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Effects of the Different Postharvest Processing Methods on the Occurrence of Ochratoxin A and Cupping Quality of Arabica Coffee

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Abstract

Postharvest processing methods of Arabica coffee affect the occurrence of fungal contaminants and green bean qualities. The common fungal contaminants on parchment coffee from the wet and honey methods and dried berries from the dry method were *Cladosporium cladosporioides, Fusarium oxysporum, Fusarium xylarioides,* and *Penicillium* spp. *Saccharomyces cerevisiae* was associated only to the wet and honey method, while *Aspergillus niger* was found only on dried berries. The remaining contaminants of green coffee beans were *Cladosporium cladosporioides, Saccharomyces cerevisiae,* and *Penicillium* spp. from wet method; *Saccharomyces cerevisiae* and *Penicillium* spp. on the honey method; and *Cladosporium cladosporioides, Fusarium oxysporum,* and *Fusarium xylarioides* on the dry method. Ochratoxin A contamination was detected only on the dry berries from the dry method. The wet and honey processed coffee attained the specialty quality standard, a superior overall cup quality than the dry-processed coffee. Moreover, the financial analysis revealed that higher returns could be obtained following the wet or honey process.

KEYWORDS

postharvest processing
microbial contamination
cup quality
financial profitability

Introduction

Arabica coffee (*Coffea arabica*) grows productively, mostly in the highlands of Benguet, owing to the suitable climate and elevation. Arabica coffee yields better in elevations of 900 to 1,200 meters above sea level (masl) unlike the other coffee species such as *C. canephora* (Robusta), *C. excelsa,* and *C. liberica* with optimum yields on elevations as low as 600 masl. The Philippines was once an exporter of coffee, but various barriers in the value chain caused the industry to decline. With the renewed goal to put the country back on the global coffee map, the government, through the Department of Agriculture (DA), classified coffee under the high-value crop development program. Thus, the coffee industry has gained attention with the vibrant support from the government by providing logistics and with the cooperation of other coffee stakeholders. This increased interest led to the crafting of the Philippine Coffee Industry Roadmap 2017-2022 with the end goal of achieving self-sufficiency. Added to this is the ambition of the coffee industry to be sustainable and globally competitive in terms of quality standards.

However, the Philippine coffee industry's revitalization posed challenges in both the production and postharvest processing sectors. Among the postharvest side's identified problems

are inadequate postharvest facilities or equipment coupled with coffee growers' improper practices during postharvest processing. These factors greatly contribute to the diminishment of the quality of coffee. In Benguet, coffee drying is mostly done through sun drying by spreading the coffee on sacks laid over pavement or on concrete roads, galvanized iron sheets, or in winnows placed over their house roofs (Tad-awan et al., 2013). These practices are laborious and pose other drawbacks such as uneven drying and prolonged drying, which may lead to over fermentation that could adversely affect the coffee flavor. Moreover, re-wetting is possible due to sudden rains and high humidity in some areas that favor growth of molds leading to deterioration and unfavorable flavors that negatively affect coffee cup quality. The current postharvest processing practices employed by coffee-growers in the locality need to be evaluated because few studies have been done to assess mycotoxin contamination of Arabica coffee beans and their impacts on coffee cup quality. Ochratoxin A (OTA), a mycotoxin, is a secondary metabolite produced by several fungal species, particularly *Aspergillus* and *Penicillium*. It is a contaminant in foods such as cereals, beans, dried fruits, and in beverages like beer, wine, and coffee. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2001 reported that OTA causes renal toxicity, nephropathy, and immunosuppression in several animal species (Walker & Larsen, 2005). The International Agency for Research on Cancer classified OTA as possibly carcinogenic to humans (Bureau of Agriculture and Fisheries Standards [BAFS], 2015). This concern is important because, despite the many processes it undergoes, coffee is ultimately appreciated or valued in its cup or beverage form. This study's results could be used as a basis for recommending appropriate practices in reducing mycotoxin contamination and enhancing the cup quality of Arabica coffee grown in the region. Thus, the study aimed to assess the different postharvest processing methods and their effects on mycotoxin contamination, cup quality, and financial profitability.

