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Fungal colonization and biochemical changes in coffee beans undergoing monsooning

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Abstract

Changes in the mycofloral composition and concentrations of proteins, reducing sugars, phenols and tannins in coffee beans were analysed during different weeks of monsooning in *Coffea arabica* L. (Arabica) and *C. canephora* Pierre ex Froehner (Robusta). The highest fungal populations occurred during the fourth to seventh week of the monsooning process and the dominant fungal species were *Aspergillus niger*, *Aspergillus tamarii*, *Aspergillus candidus*, *Penicillium* spp. and *Absidia heterospora*. The protein and reducing sugar content increased steadily while the tannin content decreased beyond the detectable limit during monsooning. The phenolic content, however, was found to decline in the case of Robusta and increase slightly in Arabica. Throughout the study the monsooned coffee beans had different mycoflora and varied biochemical composition compared to non-monsooned coffee beans.

Keywords: Coffee; Monsooning; Mycoflora; Protein; Phenolics; Reducing sugars; Tannin

1. Introduction

A speciality of Indian coffee is monsooned coffee, which is in good demand in Scandinavian countries for its characteristic aroma, flavor and cup quality, and is specially prepared to cater for the needs of buyers. It represents a solid-state fermentation process during which mycofloral colonization and a range of physical, chemical and biological changes take place (Ahmad, 2000). This coffee is produced during the southwest monsoon season in only two places in the world, namely, Mangalore and Telicherry, India.

Among the different types of processed coffee beans, monsooned coffee fetches the highest price in the international market. The process of monsooning of coffee beans was discovered accidentally when a consignment of coffee beans shipped to a Scandinavian country was found drenched due to improper storage on ship. This rejected sample was sent back, where after it was reprocessed and exported. The consumers, on account of its colour, flavour and taste, preferred the sample so sent and the process of monsooning of coffee beans is thus supposed to have started. The monsooning process is carried out after the harvested cherry coffee is dried, hulled, garbled and graded. The term monsooning is applied to this process mainly because the type of curing is done only during the monsoon (rainy) season.

Monsooning is normally carried out during the Southwest monsoon season in the Indian west coast during the period June–September. Only the best quality unwashed coffee bean (Cherry coffee) is used for monsooning. Selected grades of coffee beans are spread on the concrete floor in well-ventilated warehouses in layers of 4–5 in. so as to absorb moisture up to 15%. The beans are raked at intervals of about 3 h to mix them thoroughly. After absorption of moisture, beans swell to

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about 1 1/2 times their normal size and change colour from green to pale white or golden light brown. During a consistent rainy period, it takes about 10 days for absorbtion of the required percentage of moisture. The beans, which then reach the required size and colour, are sent for polishing when the outer silver skin is removed. The polished beans are then fumigated to prevent infestation by coffee bean weevils. After polishing, the moisture content of the beans comes down to around 13%. These beans are then garbled, graded, packed and shipped out.

The scarce scientific information that is available on monsooning of coffee dates back to the 1960s. Some of it includes studies on the storage aspects of monsooned coffee beans, to prevent them from infestation by the coffee bean weevil, *Aracerus fasciculatus* De geer (Ahmad, Rao, & Bhat, 2002; Majumder et al., 1961; Natarajan et al., 1961; Subrahmanyan, Bhatia, Natarajan, & Majumder, 1961, 1963). However, information regarding the production and accumulation of biochemical compounds during coffee bean monsooning is scarce and some of it includes the work done on chlorogenic acid and caffeine content (Balyaya & Clifford, 1995; Natarajan et al., 1961), total ash, nitrogen and microbial count (Natarajan et al., 1961) and attempts with correlate these compounds with the final beverage quality (Subrahmanyan, Bhatia, Natarajan, & Majumder, 1963).

The principal interest of this study is to evaluate changes in the concentration of proteins, phenolics, reducing sugars and tannins, during different weeks of monsooning, and to correlate it with the occurrence of mycofloral populations, as no work on these aspects has so far been carried out.

2. Materials and methods

2.1. Coffee samples

A total of 92 coffee bean samples of Arabica and Robusta varieties, which included samples belonging to different weeks of monsooning, was collected and analyzed for their mycofloral and biochemical composition. The samples, spread for monsooning over a period of 9 weeks, were collected at intervals. The study represents the data collected over a period of 2 years. In each year, two batches of each variety were monsooned. Throughout the study, the non-monsooned coffee beans served as a control against the coffee beans undergoing monsooning.

