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Fungal Contaminants and Cup Quality of Stored Roasted Arabica Coffee

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Abstract

To ensure safe and quality Arabica coffee products for consumption, prevention of microbial contamination during processing has to be guaranteed including appropriate storage practices and determining the ideal storage duration. However, there is a dearth of local information on the quality of roasted Arabica coffee and fungal contaminations as a result of storage. This study analyzed the effect of storage durations on the occurrence of fungal contaminants and its influence on the cup quality of roasted Arabica coffee beans. Wet processed green coffee beans of the Bourbon Red variety were roasted at medium level, packed at 250g in a vacuum metalized polyethylene terephthalate (VMPET) coffee pouches fitted with a one-way valve then stored for 24 months. Results show that after roasting, the coffee beans were free of fungal contaminants but these were detected after 3 months of storage. This implies that contamination could have occurred during the packaging of roasted coffee beans. Five fungal genera were isolated dominated by *Penicillium sp.*, and *Cladosporium sp.* while fungal genera identified at the species level were *Cladosporium cladosporioides* and *Saccharomyces cerevisiae*. Meanwhile, the desirable attributes of the roasted coffee lasted for 5 months of storage. The aroma was the earliest to fade while flavor exhibited slow but gradual waning. Based on these results, it is recommended that roasted Arabica coffee beans be stored using VMPET packaging material for 5 months. Also, processors should conform to Good Manufacturing Practices, specifically hygienic practices during handling and packing of roasted coffee to prevent fungal contamination. Finally, the best-before date or date of roasting should be indicated on the packaging labels of roasted coffee products.

Introduction

Coffee consumers today look for either organically produced or specialty coffee. These types of coffee have one requirement - quality. The Department of Agriculture-CAR placed Arabica coffee in the top ten high-value crops mainly to address the constraints in the production, postharvest processing, and marketing of this commodity. There were identified gaps in producing quality coffee from production to postharvest processing methods. However, less emphasis was given to maintaining quality coffee during storage, particularly roasted coffee beans.

Presently, coffee product processing and

development is not solely done by processors but also by coffee-grower groups who have access to or own processing facilities. As observed at the BSU-IHFSA coffee processing facility, local coffee processors/traders who avail of roasting services transport and store their roasted coffee beans using packaging materials such as polyethylene bags, plastic pails with covers, paper bags, or netted sacks. In Benguet and Mountain Province roasted coffees are sold in local hotels, restaurants, and public markets. Retailers at the markets use glass boxes to display roasted coffee beans while polyethylene wrapped in paper bag is used for sold ground coffee. These practices pose concern because of the potential exposure of the roasted coffee to contaminants including microbes. Due to the COVID-19 pandemic, most of the coffee retail businesses were affected and had shifted to online or off-store marketing. This led to the use low-cost paper and polyethylene laminated or aluminum-based pouches. Thus, roasted coffee had to be packed and stored using various types of packaging materials for ease in transporting the roasted products. This also made the consumers to be aware and inquisitive on where and how their cup of coffee is produced. In addition, questions on how long the quality of roasted coffee lasts persist because most packed coffee products sold locally do not have best-before dates on their labels.

Thus, there is a need to evaluate the effect of storage duration on the quality of roasted coffee due to the lack of studies done locally. In addition, assessing microbial contamination of stored coffee products is necessary because cross-contamination during post-harvest, processing, and storage is reported to occur. Thus, the result of this study shall guide to processors to further improve means on preserving the quality of their coffee products and for consumers to be knowledgeable in obtaining quality coffee. Hence, this study aimed to assess the effect of storage on the occurrence of microbial contamination and cup quality of roasted arabica coffee.

Materials and Methods

Arabica coffee berries of the 'Bourbon Red' variety were processed by a wet or washed method which involves depulping of the fresh berries, fermentation in the water of parchment coffee, washing then sun-drying until the green coffee beans (GCB) attain 9-12% moisture content. Later, the parchment coffee was dehulled to obtain the GCB and then sorted to remove defects. Then the GCB was uniformly roasted (medium roast) at 200ºC for 24 minutes using a 600g-capacity sample roaster. The roasted coffee beans (RCB) were allowed to de-gas for 24 hours before being packed in Vacuum Metalized Polyethylene Terephthalate (VMPET) packaging material fitted with a one-way valve. The packed RCBs were stored in the dark cupboards for 24 months. Sampling was intended to be done at monthly intervals but due to COVID-19 restrictions, it was done instead at 3, 5, 10, 15, 20, and 24 months of storage.