Materials and Methods

Processing Methods

This study used Red Bourbon variety of Arabica coffee grown under Benguet pine (*Pinus kesiya* Royle x Gordon) at the Benguet State University farm located at 1,300 meters above sea level. Selective picking or priming was employed to harvest ripe berries. The berries were manually sorted to select sound berries and weighed 10kg of berries for each postharvest processing method.

In the wet process, the berries were depulped using a mechanical depulper that result into fresh parchment coffee form. Fresh parchment was soaked in clean water for 36 hours to ferment, spread in drying beds. In the honey process, the berries were depulped into fresh parchment coffee and were immediately subjected to sun drying. For the dry process, the ripe berries were immediately exposed to sun drying.

Drying Structure

Drying was done inside a greenhouse-type structure measuring 18.35 meters long, 4 meters wide, and 3.5 meters high. The roof was UV-treated clear polyethylene with 0.04mm thickness, and the side walls were woven black net. Inside were drying beds made of wooden frames at three meters long, one meter wide, and 0.76 meter-high with perforated aluminum trays. Parchment coffee from the wet and honey process and berries from the dry method were separately spread over the drying trays.

Isolation and Identification of Microbial Contaminants

Fifty-gram samples of dried parchment coffee and dried berries and their green coffee bean (GCB) form were aseptically collected, placed in polyethylene zip lock pouches then brought to the BSU-Plant Health Clinic for the isolation of microbial contaminants. The samples were sterilized with 10% sodium hypochlorite (NaClO) for 30 seconds, rinsed three times with sterile distilled water, blot-dried on sterile tissue paper, and were aseptically transferred on the prepared Potato Dextrose Agar (PDA). Four whole coffee samples per treatment were loaded per plate with three replications. The Petri plates were sealed with parafilm and incubated for 5-7 days at 25 to 28ºC. Distinctive colony growths were separately sub-cultured on PDA while Malt Extract Agar (MEA) for yeast. Isolates were identified based on cultural and morphological characteristics. Cultural features were the colony color at the top and bottom of culture media. Morphological features were the septation of hyphae and fruiting

structures, shape, color, and size of conidia cell. Identification of the isolates were based on previous works of authors for specific microbial isolates. Identification of *Aspergillus* spp. followed the procedures of Klich (2003); *Cladosporium* sp. by Braun and Schubert (2007); *Fussarium* spp. by Leslie and Summerell (2006); and *Penicillium* spp. based on the key published by Frisvad and Samson (2004). Identification of yeast were based on morphological standard recommended by Kurtzman et al. (2011). Photo documentation of the isolates in pure culture and microscopic structures was done.

Ochratoxin A (OTA) Analysis

A sample of 100 grams each of parchment, dried berries and GCB were aseptically placed in polyethylene bags then sealed. These were submitted to the Regional Feed Chemical Analysis Laboratory of the Department of Agriculture-Cordillera Administrative Region (DA-CAR) for the OTA analysis. The analysis was done through enzyme-linked immunosorbent assay (ELISA) following prescribed procedures of Veratox kits for Ochratoxin®, Neogen® Corporation US. The limit of detection for Neogen® Veratox® is at 1.0 ppb.

Cup Quality Evaluation

Two kilograms of GCB from each treatment were roasted at a light level of roast using a hot air-type coffee roasting machine. The coffee cup quality evaluation was performed following the established Specialty Coffee Association (SCA) cupping protocol. A panel of three Q-graders conducted the cupping evaluation. The samples were scored in terms of fragrance/aroma, flavor, acidity, body, and balance. The descriptive and numerical scale of scores are as follows: Good: 6.00 to 6.75; Very Good: 7.00 to 7.75; Excellent: 8.00 to 8.75; and, Outstanding: 9.00 to 9.75.

Financial Analysis

The gross margin analysis was used to determine the relative profitability of postharvest processing methods. Gross margin is the difference between the total revenue or gross income and the variable production and processing costs incurred in producing the berries. For this study, unit of analysis was per 10kg fresh coffee berries.

Experimental Design and Statistical Analysis

The drying process of the postharvest processing methods were arranged in a completely randomized design with three replications. Quantitative data were subjected to analysis of variance (ANOVA), and significant means were separated by LSD at 5% using GenStat 15th Edition software.

