Evaluation of microbial load in the processing of coffee cherry relating its economic loss.

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Abstract

Coffee is an economically important agricultural product and preferred beverageworldwide. Cherries are the main yield of coffee plant. In the present study, Microbial isolation was done at different stages of coffee processing. The bacteria from coffee beans were isolated on Tryptone Soya Agar media by Pour plate method and the fungi were isolated by moisture chamber technique and by direct plating method on Sabourauds Dextrose Agar. Identification of isolated colonies were made based on microscopic observations. The bacteria isolated were all common contaminants viz *Staphylococci* and *Bacillus* species. Among the fungi isolated from coffee beans were *Aspergillus,Rhizopus* and *Penicillium* species. Both, the plate and slide method predominantly showed *Aspergillus niger* on coffee beans. Aspergillus spp. are the common contaminant as its spores are present in the soil and air. The high moisture level in coastal area, may attract more fungal spores on coffee beans. Fungal contamination accumulates mycotoxins and also drastically changes the flavor of coffee. Hence it is imperative to preserve the coffee cherry quality while processing and storage avoiding microbial contamination. This will subsequently increase the shelf life of coffee beans and save the industry from related economic loss.

Key words- Coffee;*Aspergillus* Species; Moisture; Processing; Mycotoxin.

Introduction

Coffee is one of the most popular drinks in the world prepared from roasted coffee beans which are the seeds of the berries from the coffee plant. The two most commonly grown coffees are Arabica and Robusta. Once ripe coffee beans are picked, processed and dried. Dried coffee seeds are roasted to various degrees depending on the desired flavour.

Coffee fruits and beans get contaminated by micro-organisms during different developmental stages from the crop field to storage (1).Presence of fungi in the coffee beans does not only affect quality in terms of flavour and aroma of the beverage, but also presents a safety risk for the final product due to production of mycotoxin which can be harmful to consumers at certain concentration (2,3).

The different processing methods used in the industry are likely to impact the seed microbiome in very different ways. Natural processing involves harvesting ripe coffee fruits and spreading over dry surface and allowed to dry for 18 to 24 days. This extended environmental exposure likely to results significant lot-to-lot variation in the microbial load due to difference in temperature, humidity and arthropod activity (4).

The genera*Aspergillus* comprises species that produce mycotoxins such as Aflatoxins, Ochratoxins and Patulin. In coffee grains, the most important *Aspergillus* species in terms of the risk of presenting mycotoxins belong to the genera *Aspergillus* Section *Circumdati* and Section *Nigri*. Several researches have been carried out to analyse the presence of ochratoxigenic fungi in coffee beans (5,6).The main ochratoxin A (OTA) producing species from coffee belong to genera *Aspergillus circumdati* and *nigeri*(1,7,8).In a study on Natural coffee fermentation by (9), among bacteria Bacillus was predominant genus (51%) and most of the fungi were *Aspergillus*. *A. ochraceus* is an important species producing OTA of the genera *Aspergillus (*10).It was investigated regarding OTA production during transportation. (11)

Fungal contamination and production of mycotoxins is one of the post harvest problems that influence the quality of coffee beans. Several species of *Aspergillus* and *Penicillium* do not invade seeds to any appreciable degree before harvest but they can cause severe discoloration of seed in storage resulting in germination failure, discoloured or otherwise damaged embryos or whole seeds (12). Species of *Penicillium* are found at times, usually in seed lots stored at low temperatures and above 16% moisture content. In the range of moisture content between 14.0 and 15.5% in coffee, a change of merely 0.2% makes a great difference in the rate of invasion of storagefungi resulting damage to the seeds. Preventing the invasion of these fungi and creating conditions unfavourable for their growth may help to improve quality of coffee.