2.2. Mycofloral analyses

The fungal spore load/g was estimated by following the method of Aneja (1996) and the percent incidence of fungi was estimated by following the standard blotter method according to Neergaard (1977). All treatments were carried out in triplicate and incubated at 25 ± 1 °C on PDA for up to 5–7 days for assessment of fungal count and percent incidence. Identification of fungi was done by referring to standard identification manuals.

2.3. Biochemical analyses

The protein content of the coffee beans was estimated by adopting the method of Lowry, Rosebrough, Farr, and Randall (1951) and that for reducing sugars and phenolic content by adopting the method described by Sadasivam and Manickam (1997). Tannin content in the samples was estimated by adopting the radial diffusion assay method (Hagerman, 1987).

2.4. Statistical analyses

All the treatments were carried out in triplicate except for tannins ($n = 4$). The variations between replicates, varieties, batches and years of sampling were determined using multifactorial analysis of variance (ANOVA) as well as one way and two way ANOVA, followed by a least significant test based on t distribution (lsd_t test). In the case of percent incidence of fungi, the problem of occurrence of zero values was overcome by using square root transformation (where $x = \sqrt{x+1}$) for all values, followed by analysis of variance. Statistical analysis was carried out by using Lotus-123 and Minitab release 8.3 software.

3. Results

Mycofloral analyses revealed a total of 25 fungal species, belonging to 13 genera isolated from the coffee variety Arabica, and 21 fungal species, belonging to 12 genera from Robusta coffee beans, during different weeks of monsooning (Table 1). The fungi belonged mainly to the groups of *Aspergillus* and *Penicillium*. *Aspergillus niger* was the predominant fungus in the non-monsooned samples (control) and, with the progression of the monsooning period, *Aspergillus ochraceous*, *Aspergillus tamarii* and other Phycomycetous fungi, such as *Absidia heterospora*, *Rhizopus* sp., *Syncephalastrum racemosum*, followed suit. Toward the end of monsooning, i.e. after the fifth week, fungi such as *Aspergillus versicolor*, *Aspergillus wentii*, *Aspergillus candidus*, *Syncephalis* sp., *Wallemia sebi* and yeasts predominated. Field fungi, such as *Cephalosporium acremonium*, *Myrothecium advena*, *Fusarium* sp., *Paecilomyces* sp., were found only in the initial stages of monsooning and not present thereafter. With the progression of the monsooning process, the number of fungal species (Table 1), as well as the fungal spore load, increased (Fig.

Table 1
Change in percent incidence of fungi and Actinomycetes on Arabica and Robusta coffee bean varieties during different weeks of monsooning

