Determination of microbial quality and quantity of stored cinnamon quills

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Introduction

Since Cinnamon is one of the major spicecrops in Sri Lanka, maintenance of the quality of the processed cinnamon is very important. Microbial infection of processed cinnamon quills makes lowquality final product leading the product less demandedand high post-harvest losses. The prevailing solution for the microbial infection is fumigation of sulfur and it is not acceptable in many countries due to its harmful effects for consumer health. Therefore identification of common types of microbes on quills and determination offavourable conditions for microbial growth is very important to apply effective control measures.

Methodology

Experiments were conducted to identify the particular types of fungi that thrive on cinnamon quills and the effect of moisture content, relative humidity, temperature and the storage period to the microbial growth. Cinnamon quills were collected from three different placesjust after processing. The moisture contents of the samples were measured in three days interval using gravimetricmethod. Average relative humidity and ambient temperature were also recorded each period. In quantification process, the number of colony forming units of fungi and bacteria in cinnamon quills were counted separately using colony counter.1g of powdered sample of cinnamon was dissolved in 10ml of distilled sterilized water and 200µl of the solution was poured in to the culture plates. It was allowed to incubate under the room temperature for about three days and colony count of fungi and bacteria were taken.The culture plates with microbial colonies were allowed to incubate further to obtain pure cultures of fungi by frequent sub culturing. Colony characteristics were observed with the time.The pure cultures of fungi were used to prepare slide cultures for microscopic identification highlighting the distinguishing characteristics (Funder, 1953; Cappuccino and Sherman 1996).

Results and Discussion

According to the morphological and microscopic observations, *Rhizopus sp., Penicillium sp., Aspergillus niger, Aspergillus flavus* were the most common types of fungi encountered on cinnamon quills. In addition to those types *Trichoderma sp.* was appeared in the samples taken from one farmer place. It may be happened when peeled cinnamon had been kept in ground for drying and due to bad sanitary conditions in processing place.

Rhizopus sp. was rapidly growing white coloured fungus with cottony and fuzzy aerial mycelium. The color of the colony was white initially and turned grey to yellowish brown with time. It was grown as filamentous, branching coenocytichypha without cross-walls. Sporangia were developed on the long stalks raised as groups from nodes directly above the rhizoids. Unicellular ovoid, hyaline and striated sporangiospores were produced by *Rhizopus* species and they were grown as root like rhizoids initiallyand finally grown as large mycelium. *Penicillium* colonies were initially appeared in white colourand become blue green, gray green and then gray in colour respectively. It was grown as a thallus with typical characteristic of a highly branched network of multinucleate, septate, and usually colorless.Many-branched conidia sprout on the mycelia, bearing individually constricted conidiospores. *Aspergillus flavus* colonies were initially appeared in yellowish whitecolour and changed to olive green, dark green and brownish green respectively.Hyphae grew as a thread-like structure and they were septate and hyaline. The asexual spores,conidispores, produced in conidia were rough and dark.*Aspergillus niger* colonies were initially white and become brownish with white reverse and brownish black colour respectively covering the entire plate.Morphology of *Aspergillus niger* showed large, globose, dark brown conidial heads, which become radiate. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriatewith the phialides, often septate. Conidia are globose, dark brown and rough-walled.*Trichoderma* colonies were wooly and the initialcolor is white. As the conidia are formed, yellow-green patches become visible making concentric rings.Conidiosphores were erect, smooth and penicillately branched. Globose conidia were developed on philides produced in the opposite direction in each point.

At the beginning the fungal infections of stored cinnamon were very low due to lack of enough inoculums, even though preferable higher moisture contents for fungal growth were retained in stored cinnamon quills. Next 10 days rapid increments of fungal colony forming units were observed because preferable moisture contents were retained further in cinnamon quills. Fungal infection was increased at 10 to 20 days also, but in negative rate due to desirable moisture contents were still remained in the cinnamon quills. Moisture content in the cinnamon quills were below 20 % after 20 to 30 days after processing and in this period fungal contaminations were gradually declined. When the moisture content was reached below 12% in storage cinnamon, fungal infection was at a minimum level. If the moisture level is reduced to below 12% in storage cinnamon as soon as possible, fungal infection can be controlled efficiently.
The most interesting phenomenon was the symbiotic relationship between fungal growth and bacterial growth (Figure1).There was very strong significant positive correlation between fungal and bacterial infections ($r = 0.912$, $p = 0.0001$). Bacterial contamination never had been occurred without fungal infection. Bacterial colonies were appeared in the culture plates used to have the fungal colonies separately, even though those were treated with antibiotics to retard the bacterial growth.When fungal infection is terminated by managing moisture content, the bacterial infection **is** automatically reduced (Figure 2).

