FINAL REPORT

DIPA BIOTROP 2017

POSTHARVEST QUALITY IMPROVEMENT OF ARABICA COFFEE BEANS (*Coffea arabica*) STORED UNDER WAREHOUSE CONDITIONS

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(SEAMEO BIOTROP)

2017

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4. Research Schedule : 9 (nine) months

5. Research Budget :

Bogor, 1 December 2017

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ABSTRACT

Postharvest quality improvement of Arabica coffee beans with various processing methods using two types of packaging materials to prevent the occurrence of ochratoxin A (OTA) producing fungi and OTA contamination have been conducted. Coffee beans were dried with three types of processing methods, i.e. natural, full wash, and semi wash processings. The beans were packed using two types of packaging materials, i.e polypropylene bag and polyethylene hermetic bag (4 kg/bag). They were then stored under warehouse conditions during 4 months of storage. Moisture content of coffee beans with natural processing were higher than that with full wash and semi wash processing. Grade of coffee beans in various treatments were still grade 1, but defective value of beans increased and attained maximum defective value for grade 1 (11). Taste of coffee beans in various treatments during 4 months of storage were still above total standard score for specialty grade ≥ 80. The highest total score was found in coffee bean with natural processing was packed in polyethylene hermetic bag (84). OTA producing fungi, *Aspergillus ochraceus* was only found in coffee beans with full wash and dry processing at the beginning of storage.Their population were relatively low < 3 cfu/g w.b. Population of *Aspergillus niger* of coffee beans with various processing was < 10 cfu/g w.b. The highest population of *A. niger* was found in coffee beans with semi wash processing. *Aspergillus ochraceus* and *A. niger* were also found in dried green beans with full wash processing, i.e. 13 and 10 cfu/g w.b. Although OTA producing fungi was found in coffee beans, but OTA content in all samples during storage were lower than limit detection of instrument (<1.85 ppb). At the beginning of storage, all samples were dominated by yeasts with their population $1.9 \times 10^2 - 1.2 \times 10^3$ cfu/g w.b.

Keywords : coffee beans, fungi, OTA, packaging, processing

1. INTRODUCTION

1.1. Background

In Southeast Asia Indonesia is the second ranking of coffee beans producing countries after Vietnam. In Indonesia two kinds of coffee cultivated, i.e. Robusta coffee (*Coffea canephora*) and Arabica coffee (*C. arabica*). Composition of Robusta coffee is about 83% of total coffee production, while that Arabica coffee is about 17%.

During storage coffee beans could be infested by insects, microorganisms, mites and rats. Among microorganisms, fungi are the most important cause of deterioration of stored grains or seeds. Fungal infection in grains can cause discolouration, decreases in physical quality and nutritional contents, and mycotoxin contamination (Sauer *et al*. 1992).

Ochratoxin A (OTA) contamination in coffee beans have been becoming an important issue recently since some consuming countries applying its Maximum Tolerable Limits (MTL) of the presence of OTA in some coffee products (Ismayadi *et al*. 2003). OTA is a potent nephrotoxic mycotoxin that has been linked to kidney problems in both livestock and human populations (Clark and Snedeker 2006). Ismayadi *et al.* (2005) reported that OTA content is one of the criteria in determining quality standard in the world. SNI (Indonesian National Standard) (2009) has determined MTL of OTA in *kopi sangrai* (roasted coffee) and instant coffee 5 and 10 ppb, respectively. According to Raghuramulu and Naidu (2001) Italia has determined maximum tolerable limit (MTL) of OTA in coffee beans and their processed products 8 and 4 ppb, respectively.

According to Martin *et al.* (2003) as much as 91.7% of 60 coffee bean samples collected from Brazil were contaminated by fungi. The dominant fungi were *Aspergillus niger* (83.3%), *A. ochraceus* (53.3%) and *A. flavus* (25%). The occurrence of *Cladosporium* sp. (16.6%) and *Penicillium* sp. (10%) were lower than Aspergilli section. As much as 20 samples (33.3%) of 60 samples were contaminated by ochratoxin with range and mean 0.2 – 7.3 ppb and 2.38 ppb, respectively.

Leong *et al*. (2007) reported that *A. carbonarius*, *A. niger* and yellow Aspergilli (*A. ochraceus* and related species in section *Circumdati*) were isolated by direct plating of surface-disinfected Robusta (65 samples) and Arabica (11 samples) coffee beans from southern and central Vietnam. *Aspergillus niger* infected 89% of Robusta beans, whereas *A. carbonarius* and yellow Aspergilli each infected 12-14% of beans. The maximum OTA observed in samples severely infected with toxigenic species was 1.8 ppb, however, no relationship between extent of infection and OTA contamination was observed.

According to Noonim *et al*. (2008) 32 Thai dried coffee bean (*C. arabica*) samples were collected from two growing sites of Chiang Mai Province The samples from North had an average of 78% incidence of colonization with *Aspergillus* of section *Circumdati* with *Aspergillus westerdijkiae* and *A. melleus* as the predominant species. *Aspergillus* spp. of section *Nigri* were found in 75% of samples, whereas *A. ochraceus* was not detected.

Nogaim and Gowri (2013) reported that 70 samples of *C. arabica* were collected from local markets in some Yemeni Governorates during 2010/2011. The samples were infected by *A. niger*, *A. ochraceus*, *A. fumigatus*, *A. flavus*, *A. parasiticus*, *A. ochraceus* and *A. candidus*. The percentage of samples infected by the fungi were 27.5, 17.5, 15, 12,5, 10 and 5%, respectively. The range and mean of ochratoxin A contents were 0.314-3.443 ng/g and 1.211ng/g, respectively.

de Fatima *et al*. (2013) reported a number of fungi were isolated from 30 samples of Arabica coffee. As much as 20 samples of coffee beans collected from conventional cultivation system, while 10 samples were collected from organic cultivation system. As much as 480 filamentous fungal species were isolated. They were belong to genus *Aspergillus*, group *Circumdati* and *Nigri*. Ochratoxin producing fungi isolated were *A. auricoumus*, *A. ochraceus*, *A. ostianus*, *A. niger* and agregate of *A. niger*. The most frequent species which produced ochratoxin A was *A. ochraceus* corresponding to 89.55% of samples.

In tropical regions OTA is mainly produced by *Aspergillus carbonarius*, *A. niger* and *A. ochraceus*, while in sub-tropical regions it is produced by *Penicillium verrucosum* and *P. nordicum* (Pitt *et al*. 2000). Dharmaputra *et al.* (2016) reported that *A. niger* and *A. ochraceus* had been found in some samples of Arabica coffee beans collected at certain farmer, trader and exporter levels.

The growth of *A. niger* and *A. ochraceus* is influenced among others by temperature and relative humidity of the storage, and the type of packaging. OTA production is influenced among other by moisture content of coffee beans and the strain of *A. niger* and *A. ochraceus*. A part from the most critical stage of mycotoxin production in coffee bean processing is drying. It can be affected by coffee bean processing methods. According to Ismayadi *et al.* (2006) in Indonesia there are three methods of coffee bean processing, they are dry, wet and semi-wet processing. The diagram of three coffee bean processings is presented in diagram.

