Microbiological Quality of Spices Marketed in the City of Botucatu, São Paulo, Brazil

P. L. MOREIRA,1 T. B. LOURENÇÃO,2 J. P. A. N. PINTO,2 and V. L. M. RALL1,3

1Department of Microbiology and Immunology, Institute of Biosciences, São Paulo State University, Botucatu, São Paulo, Brazil; and 2Sanitary Inspection of Animal Products, Faculty of Veterinary Medicine, São Paulo State University, Botucatu, São Paulo, Brazil

ABSTRACT

The aim of this work was to assess the microbiological quality of spices sold in Botucatu, São Paulo, Brazil. A total of 233 samples were analyzed for mesophilic bacteria, thermostolerant coliforms, Bacillus cereus, Staphylococcus aureus, and Salmonella. Data showed that 21 and 5.6% of these samples were not in agreement with the standards of Brazilian law, due to an excess of coliforms and to the presence of Salmonella, respectively. Black pepper and cumin exhibited the lowest microbiological quality, whereas bay leaf showed the highest quality. It was concluded that the seasonings possessed poor microbiological quality, and new alternatives should be taken in the primary production in order to improve this quality. Irradiation may also be a tool to assure the safety of these products.

Spices are used all over the world in food preparation for their aromatic properties and to obtain better taste (3). The term spices is applied to natural products of vegetable origin that can be sold whole, in pieces, or ground. Most spices have no nutritional value, but they possess organoleptic properties that stimulate gastric secretion and increase the tonus and motility of digestive organs (10).

Some spices such as clove, oregano, onion, garlic, and paprika, among others, are known to have antimicrobial activity, although spices should not be added to meals at high concentrations aiming to reduce microbiological contamination, since such high concentrations can alter the organoleptic characteristics of food (6).

In addition, these spices may be exposed to great microbial contamination during harvest and processing, since they are frequently dried in the sun, dispersed in an open field, and sold without any treatment to reduce contamination. In addition, spices may be in contact with dust, and feces of rodents, birds, and insects, in storage under poor hygienic conditions (3, 9). Under these circumstances, spices can be an important factor in food contamination when added to uncooked meals or after cooking, leading to serious health risks. In tropical countries such as Brazil, the temperature and elevated humidity may be associated with the presence of microorganisms in spices and foods in general (3, 9, 21).

Data from literature show several microorganisms, including Bacillus cereus, Salmonella, Escherichia coli, Aspergillus niger, Rhizopus spp., Cunninghamhamella, Clostridium perfringens, Shigella dysenteriae, Staphylococcus aureus, thermostolerant coliforms (TC), Bacillus coagulans, Bacillus polymyxa, and Bacillus subtilis are frequently isolated in spices (3, 9, 24). In 1974, 12 samples of black pepper were analyzed in the United States, and Salmonella was not detected (15). However, this pathogen was isolated in 8.2% of different types of black pepper in Australia (20) and in 50% of Brazilian samples (23). B. cereus was isolated in all samples of 33 different spices in Australia (20) and in 6 spice samples in Holland, at concentrations of 102 to 106 CFU/g (11). In Brazil, 37 samples of black pepper were analyzed, and B. cereus was observed in 85% of the samples at concentrations ranging from 1.5 × 102 to 4.6 × 104 CFU/g (8).

Mesophilic bacteria were found in ginger, black pepper, and red pepper in India (>106 CFU/g in 50% of the samples) (24). In Cuba, similar CFU numbers were found in black pepper and cumin (22). In Brazil, black pepper samples were analyzed, and higher CFU numbers were registered, which ranged from 2.8 × 105 to 1.9 × 108 CFU/g (8).

All these studies show the potential of spices as carriers of microorganisms, especially pathogens, to humans. However, there are few articles reporting the evaluation of spice quality in Brazil, which is an important producer and exporter of these seasonings.

Brazilian sanitary standards (25) allow the presence of up to 102 most probable number (MPN) of TC per g, with the absence of Salmonella in 25 g of product. The International Commission on Microbiological Specifications for Foods (13) states that the highest concentration permitted for mesophilic bacteria in spices should be 106 CFU/g. Since several works show the occurrence of B. cereus in spices, we investigated the presence of this microorganism in our samples. S aureus was also included in this study, due to its capacity to grow in low water activity (0.86).
MATERIALS AND METHODS

Sixty-six samples of oregano (Origanum vulgare), 65 samples of black pepper (Piper nigrum), 15 samples of clove (Eugenia aromatic), 15 samples of cinnamon (Cinnamomum zeylcanicum), 15 samples of cumin (Cuminum cyminum), 15 samples of basil (Ocimum basilicum), 15 samples of bay leaves (Laurus nobilis), 15 samples of dehydrated parsley (Petroselinum crispum), and 15 samples of dehydrated green onion (Allium schoenoprasum) were analyzed, totaling 236 samples from 14 different brands collected between January 2004 and April 2006 in the city of Botucatu, São Paulo, Brazil. All of these brands belong to large companies and are retailed in the entire country.

Microbiological analysis. Twenty-five grams of the samples was homogenized in 225 ml of buffered sterilized water, using a Stomach Lab Blender 400 (Seward, Sussex, UK) for 30 s. From this initial dilution (10^-1), several decimal dilutions were performed, using the same diluent. Determination of the MPN of TC was carried out according to the American Public Health Association guideline, using a three-tube serial dilution. The MPN was calculated based on gas production in tubes of E. coli broth (17).

