Research Note

GROWTH POTENTIAL OF BACILLUS COAGULANS *AND* BACILLUS MACERANS *IN SELECTED COMMERCIAL ACID AND ACIDIFIED FOODS*[1]

An acidified food is a low acid food to which an acid or acid food is added to make a product which has a final equilibrium pH of 4.6 or less and a water activity greater than 0.85.[2] The same federal regulations that define acidified foods do not require that they be thermally processed to destroy the spores of bacteria capable of growing in them. Some reports indicate that certain spore-producing bacteria of the genus *Bacillus* have grown in acid foods and raised its pH, thus creating a hazard of *Clostridium botulinum* growing and producing toxin in these otherwise safe foods.[3,4] Should spores of any of these pH-raising organisms survive the thermal process used with an acid or acidified product, a public health problem could arise unless these survivors are unable to grow in the product in question under normal conditions of storage and distribution.

To assay the existing level of risk in selected locally produced acid and acidified foods should bacterial spores survive this thermal process we made inoculation studies to determine the growth potential of *Bacillus coagulans* and *Bacillus macerans*, two bacteria that are known to grow and spoil acid foods.[5] The acidified foods studied included five commercial brands of "Recaíto" (mixture of coriander leaves, green peppers, salt, onion, garlic and citric acid), two commercial brands of "Sofrito" (basically the same ingredients as the "recaíto" plus tomato paste, sweet peppers, vegetable shortening colored with annato and vinegar), one commercial brand of minced garlic in oil (citric acid added) and one commercial brand of "Antipasto" (mixture of tuna, vegetable, vegetable oil, salt, tomato sauce, tomatoes and spices). The acid foods considered were three commercial brands of canned tomato sauce. All the above products, except the "antipasto," are used as base ingredients in Puerto Rican cuisine.

To carry out the study, we purchased samples at a local supermarket. To assure sterility, we heated in a glass flask, constantly stirring, the content of each container to a temperature that fluctuated between 90° and 97° C until aerobic plate counts of aliquots taken at different heating times indicated that the product was free of microorganisms. Heated samples were aseptically kept under refrigeration until the sterility test results were obtained.

After the product proved sterile, 25-g portions were aseptically transferred to sterile test tubes and inoculated with 0.4 ml of either a *B. coagulans* or *B. macerans* bacterial suspension in sterile 0.1% peptone water. The content of each tube was thoroughly mixed in a test tube mixer. We

[1]Manuscript submitted to Editorial Board 19 September 1990.

[2]Food and Drug Administration, 1988. 21 CFR 114. Office of the Federal Register, National Archives and Records Administration, Washington, D. C.

[3]Anderson, R. E., 1984. Growth and corresponding elevation of tomato juice pH by *Bacillus coagulans*. *J. Food Sci.* 49: 647-49.

[4]Montville, T. J., 1982. Metabiotic effect of *Bacillus licheniformis* on *Clostridium botulinum*: implications for home canned tomatoes. *Appl. Environ. Microbiol.* 44: 334-38.

[5]Stumbo, C. R., 1973. Thermobacteriology in Food Processing, 2nd ed., Academic Press, New York, N. Y.

performed an initial aerobic plate count of each tube. We used Plate Count Agar (DIFCO Laboratories, Detroit, Michigan)[#] and incubated the tubes at 35° C for 3 days. We then incubated the inoculated tubes at 35° C and performed subsequent aerobic plate counts of each tube as previously described after 1, 2 and 3 days of product incubation.

Table 1 illustrates results obtained in this study. Except for the presence of *B. coagulans* in "Recaíto" No.2, the tested products did not sustain growth of either bacteria. Plate counts diminished after only one day of incubation at 35° C, as compared to the initial inoculum, and kept diminishing or remained essentially unaltered thereafter.