SI no.	Variety	Arabica												Robusta											
		Control		1		3		5		7		9		Control		1		3		5		7		9	
			SEM		SEM		SEM		SEM		SEM		SEM		SEM		SEM		SEM		SEM		SEM		SEM
1	*Absidia heterospora* Ling-Young	1.5	1.0	4.5	6.9	8.5	1.7	10.0	2.5	2.5	0.0	2.0	0.0	0.0	0.0	8.0	2.8	7.0	5.7	0.0	0.0	2.5	2.5	0.0	0.0
2	*Aspergillus candidus* Link	0.0	0.0	10.0	2.0	5.0	7.9	11.5	5.5	17.0	4.0	15.5	6.0	3.0	2.4	6.5	6.5	1.5	1.0	7.5	3.8	16.5	2.1	4.5	2.9
3	*A. flavus* Link	1.0	1.4	0.0	2.1	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	*A. niger* van Tieghem	100.0	0.0	94.5	2.1	90.5	3.0	76.0	4.7	45.0	13.4	23.5	5.0	100.0	0.0	95.5	2.2	79.5	6.3	53.0	15.9	49.3	12.9	22.0	5.0
5	*A. ochraceous* Wilhelm	23.0	6.9	82.5	1.5	100.0	0.0	100.0	1.0	96.0	6.8	85.5	23.5	8.0	2.8	76.5	19.1	79.5	11.8	99.0	1.0	96.0	2.3	93.5	5.9
6	*A. oryzae* (Ahlb.) Cohn	1.0	1.4	0.0	0.0	0.5	0.0	0.0	0.0	0.0	7.1	0.0	0.0	0.0	0.0	6.0	4.8	1.5	1.5	0.0	0.0	0.0	0.0	1.0	1.0
7	*A. tamarii* Kita	8.5	4.0	44.5	12.0	51.0	5.9	38.5	4.5	14.5	4.5	12.0	1.5	0.5	0.5	35.5	14.3	41.0	13.3	45.5	12.7	27.5	5.9	21.5	6.8
8	*A. terreus* Thom	0.0	0.0	0.0	4.5	3.5	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	*A. versicolor* (Vuillemin) Tiraboschi	0.0	0.0	0.0	1.0	2.0	0.0	1.0	2.6	6.5	4.0	4.5	0.0	0.0	0.0	0.0	0.0	8.5	5.3	6.0	6.0	6.5	6.5	12.0	7.3
10	*A. wentii* Wehmer	0.0	0.0	0.0	2.0	12.0	7.2	4.0	4.2	8.5	4.6	6.5	0.0	0.0	0.0	0.0	0.0	5.0	3.0	11.5	3.4	13.0	4.7	7.5	4.9
11	*Fusarium* sp.	2.5	1.5	2.0	1.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	*Mucor* sp.	0.0	0.0	3.5	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	*Myrothecium advena* Sacc.	6.0	2.6	16.0	10.0	8.5	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5	4.5	9.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0
14	*Paecilomyces* sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	0.0	0.0	0.0	0.0	0.0	0.0
15	*Penicillium* sp.	0.0	0.0	5.0	14.7	20.5	7.1	9.0	5.6	14.5	5.0	16.0	7.5	11.0	3.5	8.0	4.9	2.5	2.5	2.5	2.5	10.0	6.6	3.0	3.0
16	*P. chermesianum* Biourge	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5	7.5	34.0	12.6
17	*P. chrysogenum* Thom	0.0	0.0	0.0	0.0	0.0	4.0	5.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	*P. rugulosum* Thom	13.5	1.0	14.5	15.3	14.0	7.4	24.5	5.4	16.0	4.9	9.5	0.0	16.0	2.9	27.0	3.9	18.0	6.4	16.0	3.3	21.5	4.9	20.5	7.2
19	*Rhizopus* sp.	0.0	0.0	11.0	19.6	18.5	1.9	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	2.0	1.5	1.5	0.0	0.0	3.0	3.0	0.0	0.0
20	*Stachybotrys* sp.	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	*Syncephalastrum racemosum* (Cohn) Schroeter	0.0	0.0	6.0	1.2	3.5	2.9	3.0	1.2	0.5	1.2	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0
22	*Syncephalis* sp.	0.0	0.0	0.0	0.0	0.0	7.5	2.0	9.0	15.5	16.3	38.0	0.0	0.0	0.0	0.0	0.0	8.5	8.5	15.0	11.4	34.0	9.9	38.5	17.2
23	*Trichosporonoides* sp.	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	*Wallemia sebi* (Fries) von Arx	0.0	0.0	0.0	0.0	0.0	5.0	3.5	0.0	0.0	0.0	11.5	0.0	0.0	0.0	0.0	0.0	7.5	7.5	11.0	11.0	18.0	14.3	0.0	0.0
25	*Yeast*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	3.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0	8.0	8.0	6.5	6.5	4.5	4.5
Actinomycetes[a]		4.0	2.4	4.5	9.4	12.0	5.5	19.0	9.9	24.0	8.8	9.5	0.5	0.0	0.0	3.0	3.0	1.0	1.0	6.0	3.5	24.0	4.5	42.5	14.4
Total number of species		10		15		18		16		13		13		7		12		17		13		15		13	

SEM, standard error of mean, $n = 4$.
[a] Not included under the fungal group.

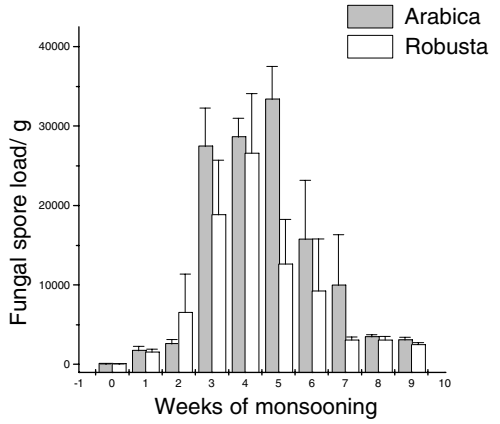

Fig. 1. Change in fungal spore load during different weeks of monsooning in Arabica and Robusta coffee beans.

