Zeitschrift: Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene = Travaux de chimie alimentaire et d'hygiène

Herausgeber: Bundesamt für Gesundheit

Band: 77 (1986)

Heft: 4

Artikel: Microbiological quality of ground cinnamon : incidence of bacillus cereus

Autor: Karapinar, M. / Aktu, S.E.

DOI: https://doi.org/10.5169/seals-983398

Mitt. Gebiete Lebensm. Hyg. 77, 520–527 (1986)
Received 21 July 1986. Accepted 18 August 1986

Microbiological Quality of Ground Cinnamon: Incidence of Bacillus cereus

M. Karapinar and *S. E. Aktuğ*
Ege University, Engineering Faculty, Food Engineering Department, Bornova-Izmir

Introduction

Spices may contain large numbers of microorganisms including sporeforming bacteria and spoilage organisms (1–6). Since the technology of spice production varies widely by product and country of origin, with production practices ranging from sanitary to unsanitary, they exhibit various microbial loads.

The microbial flora of spices is dominated by species of the genus *Bacillus,* such as *B. subtilis, B. polymyxa, B. coagulans, B. licheniformis, B. megaterium* (3, 7). *B. cereus* which is the etiological agent in food-poisoning outbreaks is also found frequently (3, 7, 8). Anaerobic sporeformers are also found, but less numerous than aerobes (6, 9). Enterococci and the members of the family Enterobacteriaceae including bacteria of public health significance occurs occasionally, sometimes in moderate levels (3, 4). The fungal flora of spices may vary with the spice to some extent, but *Aspergillus glaucus* group is usually most prevalent. However, aflatoxin producing strains of *Aspergillus* have also been reported to be a predominant component of the microflora of various spices (10–16).

The present paper reports on an investigation of the microflora of retail samples of ground cinnamon being sold in Izmir, Turkey. The recommendations of ICMSF (International Commision on Microbiological Specifications for Foods) were used as a guideline in drawing conclusions (table 1). In addition, pathogenic bacteria such as *Staphylococcus aureus, Clostridium perfringens,* and *Bacillus cereus* were also investigated in order to determine the safety of the ground cinnamon which is commonly incorporated into foods that receive no further cooking. Detection of *Salmonella* spp. was not attempted since the recovery of this organism is reported to be unusual (18).

Experimental

Samples

A total of 20 field samples of cinnamon from four different brands in approximately 30 g packages were purchased from four retail stores. The sampling plan was based on five-unit samples which is recommended by ICMSF (17).

Preparation of the samples

The content of each retail package was aseptically transferred into a sterile beaker, just before the analysis, and mixed with a sterile spatula in order to get a homogenous sample unit. After mixing within the beakers, duplicate 5 g samples were withdrawn, and transferred into a sterile 100 ml flask containing 45 ml buffered peptone water (oxoid) as a diluent. These mixtures was then thoroughly stirred by shaking for 1–2 min and left for a few minutes that the coarse material to settle down (19–21). Since many spices including cinnamon contain inhibitory substances to microorganisms, decimal dilutions down to 10^{-5} were prepared in order to overcome the effects of such compounds (2, 19, 22, 23). First series of decimal dilutions were used for making aerobic plate counts, yeast and mould counts, coliform and *E. coli* counts, and *S. aureus* counts. Whereas second decimal series were used for making anaerobic and aerobic sporeformers including *B. cereus* counts.

Microbial counts and colony isolation

Aerobic plate counts (APC) were estimated by plating 1 ml of dilutions ranging from 10^{-1} to 10^{-5} into duplicate petri dishes, poured with plate count agar (Oxoid) and incubated at 30 °C for 48 h.

Yeast and moulds were determined on potato dextrose agar (Oxoid), acidified to pH 3.5 after sterilization by means of 10% tartaric acid solution. The plates were incubated at 30 °C for 3 days.