Results and Discussion

Moisture Content, Days to Drying and Daily Moisture Loss

Before sun drying, the moisture content (MC) of the treatments were determined. The MC of fresh parchment coffee from the honey process was 59.67% and 52% from the wet process. These were similar to the technical data from the French Agricultural Research Centre for International Development (CIRAD) where the MC of Arabica coffee fresh berry is 65%, while the washed parchment coffee is 55% (Gosh & Venkatachalapathy, 2014).

Highly significant differences ($P>0.001$) were recorded in the duration of drying, with the wet and honey process being completed after six (6) days while 16 days for the dry process (Table 1). Similarly, daily moisture loss was significantly high ($P>0.001$) with the honey method having 3.63% per day followed by the wet process (2.84%) and dry process with 1.32%. The long drying period of the dry process could be attributed to the presence of the pulp, which accounts to 43.2% of the total weight and contains 77% moisture, and 4.9% is the mucilage (Bressani et al., 1972). Further, the results did not differ much from previous reports that other factors affecting sun-drying of coffee depend largely on climatic conditions and that it takes 7-15 days for parchment coffee, while 12-21 days for berries (Ghosh & Venkatachalapathy, 2014).

Isolated and Identified Fungal Contaminants

Six fungal isolates associated with the different postharvest processing methods were identified. Fungal isolates associated to parchment coffee from both wet and honey methods were *Cladosporium cladosporioides, Fusarium oxysporum,*

Table 1

Average Drying Duration (Days) and Daily Moisture Loss (%)

Treatment	Drying Duration	Average Daily Moisture Loss
Wet Process	6[a]	2.84[b]
Honey Process	6[a]	3.63[a]
Dry Process	16[b]	1.32[c]

Note: Within columns, means with the same letter are not significantly different at 5% by LSD

Fusarium xylarioides, Penicillium sp., and *Saccharomyces cerevisiae* (Table 2). Moreover, these fungal isolates, along with *Aspergillus niger*, were present on the dried berries from the dry process, except for *S. cerevisiae*. Further, in the GCB, *Fusarium* spp. were not present in the wet process. *Fusarium* spp. and *C. cladosporioides* were also not detected in the honey method, while *Penicillium* sp. and *Aspergillus niger* were eliminated from the GCB from the dry process.

The microorganisms found associated with the different processing methods were identified based on colony growth in artificial media either in test tubes or Petri plates and on microscopic examinations of their structures. The isolate with a black colony on the top and pale yellow on the bottom view and with a hyaline conidiophore stripe and conidial head bearing brown to black globous to subglobose conidia was *Aspergillus niger* (Figure 1a and c). A greyish colony on top and black pigmentation at the bottom view and exhibits hyaline oval to spherical conidia was *Cladosporium cladosporioides* (Figure 2a and c).

An isolate identified as *Fusarium oxysporum* showed white to light pink on top, while pink to violet on the bottom view and with light black sickle-shaped macroconidia that has four to five septations (Figure 3a and c). *Fusarium xlarioides* was distinguished from *F. oxysporum* by white to an orange colony on the top and bottom view but quite similar macroconidia (Figure 4a and c).

Penicillium spp. was identified based on a green colony with white mycelia along its edges on top view and light green to light yellow pigmentation at the bottom view, septated hyaline mycelium bearing conidiophores that give rise to phialides and tiny colorless single-celled conidia varying from globose to ovoid (Figure 5a and c). The isolate with white to cream and smooth colony and hyaline globose to ellipsoidal budding cells or blastoconidia was identified as *Saccharomyces cerevisiae* (Figure 6a and c).