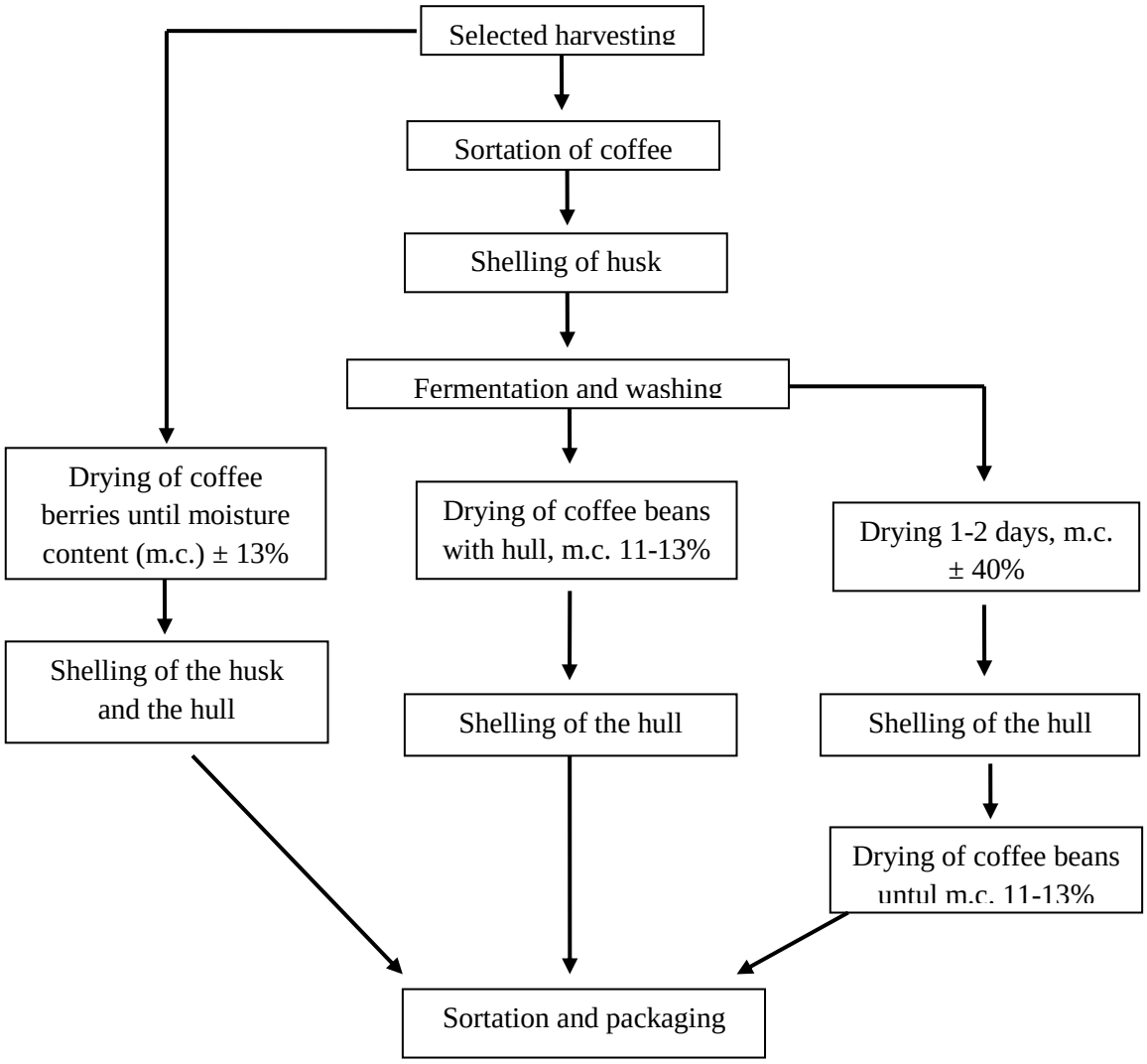


Diagram of coffee bean processing: a): dry processing, b) wet processing, c) semi-wet processing

Dharmaputra *et al.* (2016) have conducted a survey on postharvest handling of Arabica coffee beans in Tana Toraja and North Toraja Regencies, and City of Makassar. The distribution chain of the coffee beans was farmer, collector, trader, and expoter. Coffee bean processing was conducted using semi-wet processing method. The research result showed that at **farmer** level 19 % of 27 coffee bean samples were infected by *A. ochraceus* (mean population 1.4 x 10^2 cfu/g w.b), 4% samples were infected by *A. niger* (6.3 x 10 cfu/g w.b) and 11% samples were contaminated by OTA. The range and mean of OTA contents were 1.6 – 17.7 ppb and 7.2 ppb, respectively. From harvesting of coffee berries, shelling of the husk, fermentation and washing of coffee beans with hull up to

drying the beans with hull were conducted by farmers. Consequently, fungal infection and OTA contamination can occur at each level of coffee bean processing. A part from, at **big trader** level 46% of 13 coffee bean samples were infected by *A. ochraceus* (7.4 x 10^2 cfu/g w.b), 46% samples were infected by *A. niger* (4.8 x 10^2 cfu/g w.b). As much as 23% samples were contaminated by OTA with the range and mean were 1.5 – 11.3 ppb and 6.6 ppb, respectively. At **exporter** level 44% of 9 coffee bean samples were infected by *A. ochraceus* (2 x 10 cfu/g w.b) 78% samples were infected by *A. niger* (3.1 x 10^2 cfu/g w.b). As much as 33% samples were contaminated by OTA with the range and mean 2.8 – 14.7 ppb and 8.7 ppb, respectively. Collector distributed coffee beans with hull obtained from some farmers to trader and exporter. Shelling of hull, drying of coffee beans until sortation were conducted by trader and exporter.

Dharmaputra *et al.* (2016) reported that the type of packaging used by farmers, collectors and traders to store coffee beans were polypropylene bags. Packaging used by collector was that has been used by farmers, while traders used new packaging. At exporter level 25% 25 % and 50% of exporters used polypropylene bag, jute bag, and jute bag doubled with polyethylene bag, respectively.

Consequently, it was necessary to investigate the level of fungal infection (especially OTA producing fungi) and OTA contamination affected by some coffee bean processing and the type of packaging materials during storage. The research which was conducted in 2017 is the continuation of the research conducted in 2016. The research is also related to the Programme Thrust SEAMEO BIOTROP for 9th Five Year Development Plan (2012 – 2017), i.e. Tropical Biology for Community Welfare Program. It consists among others Food and Feed Security and Safety.

1.2. Objective

1. To investigate the effect of processing methods and type of packaging materials during storage on the quality of Arabica coffee beans in terms of fungal infection (especially OTA producing fungi) and OTA contamination. Moisture content and the physical quality of the beans were also analyzed, because they affect fungal infection and OTA contamination.
2. To recommend appropriate processing method and the type of packaging materials to ensure the quality of Arabica coffee beans during storage.

1.3. Expected Output

It is expected, that the research result could give :

1. Recommendation on the best method of processing and storing of Arabica coffee beans to stakeholder at each level of distribution chain.
2. Information on the effect of processing methods and type of packaging materials during storage on the quality of Arabica coffee beans in terms of fungal infection (especially OTA producing fungi) and OTA contamination.

2. BENEFIT AND IMPORTANCE OF RESEARCH

- According to International Coffee Organization (2014), Indonesia is the fourth largest of coffee producer in the world after Brazil, Vietnam, and Colombia with an estimated production reached 622 thousand metric tons per year. Indonesia is also known because have the highest coffee variants of nearly 100 types of Arabica coffee variants known since 1699, and one of them is Arabica Toraja. Indonesia also has a great opportunity to increase exports of Arabica coffee beans for the world market like Arabica coffee from Indonesia.
- Information related on fungal infection and ochratoxin A (OTA) contamination in coffee beans at various stages of the delivery chain is still very limited.
- Postharvest handling methods affect the quality of coffee beans, consequently they also affect the price of coffee beans related to foreign trade.

3. METHODOLOGY

3.1 Time and location of research and source of green coffee beans

Arabica green coffee beans Grade 1 (grade one) were obtained from a big farmer located in Pengalengan Sub-district, Bandung Regency, West Java Province. The determination of moisture content, physical quality, fungal population and OTA content were conducted at SEAMEO BIOTROP, Bogor.

3.2 Collecting of coffee beans (green beans) and drying of the beans using three different processing methods

Coffee plantation in Pengalengan Sub-district, Bandung Regency, West Java Province has an altitude ± 1200 m dpl. Ripe cherry beans were harvested in one day to

obtain homogenous sample. Cherry beans were then dried using three different processing methods, i.e. natural (N), full wash (F), and semi/mid wash (S) processings.

Natural processing is processing method with harvesting of coffee beans selectively (wet cherries), they were then dried using sun-drying (Figure 1) for 14 days until the moisture content attained ±10% (dried cherries), their husk and hull were then shelled using a huller machine. **Full wash** processing method is a processing of coffee beans by shelling of wet cherry beans after they were selected (Figure 2a), therefore they became wet green beans with hull, they were then fermented for one night and washed to eliminate their mucus (Figure 2b). Wet green beans were then dried (Figure 3) using sun-drying for 7 days until their moisture contents attained ± 10% (dried green beans), hull of the beans were then shelled (green beans). **Semi/mid wash** processing method is processing method by shelling of wet cherries after selected, then wet green beans were fermented for one night and washed to eliminate the mucus. Wet green beans were then dried using sun-drying for 1 day until the moisture content attained ± 40%, the hull were then shelled. After shelling, the beans were further dried (Figure 4) using sun-drying for 5 days until the moisture content attained ± 10% (Figure 5). Moisture content was determined using a moisture tester during drying process.