Coliform bacteria were enumerated by a 3-tube most probable number (MPN) determination in MacConkey's broth (Oxoid) by using one ml of the previously prepared 10^{-1}, 10^{-2}, 10^{-3} dilutions. Tubes were incubated at 37 °C for 48 h. For the confirmation and differentiation of coliforms a loopful of broth from each positive MacConkey's tube (displaying gas and acid) was streaked on Levine's eosine methylene blue agar (Oxoid) plates in a way to obtain discrete typical colonies, and incubated at 37 °C for 24 h (20, 24, 25). The number of tubes that provided a confirmed *E. coli* result (colonies exhibiting a greenish metallic sheen by reflected light and dark purple centres by transmitted light on EMB plates) was determined in order to obtain the MPN of *E. coli.*

Table 1. Sampling plan and recommended microbiological limits for spices (17)

Test	Type of hazard	Case	Plan class	n	c	Limit/g	
						m	M^x
Standard plate count	No direct health hazard. Utility (e. g. general contamination, reduced shelf life and spoilage)	2	3	5	2	10^4	10^6
Moulds	No direct health hazard. Utility (e. g. general contamination, reduced shelf life and spoilage)	2	3	5	2	10^2	10^4
Escherichia coli	Health hazard low, indirect (indicator)	5	3	5	2	10	10^3

n = The number of units in a sample.
c = The maximum number of marginal quality units.
m = The bacterial count that seperates good from marginal quality.
M = The bacterial count that seperates marginal from defective quality.
x = Values above M are unacceptable.

S. aureus was enumerated by spreeding 0.25 ml quantities of the dilutions onto Baird-Parker agar (Oxoid) and incubating for 24 h at 37 °C.

Total counts of *B. cereus* were determined by spreading 0.25 ml of dilutions ranging from 10^{-1} to 10^{-5} onto duplicate plates of KG agar (19) and incubating at 30 °C for 48 h. In order to estimate spores of *B. cereus,* remaining portions of decimal dilutions were pasteurized for 30 min at 80 °C and thereupon cultured onto KG agar in a similar way as mentioned previously. Both unpasteurized and pasteurized KG agar plates were observed for typical *B. cereus* colonies (rough, flat, dry, round or irregularly shaped, ground glass appearing, translucent to creamy white with a pink-red background) surrounded by a zone of turbidity (8). Typical colonies were counted and were examined microscopically. Additionally, motility test and some biochemical tests including gelatin liquefaction, nitrate reduction and Voges-Proskauer reaction were performed by applying standard techniques (27).

Total number of mesophilic aerobic spores was estimated on dextrose tryptone agar (Oxoid). One ml of pasteurized dilutions was added into 100 ml media at 45 °C and poured into a set of 5 plates in approximately equal volumes. Plates were incubated at 30 °C for 48 h.

Spores of mesophilic anaerobes were estimated by a 3-tube most probable number (MPN) technique in freshly prepared cooked meat medium (Oxoid) by using 1 ml of the previously pasteurized 10^{-1}, 10^{-2}, 10^{-3} dilutions. Tubes sealed with sterile 2% agar, were incubated at 30 °C for 4 days. A loopful from each positive culture (displaying gas and turbidity) was transferred into a tube of litmus milk for «stormy fermentation» in order to enumerate *Clostridium perfringens* (19, 28).

Results and discussion

The total number of moulds and aerobic bacteria as well as coliforms contained in 20 retail samples of cinnamon are presented in table 2. As it can be seen from the table, the aerobic plate count (APC) ranged from 5.2×10^3 to 1.2×10^5/g. Although none of the sample units had APC's greater than 10^6/g, in other words, any of them exceeded the M value (cf. table 1), only samples from source A had met the criteria of ICMSF for APC (17). By comparing the results of individual sample units as a whole with the criteria for APC, distribution of quality rates of the total 20 samples was found to be as follows: 30% in good quality, 70% in marginally acceptable quality.

The mould count ranges from 0 to 2.6×10^4 (table 2). None of the brands met the specifications set by ICMSF for mould count. However, when we considered these criteria for the result of each sample units; the acceptable, marginally acceptable and unacceptable percentages of the samples were found to be 5%, 50% and 45%, respectively.