All the fungal isolates identified in this study were also reported in other studies on postharvest processing of coffee. The common yeast genera detected in coffee, which facilitate fermentation on the wet process, include *Saccharomyces, Pichia,*

Table 2

Fungi Associated with the Different Postharvest Processing Methods, and Coffee Forms

Treatment	Coffee Form: Parchment	Coffee Form: Green Beans
Wet Process	*C. cladosporioides, F. xylarioides, Penicillium spp., S. cerevisiae, F. oxysporum*	*C. cladosporioides, S. cerevisiae, Penicillium spp.*
Honey Process	*C. cladosporioides, F. xylarioides, Penicillium spp. S. cerevisiae, F. oxysporum*	*S. cerevisiae, Penicillium spp.*
Dry Process	*A. niger, Penicillium spp. C. cladosporioides, F. oxysporum, F. xylarioides*	*C. cladosporioides, F. xylarioides, F. oxysporum*

Figure 1

Pure Culture Top (a) and (b) Bottom View, and (c) Structure (40x) of Aspergillus niger

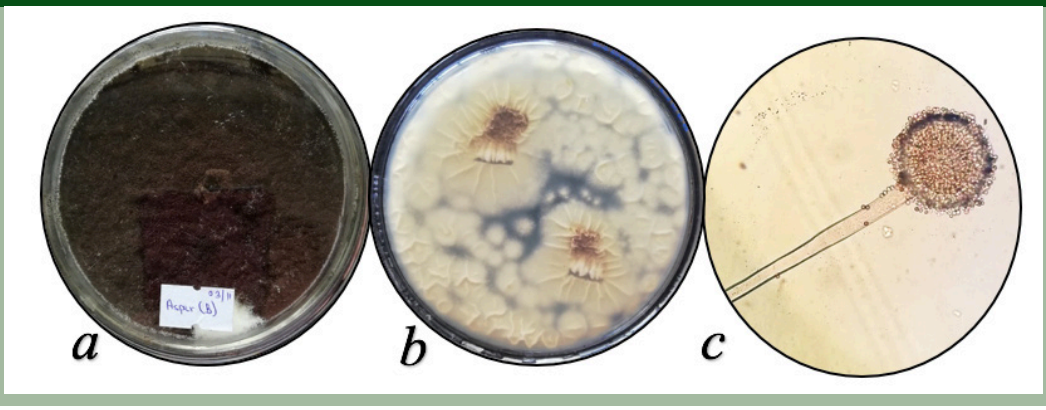


Figure 2

Pure Culture Top (a) and (b) Bottom View, and (c) Structure (40x) of Cladosporium cladosporioides

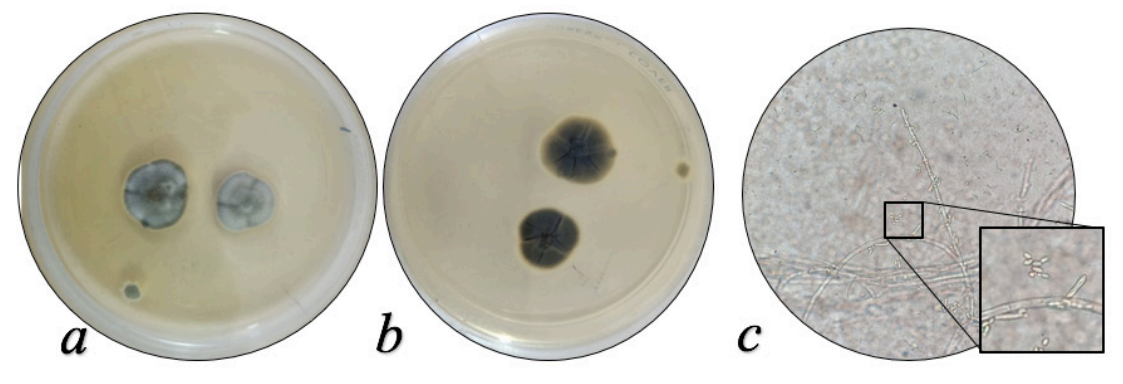


Figure 3

Pure Culture Top (a) and (b) Bottom View, and (c) Structure (40x) of Fusarium oxysporum