The room temperature and relative humidity were monitored during the storage period. The recorded mean room temperature was 24.5ºC with minimum at 21.4ºC and while maximum of 25.5ºC. Meanwhile, the mean relative humidity was 76.4% with a minimum of 45.6% while the highest recorded was 86.6%.

Final Weight

The RCB was packed at 250g in Vacuum Metalized Polyethylene Terephthalate (VMPET) type packaging materials fitted with one-way valves (ZPWV-K009 Coffee Pouch). Final weights of the RCB were obtained using a digital weighing scale after 3, 5, 10, 15, 20, and 24 months of storage.

Determination of Fungal Contaminants

Isolation of microorganisms from coffee samples into culture media was done at the BSU-Plant Health Clinic. Isolated microorganisms was observed from 2 to 7 days of incubation. Microbial growth or structures were observed using a compound microscope and were subcultured separately into pure culture for identification. Potato Dextrose Agar media was used for filamentous fungal isolates, while Malt Extract Agar for unicellular fungus like yeast.

Identification of Associated Fungi

The identification of isolated fungi to the genus and species level were done using as reference the cultural and morphological characteristics as described by the following authors: *Penicillium spp*. by Frisvad and Samson (2004), Pitt (2000), Raper and Thom (1949), Thom (1930); while the works of Kurtzman et al. (2011), Martini and Martini (1993), Kreger-van-Rij (1984) for yeast.

Cup Quality

The coffee cup quality evaluation or referred to as coffee cupping was performed following the established Specialty Coffee Association (SCA) cupping protocol. The protocol recommends standards and a set of guidelines to accurately evaluate the quality of coffee. It contains the necessary equipment, forms, and other paperwork, cupping environment conditions, preparation of coffee samples, sample evaluation, and description of procedures. Further, it provides guide on assigning individual scores of positive attributes namely, Fragrance/Aroma, Flavor, Aftertaste, Acidity, Body, Balance, Sweetness, Uniformity, and Cleanliness and Overall using a quality scale: Good (6.0-6.75); Very Good (7.0-7.75); Fine (8.0-8.75); and, Outstanding (9.0-9.75). The final score is calculated by summing the individual scores given for each attribute and then subtracting any defects to arrive at a final score. The final score and its quality classification are as follows: <80 and below = below specialty (not specialty); 80 to 84.99 = very good (specialty); 85 to 89.99 = excellent (specialty); 90 to 100 = outstanding (specialty). A panel of three (3) trained cuppers and a Q-grader conducted the cupping.

Design and Data Analysis

The experiment was arranged following the completely randomized design with four replications. Quantitative data were subjected to analysis of variance (ANOVA) at 5% level of significance and significant means were separated by Fisher's protected LSD at 5% probability using GenStat 15th edition software.

Results and Discussion

Fungal Contaminants

After roasting, no fungal contaminants were isolated from the coffee beans. However, mycological analysis revealed that RCB samples obtained beginning from three (3) months of storage were contaminated with fungi of several different genera (Table 1). The highest percent contamination (83%) was recorded from samples obtained at 15 months of storage. The result affirms the previous findings that roasting eliminates microbial contaminants. Alvindia and Acda (2010) reported that the roasting process at 218ºC for 30 minutes decreased the total fungal load in coffee beans by 93-97%. Basalong et al. (2023) also found fungal species *Saccharomyces cerevisiae* and *Penicillium sp.* in the green coffee beans but these were not detected after roasting. The result further shows that despite the use of good packaging material, the growth of fungal contaminants could arise in the stored RCB. It confirms the findings that contamination can still occur at different stages of coffee growing, harvesting, processing, transport, and storage as a result of improper harvesting procedures, precarious drying, and inadequate storage conditions combined with unfavorable climate (Noonim et al., 2008; Taniwaki et al., 2003).

Table 1

Total Fungal Count from the Different Storage Durations

Months	Number of Contaminated Samples (N=12)	Percent of Contaminated Sample, %
0	0	0
3	7	58
5	8	67
10	8	67
15	10	83
20	7	58
24	8	67

At least five (5) fungal genera were isolated from the stored RCB at different storage periods (Table 2). Of the 52 total isolates, the highest contaminants found were *Penicillium sp.* (40%) followed by *Cladosporium sp.* (33%), and *Saccharomyces sp.* (11). *Mucor sp.* and *Rhizopus sp.* were also detected but at lower counts. Two fungal contaminants were identified to the species level namely, *Cladosporium cladosporioides* and *Saccharomyces cerevisiae*. *Penicillium, Mucor* and *Rhizopus* species were difficult to differentiate through cultural and morphological means only; thus, these isolates were not identified to the species level. The isolation of these fungi from stored RCB pose a risk, especially *Penicillium sp.*, because it is known to produce mycotoxins harmful to humans with compromised immune systems. Although the mere presence of a

toxigenic fungi does not automatically mean that mycotoxins such as Ochratoxin-A is present (Pardo et al., 2004), it is imperative to strictly adhere to Good Manufacturing Principles (GMP) to reduce health risks.

