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Changes in Microbial Load of Black Pepper (*Piper nigrum* L.) During Processing

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The qualitative and quantitative changes in microbial flora in 2 varieties of black pepper, ('KS 27' and 'P 24') during processing by traditional method (sun-drying), blanching followed by sun-drying and mechanical drying and during storage were studied. The moisture content of berries after 3 days of traditional method of drying was 15% whereas in blanching followed by drying and mechanical drying, the moisture contents were 8.5% and 7.5%, respectively. The microbes were enumerated on nutrient agar (NA), Luria Bertani's (LBA, for coliforms), Rose Bengal (RBA) and *Aspergillus flavus* agar medium (AFM). In traditional method of drying, there was no significant reduction in bacterial population after 4 days of drying in both the varieties. Blanching followed by drying showed no reduction in coliform, but there was significant reduction in bacterial population. Fungi were completely eliminated after blanching but reappeared during drying probably as contaminants from the atmosphere. In the mechanically dried samples, bacterial population was high in fully dried samples of 'P 24' and 'KS 27'. The 'P 24' berries harbored more fungi in partly dried sample (6 h), while 'KS 27' contained more fungi in the fully dried sample (12 h). The results indicated the effectiveness of mechanical dryer for producing black pepper free from bacteria and fungi for export.

Keywords: Black pepper, *Piper nigrum* L., Processing, Microflora, *Aspergillus flavus*, Blanching, Sun-drying

Black pepper *(Piper nigrum* L.), the king of spices is one of the most important and widely used spice in the world. The pungency and flavour of black pepper makes it an important ingredient in many food preparations (Ravindran et al. 2000). Black pepper is evaluated on the basis of its appearance, pungency level, aroma and flavour quality. The appearance of the berries is important for export and dark brown to black colour berries fetch the best prices. Black pepper is a major foreign exchange earner for India and so it is important to maintain all aspects of quality including free from microbial contamination (Pruthi 1992; Peter 1997). The aroma and flavour imparted by the volatile oil is of great significance when black pepper is sold for domestic and culinary purposes.

Processing plays an important role in determining the quality of black pepper. Among the different existing methods, the

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traditional method (sun-drying) is followed widely by the native farmers. The unhygienic practices followed by the native farmers (drying on cow dung plastered soil or mats), add microbes and extraneous matter on the berries. In spite of increased attention to microbial contamination of imported spices and the imposition of limits on filth, the pattern of microbial contamination remains largely unchanged (Subbulakshmi and Mridula Naik 2002). Improper drying and the presence of excess moisture (>10%) results in microbial deterioration during storage. A microbial toxin such as aflatoxin in the stored produce reduces its marketability. This also reduces the quality of the spice and its export potential. Sometimes drying is performed on drying yard, which is plastered with cement, fenugreek coated bamboo mats or polythene sheets.

Blanching is another procedure that ensures black colour and reduces the drying time. The black shining product obtained is due to the activity of the enzyme phenolase, which catalyses the oxidation of colourless phenolic compounds to black polymeric compounds. Solar dryers and mechanical dryers are also used for drying pepper. Although costly, it reduces the drying time drastically. With this background, the present investigation was conducted to estimate the moisture levels of pepper in different methods of drying, and to enumerate microflora associated with black pepper and their population dynamics during drying by three methods.

Fresh berries were collected from two cultivars of black pepper 'KS 27' and 'P 24' cultivated in the research farm of the Indian Institute of Spices Research at Peruvannamuzhi, Calicut. The samples were packed in polythene bags and brought to the laboratory for studying microbial load and population dynamics of microflora in black pepper during processing/drying by serial dilution technique (Johnson and Curl 1972). Moisture content was estimated by drying 10 g of sample in hot air oven, at 65°C to constant weight. The processing of black pepper was carried out using three methods of drying: i) traditional method – sun-drying on clean transparent polythene sheet (20 gauges) for 4 days at 8 h /day, ii) blanching – immersing the berries in hot water for 2 min, followed by drying on clean transparent polythene sheet