3.3 Methods of packaging and storing

Coffee beans with ± 10% moisture content were packed using two types of packaging materials, i.e polypropylene bag and polyethylene hermetic bag. Each bag contained 4 kg of green beans.

Coffee beans were stored under warehouse conditions for two and four months (Figure 6). The temperature and relative humidity of the storage were determined using a thermohigrometer.

Three replications were used for each treatment. Thus, the number of unit experiment were 3 x 2 x 3 x 3 = 54 (3 = processing methods; 2 = type of packaging materials; 3 = storage durations including at beginning of storage; 3 = replications).

3.4 Sampling methods

Coffee bean samples were collected from each processing method, i.e. 2 samples from **natural** processing (1 sample wet cherry and 1 sample dried cherry), 2 samples from **full wash** processing (1 sample wet green bean with hull and 1 sample dried green bean

with hull), and 1 sample from **semi/mid wash** processing (wet green bean with hull after drying for 1 day). Samples of coffee beans were also obtained from each bag at the beginning of storage, subsequently after 2 and 4 months of storage (± 1 kg/bag). Each sample was then divided three times using a box divider to obtain working samples for moisture content, physical quality, cupping test, fungal population and OTA content determinations.

3.5 Determination of moisture content, physical quality, fungal population, and OTA content

Moisture content of green coffee beans (based on wet basis) was determined using the oven method (SNI 2008). Physical quality of green beans were determined based on the number of defective beans from 300 g of sample (SNI 2008). Kind of defective, defective value, quantitative requirements of Arabica coffee are presented in Appendices 1 and 2.

Cupping testing of the samples used Standard Cupping Protocol given by Coffee Quality Institute and Specialty Coffee Association of America (2015). The panelists have a certificate from CQI Q grader. Cupping testing was conducted at Cupping Testing of Coffee Laboratory PT Kemenady Industri Mandiri, Bogor.

Fungi were isolated using serial dilution method, followed by pour plate method on Dichloran 18% Glycerol Agar (DG18) (Hocking and Pitt 1980, Pitt and Hocking 2009). Fungal population were determined from the number of fungal colonies on DG18 from certain dilution factor. Each fungal species was identified using Pitt and Hocking (2009) as the main reference. OTA contents were determined using High Performance Liquid Chromatography (HPLC) method (AOAC 2012). Two replicates were used for each sample. Ground coffee was extracted using methanol : 1% sodium bicarbonate (70:30). Sample extract was diluted using PBS 0.01% Tween 20, it was then eluted using Ochra Test Immunoaffinity Column. Detection was determined using HPLC-FL detector.

3.6 Statistical analyses

The data was analyzed using Completely Randomized Factorial Design with three factors. The 1st, 2nd and the 3rd were processing methods, type of packaging materials, and duration of storages, respectively.

Three replications were used for each treatment. Thus, the number of unit experiment were 3 x 2 x 3 x 3 = 54 (3 = processing methods; 2 = type of packaging materials; 3 = storage durations including at beginning of storage; 3 = replications).

4. RESULT AND DISCUSSIONS

4.1 Moisture content and fungal population

Coffee beans used in this research were dried coffee beans (moisture content ± 10%). Cherry pulp, husk, and hull were shelled. The beans with moisture content ± 10% were called green beans. Coffee beans derived from wet cherries that were processed in some grades of processing. They were two processing methods of wet cherry beans to become green beans, i.e. full wash and dry (natural) processing. The main difference between the two methods was the time to shell cherry fruit, husk, and hull. In natural processing, shelling of cherry fruit, husk and hull were conducted after the cherry was dried, while in full wash processing, the shelling of cherry fruit was conducted when the cherry was still wet.

The aim of collecting samples in every stage of processing methods was to get information the condition of coffee beans before they were dried and stored. Moisture content of **wet cherry beans** was 49.42%, they were then dried for 15 days using sun-drying until their moisture contents attained 10.28% (**dried cherry beans**) (Table 1). **Wet green bean** with hull was collected after fermentation and washing process, consequently its moisture content was 49.69%. Wet green bean with hull was then dried using sun-drying for 7 days until moisture content attained 8.09% (**dried green bean)**. In semi/mid wash processing, wet green bean with hull was dried using sun-drying for 1 day until moisture content attained 42.73% (**dried green bean after 1 day)**, the hull was then shelled and further dried until its moisture content attained safe moisture content to be stored.

Fungal species and its population isolated from each stage of processing methods are presented in Table 2. Yeast A was a dominant fungus in wet or dried cherry bean samples, and wet or dried green bean with hulls collected from each stage of processing. The yeast was found in wet cherry before fermentation. Its population increased in dried green bean derived from full wash processing (F). In dried green bean the number of filamentous fungi (fungi instead of yeast) increased from 3 became 11 species (Table 2).

Silva et al. (2008) reported that the number of fungi were isolated from coffee beans during fermentation and drying. Total fungal population in coffee beans were picked from the tree was 1.5 x 10^3 cfu/g w.b. The fungi increased during fermentation and drying (22 days) attained 2 x 10^5 cfu/g w.b. As much as 263 filamentous fungal isolates were found in the samples. *Aspergillus* spp. were dominant fungi. The other fungi were also found, i.e. *Pestalotia* (4 isolates), *Paecilomyces* (4 isolates), *Cladosporium* (26 isolates), *Fusarium* (34 isolates), *Penicillium* (81 isolates), *Aspergillus* (112 isolates) and 38 other fungal species.

4.2 Moisture content, physical quality and taste of coffee beans in the different type of packaging materials and processing during storage

Two type of packaging materials, i.e. polypropylene and polyethylene hermetic bags were used, because according to Dharmaputra *et al.* (2016) polypropylene bag is type of packaging material used by farmers, collectors, and big traders in Tana Toraja and North Toraja Regencies to store coffee beans. In Makassar, in general exporters used jute bag double with polyethylene hermetic bag to store coffee beans.

At the beginning of storage, moisture content of coffee bean in natural processing was higher than full wash and semi/mid wash processing (Table 3). In natural processing, drying of coffee bean was not maximal because it was protected by the pulp of cherry bean. Moisture content of coffee beans was in equilibrium with the relative humidity of the warehouse after 2 and 4 months of storage (Table 4). The moisture content of coffee beans packed in polypropylene and polyethylene hermetic bags is presented in Table 3. Aeration of polypropylene bag was higher than that polyethylene hermetic bag, consequently moisture content of coffee beans packed in polypropylene was higher than that packed in polyethylene hermetic bag. Determination of coffee beans grades based on defective value system is presented in Appendix 1, while determination of the number defective coffee beans is presented in Appendix 2 (SNI 2008). Grade of coffee beans was still grade 1 (Table 5). It means that grade of coffee beans did not decrease, but its defective value increased after 4 months of storage (Table 5) and attained maximum defective value 11 for grade 1 (Appendix 2). Increasing of defective value in natural processing was caused by increasing of brown bean. Drying process in natural processing was drying of cherry bean to become dried cherry bean with long duration (15 days), consequently the color of the beans was brown. In full wash processing, increasing of

defective value was caused by increasing of brown bean and broken bean, while in semi/mid wash processing was caused by increasing of broken bean. In semi/mid wash processing, green bean was dried after shelling of hull, therefore drying process was relatively short duration and caused the increasing of broken bean.

At present, some importer countries or coffee lovers still commented that the best quality of coffee was the coffee which had high value for cupping test. Organoleptic / cupping test is determination objectively from sensoric, using skin, tongue, nose, ears, and eyes (Meilgard *et al.* 2007). In cupping test, panelist determined and gave the value on product test such as flavor, taste of food or beverages, morphological of food, and other parameters which could not be determined with tool or equipment. Panelist only used hand/skin, tongue, nose, ears, and eyes as a determinator. Cupping test has weakness, i.e. the subjectivity of giving value was conducted by panelist due to preference or other factors. Therefore, cupping test had a specific method and step, included selecting of good panelist. Panelist who work for cupping test is called Q-grader.