Further, after 3 months of storage, *Penicillium spp.* and *Cladosporium cladosporioides* were detected until 24 months of storage (Table 3). *Saccharomyces cerevisiae* were found at 5 to 20 months, *Rhizopus spp.* at 10 months while *Mucor spp.* at 15 and 20 months of storage.

The dominance of *Penicillium* and *Cladosporium* in the stored RCB in this study corroborates the findings of numerous studies on fungal contaminants of coffee. *Aspergillus, Penicillium, Fusarium,* and *Cladosporium* are known as natural coffee contaminants and are present from the field to storage (Mislivec et al., 1983; Nakajima et al., 1997; Silva et al., 2000; Bucheli & Taniwaki, 2002) while *Saccharomyces cerevisiae* is associated to coffee fermentation of the wet process. The occurrence in the stored RCB could be ascribed to other factors because these were eliminated during the roasting process. The conidia or spores of *Penicillium* and *Aspergillus* are not heat resistant and are usually destroyed by heat processes (Splittstoesser & King, 1984) while the spores of *Cladosporium* are common air contaminants and are found on walls of processing facilities. Meanwhile, *Mucor* and *Rhizopus* were also reported as natural contaminants of coffee (Alvindia & Acda, 2010). The humidity and chemical composition of the coffee beans, environmental conditions, and crop and product management can influence development of microorganisms and their metabolic activity (Silva et al., 2008). Thus, the incidence of these fungal organisms is an indication of contamination through the air which could be traced to the coffee processing facility, packaging room

Table 2

Profiles of Fungal Contaminants Detected in Stored Coffee Beans

Fungal Genera	Species	Number of Isolates	Percentage
Penicillium sp.	Unidentified	21	40%
Cladosporium sp.	*Cladosporium cladosporioides*	17	33%
Saccharomyces sp.	*Saccharomyces cerevisiae*	11	21%
Mucor sp.	Unidentified	2	4%
Rhizopus sp.	Unidentified	1	2%
	TOTAL	52	100

Table 3

Storage Duration and Occurrence of Fungal Contaminants

Months	Fungal Contaminants				
	Penicillium spp.	*Saccharomyces cerevisiae*	*Cladosporium cladosporioides*	*Rhizopus spp.*	*Mucor spp.*
0					
3	/		/		
5	/	/	/		
10	/	/	/	/	
15	/	/	/		/
20	/	/	/		/
24	/		/		

premises, and equipment. It was noted that the recorded mean room temperature was 24.5ºC while the mean relative humidity was 76.4% which is favorable to the growth of the fungal contaminants. Al-Abdalall (2014) found that fungal contaminants were present at 10% to 45% relative humidity on stored ground coffee while various research reported a maximum growth of most fungi at 92.5% to 100% relative humidity. Hence, the point of contamination could have occurred during the packaging of RCB. Therefore, adherence to Good Manufacturing Practices (GMP) at the coffee processing facilities should be properly observed. This includes location, design and construction of the facility and its interior, personal hygiene, cleaning, and sanitizing and equipment maintenance, among others.

From the isolated fungal contaminants, *Saccharomyces cerevisiae* and *Cladosporium cladosporioides* are known to have a positive effect on the quality of wet-processed coffee. Meanwhile, *Rhizopus* and *Mucor* are confirmed common natural contaminants of coffee in the Philippines (Alvindia & Acda, 2010) but some species of these have the potential as starter cultures in coffee fermentation (Tang et al., 2021; Lee et al., 2016). Silva et al. (2013) found that the introduction of *Saccharomyces cerevisiae* resulted in the production of high-quality coffee beverages with a special aroma of caramel, herbs, and fruits. Meanwhile, *Cladosporium cladosporioides* is called a "bio protector" as it prevents the development of harmful fungi that affect coffee quality and promotes an increase in the physical and chemical composition and sensory quality of coffee (Paiva et al., 2023).