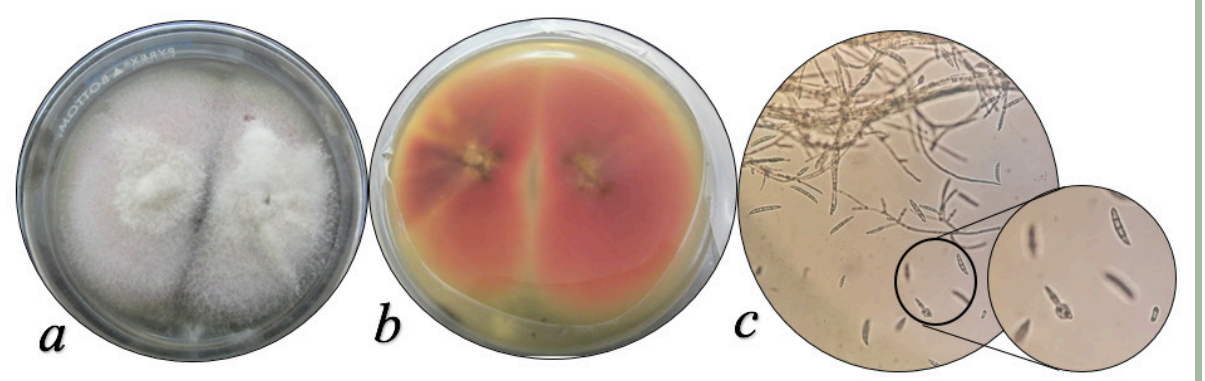


Figure 4

Pure Culture Top (a) and (b) Bottom View, and (c) Structure (40x) of Fusarium oxysporum

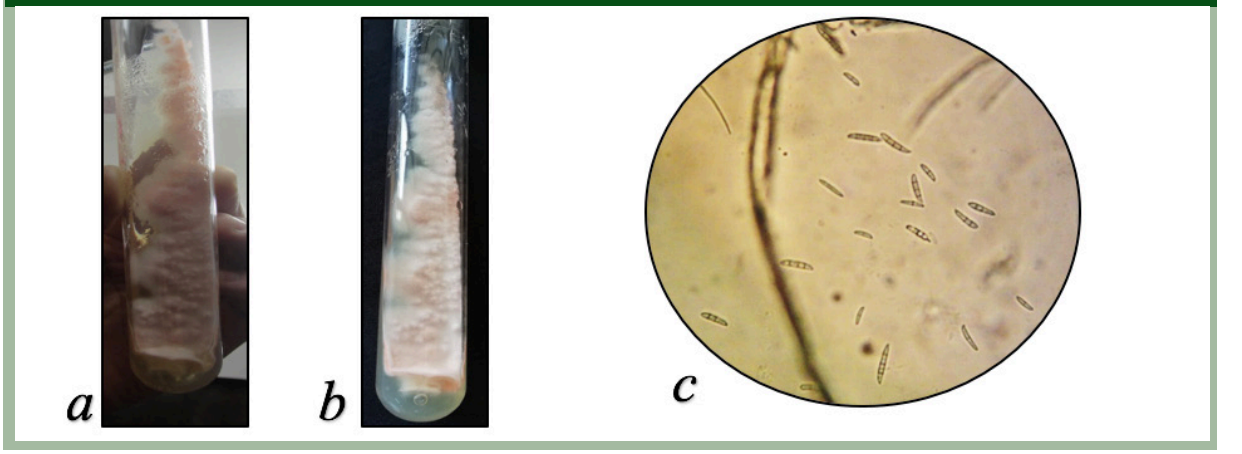


Figure 5

Pure Culture Top (a) and (b) Bottom View, and (c) Structure (40x) of Penicillium spp.