Q-grader is certified panelist who was tested and calibrated by Specialty Coffee Association (SCA), so he/she has a capability to give value on quality of Arabica coffee bean based on cupping. In cupping test, taste of coffee was determined based on aroma, flavor, acidity, body, balance, sweetness, clean up, and uniformity. Coffee bean that was used for testing should be followed standardized protocols, therefore that it had no effect of process such as roasting and brewing in flavor. In other words, taste or flavour was determined in coffee was pure flavor otentically from the green bean. Cupping produced a score that became the reference label quality and specialty coffee level. In the specialty coffee market, cupping value is representative of the quality of coffee that determines the selling price of coffee. Therefore, cupping is one of the important Quality Control (QC) processes in specialty coffee. Decreasing of quality and long shelf life in coffee beans were caused by temperature and relative humidity of the storage, and condition of packaging materials during storage. According to Specialty Coffee Association of America (SCAA), specialty coffee bean was not accepted if the cupping score was lower than 80.

According to Atmawinata (2002) in general coffee was not consumed because of its nutritional value, but it was caused by the value of flavor and physiological influences that caused people to stay awake, add freshness, reduce fatigue and create a feeling more excited. Saepudin (2005) reported that the value of coffee beans was not only determined by physical quality, but more determined by the value of flavor so that some coffee importing countries determined the quality of coffee by cupping test.

Qualification of coffee beans based on final score according to SCAA (2015) was divided into 4 categories, i.e. outstanding (90-100), excellent (85-89.99), very good (80-84.99), dan below specialty quality (below 80). Coffee bean with outstanding, excellent and very good categories or had final score (80-100) into specialty qualification and coffee bean with below specialty category into not specialty qualification but it may still consumed. According to panelist, coffee bean could not be consumed if the final score was ≤ 30.

The flavor of coffee beans in three processing methods and packed in polyethylene hermetic bag and polypropylene bag for 4 months of storage were still above the total score specialty grade ≥ 80 (Table 6). The highest total score was coffee beans with natural processing and were packed in polyethylene hermetic bag (84). Borem *et al.* (2013) reported that polyethylene hermetic bag is also impermeable to water and gases including CO_2, O_2 and N_2. Ribeiro *et al.* (2011) explained this package could maintain the sensory qualities of materials such as volatile compounds, textures, and colors. It is suitable for certain products that require the manipulation of atmospheric conditions, therefore it could maintain the sensory quality especially the aroma of packaged material.

Murthy and Naidu (2011) found that taste of coffee bean produced from full wash processing (wet processing) was better than that natural processing (dry processing), because the aroma of volatile taste in roasting of coffee bean with wet processing was better than that the bean with dry processing. According to Puslitkoka (2007) taste of coffee bean was also formed in natural processing (dry processing) without fermentation, because it contained volatile precursor for taste former.

4.3 OTA producing fungi and OTA content

de Fatima *et al*. (2013) reported a number of fungi were isolated from 30 samples of Arabica coffee. As much as 20 samples of coffee beans collected from conventional cultivation system, while 10 samples were collected from organic cultivation system. As much as 480 filamentous fungal species were isolated. They were belong to genus *Aspergillus*, group *Circumdati* and *Nigri*. Ochratoxin producing fungi isolated were *A. auricoumus, A. ochraceus, A. ostianus*, *A. niger* and agregate of *A. niger*. The most frequent species which produced ochratoxin A was *A. ochraceus* corresponding to 89.55% of samples. According to Pitt et al. (2000) in tropical regions OTA is mainly produced by

Aspergillus carbonarius, *A. niger* and *A. ochraceus*, while in sub-tropical regions it is produced by *Penicillium verrucosum* and *P. nordicum*.

Based on the result of the research, *Aspergillus ochraceus* was only found in sample with wet (full wash) and dry (natural) processing at the beginning of storage with its population was relatively low, i.e. 1 and 3 cfu/g w.b (Appendix 4). Population of *A. niger* in coffee beans in three different processing were relatively low < 10 cfu/g w.b (Table 7). The highest population of *A. niger* was found in coffee bean in semi/ mid wash (mid wet) processing (Table 2).

Batista et al. (2009) reported that genus of *Aspergillus* Section *Circumdati* and Section *Nigri* were found in cherry and green beans when they were harvested and during processing. *Aspergillus* section *Circumdati* was found in cherry and green bean, i.e. 80% and 41%, but its population decreased after first processing. As much as 33% of 12 sample coffee beans with wet processing (F) was contaminated by fungi, it was *Aspergillus* section *Circumdati*. As much as 73% *Aspergillus* section *Nigri* was found in cherry beans and 25% of it was found in processed cherry beans.

OTA content in all samples during storage were lower than limit detection of instrument (< 1.85 ppb). Samples were dominated by yeast at the beginning of storage with its population was $1.9 \times 10^2 - 1.2 \times 10^3$ *cfu*/g w.b (Appendix 4). Yeast was found in wet cherry beans (Table 2). The yeast may inhibit the growth of OTA-producing fungi and reduce OTA production. According to Masoud et al. (2005), volatile compounds produced during coffee processing by *Pichia anomala, P. kluyveri,* and *Hanseniaspora uvarum* (ethyl acetate, isobutyl acetate, 2-phenyl ethyl acetate, ethyl propionate and isoamyl alcohol) inhibited OTA producing fungi (*A. ochraceus*) and prevented OTA production.

Total yeast population decreased during storage (Table 8). It was assumed that yeast compete with filamentous fungi. Filamentous fungi (except *A. niger* and *A. ochraceus*) isolated from coffee beans, i.e. *A. flavus, A. penicillioides, A. tamarii, Cladosporium cladosporioides, Eurotium chevalieri, Fusarium avenaceum, Fusarium* sp., *Penicillium citrinum, Wallemia sebi* (Appendix 4). Fungi isolated from stored coffee beans and dried green beans with hulls, i.e. *A. flavus, A. niger,* and *A. ochraceus* (Table 2). Munyendo *et al.* (2017) reported that fungal infection in coffee beans with dry processing (N) were higher than those with wet processing (F). Although some samples were contaminated with fungi, but OTA was not detected.

5. CONCLUSIONS

Moisture content of coffee beans with natural processing was higher than that with full wash and semi wash processing, because coffee beans with natural processing were still protected by pulp of cherry that was thicker than its hull. Aeration of polypropylene bag was higher than that polyethylene hermetic bag, consequently moisture content of coffee beans packed in polypropylene bag was higher than that in polyethylene hermetic bag.

Grades of coffee beans in various treatments did not decrease, i.e. they were still grade 1, but defective value of beans increased and attained maximum defective value for grade 1 (11). Increasing of defective value was caused by increasing of brown and broken beans.

Taste of coffee beans in various treatments during 4 months of storage were still above total standard score for specialty grade ≥ 80. The highest total score was found in coffee bean with natural processing and packed in polyethylene hermetic bag (84).

Aspergillus ochraceus was one of OTA producing fungus that was only found in coffee beans with full wash and dry processing at the beginning of storage. Its population was relatively low (< 3 cfu/g w.b). *Aspergillus niger* was also OTA producing fungus was only found in coffee beans with various processing. Its population was < 10 cfu/g w.b. The highest population of *A. niger* was found in coffee beans with semi wash processing. *Aspergillus ochraceus* and *A. niger* were found in dried green beans with hulls in full wash processing. Their populations were 13 and 10 cfu/g w.b, respectively.

Although OTA producing fungi was found in coffee beans, but OTA content in all samples during storage were lower than limit detection of instrument (<1.85 ppb). At the beginning of storage, all samples were dominated by yeasts with their population 1.9×10^2 – 1.2×10^3 cfu/g w.b. Yeasts were also found in wet cherry beans. It was assumed that yeasts may inhibited the growth of OTA producing fungi, consequently the yeasts inhibited OTA production.

6. RECOMMENDATION

To obtain good quality of Arabica coffee beans stored under warehouse conditions we should conduct an appropriate postharvest handling method (Good Handling Practice). The result of this research recommend appropriate postharvest handling process of coffee

beans, i.e.: 1) drying with full wash, semi wash, and natural processing are available; 2) coffee beans should be packed in polyethylene hermetic bag to be stored under warehouse conditions ; and 3) coffee beans is still good to be consumed until 4 months of storage.

7. REEFERENCES

AOAC Official Method 2004.10. 2012. Ochratoxin A in green coffee. 19th Edition. Latimer GW, Editor. Official Methods of Analysis of AOAC International. Gaithersburg (US): AOAC International. Vol II, Chapter 49: 67-69.

Atmawinata, O. 2002. Peranan uji citarasa dalam pengendalian mutu kopi. Materi pelatihan Uji citarasa kopi. Pusat penelitian kopi dan kakao. Jember. 39 hlm.