TABLE 1.—*Growth of* B. coagulans *and* B. macerans *in selected acid and acidified foods*

Organism	Product	pH[1]	Days of Incubation at 35° C			
			0	1	2	3
			CFU[2]			
B. coagulans	Recaíto No. 1	4.78	2.5×10^{1}	3.1×10^{3}	3.2×10^{3}	2.2×10^{3}
	" " 2	4.75	1.7×10^{4}	2.2×10^{4}	2.5×10^{4}	2.4×10^{4}
	" " 3	4.34	2.0×10^{4}	5.3×10^{3}	6.5×10^{3}	4.9×10^{3}
	" " 4	4.36	2.7×10^{4}	1.8×10^{4}	1.6×10^{4}	1.1×10^{4}
	" " 5	3.87	2.3×10^{4}	1.3×10^{4}	1.4×10^{4}	1.0×10^{4}
	Sofrito No. 1	4.01	3.2×10^{4}	1.3×10^{4}	1.5×10^{4}	9.6×10^{3}
	" " 2	4.25	3.1×10^{4}	2.6×10^{4}	2.5×10^{4}	2.1×10^{4}
	Minced Garlic	4.52	6.6×10^{4}	9.0×10^{3}	8.6×10^{3}	———
	Antipasto	4.45	4.8×10^{5}	9.0×10^{3}	1.1×10^{4}	7.2×10^{3}
	Tomato Sauce No. 1	4.09	1.4×10^{4}	1.5×10^{3}	2.0×10^{3}	2.1×10^{3}
	" " " 2	4.22	6.4×10^{4}	2.5×10^{3}	2.6×10^{3}	2.4×10^{3}
	" " " 3	4.27	5.1×10^{4}	2.5×10^{3}	2.2×10^{3}	1.8×10^{3}
B. macerans	Recaíto No. 1	4.78	6.7×10^{1}	1.4×10^{4}	4.9×10^{3}	1.0×10^{3}
	" " 2	4.75	5.0×10^{4}	4.4×10^{4}	3.0×10^{4}	1.1×10^{4}
	" " 3	4.34	7.1×10^{4}	9.1×10^{3}	8.2×10^{3}	2.4×10^{3}
	" " 4	4.36	2.5×10^{4}	1.9×10^{4}	1.9×10^{4}	6.1×10^{3}
	" " 5	3.87	4.2×10^{4}	2.1×10^{4}	2.1×10^{4}	4.0×10^{3}
	Sofrito No. 1	4.01	5.4×10^{4}	5.4×10^{4}	2.0×10^{4}	3.2×10^{4}
	" " 2	4.25	4.8×10^{4}	3.3×10^{4}	3.6×10^{4}	3.6×10^{4}
	Minced Garlic	4.52	2.6×10^{5}	2.7×10^{4}	2.7×10^{4}	———
	Antipasto	4.45	2.5×10^{5}	2.3×10^{4}	2.4×10^{4}	7.6×10^{3}
	Tomato Sauce No. 1	4.09	6.5×10^{4}	4.0×10^{3}	7.2×10^{3}	6.4×10^{3}
	" " " 2	4.22	2.5×10^{5}	7.0×10^{3}	6.8×10^{3}	6.0×10^{3}
	" " " 3	4.27	4.3×10^{5}	5.0×10^{3}	7.2×10^{3}	6.9×10^{3}

[1]Values are the average of two samples.

[2]Colony-Forming Units per gram of product. Counts represent the average of duplicate plates for each of two different product samples inoculated on different days.

[#]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment of materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

The very slight increase in *B. coagulans* counts in Recaíto No. 2 can be attributed in part to the higher pH value of this product over that of the others, except for "Recaíto" No. 1. "Recaíto" No. 2 was a refrigerated product whereas "Recaíto" No. 1 was frozen. This difference may explain why they had higher pH values than the rest of the products tested. Since low temperature storage and distribution is an additional barrier to curtailing microbial growth, pH of the product becomes less critical for these foods. However, refrigerated foods should contain sufficient acidity or other means of preservation to avoid growth of non-proteolytic, psychrotrophic strains of *Clostridium botulinum*. This need becomes more so if we consider the probability of product temperature abuse during handling, distribution, retail and consumer use.

The data obtained in this study indicate that the locally produced acid and acidified products tested are not a potential botulinum hazard due to raising of pH by the bacteria considered if these bacteria survive the thermal process.

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