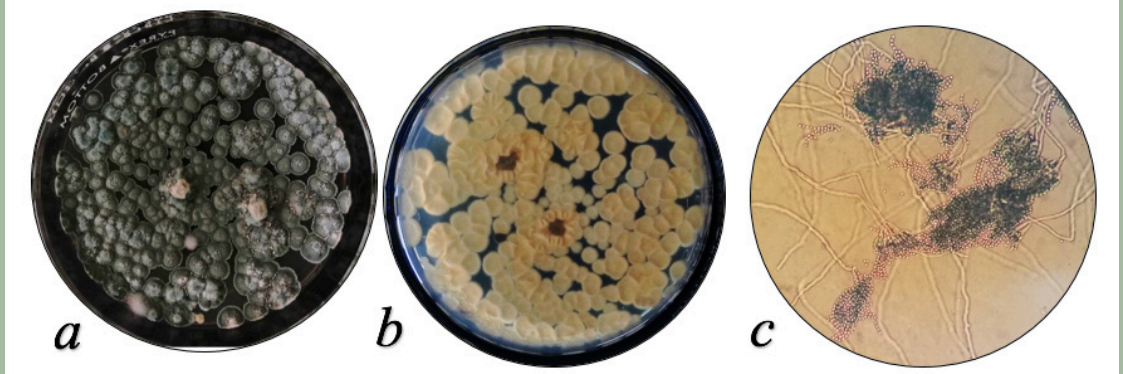
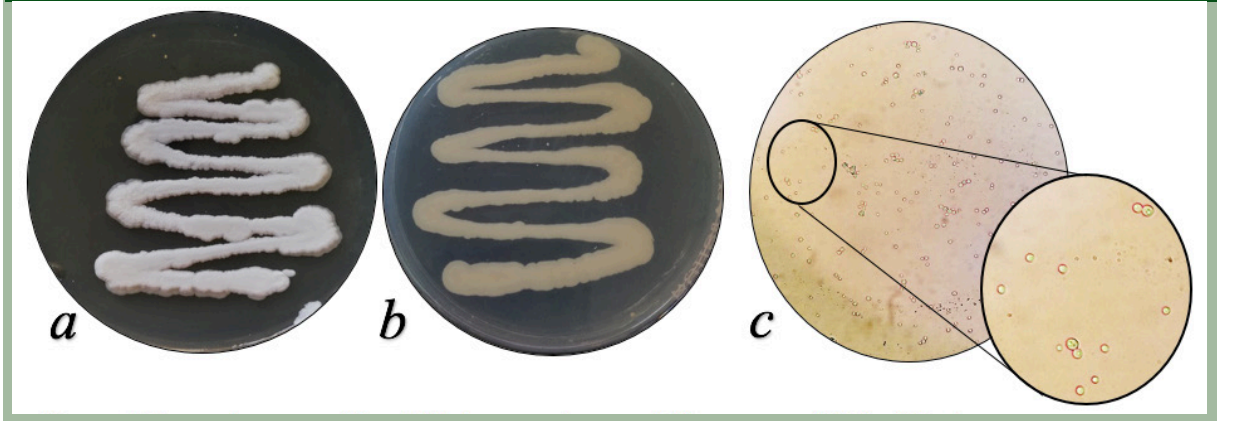


Figure 6

Pure Culture Top (a) and (b) Bottom View, and (c) Structure (40x) of Sacharomyces cerevisiea



Kluyveromyces, and Candida (Masoud et al., 2004; Silva et al., 2000). These yeasts were reported to produce pectinolytic enzymes (Agate & Bhat, 1966). *Penicillium* spp. also were reported for their pectinolytic ability (Mamma et al., 2008). Moreover, diverse filamentous fungi genera such as *Aspergillus, Cladosporium, Fusarium,* and *Penicillium* were reported in coffee berries, the natural fermentation method, and even in coffee beans in storage (Silva et al., 2008).

Ochratoxin A (OTA) Contamination

Both parchment coffee and GCB from the wet and honey process and GCB from the dry process had no OTA contamination. Meanwhile, dried berries from the dry process were positive for OTA contamination at 2.9 ppb (Table 3). The OTA contamination of dried berries is correlated to the presence of *A. niger*, an OTA producing fungus. In this study, the *Penicillium* spp. were presumed to be the non-OTA producing species due to the absence of OTA on samples where they were associated. The result of this study conforms with the findings of Suarez-Quiroz et al. (2004) that the dry process seemed to promote the presence of *A. niger* as compared to the other postharvest processes. Further, Culliao and Barcelo (2015) found fungal and mycotoxins of coffee beans from Benguet with the predominance of *Aspergillus* and OTA was highest in dried whole berries at 193.7 µg/kg. Nevertheless, the OTA level detected in this study (2.9 ppb) was lower than the maximum level for OTA which is 5.0 ppb for food and spices, in reference to the CODEX Standard 193-1995.