Batista LR, SM Chalfoun, CF Silva, M Cirillo, EA Varga, RF Schwan. 2009. Ochratoxin A in coffee beans (*Coffea arabica* L.) processed by dry and wet methods. Food control 20: 784-790. Elsevier.

Borem FM, Riberio FC, Figueiredo LP, Giomo GS, Fortunato VA, Isquerdo EP. 2013. Evaluation of the sensory and color quality of coffee beans stored in hermetic packaging. *J of stored product research* 52:1 – 6.

Clark HA, Snedeker SM. 2006. Ochratoxin A: Its cancer risk and potential for exposure. Journal of Toxicology and Environmental Health B. 9:265-296.

Dharmaputra OS, Ambarwati S, Retnowati I, Nurfadila N. 2016. Fungal infection and ochratoxin A contamination in stored Arabica coffee beans (*Coffea arabica*) at various stages of the delivery chain in Tana Toraja Regency, South Sulawesi Province. SEAMEO BIOTROP. Internal Report.

de Fatima RE, Borges JG, Cirillo MA, Prado G, Paiva LC, Batista LR. 2013. Ochratoxigenic fungi associated with green coffee beans (*Coffea arabica* L.) in conventional and organic cultivation in Brazil. Brazilian Journal of Microbiology 44 (2): 377-384. DOI: 10.1590/51517-83822013 0002 00006.

Hocking AD, Pitt JI. 1980. Dichloran-glycerol medium for enumeration of xerophilic fungi from low moisture foods. Applied and Environmental Microbiology 39:488-492.

Ismayadi C, Zaenudin. 2003. Pola produksi, infestasi jamur, dan upaya pencegahan kontaminasi ochratoxin-A pada kopi Indonesia. Warta Pusat Penelitian Kopi dan Kakao Indonesia 19(1):45-60.

Ismayadi C, Silviana J, Nisa FC, Zubaidah E, Zaenudin. 2005. Kemampuan produksi ochratoxin A secara *in vitro* spesies jamur yang diisolasi dari biji kopi. Pelita Perkebunan 19(2):78-89.

Ismayadi C, Sumartono B, Susila WR, Mawardi S. 2006. Penguatan agribisnis kopi menuju peningkatan mutu, diversifikasi produk dan perluasan pasar. Prodising Simposium Kopi, Surabaya, Jawa Timur, 2 – 3 Agustus 2006.

Leong SL, Hien LT, An TV, Trang NT, Hocking AD, Scott ES. 2007. Ochratoxin A-producing Aspergilli in Vietnamese green coffee beans. Letters in Applied Microbiology 45:301-306.

Martins ML, Martins HM, Gimeno A. 2003. Incidence of microflora and ochratoxin A in green coffee beans (*Coffea arabica*) Food Additives and Contaminants 20 (12): 1127-1131.

Masoud W, L Poll, M Jakobsen. 2005. Influence of volatile compounds produced by yeasts predominant during processing of *Coffea arabica* in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus ochraceus.* Yeast 2005: 22: 1133-1142. John Wiley & Sons Ltd.

Morten C, B Meilgaard, T. Carr, GV Civille. 2006. Sensory Evaluation Technique. Detroit. United States. CRC Press.

Munyendo LM, R Koskei, M Mburu. 2017. Moulds and ochratoxin A associated with green coffee (*Coffea arabica*) beans processed by dry and wet methods in Nyeri County. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e- ISSN: 2319-2402,p-ISSN: 2319-2399, Vol 11, Issue 6 Ver. I (June 2017)PP 66-72. www.iosrjournals.org.

Murthy, PS. and MM Naidu. 2011. Improvement of robusta coffee fermentation with microbial enzymes. *European Journal of Applied Sciences* 3(4): 130-139.

Nogaim QA, Gowri PM 2013. Determination of ochratoxin A in Yemeni Green Coffee. Scholars Academic Journal of Bioscience (SAJB) 1(6):253-262.

Noonim P, Mahakarnchanakul W, Nielsen KF, Frisvad JC, Samson RA. 2008. Isolation, identification and toxigenic potential of ochratoxin A-producing *Aspergillus* species from coffee beans grown in two regions of Thailand. International Journal of Food Microbiology 128(2):197-202.

Pitt JI, Basilico JC, Abarca ML, Lopez C. 2000. Mycotoxins and toxigenic fungi. Medical Mycology 38:41-46.

Pitt JI, Hocking AD. 2009. Edisi ke-3. *Fungi and Food Spoilage.* New York: Springer.

Puslitkoka, 2007. Pengolahan biji kopi sekunder. Leaflet, Pusat penelitian kopi dan kakao Indonesia, Jember.

Raghuramulu Y, Naidu R. 2001. The Ochratoxin-A Contamination in Coffee and Its in Food Safety Issues. http://www.indiacoffee.org/newsletter/9/coverstory.html-16k. [14 April 2015]

Ribeiro FC, Borem FM, Giomo GS, Lima RRD, Malta MR, Figueiredo LP. 2011. Storage of green coffee in hermetic packaging injected with CO2. J of stored product research 47:341 – 348

Saepudin A. 2005. Evaluasi faktor-faktor yang mempengaruhi citarasa kopi Arabika dengan menggunakan Manova dan analisis profil. Skripsi Fakultas Matematika dan Ilmu Pengetahuan Alam IPB, Bogor. 17 hlm.

Sauer DB, Meronuck RA, Christensen CM. 1992. Microflora. *Dalam* Sauer DB (editor). *Storage of Cereal Grains and Their Product*. 4th ed. Minnesota: American Association of Cereal Chemist. pp. 313 – 340.

Silva CF, LR Batista, RF Schwan. 2008. Incidence and distribution of filamentous fungi during fermentation, drying and storage of coffee (*Coffea arabica* L.) beans. Braz.J. Microbiol. 39 (3): 521-526.

SNI. (Standar Nasional Indonesia). 2008. *Biji Kopi*. Jakarta: Badan Standardisasi Nasional.

SNI. (Standar Nasional Indonesia). 2009. *Batas Maksimum Kandungan Mikotoksin dalam Pangan*. Jakarta: Badan Standardisasi Nasional.

APPENDICES

Appendix 1. Determination of the number defective coffee beans

No	Kind of defective	Defective value
1.	1 (one) black bean	1
2.	1 (one) a half of black bean	½
3.	1 (one) broken black bean	½
4.	1 (one) cherry bean	1
5.	1 (one) brown bean	¼
6.	1 (one) big husk	1
7.	1 (one) middle husk	½
8.	1 (one) small husk	1/5
9.	1 (one) bean with hull	½
10	1 (one) big hull	½
11	1 (one) middle hull	1/5
12	1 (one) small hull	1/10
13	1 (one) broken bean	1/5
14	1 (one) immature bean	1/5
15	1 (one) bean with one hole	1/10
16	1 (one) bean with more than one hole	1/5
17	1 (one) bean with spot	1/10
18	1 (one) big branch, soil, or gravel	5
19	1 (one) middle branch, soil, or gravel	2
20	1 (one) small branch, soil, or gravel	1

Source: Indonesian National Standard (SNI 01-2907-2008)

Notes : If one bean has more than one kind of defective, the defective value was determined based on the highest defective value.

Appendix 2. Determination of coffee beans grades based on defective value system

Grade	Criteria of grade
Grade 1	Maximum number of defective value 11
Grade 2	Number of defective value 12 – 25
Grade 3	Number of defective value 26 – 44
Grade 4-A	Number of defective value 45 – 60
Grade 4-B	Number of defective value 61 – 80
Grade 5	Number of defective value 81 – 150
Grade 6	Number of defective value 151 – 225

Source: Indonesian National Standard (SNI 01-2907-2008)

Notes : The number of defective beans was determined based on Appendix 1.

Appendix 3. Explanation of attributes criteria in cupping test of coffee beans

No.	Attribute	Explanation
1	Fragrance	The aromatic aspects include Fragrance (defined as the smell of the ground coffee when still dry) and Aroma (the smell of the coffee when infused with hot water). One can evaluate this at three distinct steps in the cupping process: (1) sniffing the grounds placed into the cup before pouring water onto the coffee; (2) sniffing the aromas released while breaking the crust; and (3) sniffing the aromas released as the coffee steeps. Specific aromas can be noted under "qualities" and the intensity of the dry, break, and wet aroma aspects noted on the 5-point vertical scales. The score finally given should reflect the preference of all three aspects of a sample's Fragrance/Aroma.