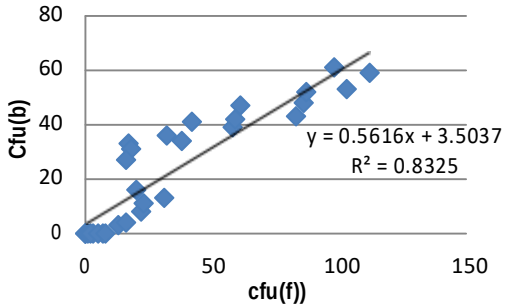

Figure 1. Relationship between number of fungal colony forming units [cfu(f)] and bacterial colony forming units [cfu(b)] during the period of storagein cinnamon

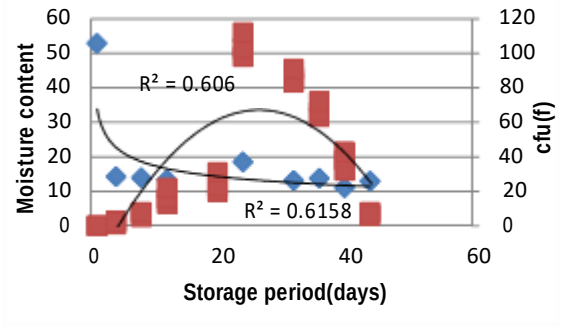

Figure 2. Relationship of moisture content and Fungal colony forming units during the period of storage

The number of fungal and bacterial colonies has been increased with the increment of relative humidity. These relationships were stronger in sample collected from processing center and farmer's place1 than farmer's place 2. Cultivated cinnamon was not belonging to individual variety and some cinnamon plants may have more resistance to microbial contaminants. Furthermore, fungal growth had stronger correlation with relative humidity than bacterial growth. In general, fungi and bacteria both were well grown and the spread in high relative humidity levels and it provided more favorable conditions for microbes' development in stored cinnamon.

The number of fungal and bacterial colony forming units makes a moderate negative relationship with temperature. Maximum number of fungal and bacterial colonies can be observed within the temperature range of 28 to 31 C and the most favorable temperature range for post-harvest microbes on cinnamon quills may be 28 to 31 C. This experiment was conducted Matara and the fluctuation of temperature was very low. Daily temperature was retained at 28 to 31 C mostly during the study period and this range of temperature was optimum to growth of fungus in stored cinnamon. High temperature (35 C) with low relative humidity (20 %) and low temperature (20 C) with low relative humidity (10%) are the most suitable conditions for keeping insects and microbial contaminants away from stored cinnamon quills (Jayasinghe, 2012). Ambient temperature alone not affected microbial growth in stored products. When it is unified with other factors such as RH, moisture content, it will play major role to increase or decrease the microbial contaminants in stored cinnamon. In other hands, microbial contaminants attract the invertebrates concurrently such as frugivorous mites when the cinnamon is contaminated with fungus making the quality of the cinnamon quills lower furthermore.

Conclusion

Rhizopus sp., Penicillium sp., Aspergillus niger, Aspergillus flavus were the most common types of fungi encountered on cinnamon quills. The most favourable conditions for microbial growth was 80% to 90% of relative humidity, 28 to 310C of temperature, moisture levels above 20% and 20 to 30 days of storage period.

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References

Cappuccino,J. and Sherman, N. 1996.Microbiology, A laboratory manual. 4th ed. New York: Rockland community college.P 22-26.

Funder, S. 1953 .Practical mycology, manual for identification of fungi. 3rd ed. New York: Hafner publishing company. P 16- 23.

Jayasinghe, G. G. 2012. Identification and management of stored product pest of cinnamon quills (CinnamomumzeylanicumBlume). Proceeding of the symposium on Minor Export Crops (Ed: B. Marambe), 16 – 17 August 2012, Peradeniya, Sri Lanka, P95 -100.