

BACTERIAL AND YEAST COUNTS IN BRAZILIAN COMMODITIES AND SPICES

Francisco das Chagas Oliveira Freire^{1*}; Lisa Offord²

¹Embrapa Agroindústria Tropical, Laboratório de Fitopatologia, Fortaleza, CE, Brasil. ²CABI Bioscience, UK Centre, Egham, England.

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SHORT COMMUNICATION

ABSTRACT

A total of thirteen genera of bacteria and two genera of yeasts were detected in surface sterilized and unsterilized Brazilian commodities and spices such as cashew kernels, Brazil nut kernels, black and white pepper. The genus *Bacillus* with eight species was by far the most common. The yeasts isolated were *Pichia* sp., *P. guilliermondii* and *Rhodotorula* sp. *Bacillus cereus*, *Salmonella typhimurium* and *Staphylococcus aureus* were detected in cashew and Brazil nut kernels.

Key words: cashew kernels, Brazil nut kernels, black and white pepper, microbiology.

Commodities such as cashew kernels, Brazil nut kernels and spices as black and white pepper are subjected to microbial contamination at various stages. The traditional methods of harvesting and preparations of these products result in heavy contamination. Growth of bacteria and yeasts may take place if the environmental conditions are damp, when equipment and workers may contribute to increase the levels of contamination.

Although being an excellent substrate for microorganisms growth, occurrence of bacterial infection on cashew kernels has rarely been reported. Studies conducted in South Africa (20) revealed light contamination of processed and non-processed cashew kernels by *Bacillus* spp. As for Brazil nut kernels, bacteria associated with decaying was first reported in 1921 (17). Mesophile bacterial counts on roast and salted Brazil nut kernels ranged from 5.3×10^3 to 1.2×10^4 cfu/g during storage period. Yeasts occurred in very low counts. The authors, however, did not identify bacteria (16). Concerning to black pepper, which accounts for some 35% of the world trade in ground spice, counts are by far the highest among the spices most used. Total number of bacteria ranging from 8.3×10^6 to 7.09×10^8 cfu/g (mean 1.9×10^8) has been confirmed (5). Other workers have also found high levels of bacterial contamination

on black pepper, although yeast populations had always been low (6,12,13,14). White pepper, to a lesser extent, can also be a source of contamination (2). Bacteria such as *Bacillus* spp., *Escherichia freundii*, *E. coli*, *Klebsiella* sp., *Serratia* sp., *Staphylococcus* spp. and *Streptococcus* spp. have been identified on black and white pepper. A comprehensive review on spices microbiology has been provided (7).

In the present paper an attempt has been made to survey the species of bacteria and yeasts on cashew nuts, Brazil nuts, black and white pepper imported from Brazilian producers.

A total of 4 samples of black pepper (500 g each), 4 samples of white pepper (500 g each) and 2 samples of Brazil nut kernels (2 kg each) were purchased from two retail suppliers in Belem city (State of Pará), in the Brazilian Amazonian Region. Ten kilograms of cashew kernels of common type (5 kg) and dwarf type (5 kg) were obtained from farmers of the State of Ceará (Pacajus county) in the Brazilian Northeastern Region. All samples were brought to CABI Bioscience (Egham, England) where commodities and spices were separated into visually healthy and infected. Sub-samples of 1 g (for black and white pepper) and 3 g (for common and dwarf cashew types), healthy and infected, were taken for microbiological analysis.

* Corresponding author. Mailing Address: Embrapa Agroindústria Tropical, Laboratório de Fitopatologia, Rua Dr^a Sara Mesquita, 2270, Planalto Pici. 60511-110, Fortaleza, CE, Brasil. E-mail: freire@cnpat.embrapa.br

The first batch of samples was placed in sterile universal vials and covered with 30% hydrogen peroxide for 5 minutes. After removal from H₂O₂ samples were rinsed in sterile distilled water and ground up with pestle and mortar in 5 ml of sterilized distilled water. Progressive dilutions (10⁻¹, 10⁻² and 10⁻³) were prepared and 0.1 ml of each was spread onto the nutrient agar surface in five 9cm diameter disposable Petri dishes. Plates were kept at laboratory temperature (18°C to 25°C) and colonies counted after 5 days. For the second batch samples were ground up without previous surface sterilization and dilutions were up to 10⁻⁵.