Appendix 3. Explanation of attributes criteria in cupping test of coffee beans (Continued)

No.	Attribute	Explanation
2	Flavor	Flavor represents the coffee's principal character, the "mid-range" notes, in between the first impressions given by the coffee's first aroma and acidity to its final aftertaste. It is a combined impression of all the gustatory (taste bud) sensations and retro-nasal aromas that go from the mouth to nose. The score given for Flavor should account for the intensity, quality and complexity of its combined taste and aroma, experienced when the coffee is slurped into the mouth vigorously so as to involve the entire palate in the evaluation.
3	Aftertaste	Aftertaste is defined as the length of positive flavor (taste and aroma) qualities emanating from the back of the palate and remaining after the coffee is expectorated or swallowed. If the aftertaste were short or unpleasant, a lower score would be given.
4	Acidity	Acidity is often described as "brightness" when favorable or "sour" when unfavorable. At its best, acidity contributes to a coffee's liveliness, sweetness, and fresh- fruit character and is almost immediately experienced and evaluated when the coffee is first slurped into the mouth. Acidity that is overly intense or dominating may be unpleasant, however, and excessive acidity may not be appropriate to the flavor profile of the sample. The final score marked on the horizontal tick-mark scale should reflect the panelist's perceived quality for the Acidity relative to the expected flavor profile based on origin characteristics and/or other factors (degree of roast, intended use, etc.). Coffees expected to be high in Acidity, such as a Kenya coffee, or coffees expected to be low in Acidity, such as a Sumatra coffee, can receive equally high preference scores although their intensity rankings will be quite different.

Appendix 3. Explanation of attributes criteria in cupping test of coffee beans (Continued)

No.	Attribute	Explanation
5	Balance	How all the various aspects of Flavor, Aftertaste, Acidity and Body of the sample work together and complement or contrast to each other is Balance. If the sample is lacking in certain aroma or taste attributes or if some attributes are overpowering, the Balance score would be reduced.
6	Body	The quality of Body is based upon the tactile feeling of the liquid in the mouth, especially as perceived between the tongue and roof of the mouth. Most samples with heavy Body may also receive a high score in terms of quality due to the presence of brew colloids and sucrose. Some samples with lighter Body may also have a pleasant feeling in the mouth, however. Coffees expected to be high in Body, such as a Sumatra coffee, or coffees expected to be low in Body, such as a Mexican coffee, can receive equally high preference scores although their intensity rankings will be quite different.
7	Uniformity	Uniformity refers to consistency of flavor of the different cups of the sample tasted. If the cups taste different, the rating of this aspect would not be as high. 2 points are awarded for each cup displaying this attribute, with a maximum of 10 points if all 5 cups are the same.
8	Clean Cup	Clean Cup refers to a lack of interfering negative impressions from first ingestion to final aftertaste, a "transparency" of cup. In evaluating this attribute, notice the total flavor experience from the time of the initial ingestion to final swallowing or expectoration. Any non-coffee like tastes or aromas will disqualify an individual cup. 2 points are awarded for each cup displaying the attribute of Clean Cup.

Appendix 3. Explanation of attributes criteria in cupping test of coffee beans (Continued)

No.	Attribute	Explanation
9	Sweetness	Sweetness refers to a pleasing fullness of flavor as well as any obvious sweetness and its perception is the result of the presence of certain carbohydrates. The opposite of sweetness in this context is sour, astringency or "green" flavors. This quality may not be directly perceived as in sucrose-laden products such as soft drinks, but will affect other flavor attributes. 2 points are awarded for each cup displaying this attribute for a maximum score of 10 points.
10	Overall	The "overall" scoring aspect is meant to reflect the holistically integrated rating of the sample as perceived by the individual panelist. A sample with many highly pleasant aspects, but not quite "measuring up" would receive a lower rating. A coffee that met expectations as to its character and reflected particular origin flavor qualities would receive a high score. An exemplary example of preferred characteristics not fully reflected in the individual score of the individual attributes might receive an even higher score. This is the step where the panelists make their personal appraisal.

Appendix 3. Explanation of attributes criteria in cupping test of coffee beans (Continued)

No.	Attribute	Explanation
11	Defect	Defects are negative or poor flavors that detract from the quality of the coffee. These are classified in 2 ways. A taint is an off-flavor that is noticeable, but not overwhelming, usually found in the aromatic aspects. A "taint" is given a "2" in intensity. A fault is an off-flavor, usually found in the taste aspects, that is either overwhelming or renders the sample unpalatable and is given an intensity rating of "4". The defect must first be classified (as a taint or a fault), then described (sour, rubbery, ferment, phenolic for example) and the description written down. The number of cups in which the defect was found is then noted, and the intensity of the defect is recorded as either a 2 or 4. The defect score is multiplied and subtracted from the total score according to directions on the cupping form.
12	Final Scoring	The Final Score is calculated by first summing the individual scores given for each of the primary attributes in the box marked "Total Score." Defects are then subtracted from the "Total Score" to arrive at a "Final Score." The following Scoring Key has proven to be a meaningful way to describe the range of coffee quality for the Final Score.

Sumber: *Specialty Coffee Association of America* (2015)

Appendix 4. Fungal population in coffee beans with various treatments during at the beginning of storage, after 2 and 4 months of storage

Treatment	Species	Fungal population (cfu/g wet basis)		
		0 month	2 months	4 months
FH	*Aspergillus flavus*	0	0	3
	Aspergillus niger	2	2	1
	Aspergillus ochraceus	1	0	0
	A. penicillioides	0	0	7
	Aspergillus tamarii	0	1	0
	Cladosporium cladosporioides	3	3	3
	Eurotium chevalieri	330	3	40
	Fusarium avenaceum	8	0	0
	Fusarium sp.	0	11	0
	Penicillium citrinum	9	17	3
	Wallemia sebi	0	0	11
	Yeast A	219	0	38
	Yeast B	43	822	0
	Yeast B2	0	11	3

N = coffee bean with dry processing (natural processing)
F = coffee bean with wet processing (full wash processing)
S = coffee bean with semi wet processing (semi wash processing)
H = coffee bean was packed in polyethylene hermetic bag
P = coffee bean was packed in polypropylene bag

Appendix 4. Fungal population in coffee beans with various treatments during at the beginning of storage, after 2 and 4 months of storage (Continued)

Treatment	Species	Fungal population (cfu/g wet basis)		
		0 month	2 months	4 months
SH	*Aspergillus flavus*	0	2	10
	Aspergillus niger	3	9	4
	A. penicillioides	0	4	1
	Cladosporium cladosporioides	0	7	1
	Eurotium chevalieri	38	7	5
	Fusarium sp.	0	73	0
	Penicillium citrinum	7	7	0
	Yeast A	178	0	0
	Yeast B	9	115	0
	Yeast B2	0	55	2
NH	*Aspergillus flavus*	1	11	2
	Aspergillus niger	1	1	0
	Aspergillus ochraceus	6	0	0
	A. penicillioides	0	2	1
	A. tamarii	0	9	0
	Cladosporium cladosporioides	0	6	4
	Eurotium chevalieri	19	0	2
	Fusarium avenaceum	7	0	0
	Penicillium citrinum	2	2	3
	Yeast A	89	0	0
	Yeast B	8	0	0

N = coffee bean with dry processing (natural processing)
F = coffee bean with wet processing (full wash processing)
S = coffee bean with semi wet processing (semi wash processing)
H = coffee bean was packed in polyethylene hermetic bag
P = coffee bean was packed in polypropylene bag

Appendix 4. Fungal population in coffee beans with various treatments during at the beginning of storage, after 2 and 4 months of storage (Continued)

Treatment	Species	Fungal population (cfu/g wet basis)		
		0 month	2 months	4 months
FP	*Aspergillus flavus*	3	0	1
	Aspergillus niger	3	1	2
	Aspergillus ochraceus	3	1	0
	A. penicillioides	0	3	0
	A. tamarii	0	1	0
	Cladosporium cladosporioides	0	3	2
	Eurotium chevalieri	31	7	10
	Fusarium avenaceum	8	0	0
	Fusarium sp.	0	7	0
	Penicillium citrinum	22	50	0
	Yeast A	584	0	0
	Yeast B	562	20	0
	Yeast D	39	0	0
SP	*Aspergillus niger*	0	2	3
	A. penicillioides	0	4	0
	Cladosporium cladosporioides	1	8	2
	Eurotium chevalieri	17	2	2
	Penicillium citrinum	1	0	1
	Yeast A	112	0	0
	Yeast B	30	24	0
	Yeast B2	0	1	0
	Yeast D	300	0	0