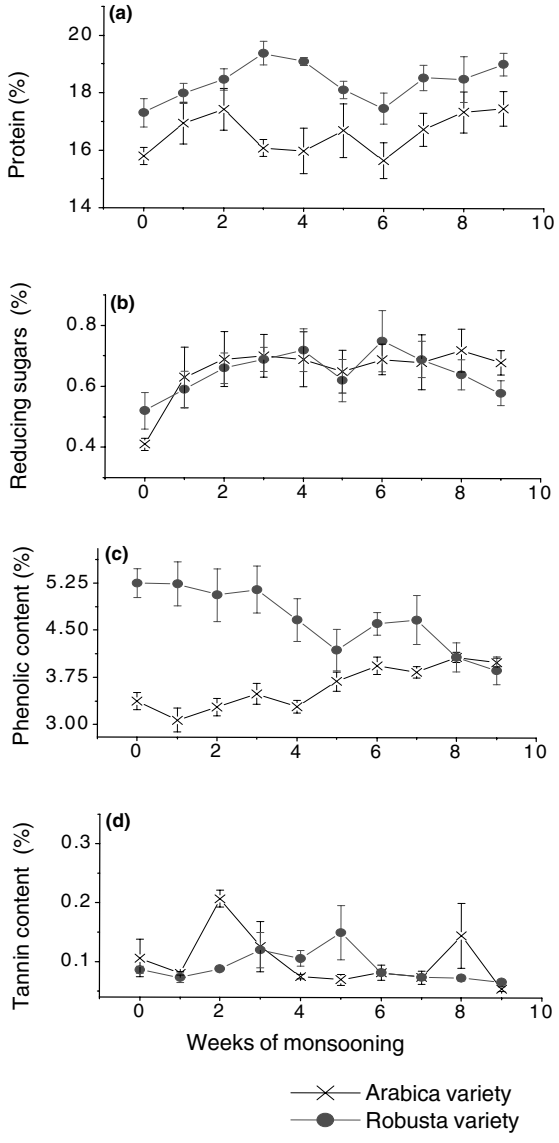

Fig. 2. Change in protein, reducing sugars, phenolic and tanin contents in the Arabica and Robusta coffee beans during different weeks of monsooning.

1), the highest being between the third and fifth week. Significant differences were observed between batches, varieties and years of sampling at the $P < 0.05$ level of significance but the data have been represented in the combined form, omitting batches and years of sampling, for convenience.

The protein content during monsooning, varied from 15.8% to 17.5% in Arabica and from 17.3% to 19.4% in Robusta (Fig. 2(a)) and the reducing sugar content varied from 0.41% to 0.72% in Arabica and from 0.52% to 0.75% in Robusta (Fig. 2(b)). In both varieties, there was an increase in protein content and reducing sugar content during the process of monsooning. The phenolic content varied from 3.07% to 4.08% in Arabica and from 3.87% to 5.25% in Robusta during monsooning. There was seen an overall decrease in the phenolic content during monsooning in Robusta coffee but, in Arabica coffee, there was a slight increase (Fig. 2(c)). The tannin content during monsooning, varied from 0.06% to 0.21% and from 0.07% to 0.15% in Arabica and Robusta, respectively. In both varieties, the tannin content diminished during the course of monsooning (Fig. 2(d)).

4. Discussion

4.1. Mycofloral changes

The predominance of the different groups of mycoflora was the same in both Arabica and Robusta coffee varieties, indicating no varietal difference in the substrate composition of the beans with regard to fungal incidence. Though a rough pattern of occurrence of different fungi was seen during the course of monsooning, it was not very clear cut. This could be because the samples were collected during the traditional method of monsooning and not under controlled conditions where several factors, such as temperature, moisture content, rainfall and humidity, were fluctuating. Further studies are therefore needed to establish a sequential pattern of fungal species occurrence and to correlate it with the factors responsible for it, by carrying out the experiment under controlled conditions. The occurrence of different fungal groups, however, was in the following order of percent incidence: Deuteromycetes (73%), Phycomycetes (19%), Ascomycetes and Actinomycetes (4%), Basidiomycetes (0%). Actinomycetes, though not belonging to the fungal group, have been included in the results of the present study owing to their frequency of occurrence. Actinomycetes were represented by two species, namely, *Streptomyces* and *Nocardia*.

The highest spore load as well as number of fungal species was observed during the initial stages of monsooning, which being a solid-state fermentation process proves the theory put forward by Christensen (1981) that an increase in number of species through early

stages of fungal degradation of single substrates was the norm. The disappearance of the field fungi was due to the changed environmental conditions during processing.

4.2. Biochemical changes

The differences in the chemical composition of plant tissues are found to be selective for particular groups of fungi, as fungi have specific relationships with various kinds of substrates as a source of nutrition and energy. Information pertaining to the interaction of coffee seed mycoflora with chemical constituents is not available.