Fresh bacterial and yeast colonies (24 – 48 h old) were suspended in sterilized distilled water and processed in a Hewlett Packard 5890 Series II Gas Chromatograph for the extraction of the fatty acids. The organisms (bacteria/yeast) were identified using the MIDI microbial identification system where fatty acids profiles are compared with a database. The whole process from initial sub-culture to final identification was completed in 72 h.

The results of microbial load in cashew and Brazil nut kernels, in black and white pepper, with and without surface sterilization, are given in Tables 1 and 2, respectively. A total of thirteen

Table 1. Bacterial and yeast counts of surface sterilized Brazilian commodities and spices.

Source	Organisms	cfu/samples
Common cashew (healthy)	<i>Bacillus macerans</i>	0.20 x 10 ²
	<i>B. pumilus</i>	0.40 x 10 ²
Common cashew (infected)	<i>B. macerans</i>	0.10 x 10 ²
	<i>B. pumilus</i>	0.15 x 10 ²
	<i>Micrococcus luteus</i>	0.10 x 10 ²
Dwarf cashew (healthy)	<i>B. macerans</i>	0.15 x 10 ²
	<i>B. pumilus</i>	0.70 x 10 ²
	<i>Gordona bronchialis</i>	1.2 x 10 ²
	<i>Staphylococcus aureus</i>	1.7 x 10 ²
	* <i>Pichia guillermondii</i>	3.2 x 10 ²
Dwarf cashew (infected)	<i>B. macerans</i>	0.14 x 10 ²
	<i>B. pumilus</i>	0.70 x 10 ²
	<i>B. spaericus</i>	0.16 x 10 ²
	<i>S. aureus</i>	0.20 x 10 ²
Black pepper	<i>B. macerans</i>	2.1 x 10 ³
	<i>B. pumilus</i>	2.9 x 10 ³
White pepper	<i>B. macerans</i>	0.32 x 10 ³
	<i>B. pumilus</i>	0.53 x 10 ³
Brazil nut	<i>B. macerans</i>	0.1 x 10 ²
	<i>B. pumilus</i>	0.1 x 10 ²
	<i>S. aureus</i>	0.64 x 10 ²
	* <i>Pichia</i> sp.	9.14 x 10 ²

* yeasts.

Table 2. Bacterial and yeast counts of Brazilian commodities and spices without sterilization.

Source	Organisms	cfu/samples
Common cashew (healthy)	<i>Bacillus macerans</i>	0.40 x 10 ²
	<i>B. megaterium</i>	0.25 x 10 ²
	<i>B. mycooides</i>	0.15x 10 ²
	<i>Enterobacter sakazakii</i>	0.38 x 10 ³
	<i>Klebsiella pneumoniae</i>	0.60 x 10 ²
	<i>Micrococcus luteus</i>	0.15 x 10 ²
Common cashew (infected)	<i>Xanthomonas maltophilia</i>	0.14 x 10 ²
	<i>Acinetobacter baumannii</i>	0.10 x 10 ²
	<i>B. cereus</i>	0.17 x 10 ²
	<i>B. macerans</i>	0.13 x 10 ²
	<i>B. subtilis</i>	0.12 x 10 ²
	<i>E. cloacae</i>	0.08 x 10 ²
	<i>E. sakazakii</i>	0.21 x 10 ²
	<i>M. luteus</i>	0.19 x 10 ²
	<i>Rhodobacter capsulatus</i>	0.10 x 10 ²
	<i>Staphylococcus hominis</i>	0.10 x 10 ²
Dwarf cashew (healthy)	<i>X. maltophilia</i>	0.10 x 10 ²
	<i>B. macerans</i>	0.20 x 10 ²
	<i>B. megaterium</i>	0.09 x 10 ²
	<i>B. mycooides</i>	0.12 x 10 ²
Dwarf cashew (infected)	<i>E. sakazakii</i>	0.10 x 10 ²
	<i>B. cereus</i>	0.18 x 10 ²
	<i>B. macerans</i>	0.18 x 10 ²
	<i>B. mycooides</i>	0.10 x 10 ²
	<i>B. pumilus</i>	0.12 x 10 ²
Black pepper	<i>E. sakazakii</i>	0.20 x 10 ²
	<i>K. pneumoniae</i>	0.16 x 10 ²
	<i>B. alvei</i>	69.6 x 10 ³
	<i>B. pumilis</i>	36.8 x 10 ³
	<i>B. subtilis</i>	13.0 x 10 ³
	<i>Clavibacter michiganense</i>	1.8 x 10 ³
White pepper	<i>Erwinia herbicola</i>	0.10 x 10 ²
	<i>M. kristinae</i>	0.30 x 10 ²
	<i>B. macerans</i>	0.15 x 10 ²
Brazil nut	<i>B. pumilis</i>	0.80 x 10 ²
	<i>B. sphaericus</i>	0.10 x 10 ²
	<i>B. thuringiensis</i>	0.12 x 10 ²
	<i>M. luteus</i>	0.17 x 10 ²
	<i>Acinetobacter baumannii</i>	2.2 x 10 ³
Brazil nut	<i>B. cereus</i>	4.0 x 10 ³
	<i>B. macerans</i>	5.1 x 10 ³
	<i>B. subtilis</i>	3.2 x 10 ²
	<i>Escherichia coli</i>	1.3 x 10 ²
	<i>E. sakazakii</i>	4.2 x 10 ²
	* <i>Pichia</i> sp.	6.4 x 10 ⁴
	<i>Rothayibacter tritici</i>	1.1 x 10 ²
	* <i>Rhodotorula</i> sp.	10.7 x 10 ⁴