S. aureus enumeration. Serial dilutions of food homogenates were plated on Baird-Parker agar with 5% egg yolk tellurite emulsion, and incubated at 35°C for 48 h. Afterward, characteristic colonies were counted and transferred to tubes containing nutrient agar. The colonies were tested for catalase and coagulase production by using the Staphytect Plus Dry Spot Kit (Oxoid, Ltd., Basingstoke, UK) (18).

Mesophilic bacteria. The pour plate method was used for bacterial enumeration. One milliliter of each dilution was dispensed in a dish and mixed with 20 ml of plate count agar. CFU were counted after incubation at 35°C for 24 h. In order to calculate the final concentration, the number of CFU was multiplied by the inverse of the dilution factor of the respective plate (19).

Detection of Salmonella. Two hundred twenty-five milliliters of buffered peptone broth and 25 g of spices were homogenized in a Stomach Lab Blender 400 for 1 min and incubated for 18 to 20 h at 37°C. After incubation, 0.1 ml of the homogenate was transferred to 10 ml of Rappaport-Vassiliadis broth, and 1 ml of the homogenate was transferred to 10 ml of tetraphionate brilliant green broth. These suspensions were incubated at 42 and 35°C for 24 h, respectively. After incubation, a loopful of each suspension was plated onto xylose-lysine-deoxycholate agar and Salmonella-Shigella agar. After incubation at 35°C for 24 h, five typical colonies from each agar plate were biochemically tested, using triple sugar iron agar and the API 20E test kit (bioMérieux, l’Etoile, France). The colonies were also submitted to serological tests, using polyvalent somatic and flagellar antisera (Probac, São Paulo, Brazil) (1).

B. cereus group. Using the spread method, 0.1 ml of serial dilutions was plated in Mossel agar and incubated at 35°C for 24 h. Typical colonies were tested for Gram staining, catalase production, rhizoid growth, motility, hemolysis, and tyrosine decomposition (4).

All media were obtained from Difco, Becton Dickinson (Sparks, MD), except when otherwise specified.

RESULTS AND DISCUSSION

In this study, 236 samples of spices were analyzed, and 60 (25.8%) of them did not comply with the standards for mesophilic bacteria, according to the International Com-
one sample (6.7%) positive for each microorganism group. Other researchers did not detect *Salmonella* in this spice (3, 16, 20, 22), whereas *B. cereus* (3, 16, 20) and *S. aureus* (16) were isolated. Previous studies on the microbiological quality of cinnamon have found mesophilic bacteria frequencies lower (22), similar (16), or higher (3, 20) than those found in this work.

Basil samples did not show contamination by pathogens, but five (33.3%) samples were positive for TC. Other authors did not detect dangerous microorganisms in this spice, but the presence of mesophilic bacteria at high concentrations (2.6 × 10⁶ CFU/g) was reported (16).

A small percentage of clove samples did not comply with the Brazilian standards regarding TC and mesophilic bacteria; only two (13.3%) samples were positive for microorganisms belonging to both groups. In addition, *Salmonella* and *S. aureus* could not be isolated, and *B. cereus* was detected in a single sample. In Australia, no pathogen or mesophilic bacteria was detected in the samples (20). In Austria, *S. aureus* was found in clove, whereas *B. cereus* was found to be present at low concentrations, and *Salmonella* could not be isolated (16). Researchers reported the presence of *B. cereus* in clove samples tested in India, and also observed that half of them exhibited mesophilic bacteria concentrations up to 10⁶ CFU/g. No TC or *Salmonella* could be detected (3).

In this study, 15 samples of dehydrated parsley were analyzed and only 1 was positive for *B. cereus*; 6 (40%) and 1 (6.7%) showed an excess of TC and mesophilic bacteria, respectively. All of them were negative for *Salmonella*.

Dehydrated green onion was positive for *B. cereus* in 8.3% of the cases, but no contamination by *Salmonella* or *S. aureus* was detected. Two samples (16.6%) showed excess of TC.

Oregano was frequently contaminated by mesophilic bacteria, with concentrations above the upper standard limits in 14 samples (21.5%). Only 3 (4.6%) of 65 oregano samples were improper for consumption due to TC; all of the samples were negative for *Salmonella, B. cereus,* and *S. aureus.* The microbiological data found in the literature regarding this spice are quite variable. As with work by other authors, *Salmonella* could not be detected (2, 9, 15, 20, 22). In Argentina, *B. cereus* was not detected in oregano (14); however, this microorganism was present in 42% of the samples analyzed in Australia (20). Data concerning the indicator microorganisms are also highly variable. In other studies, mesophilic bacteria counts were <10² CFU/g in all samples (15). In contrast, several researchers found that 7 to 73.3% of the samples contained concentrations above 10⁶ CFU/g (2, 9, 20). Some researchers were unable to detect any TC in their samples (15, 20); on the other hand, it was reported that 20% of the samples were contaminated by these microorganisms, which were present at concentrations above 10³ MPN/g (2).

Our data confirm the poor hygienic quality of most of the spices produced not only in Brazil, but also around the world. Among those products, black pepper was found to present the largest problem. Initiatives in the primary production should be taken to improve this quality. The irradiation process may also be a tool to assure the safety of these products.

**ACKNOWLEDGMENTS**

The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo for the scientific initiation fellowship to P. L. Moreira, and José Maurício Storcin for the critical review of the manuscript.

**REFERENCES**


