greater than dried fermented cocoa beans by the study of Karisma [25].

Supposedly, the amount of contamination decreases due to the processing using high temperatures capable of inactivating all the fungus conidia. According to Copetti *et al.* [27] high temperatures (85-115 °C) and the alkalization applied during cocoa processing can kill the microbial vegetative cells, so it is not expected to appear contamination of fungi in chocolate products. So the high contamination of fungus in chocolate candy is thought to come from the raw material of cocoa beans. In addition, it is suspected that the fermented cocoa beans used in the processing of chocolate candy have been stored for two months.

The authors also compared the data obtained with the results of fungal contamination research on chocolate powder that was conducted by Monica [26]. In Table 4, it can be seen that the amount of

contamination of fungus on chocolate candy is greater than that of chocolate powder in all treatments. In fact, the chocolate candy processing is longer such as ballmill process for 10-11 h at 60 °C and uses a high temperature treatment to 130 °C on the roasting process.

When the amount of fungus contamination was compared between treatments, there was a similar trend between chocolate candy, dried cocoa beans and chocolate powder. Fungus contamination in old box samples is bigger than new box samples. This indicates a reduction of fungus contamination with the use of a new fermentation box. The contaminations of fungi on chocolate candy processed from cocoa beans fermented with *L. plantarum* HL-15 culture both OBS and NBS were reduced to approximately 1 log cycle compared with chocolate candy from cocoa beans fermented without *L. plantarum* HL-15 culture so that the addition of *L. plantarum* HL-15 starter can reduce the contamination of fungi. This supports the theory of anti-fungal activity of *L. plantarum* HL-15 in the

fermentation process.

4. Conclusions

Based on the result, it can be concluded that the addition of *L. plantarum* HL-15 as starter culture during fermentation did not affect the chemical quality but affected microbiological quality. From the microbiological aspect, chocolate candy produced using new box fermented cocoa beans and the addition of *L. plantarum* HL-15 culture had lower fungal contamination which was $< 10^2$ colonies/g. Thus further research is needed on the effect of adding *L. plantarum* HL-15 during fermentation to inhibition of mycotoxin-producing fungi during storage of cocoa beans.

Acknowledgments

Authors show their thanks to Indonesia Agency for Agricultural Research and Development that has supported funding for this work through research collaboration project entitled "The Control Technology of Mycotoxin Contaminants Using Lactic Acid Bacteria to Improve Cocoa Beans Quality".

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