Cup Quality

Cup quality of Arabica coffee had highly significant differences ($p=<0.001$) between the postharvest processing methods (Table 4). The honey process had the highest cupping score with 83.25, while the wet-processed coffee had 82.75. Meanwhile, coffee from the dry process scored the lowest with 79.25. The cupping scores of coffees from the wet and honey process were classified as specialty grade coffee based on the SCA cupping protocol. Specialty grade coffee has scores of 80-84.99 (very good), 85-89.99 (excellent), and 90-100 (outstanding), while less than 80.0 are classified as below specialty quality.

The honey process having a significantly higher cupping score over the other processes validates that honey processing methods, which have become common in other countries, can be adopted to meet market requirements for coffee aroma and produce specialty coffees (Poltronieri & Rossi, 2016). Besides, washed coffees also have been known to present better quality, less body, higher acidity, and more aroma than the unwashed coffees (Mazzafera & Padilha-Purcino, 2004). Further, Food and Agriculture Association [FAO] (2010) reported that the wet or washed coffee method produces a superior acidity in the brewed coffee, fewer defects, and a cleaner finish.

Based on the Q-graders' descriptions, honey-processed coffee was described as having a sweet aroma and juicy fragrance, while the flavor had hints of mashed fresh fruits like passion fruit, strawberry, and green apple. Further, it had a heavy and smooth body with a crisp and wine-like brightness, clean and no off odor. On the other hand, the wet-processed coffee had a juicy aroma, but had jackfruit-like and vegetal fragrance; no off flavor was sensed and had hints of mild black tea, and the body is smooth rich and had dried lemon or citrusy acidity. Meanwhile, the dry-processed

Table 3

Ochratoxin A (OTA) Contamination of Coffee from the Different Postharvest Processing Methods

Treatment	Coffee Form	Ochratoxin A (ppb)
Wet Process	*Parchment*	*Below detection limit*
	Green Beans	*Below detection limit*
	Dried Berries	*2.9*
Dry Process	*Green Beans*	*Below detection limit*
	Parchment	*Below detection limit*
Honey Process	*Green Beans*	*Below detection limit*

Note: Maximum level for Ochratoxin A is 5.0 ppb for food and spices (Codex Standard 193-1995)

Table 4

Cup Quality of Arabica Coffee as Influenced by the Different Postharvest Processing Methods

Treatment	Cupping Score	Attributes			
		Fragrance/Aroma	Flavor	Body	After Taste
Wet Process	82.75[b]	Juicy, muscovado, jackfruit, vegetal	Mild black tea	Smooth, rich with dried lemon feel, citrus acidity	Clean
Dry Process	79.25[c]	Sweet, overripe jackfruit	Milky, chocolaty	Creamy and heavy	Tangy, fermented
Honey Process	83.25[a]	Juicy, sweet	Fresh juicy fruits, passion fruit, strawberry, green apple	Smooth and heavy	Fermented fruit, crisp, wine-like, clean

Note: For cupping score, means with the same letter are not significantly different at 5% by LSD

coffee had a sweet fragrance, milky, and chocolaty aroma. However, it had hints of overripe jackfruit fragrance due to the prolonged drying that promoted the beans' fermentation inside the berry during the drying; body is creamy and heavy with lime-like acidity and had fermented and tangy flavors.

Coffee flavor is the most important factor in determining quality (Van Der Vossen, 2009). Positive flavors are described in terms of winey, spicy, and fragrant or floral; while negative or off-flavors are grassy, onion, woody, and earthy as some descriptions (International Trade Center [ITC], 2002).

Financial Analysis

The financial analysis of the three postharvest processing methods was done by calculating the gross margin for each processing method (Table 5). The cost of production and postharvest processing of Arabica coffee was based on the present costs and returns analysis for Arabica coffee under the pine-based Agroforestry system of the Benguet State University. All revenues and costs were computed based on per 10kg berries.

The gross margin is affected much by the selling price per kilogram of GCB products and the cost of processing protocols. The dry process has the highest estimated total cost of production and processing at Php449.29, followed by the wet process at Php382.90, and the lowest is from the honey process at Php342.40. High labor cost in drying coffee in the dry process is attributed to the long duration of attaining the desired 11-12% moisture content. The wet and honey process cuts labor costs in drying. However, the higher cost of the wet process over the honey process is on the added cost of water used for fermentation and washing.