* yeasts.

genera of bacteria and two genera of yeasts were encountered among the microorganisms isolated. The genus *Bacillus* with eight species was by far the most common. The yeasts *Pichia guillermondii*, *Pichia* sp. and *Rhodotorula* sp. were present in higher populations, compared to bacteria. Yeasts were detected in cashew and Brazil nut kernels.

Among the commodities and spices surface sterilized, healthy dwarf cashew kernels showed the highest variety of microorganisms isolated. The higher populations, however, was detected in black pepper. When samples were examined without surface sterilization Brazil nut kernels yielded greater variety of species and the highest population of yeasts. Black pepper had the highest bacterial contamination. *Erwinia herbicola* was the only bacteria isolated which can behave as plant pathogen. *B. cereus* and *Staphylococcus aureus* have been reported as food poisoning agents.

As was expected, samples without surface sterilization yielded higher numbers of bacterial and yeast colonies as well as greater variety of microorganisms species. *Bacillus* spp. were the most common bacteria isolated, being present in all samples either with or without sterilization. Among the species isolated only *E. herbicola* and *Clavibacter michiganense* have been recognized as a plant pathogen. *X. maltophilia*, although belonging to a genus which encompasses some plant pathogens is actually a saprophyte. Most of the species isolated are ubiquitous saprophytes, commonly found in soil, water and on plant surfaces (3). Their presence in the commodities and spices examined revealed mainly contamination due to inadequate handling and storage.

Spices are known to be heavily contaminated with microorganisms. Ground black pepper may carry as many as 10^8 bacteria/g. White pepper, to a lesser extent, may also be infected (2,5,6,11). Most bacteria present in spices are aerobic spore-former. Baxter and Holzapfel (2) found that approximately 90% of colonies in viable counts were *Bacillus* spp. In the present study we have obtained similar results, with six species of *Bacillus* being identified. Our counts, however, are far lower compared to the workers above. Among the species we isolated only few of them have been associated with food poisoning. Indeed, *B. cereus* has been recognized as the etiological agent of food poisoning outbreaks in Europe as far back as 1906 (8). In USA and United Kingdom foodborne illness due to this species has been reported between 1968 and 1975 (18,19) According to Silliker (15), black pepper contaminated with aerobic spore formers enormously increased the microbial count of sausage. Aerobic spore formers have also been detected in white pepper powder (9). Coliforms and faecal streptococci were present in significant numbers in black and white pepper (2,13). We did not isolate faecal species from our black and white pepper samples but *Salmonella* has been found in black pepper produced in the Brazilian Amazonian Region (Dr. Maria L. Duarte, personal communication). Interestingly no yeasts were

detected in our black and white pepper samples. This may have occurred due to the inhibitory effects of essential oils present in these spices. Guarino (10) noted that yeasts were either entirely absent or present only in insignificant numbers in spices. Working with Indian spices Krishnaswamy *et al.* (13) identified the species *Candida huminicola*, *C. parapsilosis* and *C. tropicalis*.