TABLE 1. MOISTURE CONTENT (%) OF BLACK PEPPER BERRIES AFTER DIFFERENT STAGES OF DRYING

		Drying period, h									
		Traditional Method			Blanching & drying				Mechanical drying		
Variety	Fresh berries	8*	16	24	32	8	16	24	32	6	12
'P 24'	75	48	21	15	4	69	44	28	4.8	15	7.6
'KS 27'	72	52	36	15	4	70	45	33	8.5	15.5	7.5

Drying period in hours from 9:00 AM to 5:00 PM.

TABLE 2. PERCENTAGE REDUCTION IN MICROBIAL POPULATION UPON DIFFERENT METHODS OF DRYING OF BLACK PEPPER

	*Reduction (%) in microbial count after drying			
Method of drying	Bacteria	Coliform	Fungi	*Aspergillus*
Traditional method**	21.8	49.7	67.0	0.1
Blanching and drying**	97.7	65.0	90.5	84.3
Mechanical drying+	91.3	99.1	95.0	65.1

* Mean of two varieties 'P 24' and 'KS 27'; ** After 32 h; + After 12 h.

(2 gauges) for 3 days and iii) drying in a mechanical dryer (6 h and 12 h at 65-70°C). Bacteria, coliform, fungi and *Aspergillus* populations were determined on NA, (Dhingra and Sinclair 1983), LBA (Ausubel et al. 1995), RBA (Martin 1950) and AFM medium (Pitt et al. 1983), respectively. The populations of microbes were expressed as cfu/g of black pepper.

One of the major post-harvest operations in black pepper is reduction in moisture by sun-drying during which there is a change in the microbial load. The moisture content of fresh berries was 72-75% and 4 days dried sample contained 4% of moisture during the traditional method of drying. Normally the moisture content range between 8 and 10%. When blanching and drying method was adopted, the moisture was reduced to 4.8% from 75% in the case of 'P 24' and to 8.5% from 72% in 'KS 27', the moisture content of berries processed by mechanical dryer were 7.6 and 7.5% (Table 1). It is evident that the blanching followed by sun-drying is efficient in reducing the moisture in black pepper.

Population dynamics of microbes during traditional method of drying: The percentage reduction of bacteria, coliform, fungi and *Aspergillus* was 21.8%, 49.7%, 67.4% and 0.1%, respectively (Table 2 and 3). This ineffectiveness of the traditional method could be due to sublethal temperature that existed at the time of drying. During different stages of drying, the fungi often reappeared mostly as contaminants, probably from the drying yard atmosphere (Table 3). The predominant fungi were *Cephalosporium* sp. *Penicillium* sp. and *Aspergillus* sp. Enumeration on AFM showed the presence of *Aspergillus flavus* and *Asp. niger* after 24 h of drying. The observation is consistent with that of Aziz et al (1998), who recorded *Asp. flavus* as the major contaminant in different spice samples. *Asp. flavus* and *Asp. parasiticus* are found to be temperature tolerant fungi (Davis and Diener 1983). Post-processing contamination by pathogenic and non-pathogenic microbes such as bacteria, thermophilic aerobic bacteria, yeast and molds has been reported in whole and powdered spices (Rani and Singh 1990; Hafez and Said 1997). The green coloured *Aspergillus* sp, which was encountered on fresh berries of black pepper, was not present after drying. In the dried samples, mostly black, *Asp. niger* was predominant that is chiefly responsible for mycotoxins in stored produce including spices and herbal drugs (Roy and Chourasia 1990).