Final Weight

The final weight of stored 250g RCB highly differed (p=<.001) among the storage durations (Table 4). After three (3) months of storage, the stored RCB weighed 245.64g or 2% of weight lost. A gradual decrease in weight was observed with 208.43g recorded after 24 months of storage which accounts for 17% weight loss. The weight loss in the RCB could be explained by the gradual release of gas from the RCB during storage. It was reported that about 1% of the weight of freshly roasted coffee is carbon dioxide (CO_2). CO_2 is produced by both the Strecker degradation reaction (Maillard reaction) and carbohydrate pyrolysis during high temperature roasting of coffee and trapped in the fresh roasted or ground coffee (Baggenstoss et al., 2007; Shimoni & Labuza, 2000; Smrke et al., 2018; Wang & Lim, 2014). The release of gas, or degassing, occurs for about one month from whole bean coffee (Smrke et al., 2018). However, results of the study indicated that degassing continued until the 20th month of storage as indicated by a gradual decrease in weight and stopped at the aforesaid period. The packaging material used in this study is fitted with a one-way valve that prevents the entry of oxygen from the air but allows the release of CO_2. This type of packaging material is common in the coffee industry as it helps prevent or slow down oxidative degradation of coffee aroma, subsequently reducing loss of freshness (Gloss et al., 2014) However, even the best packaging and oxygen-free conditions cannot prevent loss of freshness (Clarke, 1985), since coffee is intrinsically an unstable product. The results of this study agree with these findings.

Table 4

Final Weight and Percent Weight Loss as Affected by Storage Durations

Months	Final Weight	Weight Loss (%)
3	245.64^{a}	2
5	242.60^{b}	3
10	233.72^{c}	7
15	225.48^{d}	10
20	216.45^{e}	13
24	208.43^{e}	17

Cup Quality

Following the Specialty Coffee Association (SCA) cupping protocol, the primary components or attributes of quality coffee beverages namely, aroma, flavor, aftertaste, acidity, and body were assessed at the different storage periods. Feria-Morales (2002) stated that these attributes are important for the acceptance and definition of the bean quality. Results revealed a decreasing trend in all the primary attributes of the RCB stored at various durations (Figure 1). The aroma intensity faded first with a steep decline from 7.25 to 6.25 points after 5 months of storage and had the lowest (6.0) points after 15 months. In contrast, flavor intensity was observed to exhibit the slowest

but most gradual deterioration followed by the body and acidity. Nevertheless, flavor, along with the other coffee quality attributes had reached the lowest score after 20 months of storage.

The fading of primary attributes is a result of the gradual deterioration of the stored RCB as reflected in the decreasing cup score (Table 5). After roasting and before storage, the RCB had an 81.50 cup score classified as specialty-grade coffee. However, after 3 months of storage, the cup score fell to 79.75 or, "not specialty coffee" then reached a final score of 72.00 after 24 months of storage. At 24 months of storage, the coffee aroma was still perceptible but had the smell of oxidized oil, cardboard and paper which are undesirable traits of old coffee. It also had an unpleasant rancid aftertaste, weak and sour acidity, and a body described as weak and rough. The smell of oxidized oil is an indicator of oxidation which means the coffee has lost its freshness. Oxidation with O_2 is a chemical reaction and is one of the two main pathways on how coffee loses its freshness (Gloss et al., 2014) besides the loss of highly volatile compounds.

Results further indicate that the Vacuum Metalized Polyethylene Terephthalate (VMPET) packaging material used in this study was able to prevent the entry of oxygen but not for more than 5 months. Gloss et al. (2014) recommended packaging materials containing an aluminum layer and thickness of 0.03–0.05 mm which offer better protection for 46 weeks (11.5 months) of storage. As the transformation of chemical contents happens not only during roasting but also during storage, roasted beans are thus susceptible to further chemical and physical changes that affect the sensory quality of coffee beverages. Water (moisture), air (oxygen), light, and extraneous odors have the greatest influence on the quality of coffee during storage. Massive odor and flavor losses are the consequence of the water solubility of essential oils of coffee and the formation of volatile flavoring substances with oxygen (Ross et al., 2006; Makri et al., 2011; Toci et al., 2013). Hence, results imply that the VMPET could be used for RCB but should not be stored for more than five months. Further, declaration of the best-before date and or the roasting date of coffee should be required to be indicated in the coffee packaging as a guide to consumers.