N = coffee bean with dry processing (natural processing)
F = coffee bean with wet processing (full wash processing)
S = coffee bean with semi wet processing (semi wash processing)
H = coffee bean was packed in polyethylene hermetic bag
P = coffee bean was packed in polypropylene bag

Appendix 4. Fungal population in coffee beans with various treatments during at the beginning of storage, after 2 and 4 months of storage (Continued)

Treatment	Species	Fungal population (cfu/g wet basis)		
		0 month	2 months	4 months
NP	*Aspergillus flavus*	1	7	0
	A. niger	0	1	2
	A. penicillioides	0	7	0
	A. tamarii	0	7	0
	Cladosporium cladosporioides	0	9	2
	Eurotium chevalieri	11	0	3
	Fusarium avenaceum	1	0	0
	Penicillium citrinum	7	1	0
	Yeast A	152	0	0
	Yeast B	8	0	0
	Yeast D	12	0	0

N = coffee bean with dry processing (natural processing)
F = coffee bean with wet processing (full wash processing)
S = coffee bean with semi wet processing (semi wash processing)
H = coffee bean was packed in polyethylene hermetic bag
P = coffee bean was packed in polypropylene bag

Appendix 5. Analyses of variance on the effects of processing, packaging materials and duration of storage on the moisture content in coffee beans

Source of variance	**df**	**SS**	**MS**	**F-value**
Processing (A)	2	2.69148148	1.34574074	62.65**
Packaging material (B)	1	18.49185185	18.49185185	860.83**
Duration of storage (C)	2	9.52259259	4.76129630	34.41**
AB	2	1.47814815	0.73907407	221.65**
AC	4	0.03629630	0.00907407	0.42
BC	2	4.34481481	2.17240741	101.13**
ABC	4	0.25185185	0.06296296	2.93*
Error	36	0.77333333	0.02148148	

* Significantly different at 95% confidence level
** Very significantly different at 99% confidence level

Appendix 6. Analyses of variance on the effects of processing, packaging materials, and duration of storage on the **population of *Aspergillus niger*** in coffee beans with transformation log (x+1)

Source of variance	df	SS	MS	F-value
Processing (A)	2	1.03444444	0.51722222	4.71*
Packaging material (B)	1	0.42666667	0.42666667	3.89
Duration of storage (C)	2	0.28000000	0.14000000	1.27
AB	2	0.68777778	0.34388889	3.13
AC	4	0.16888889	0.04222222	0.38
BC	2	0.65333333	0.32666667	2.97
ABC	4	0.05555556	0.01388889	0.13
Error	36	3.95333333	0.10981481	

* Significantly different at 95% confidence level

** Very significantly different at 99% confidence level

Appendix 7. Analyses of variance on the effects of processing, packaging materials, and duration of storage on the **total yeast population** in coffee beans with transformation log (x+1)

Source of variance	df	SS	MS	F-value
Processing (A)	2	6.62925926	3.31462963	8.96**
Packaging material (B)	1	0.28166667	0.28166667	0.76
Duration of storage (C)	2	35.06481481	17.53240741	47.41**
AB	2	3.49000000	1.74500000	4.72*
AC	4	2.02296296	0.50574074	1.37
BC	2	2.19444444	1.09722222	2.97
ABC	4	4.63555556	1.15888889	3.13*
Error	36	13.31333333	0.36981481	

* Significantly different at 95% confidence level

** Very significantly different at 99% confidence level

TABLES

Table 1. Moisture content of coffee beans in each stage of processing

Kind of sample	Moisture content (%)
Wet cherry-N	49.92
Dried cherry-N	10.28
Wet green bean-F	49.69
Dried green bean-F	8.09
Dried green bean after 1 day-S	42.73

Notes :

N = coffee bean with dry processing (natural processing)

F = coffee bean with wet processing (full wash processing)

S = coffee bean with semi wet processing (semi wash processing)

Table 2. Fungal population in coffee beans with various stage of processing

No.	Kind of sample	Fungi	Fungal population (cfu/g wet basis)
1	Wet cherry-N	*Acremonium* sp.	$2,3 \times 10^2$
		Geotrichum candidum	3.3×10
		Yeast A	5.4×10^3
		Yeast B	$9,0 \times 10^2$
2	Dried cherry-N	*Eurotium chevalieri*	$2,1 \times 10^3$
		Yeast A	$1,0 \times 10^3$
		Yeast B	$8,7 \times 10^2$
3	Wet green bean-F	*Acremonium* sp	$5,0 \times 10^2$
		Geotrichum candidum	$4,3 \times 10^2$
		Yeast A	$4,9 \times 10^3$

Notes :
N = coffee bean with dry processing (natural processing)
F = coffee bean with wet processing (full wash processing)
S = coffee bean with semi wet processing (semi wash processing)

Table 2. Fungal population in coffee beans with various stage of processing (Continued)

No.	Sample	Fungi	Fungal population (cfu/g wet basis)
4	Dried green bean-F	*Aspergillus flavus*	1,3 x 10
		A. niger	1,0 x 10
		A. ochraceus	1,3 x 10
		Acremonium sp.	1,3 x 10^3
		Fusarium solani	6,7 x 10
		Mucor sp	3,0 x 10^2
		Penicillium sp. 1	1,3 x 10^3
		Penicillium sp. 3	3.0 x 10^2
		Isolate sp 5	6,7 x 10
		Isolate sp 7	2,7 x 10^2
		Yeast A	1,2 x 10^4
		Geotrichum candidum	3,3 x 10^2
5	Dried green bean after 1 day-S	*Penicillium* sp 2	1,4 x 10^3
		Yeast A	9,3 x 10^3
		Yeast B	1,2 x 10^3
		Isolate sp 9	6,0 x 10^2

Notes :

N = coffee bean with dry processing (natural processing)

F = coffee bean with wet processing (full wash processing)

S = coffee bean with semi wet processing (semi wash processing)

Table 3. Moisture content of coffee beans with various treatments at the beginning of storage, after 2 and 4 months of storage

Processing	Moisture content (%)					
	0 month		2 months		4 months	
	Polyethylene hermetic bag	Polypropylene bag	Polyethylene hermetic bag	Polypropylene bag	Polyethylene hermetic bag	Polypropylene bag
Natural process / dry (N)	9.5 ± 0.1 be	9.6 ± 0.1 bc	9.8 ± 0.1 c	10.4 ± 0.3 d	9.8 ± 0.2 c	11.2 ± 0.0 h
Full wash / wet (F)	8.7 ± 0.2 a	9.2 ± 0.1 ef	8.8 ± 0.1 a	10.3 ± 0.1 d	8.9 ± 0.2 ag	11.2 ± 0.2 h
Semi wash / semi wet (S)	8.8 ± 0.1 a	9.6 ± 0.1 bc	9.1 ± 0.1 fg	10.3 ± 0.1 d	9.2 ± 0.3 ef	11.2 ± 0.1 h

Notes :

N = coffee bean with dry processing (natural processing)

F = coffee bean with wet processing (full wash processing)

S = coffee bean with semi wet processing (semi wash processing)

0 = coffee bean at the beginning of storage

2 = coffee bean after 2 months of storage

4 = coffee bean after 4 months of storage

Table 4. Range and mean of temperature and relative humidity in the warehouse for storing of coffee beans during storage

Duration of storage (months)	Range and mean of temperature (^oC)	Range and mean of relative humidity (%)
0 - 2	(24.8 – 29.3) 28.2 ± 0.7	(59.0 – 77.4) 67.8 ± 4.5
2 - 4	(26.5 – 29.8) 28.6 ± 0.7	(60.0 – 78.3) 69.7 ± 5.1

Table 5. Grade of physical quality in coffee beans with various treatments at the beginning of storage, after 2 and 4 months of storage, based on defective value

Treatment	Total defective value			Grade
	0 month	2 months	4 months	
NH	3	6	8	1
NP	1	6	9	1
FH	4	7	8	1
FP	5	7	8	1
SH	5	8	9	1
SP	5	8	10	1

Notes :

N = coffee bean with dry processing (natural processing)

F = coffee bean with wet processing (full wash processing)

S = coffee bean with semi wet processing (semi wash processing)

H = coffee bean was packed in polyethylene hermetic bag

P = coffee bean was packed in polypropylene bag

Table 6. Result of cupping test in coffee beans with various treatments after 2 and 4 months of storage