Population dynamics of microbes during blanching: Total fungal propagules on fresh 'KS 27' berries were 7.1×10^3 cfu/g and after blanching fungi were not detected. The fresh berries of 'P 24' harboured 31.4×10^4 cfu/g, and the fungal population was declined to undetectable level after blanching. This could be due to the complete elimination of fungi during heat treatment. In both the varieties of black pepper 100 -fold reduction in population was recorded after 24 h of drying. This could be due to desiccation and heat generated by way of drying the sample. It is interesting to note that the coliform was not significantly reduced even after 3 days of drying. The persistence of coliform might be due to the occurrence of temperature tolerant coliform in black pepper. This observation is in agreement with Pruthi (1992), who reported that coliform contamination is very high in black pepper. Blanching followed by drying could eliminate 97.7% of the bacteria present on fresh black pepper berries. The percentage reduction of coliform, fungi and *Aspergillus* was 65%, 90.5% and 84.3% respectively (Table 2).

Population dynamics of microbes in mechanically dried sample: In mechanically dried samples 91.3% of bacterial and 99.1% coliform population got eliminated after 12 h of drying and over 95% of the fungi were also eliminated (Table 2). This could be due to inactivation of bacterial cells and fungal spores residing on fresh berries due to heat treatment (65°C) in a closed chamber. Mechanically dried black pepper harboured no or very little standard plate count (SPC) of microbes indicating that the produce is in good hygiene. These results clearly indicate the effectiveness of mechanical dryer for producing black pepper free from pathogenic bacteria and fungi for export. However, heat is generally considered as inapplicable for sterilizing spices, as the temperature ensures microbiological safety and it always leads to loss of some of the volatile oil, and consequently to a marked reduction in aroma and quality (Gottschalk 1977). Therefore, it is evident from this investigation that mechanical dryer is superior to other methods of drying black pepper. Although the initial costs are high, clean and safe black pepper berries can be obtained using this method. Proper handling, packaging and storage of processed samples after mechanical drying would ensure high quality black pepper for local and international trade.

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TABLE 3. POPULATION DYNAMICS OF MICROORGANISMS ON BLACK PEPPER BERRIES UPON TRADITIONAL METHOD OF DRYING

	Bacteria		Coliform		Fungi		*Aspergillus*	
Duration of drying, h	P24 (CFU × 10^5)	KS 27 (CFU × 10^6)	P 24 (CFU × 10^5)	KS 27 (CFU × 10^5)	P24 (CFU × 10^4)	KS 27 (CFU × 10^4)	P 24 (CFU × 10^4)	KS 27 (CFU × 10^4)
Fresh	13.11 $(6.060)^D$	40.225 $(7.470)^B$	6.45 $(5.783)^{DE}$	66.5 $(6.751)^{AB}$	3.1 $(4.455)^A$	7.1 $(3.658)^{AB}$	1.806 $(2.868)^{ABC}$	0.258 $(3.194)^{AB}$
8	80 $(6.711)^C$	57.85 $(7.746)^{AB}$	13.95 $(5.985)^{CD}$	116 $(6.988)^A$	0 $(0.0)^D$	1.590 $(3.951)^{AB}$	0.0500 $(1.500)^{BC}$	1.75 $(4.047)^A$
16	70.75 $(6.793)^C$	102.65 $(7.928)^A$	12.10 $(5.997)^{CD}$	101.75 $(6.912)^{AB}$	2.775 $(4.424)^C$	8.425 $(4.742)^A$	1.275 $(4.083)^A$	0.035 $(1.452)^{BC}$
24	9.03 $(5.930)^D$	53.750 $(7.659)^{AB}$	5.08 $(5.679)^{DE}$	164.0 $(7.669)^D$	0.230 $(3.126)^B$	1.5 $(4.103)^{AB}$	0.015 $(1.226)^C$	0.890 $(3.836)^A$
32	10 $(5.925)^D$	32.250 $(7.503)^{AB}$	3.35 $(5.410)^E$	32.33 $(6.374)^{BC}$	0.100 $(1.651)^C$	4.450 $(4.509)^A$	0.2 $(1.801)^{BC}$	2.120 $(4.209)^A$

Figures in parenthesis are log-transformed values; Data superscripted with the same letter are not different according to Duncan's Multiple Range Test at p= 0.05. CFU: Colony Forming Units

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