Low numbers of coliforms were found and the counts exceeded 10/g in only 5 samples (table 3). However, their presence does not indicate a health hazard since *E. coli* was not found in any sample tested. These results agree with the results of others who reported that coliforms are not a necessary ingredient of spices and the occurence of *E. coli* in spices is rare and very sporadic (2, 3, 5, 26).

The total numbers of anaerobes varied between 0 to 95/g and the counts exceeded 50/g in only two samples (table 2). None of the samples contained *Cl. perfringens*. This is in contrast with the report of *Powers et al.* (2), who reported the relatively high incidence of *Cl. perfringens* in cinnamon, of which 14 out of 18 samples contained *Cl. perfringens.*

Table 3 indicates that the predominant types of organisms in the samples of the brands A, B, and C were aerobic sporeformers and these organisms accounted for between 23% and 91% of the all organisms found in the samples tested (cf. table 2). However, the samples obtained from firm D exhibited a different pattern, in which the percentages of sporeformers varied from ca. 1 to 6%. Considerable variations were also observed in total numbers and distributions of *B. cereus,* not only between the samples of the same brand, but especially between samples of the different brands. *B. cereus* was found in all samples ranging from 40 to 6800/g (table 3).

Table 2. Microbiological quality of retail samples of ground cinnamon

Brand	Sample No	Number of organisms/g			
		APC	Coliforms (MPN)	Moulds	Mesophilic anaerobe spores
A	1	5.2×10^3	<3	3.0×10^2	4
	2	6.9×10^3	7	4.0×10^2	4
	3	4.6×10^3	15	5.0×10^2	4
	4	7.4×10^3	250	4.0×10^2	<3
	5	7.3×10^3	45	5.0×10^2	4
B	1	4.3×10^4	30	1.6×10^4	14
	2	3.3×10^4	11	1.7×10^4	25
	3	2.6×10^4	4	1.2×10^4	75
	4	4.4×10^4	3	1.5×10^4	95
	5	2.8×10^4	<3	1.0×10^3	9
C	1	1.0×10^4	<3	1.7×10^4	9
	2	1.0×10^4	<3	2.6×10^4	7
	3	9.2×10^3	<3	1.9×10^4	15
	4	1.2×10^4	<3	1.6×10^4	15
	5	1.6×10^4	7	2.4×10^4	25
D	1	2.6×10^4	<3	2.0×10^3	<3
	2	3.5×10^4	<3	1.0×10^3	<3
	3	2.9×10^4	<3	1.0×10^2	<3
	4	1.2×10^5	<3	0	<3
	5	9.4×10^4	<3	1.0×10^3	<3

S. aureus and the yeast were not detected in any samples tested.

The results of this investigation and the earlier reports of others (1, 5, 13, 26) showed that spices including the cinnamon may be a source of contamination in the food industry and the kitchen.

The incidence of *B. cereus* in all samples analysed must be considered as a potential health hazard, because they may grow in foods, which are seasoned or garnished with it and not adequatly cooked or properly refrigerated. Our findings and earlier reports of others (7, 8) bring us to a conclusion that incidence of *B. cereus* in spices is considerably high as compared to other pathogens such as salmonella, coagulase positive staphylococci, and thereby the necassary precautions must be taken into consideration in preparation and handling of the foods that are highly seasoned.

Table 3. Relationship between the total number of aerobic sporeformers and B. cereus in ground cinnamon (numbers per gram)