The price per kilogram of GCB was based on prevailing selling and buying price and influenced by the GCB physical quality. Wet and honey processed coffee after sorting are priced at Php400/kg, while dry-processed coffee is sold at Php250/kg. Henceforth, the computed gross margin or estimated income left for the farmer after deducting all variable expenses is highest for the honey method of processing Arabica coffee at Php325.00, followed by the wet process at Php285.10 per 10kg berries. Despite being the simplest method, the dry process gave a negative gross margin, which means the gross income was not enough to cover the variable costs for producing the berries indicating non-profitability. The gross margin percentage further shows that the honey method is relatively more economical than the wet method.

Processing methods influence the pricing of GCB, such that Tiwari (2009) claimed that shifting from dry processing to wet method improved the quality of coffee and increased the income of

Table 5

Gross Margin Analysis of the Different Postharvest Processing Methods

Description	Material Balance of Coffee Processing	Price or Cost (Php/10kg berries), by Processing Method		
		Wet Method	Honey Method	Dry Method
A. Gross Return				
Green coffee bean vol., kg		1.67	1.67	1.67
Green coffee bean price, Php		400.00	400.00	200.00
Total Gross Return		668.00	668.00	334.00
B. Cost				
1. Production Cost of Coffee berry		46.50	46.50	46.50
2. Processing Cost of Green coffee beans	Wet/Honey: Dry Method			
Depulping	10 kg fresh berries	50.00	50.00	
Water Cost		40.50		
Drying	4.5 kg wet parchment 10 kg fresh berries	87.50	87.50	233.19
Dehulling	2.3 kg dry parchment: 3.7 kg dry berries	18.50	18.50	29.60
Sorting	1.9 kg green coffee beans	140.00	140.00	140.00
Total Costs		382.90	342.40	449.29
Gross margin (Php/10kg berries)		285.10	325.60	-115.29
Gross margin (%)		43%	49%	-35%

coffee growers. On the other hand, Subedi (2011) reported that dry processing of coffee in Nepal had a higher benefit-cost ratio than the wet method, but GCB quality was poor due to high MC because of insufficient drying. Thus, wet-processed coffee fetched better pricing and is popular among coffee growers, processors, and exporters.

The financial analysis suggests that it is more profitable for Arabica coffee grown in areas similar to La Trinidad, Benguet conditions to be processed following either the wet or honey process. These methods would provide greater returns per kilogram of GCB. In areas where water is scarce, and the labor requirement is inadequate, the honey method is recommended.

Conclusions

The study results showed that postharvest processing methods, namely dry, wet, and, honey method affected the fungal occurrence and ochratoxin A (OTA) contamination. Fungi found associated with parchment coffee of wet and honey process were *Cladosporium cladosporioides, Fusarium oxysporum, Fusarium xylarioides, Penicillium sp.,* and *Saccharomyces cerevisiae,* while *Aspergillus niger, Cladosporium cladosporioides, Fusarium oxysporum, Fusarium xylarioides,* and *Penicillium sp.* were found on dried berries of the dry process. Meanwhile, OTA was only detected on the dried berries from the dry process but none in its green coffee bean. This detection could be correlated to the presence of *A. niger* that may have contaminated the pulp during harvest. Thus, the dry process, if improperly done, may pose a risk of OTA contamination.

Meanwhile, the wet and honey process produced superior coffee cup quality than the dry process. Finally, the financial analysis indicated that higher returns would be obtained when Arabica coffee is processed either through the wet or honey method.

Recommendations

This study recommends that the wet and honey methods of processing be adapted in Benguet conditions to eliminate ochratoxin A-producing contaminants. In areas with limited safe water for fermentation and washing, the use of the honey method is appropriate.

It is further recommended that a study on isolation of microbial contaminants before processing be done to determine the sources of contaminants. Moreover, contaminants identified only to the genus level be identified using molecular parameters, especially those genera with species known to be producing mycotoxins.

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