Figure 1

Effect of Storage Durations on the Primary Cup Quality Attributes of Arabica Coffee

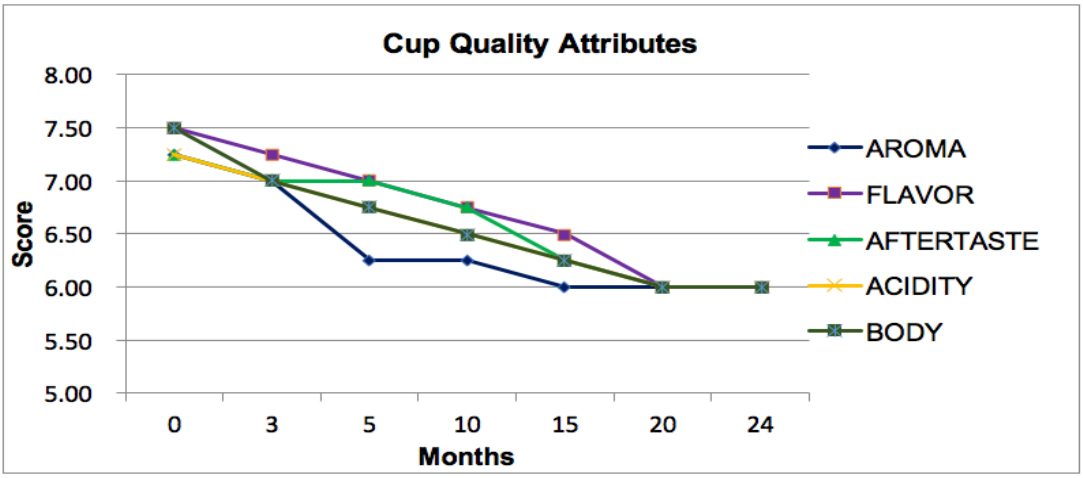

Table 5

Cup Quality of Roasted Arabica Coffee Beans Stored at Different Durations

Months	Cup score	Attributes				
		Fragrance/Aroma	Flavor	Aftertaste	Acidity	Body
0	81.50	Floral, black tea, honey, peanut, fermented, sweet, pear, peach, biscuit, cocoa, vegetal	Biscuit, tangerine, choco, nutty, peanut, barley tea, straw, strawberry	Nutty, dark choco, hazelnut, graham, cracker	Apple, tamarind, candy, raisin, citrusy	Smooth, juicy
3	79.75	Fruity, milk choco	Tomato, papaya, cacao, caramel, ponkan, barley tea, melon	Hazelnut, sweet	guava, citrus, red berries, grape skin	Watery, rough
5	77.50	Cacao, biscuit, floral, raisin, roasted, almond, molasses, straw, black tea, pandan	Barley tea	Nutty, cacao	Weak, sour	Weak, rough
10	76.25	Straw, toast, choco, cherry, papery	Old coffee	Unpleasant	Sour	Weak, rough
15	73.75	Oxidized oil, dusty, papery, molasses, peanut	Old coffee	Unpleasant	Sour	Weak, rough
20	72.00	Papery, cardboard, oxidized oil, soya, honey	Old coffee	Unpleasant	Sour	Weak, rough
24	72.00	Oxidized oil, papery, cardboard, peanut	Old coffee	Unpleasant	Sour	Weak, rough

Conclusions

The study was conducted to determine the cup quality and occurrence of microbial contaminants or growths on stored roasted coffee beans (RCB). Freshly roasted coffee beans were negative of fungal contaminants, but these were detected after 3 months of storage implying that the point of contamination could occur during handling and packing of roasted coffee. Five fungal genera were isolated, the highest contaminants were *Penicillium sp., Cladosporium sp.*, followed by *Saccharomyces sp.* Meanwhile, *Mucor sp.* and *Rhizopus sp.* were also detected but at lower counts. Two fungal contaminants identified at the species level were *Cladosporium cladosporioides* and *Saccharomyces cerevisiae*.

Results further reveal that the cup quality of the RCB falls to below specialty grade after three months of storage and further declines as storage is prolonged. The earliest to fade was the aroma intensity while flavor exhibited the slowest but gradual deterioration followed by the body and acidity. All primary attributes reached the lowest intensity score after 20 months of storage. Furthermore, VMPET material fitted with a one-way valve could preserve desirable coffee aroma and flavors of RCB but not beyond 5 months.

Recommendations

Adherence of processors to Good Manufacturing Practices and hygienic practices is recommended to reduce fungal contamination to ensure the coffee products are safe and quality. In addition, the VMPET material fitted with a one-way valve is recommended for the packaging of roasted coffee beans and storage of not more than five (5) months. Also, the best-before date or date of roasting should be indicated in the labels of roasted coffee products to serve as a guide for consumers. Finally, further studies on the preservation of cup quality by evaluating highly volatile compounds present on the RCB and during storage are endorsed.

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