Brand	Sample no.	Aerobic mesophilic spores	B. cereus	
			Before pasteurization	After pasteurization
A	1	3.8×10^3	6.0×10^2	3.0×10^2
	2	3.7×10^3	5.6×10^2	1.3×10^2
	3	4.2×10^3	7.2×10^2	2.8×10^2
	4	6.4×10^3	1.0×10^3	4.4×10^2
	5	3.2×10^3	7.4×10^2	2.6×10^2
B	1	1.8×10^4	2.2×10^3	1.4×10^2
	2	1.5×10^5	2.2×10^3	6.4×10^2
	3	1.5×10^4	2.3×10^3	1.4×10^3
	4	9.9×10^3	1.2×10^3	9.6×10^2
	5	6.4×10^3	2.4×10^2	8.0×10
C	1	4.9×10^3	3.6×10^3	7.6×10^2
	2	7.6×10^3	1.2×10^3	2.4×10^2
	3	8.2×10^3	6.8×10^3	3.2×10^2
	4	9.5×10^3	1.2×10^3	1.2×10^2
	5	7.9×10^3	1.2×10^3	4.0×10^2
D	1	1.6×10^3	4.0×10	4.0×10
	2	1.4×10^3	8.0×10	4.0×10
	3	1.3×10^3	4.0×10	4.0×10
	4	1.6×10^3	2.0×10^2	4.0×10
	5	1.0×10^3	2.8×10^2	4.0×10

Summary

The microbiological quality of ground cinnamon purchased locally was studied. The total number of aerobic bacteria and moulds ranged from 5200–120000/g and 0–26000/g, respectively. In general, the microflora of samples from 4 different brands varied widely. The incidence of *Bacillus cereus* was high (100%) and counts varied from 40 to 6800 per gram. No other bacteria of public health significance were found.

Zusammenfassung

In der vorliegenden Arbeit wurden die mikrobiologischen Eigenschaften von Zimtproben aus dem lokalen Handel untersucht. Die Gesamtkeimzahlen von aeroben Bakterien und Schimmelpilzen schwankten zwischen 5200–120000/g resp. 0–26000/g. Die Proben von vier verschiedenen Herstellern zeigten grosse Unterschiede bezüglich mikrobiologi-

scher Qualität. In allen Zimtproben wurde *Bacillus cereus* gefunden (40–6800 Keime/g). Andere pathogene Bakterien waren hingegen nicht nachweisbar.

Résumé

Nous avons procédé à la recherche qualitative et quantitative des microorganismes qui contaminent la cannelle en poudre achetée dans notre région. Le nombre total des bactéries aérobies et des moisissures se situe entre 5200 à 120000/g et entre 0 à 26000/g respectivement. En général, la microflore des échantillons provenant de 4 firmes différentes est assez variée. L'incidence de *Bacillus cereus* était la plus élevée (100%) et leur nombre variait entre 40 et 6800 par gramme. D'autres bactéries pathogènes n'ont pas pu être décelées dans nos échantillons.

Literature

1. *Jay, J. M.:* Modern food microbiology, 1st edition, p. 44–49. Van Nostrand Reinhold Company, New York 1970.
2. *Powers, E. M., Lawyer, R.* and *Masuoka, Y.:* Microbiology of processed spices. J. Milk Food Technol. **38**, 683–687 (1975).
3. *Baxter, R.* and *Holzapfel, W. H.:* A microbial investigation of selected spices, herbs and additives in South Africa. J. Food Sci. **47**, 570–574, 578 (1982).
4. *Seenappa, M.* and *Kempton, A. G.:* Bacteriological quality of black pepper in retail stores in a Canadian city. J. Food Sci. Technol. **17**, 130–133 (1980).
5. *Schwab, A. H., Harpestad, A. D., Swartzentruber, A., Lanier, J. M., Wentz, B. A., Duran, A. P., Barnard, R. J.* and *Read, Jr., R. B.:* Microbiological quality of some spices and herbs in retail markets. Appl. Environ. Microbiol. **44**, 627–630 (1982).
6. *ICMSF:* Microbiological ecology of foods II, 1st Edition, p. 731–751. Academic Press, New York 1980.
7. *Seenappa, M.* and *Kempton, A. G.:* A note on the occurrence of Bacillus cereus and other species of Bacillus in Indian spices of export quality. J. Appl. Bacteriol. **50**, 225–228 (1981).
8. *Powers, E. M., Latt, T. G.* and *Brown, T.:* Incidence and levels of Bacillus cereus in processed spices. J. Milk Food Technol. **39**, 668–670 (1976).
9. *Inal, T., Keskin, S., Tolgay, Z.* und *Tezcan, I.:* Gewürzsterilisation durch Anwendung von Gammastrahlen. Fleischwirtschaft **55**, 675–677 (1975).
10. *Moreno-Martiner, E.* and *Christensen, C. M.:* Fungus flora of black and white pepper (Piper nigrum L.) Rev. Latino-Americ. Microbiol. **15**, 19–22 (1973).
11. *Flannigan, B.* and *Hui, S. C.:* The occurence of aflatoxin-producing strains of Aspergillus flavus in the mould floras of gound spices. J. Appl. Bacteriol. **41**, 411–418 (1976).
12. *Pal, N.* and *Kundu, A. K.:* Studies on Aspergillus spp. from Indian spices in relation to aflatoxin production. Sci. Culture **38**, 252–254 (1972).
13. *Christensen, C. M., Fanse, H. A., Nelson, G. H., Bates, F.* and *Mirocha, C. J.:* Microflora of black and red pepper. Appl. Microbiol. **15**, 622–626 (1967).
14. *Hitokoto, H., Morozumi, S., Wauke, T., Sakai, S.* and *Kurata, H.:* Fungal contamination and mycotoxin detection of powdered herbal drugs. Appl. Environ. Microbiol. **36**, 252–256 (1978).