As far as cashew kernels are concerned the only report on bacterial occurrence was the isolation of *Bacillus* spp.(20). In our study *Bacillus* spp. were too the most common organisms found but eight other different genera of bacteria and the yeast *P. guillermondii* were also detected. Among them a considerable number of coliforms such as *E. cloacae*, *E. sakazakii* and *K. pneumoniae* were found. Species associated with food poisoning as *B. cereus* and *S. aureus* occurred commonly on the commodity. As no case of food poisoning with cashew kernels have ever been reported, the possible explanation is that strains occurring on cashew kernels are mainly saprophytic, thus unable to produce enterotoxins, or because they have always been isolated in low numbers. Besides, the kernels examined were fresh and non-processed. As the processing involves heating and they are usually salted, these stages would be sufficient to kill the bacteria. The only yeast identified, *P. guillermondii*, is harmless and may be using the kernels as an occasional food source.

Concerning Brazil nut kernels, as far as we are aware, there has been no reports on bacterial or yeast identification. Although the presence of bacteria in decaying Brazil nut had been detected since 1921(17), no attempt was made to identify them. In Brazil a microbiological evaluation of roasted and salted Brazil nut kernels stored at room temperature revealed the presence of low population of unidentified mesophilic, thermophilic, proteolytic and lipolytic bacteria (16). Counts of the yeasts *Pichia* sp. and *Rhodotorula* sp. were higher in Brazil nut compared to cashew kernel samples. Counts of bacteria such as *Bacillus* spp., *E. coli* and *S. typhimurium* were not high but these findings indicates the unsuitable conditions the nuts were handled during processing. The samples used in the present study were purchased already shelled. This obviously exposed them to manual contact which may have facilitated their microbial contamination. It is well known that the hard shell can protect them from external invaders during storage and shipment (1,4).

Despite the presence of *B. cereus*, *S. typhimurium* and *S. aureus* in cashew and Brazil nut kernels, there is no reason for concern about the possibility of these commodities being a source of pathogenic bacteria, provided the processed kernels are properly packed and stored. Most of these strains are saprobes, thus unable to produce enterotoxins. Probably the strains we isolated are typically saprophytic, posing no threat to consumers' health.

As for black and white pepper the results of this investigation show that no organism posing a public health hazard was found.

The common occurrence of populations of *Bacillus* spp., *C. michiganense*, *E. herbicola* and *Micrococcus* spp. may also be expected since hundreds of small farmers with diverse growing and harvesting techniques are involved in the production of these spices. It must be pointed out, however, that spices such as black and white pepper may be a source of contamination if they are allowed to harbour sufficient population of toxigenic bacteria, mainly when incorporated into uncooked convenience foods. These microorganisms do not present a health hazard when spices are consumed in food which is cooked directly after addition of the spices. More recently major producing countries like Brazil are employing modern artificial drying equipment for commercial purpose. Such through-flow hot-air dryers can provide the market with pepper of better microbiological standard.

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RESUMO

Bactérias e leveduras associadas a amêndoas de cajueiro, de castanha-do-Brasil e à pimenta-do-reino preta e branca

Um total de 13 gêneros de bactérias e 2 de leveduras foi detectado a partir de amêndoas de castanha de cajueiro, de amêndoas de castanha-do-Brasil e de pimenta-do-reino preta e branca produzidas no Brasil. O gênero bacteriano mais freqüentemente encontrado foi o *Bacillus*, com 8 espécies. As leveduras *Pichia* sp., *P. guilliermondii* e *Rhodotorula* sp., isoladas somente a partir de amêndoas de cajueiro, apresentaram populações mais elevadas que as de bactérias. *Bacillus cereus*, *Salmonella typhimurium* e *Staphylococcus aureus* foram também detectadas em amêndoas de cajueiro e de castanha-do-Brasil.

Palavras-chave: população microbiana, amêndoas de cajueiro, castanha-do-Brasil, pimenta-do-reino, bactérias, leveduras.

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