The protein content increased during monsooning with a corresponding increase in the number of fungi. These results are similar to those reported by Ahmad (1999, 2000). Correlation studies revealed that fungi, such as *A. niger*, *Absidia heterospora*, *Penicillum* sp., and *Penicillum rugulosum*, were negatively related to the increase in protein content while *Syncephalis* sp. and *A. ochraceous* were positively correlated. It can be surmised that the increase in protein content during monsooning was due to the activity of the fungi, which has also been reported by different workers on other crops (Chahal & Gray, 1971; Dube, Shukla, & Tripathi, 1988; Giridhar & Reddy, 1997; Reddy, Surekha, & Rajakumari, 1994).

The increase in reducing sugar content during monsooning varied from 0.41 to 0.75 mg/100 mg. A positive correlation between increase in reducing sugars and occurrence of *A. ochraceous*, *A. candidus*, *A. wentii*, *Syncephalis* sp. and Actinomycetes was observed whereas *Aspergillus niger*, *Absidia heterospora* and *P. rugulosum* showed a negative correlation. The increase in reducing sugar content is attributed to the breaking down of complex carbohydrates, resulting in an increase of reducing sugars during monsooning since reducing sugar content and number of fungal species were positively correlated. Ahmad (1999, 2000), however, reported an initial increase and then a gradual decrease in the amount of reducing sugars during monsooning. Dube et al. (1988) and Bhadraiah, Singh, and Teja (2001) also recorded an increase in the reducing sugars upon fungal infestation on other crops and have arrived at similar conclusions. This suggests that the activity of fungi capable of breaking down complex polysaccharides, such as cellulose, paves the way for the next set of fungi capable of utilizing this ready made source of sugars, namely the 'sugar fungi', a name coined by Burges in (1939).

The phenolic content during monsooning decreased in Robusta but showed a slight increase in Arabica (Fig. 2(c)). Increase in phenolic content showed a decline in the occurrence of fungi, such as *A. niger*, *A. tamarii*, *A. candidus*, *A. ochraceous*, *A. wentii*, *Syncephalis* sp., *P. rugulosum* and Actinomycetes, with the exception of *Absidia heterospora*. Reddy et al. (1994), Giridhar and Reddy (1997) and Bhadraiah et al. (2001) have also reported an increase in the phenolic content on other crops with progress of incubation. Robusta showed a higher average phenolic content (4.50) than Arabica (3.62) and, in the former, the phenolic production was negatively correlated with the number of fungal species occurring, indicating that the higher growth of fungi was due to the depleted phenolic content.

During monsooning, the tannin content was found to diminish beyond the detectable limit. Tannins have been reported to be immediately toxic to the fungus or to reduce the nutritive capacity of the substrate (Van Sumere, Albrecht, Dedonder, De Pooter, & Irma, 1975) and studies on litter showed a decreasing trend in tannin content upon decomposition (Frankland, 1974). From the correlation studies, it could be deduced that early colonists, such as *A. niger* and *Absidia heterospora*, were tolerant to tannins whereas the late colonists, such as *A. tamarii*, *A. wentii* and *P. rugulosum*, were inhibited, which is supported by the views of Harrison (1971).

The impact of a simple phenolic such as chlorogenic acid on the taste of coffee and ciders has long been recognized and the astringency of tannins, i.e. the ability to cause a dry puckery sensation in the month, is well known. Considering all these facts, the loss in astringency leading to a mellowed syrupy feeling in monsooned coffee, may be attributed to the reduction in tannins and other phenolic compounds during this process though this assumption has to be proved by further studies.

Despite monsooning being in practice for a long time, traditional and unscientific methods are adopted in its processing, as a result of which the quality of coffee differs from batch to batch. Further, the conditions under which monsooning is carried out favour the growth of toxin-producing fungi such as *A. ochraceous*, a fact which needs investigation so as to prevent the occurrence of toxins in the monsooned coffee, especially considering that it is the most highly priced of Indian coffees. The results suggest that factors, external as well as internal to the immediate microenvironment of the coffee bean undergoing monsooning, have an influence on the growth of its fungal community. Rapid changes in the chemical constituents of the coffee bean cause an enhancement in quality with regard to the increase in protein and reducing sugars, and reduction in phenolics and tannins, whereby the unique mellow fruity flavour of monsooned coffee, favoured by the connoisseurs of coffee, is obtained. However, in our earlier studies (Ahmad, Tharappan, & Bongirwar, 2003) on irradiated coffee beans, it was found that microbes were playing a minimal role in the monsooning process and so further studies, under controlled conditions, are necessary to elucidate the role of fungi in altering the biochemical composition of the monsooned coffee beans and monitor the quality of the monsooned coffee.

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