Attribute*)	*Score*											
	FH		FP		SH		SP		NH		NP	
	2 mnt	4 mnt	2 mnt	4 mnt	2 mnt	4 mnt	2 mnt	4 mnt	2 mnt	4 mnt	2 mnt	4 mnt
Fragrance	7.75	7.5	7.75	7.75	7.5	7.5	7.5	7.5	7.75	7.75	7.5	7.5
Flavor	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.25	8	7.75	7.75	7.75
Aftertaste	7.25	7.25	7.5	7.5	7.5	7.5	7.5	7.5	7.75	7.75	7.5	7.5
Acidity	7.75	7.5	7.75	7.75	7.75	7.5	7.5	7.25	7.75	7.75	7.75	7.75
Body	7.75	7.75	7.75	7.75	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Balance	7.5	7.5	7.75	7.75	7.5	7.5	7.5	7.5	7.75	7.75	7.5	7.5
Uniformity	10	10	10	10	10	10	10	10	10	10	10	10
Clean Cup	10	10	10	10	10	10	10	10	10	10	10	10
Sweetness	10	10	10	10	10	10	10	10	10	10	10	10
Overall	7.75	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Total score	83.25	82.5	83.5	83.5	82.75	82.5	82.5	82.5	84	83.75	83	83
Defect	0	0	0	0	0	0	0	0	0	0	0	0
Final score	83.25	82.5	83.5	83.5	82.75	82.5	82.5	82.5	84	83.75	83	83

Tester Laboratory : *Coffee Laboratory* PT Kemenady Industri Mandiri, Bogor
* keterangan skor : 0= Not present, 1= Unacceptable, 2= Verry Poor, 3= Poor, 4=Fair, 5= Average, 6= good, 7= Verry good, 8= Excellent, 9= Outstanding, 10= Exceptional
** Specialty grade ≥ 80
*) The explanation can be seen in Appendix 3

Notes :

N = coffee bean with dry processing (natural processing)

F = coffee bean with wet processing (full wash processing)

S = coffee bean with semi wet processing (semi wash processing)

H = coffee bean was packed in polyethylene hermetic bag

P = coffee bean was packed in polypropylene bag

Table 7. Population of *A. niger* in coffee beans with various processing methods

Processing method	Population of *A. niger* (cfu/g wet basis)
Natural process / dry (N)	1 (1 – 2) b
Full wash / wet (F)	2 (1 – 3) ab
Semi wash / semi wet (S)	4 (2 – 9) a

Notes :

N = coffee bean with dry processing (natural processing)

F = coffee bean with wet processing (full wash processing)

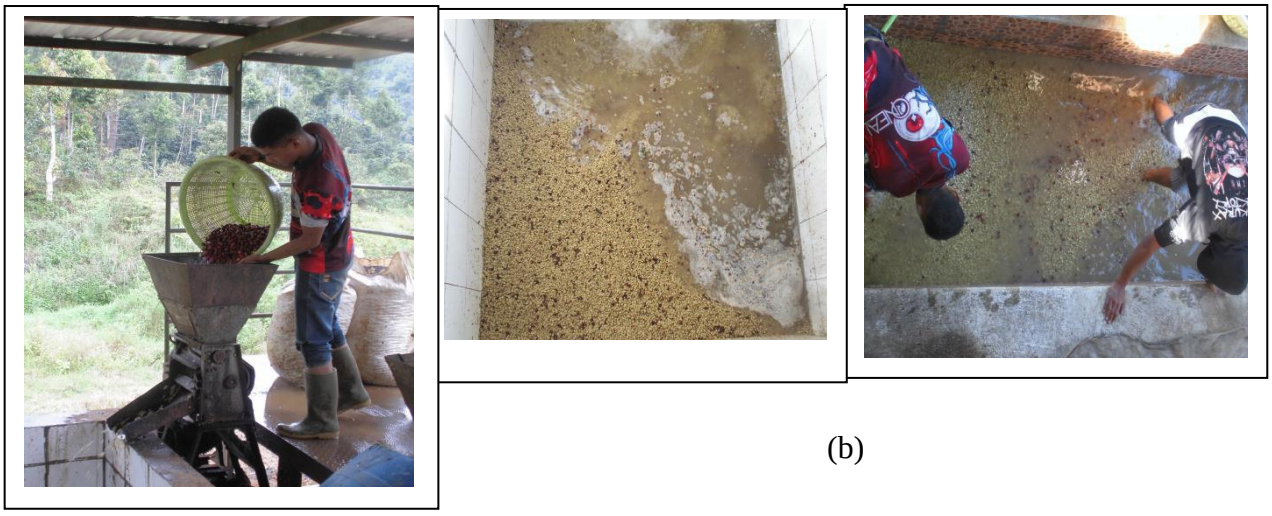
S = coffee bean with semi wet processing (semi wash processing)

Table 8. Total yeast population in coffee beans caused by various treatments

Processing method	Populasi total khamir (*cfu*/g berat basah)					
	0 month		2 months		4 months	
	Polyethylene hermetic bag	Polypropylene bag	Polyethylene hermetic bag	Polypropylene bag	Polyethylene hermetic bag	Polypropylene bag
Natural process / dry (N)	97 (30-174) abcd	152 (40-370) abcd	0 f	0 f	0 f	0 f
Full wash / wet (F)	262 (33-533) a	1 185 (397-1683) abcd	534 (213-1 143) ab	24 (3-60) def	39 (10-57) bcd	0 f
Semi wash / semi wet (S)	187 (120-283) abcd	442 (136-986) abc	170 (40-387) abcd	25 (17-37) cde	2 (0-7) ef	0 f

FIGURES

Figure 1. Drying of coffee beans with natural processing



(a)

(b)

Figure 2. (a) Shelling of cherry beans and (b) fermentation and washing of green beans with hull in full wash and semi wash processing.

Figure 3. Drying of wet green beans with hulls in full wash and semi wash processing.

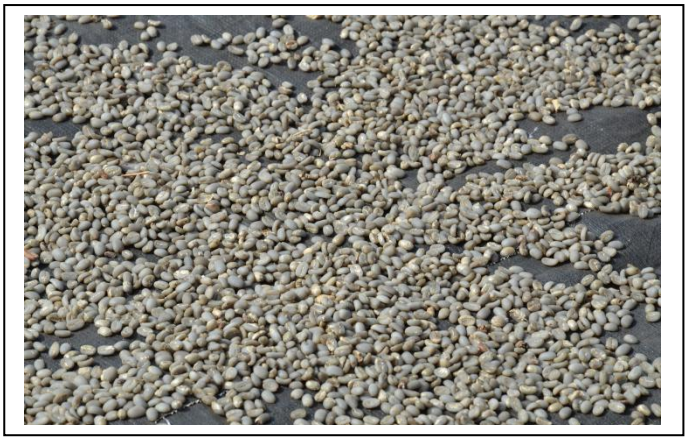


Figure 4. Drying of green beans in semi wash processing

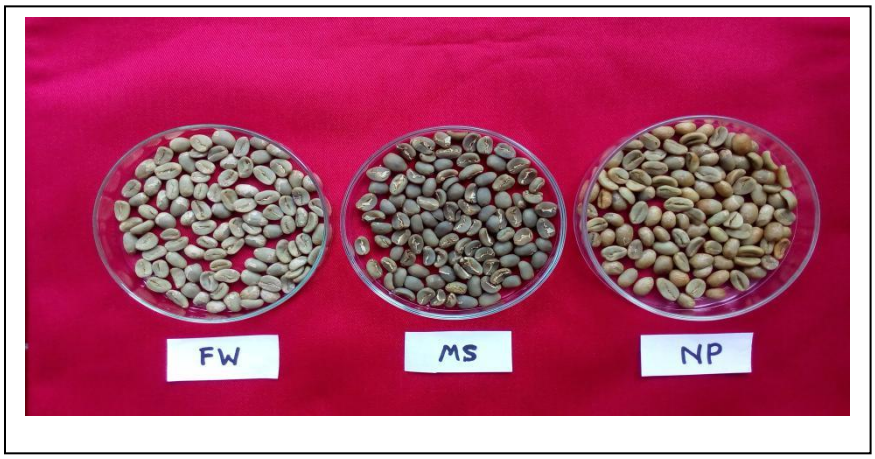


Figure 5. Coffee beans with various processing methods.

Figure 6. Storing of coffee beans were packed in polyethylene hermetic bags and polypropylene bags in the warehouse condition.