15. *Seenappa, M., Stabbs, L. W.* and *Kempton, A. G.:* The role of insects in the biodeterioration of Indian Red Peppers by fungi. Int. Biodeterior. Bull. **15**, 96–102 (1979).
16. *Seenappa, M., Stabbs, L. W.* and *Kempton, A. G.:* Aspergillus colonization of Indian Red Pepper during storage. Phytophatology **70**, 218–222 (1980).
17. *ICMSF:* Microorganisms in foods, II. Sampling for Microbiological analysis: Principles and specific applications, 2nd reprint. University of Toronto Press, Toronto 1978.
18. *Wilson, C. R.* and *Andrews, W. H.:* Sulfite compounds as neutraliezers of spice toxicity of Salmonella. J. Milk Food Technol. **39**, 464–466 (1976).
19. *Speck, M. L.:* Compendium of methods for the microbiological examinations of foods, 2nd edition. American Public Health Association, Washington 1976.
20. *Harrigan, W. F.* and *McCance, M. E.:* Laboratory methods in foods and dairy microbiology, 2nd printing. Academic Press Inc., Ltd., London 1976.
21. *Hersom, A. C.* and *Mulland, E. D.:* Canned foods, 6th edition, p. 279–280. J. and A. Churchill, London 1969.
22. *Bullerman, L. B:* Inhibition of aflatoxin production by cinnamon. J. Food Sci. **39**, 1163–1165 (1974).
23. *Bullerman, L. B., Lieu, F. Y.* and *Seier, S. A.:* Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. J. Food Sci. **42**, 1107–1109, 1116 (1977).
24. *ICMSF:* Microorganisms in foods, I. Their significance and methods of enumeration, 2nd edition. University of Toronto Press, Toronto 1978.
25. *The Oxoid Manual:* Culture media, ingredients and other laboratory services, 4th Edition. Oxoid Ltd. Basingstate 1973.
26. *Dagneau, E. L.* and *Mossel, D. A. A.:* The microbiological condition of dried soups. In: Microbiology of dried foods. Proceedings 6th Int. Sym. on food microbiology, (ed.) Kampelmacher, E. H., Ingram, M. and Mossel, D. A. A., p. 411–425. Central Institute for nutrition and food research, Inc. Zeiod, The Netherlands 1968.
27. *Refai, M. K.:* Manuals of food quality control, 4: Microbiological analysis. FAO, Rome 1979.
28. *Anonymous:* The bacteriological examination of water supplies. Reports on public health and medical subjects. No. 71, 4th Edition, p. 32–33. Her Majesty's Stationary Office, London 1969.

Dr. M. Karapinar
S. E. Aktuğ
Ege University
Engineering Fakulty
Food Engineering Department
Bornova-Izmir
Turkey