The current work is envisaged at analysing the microbial load from four samples of *Coffee arabica* cherryobtained from the coffee cherry processing industry of Mangalore. These sampleswere with different moisture levels in mansooning process. The coffee beans were wet and dry processed. The study was done to in response to the repeated discolouration and change in the flavour of stored coffee beans.Such analysis was felt necessaryto restore the best quality and flavour of regional coffee crop.

Materials and Methods

Collection of*Coffee arabica* cherry sample

The samplesof *Coffee arabica* cherry were provided by the coffee processing unit at Mangalore, Karnataka.At the farm level, Parchment coffee is obtained when wet processing method is employed. When dry processing is employed the dried coffee is obtained that has dried fruit skin which is subsequently hulled. These coffees are called cherry coffees. In both cases the washed coffee (parchment coffee) and dry cherries go through the dry mill and are cleaned of extraneous matter, graded size wise, polished and sorted. Here the parchment from the washed coffee during polishing or the dried skin from the dried cherries is removed in the hulling process. These *Coffee Arabica*cherry beans with different moisture levels were graded and considered for microbial analysis.

Microbial Analysis:

Bacterial isolation

Bacterial load on each produce was enumerated by plating 1ml of 10^{-1}dilutions of the wash waters on the Tryptone Soya Agar and the colonies counted after 24-hour incubation at 37^0C and colonies were identified by Gram staining.(13)

Fungal isolation

The fungi were isolated by direct method by using moisture chamber and by directly plating on Sabourauds Dextrose Agar. In moisture chamber method the specimen is slightly moistened with sterile distilled water and placed on a filter paper. This helps in direct isolation of fungi. For this, filter paper was moistened with distilled water and placed in Petri plate and sterilized. Selected coffee beans were placed in it and incubated at room temperature for three days. In plate method coffee beans were placed on sterile Sabourauds Dextrose Agar using forceps. The plates were incubated at room temperature for three days. Fungi were identified by Lactophenol cotton blue staining using Pictorial Atlas of Soil and Seed fungi (14)

Results:

Sample A was Complete monsooned with 18.5% moisture, Sample B was complete monsooned with 17% moisture, Sample C was midway through with 19.2% moisture and. Sample D was with 13.8% moisture content

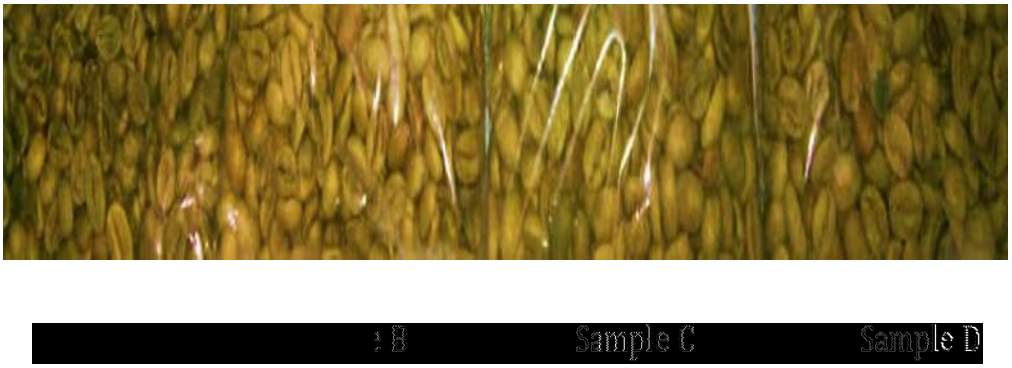
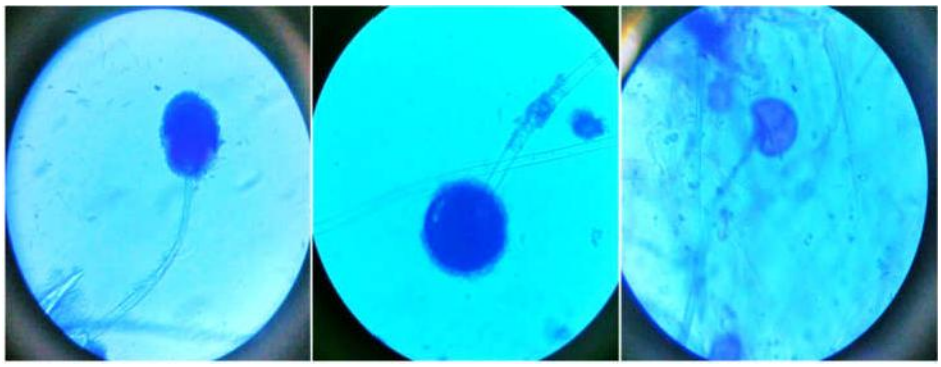


Fig 1. Four different Coffee beans samples used for microbial analysis

Sample A Complete monsooned with 18.5% moisture had only one bacterial colony whereas Sample B Coffee beans with 17% moisture had too Numerous to Count bacterial colonies. Colony characteristics of selected bacterial colonies on Tryptone Soya Agar were studied. The studies showed that Sample A had Gram Positive Bacillus species. Sample B and Sample D had both Gram Positive Bacilli and Cocci. Sample C had only Gram Positive Cocci. In isolation of Fungi by 'Moisture Chamber Method' all the four Coffee beans samples showed the presence of *Aspergillus niger* as the predominant isolate. (Table 1.)

Table 1: Fungalisolates by Moisture Chamber Method

Sample	Fungi isolated
Sample A	*Aspergillus niger*
Sample B	*Aspergillus niger* *Aspergillus ochraceus*
Sample C	*Rhizopus spp,* *Aspergillus niger* *Aspergillus ochraceus*
Sample D	*Rhizopus spp,* *Aspergillus niger* *Aspergillus ochraceus*



Aspergillus ochraceus ***Aspergillus niger*** ***Rhizopus spp***

Fig 2: Microscopic view of the fungi isolated from coffee bean samples

Discussion

One hundred and eighty isolates comprised of *Aspergillus* sections and eight species of *Penicillium* were found in the work carried on Toxogenic fungi associated with processed (green) coffee beans (7). In the 30 samples of coffee beans analysed for ochratoxegenic fungi in conventional and organic cultivation 480 filamentous fungi of the Genera *Aspergillus* of the *circumdati* and*nigri were found.*

In our study, *Aspergillus* species were the predominant isolate. Samples in different stages of processing yielded A.niger and A.*ochraceus* (Table 1). All these groups contain species that are well recognized as toxin producers (15,16,17,18) and their presence in coffee beans must be considered a matter of concern, as coffee is commonly consumed by millions of people all around the world.

The bacteria isolated were Gram Positive Cocci mainly Staphylococci which might be from the coffee bean handlers and air flora. The fungi isolated were *Aspergillus* and *Rhizopus* species. Our findings correlates with those reported by similar such studies. It is worth noting that fungal presence is an important indicator of potential mycotoxin contamination, but the absence of microorganisms is not enough for discarding the presence of toxins. Many studies demonstrated the presence of mycotoxins in commodities without fungal isolation, as for example pulses, cereals and wine (19,20). This means that the presence of fungal strains is not necessary for a food to be potentially dangerous: if those strains produced toxins at some point of their growth stage, toxins can last on substrate well even after the mycelium has disappeared.

Mycotoxins are quite resistant and it is possible they remain chemically stable in stored commodities for long time. It has been shown that they can even survive extreme heating (21), which is an additional problem to coffee beans.

Conclusion To safeguard coffee beans from the damage incurred by microbial contamination, discolouration and mycotoxin accumulation, it is necessary to preserve the coffee bean quality while processing and storage. By avoiding external contamination and keeping the minimal contact of coffee beans with ground or soil while drying, it is possible to reduce fungal contamination to beans.This will subsequently increase the shelf life of coffee beans and save the industry from related economic loss even with prolonged storage.

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