Chlorine Tolerance of Microorganisms Found in Wheat and Flour[1]

C. P. KURTZMAN and C. W. HESSELTINE, Northern Regional Research Laboratory, Peoria, Illinois

ABSTRACT

Wheat is frequently washed and tempered in chlorinated water before milling to reduce the microbiological population, but complete destruction of the microorganisms does not occur. Microorganisms commonly found in wheat and flour were tested to determine if they were resistant to the action of chlorine. Molds, yeasts, actinomycetes, and most bacteria were destroyed by low (5- to 25-p.p.m.) levels of chlorine. Only species of *Bacillus* were able to survive chlorine concentrations of 100 p.p.m. or greater. Consequently, survival of microorganisms during the washing and tempering of wheat with chlorinated water generally does not result from the microorganisms' having a high tolerance to chlorine.

Before milling, wheat is frequently washed and tempered in chlorinated water to reduce the microbiological population. The chlorine concentration usually ranges between 100 and 300 p.p.m. (1,2). However, treatment with chlorine seldom reduces the microbiological population by more than half (2). This study was undertaken to determine what effect chlorine has on typical wheat and flour microorganisms, and to ascertain if resistance to the action of chlorine might be responsible for their survival during the washing and tempering processes.

MATERIALS AND METHODS

Recent studies by Graves et al. (3,4) enumerated the microflora of wheat and flour from major domestic growing areas, and molds, yeasts, bacteria, and actinomycetes isolated during that work were used in the present investigation. Frequently, several isolates of the more common species were tested.

The chlorine solutions employed were made by bubbling chlorine gas through 0.05M monobasic potassium phosphate solution and the pH was set at 7.0 with 3N potassium hydroxide. The chlorine solutions were then standardized against 0.1N sodium thiosulfate (5).

One million cells of each microorganism tested were suspended in distilled water and added to the appropriate chlorine solution for a final volume of 10 ml. For molds and actinomycetes, spores were used. The chlorine concentrations and treatment times were 5, 25, 50, and 100 p.p.m. for 1 min. and 200 p.p.m. for 1 and 5 min.

After treatment, the cells were collected on a membrane filter (0.45-μ pore size, Millipore Corp., Bedford, Mass.) and washed with 25 ml. of sterile distilled water to remove any remaining chlorine. Filters with mold or actinomycete spores were placed upright on the surface of glucose-yeast extract agar (5 g. glucose, 5 g. yeast extract, 20 g. agar, and 1 liter distilled water) in 90-mm. Petri plates. Quantitative estimates of surviving yeasts and bacteria were made by shaking the filters in 100

---

[1]Contribution from the Northern Regional Research Laboratory, Peoria, Illinois 61604. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Reference to a company or product name does not imply approval or recommendation by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

ml. of sterile distilled water and plating the dilutions on the above agar medium. Control platings showed that suspension in either phosphate buffer or distilled water did not affect survival of the organisms tested.

Plates of inoculated media were then incubated for 24 hr. at 25°C., the percentage of germination was determined for the molds and actinomycetes, and the number of yeast and bacterial colonies was counted. Observation of the mold and actinomycete spores was facilitated by staining the spores on the filters with methylene blue, drying the filters at room temperature, and then mounting filter sections in immersion oil on standard microscope slides.

RESULTS

Many of the microorganisms common to wheat and flour were unable to survive an exposure to 5 p.p.m. chlorine for 1 min. These included the molds *Aspergillus candidus, A. amstelodami, Cladosporium cladosporioides,* and *Aureobasidium pullulans*; the yeasts *Hansenula anomala, Pichia farinosa, Cryptococcus albidus,* and *Pichia burtonii*; and the following bacteria: *Aerobacter cloacae, Erwinia* sp., *Escherichia coli, Flavobacterium* sp., *Pseudomonas* sp., *Paracolobactrum aerogenoides,* and *Micrococcus candidus*. Ascospores were present in the cultures of *Hansenula anomala* and *Pichia farinosa* but did not enhance survival.

Approximately half of the spores of *Penicillium cyclopium, P. urticae,* and *P. citrinum* survived a chlorine concentration of 5 p.p.m. for 1 min., but none survived a concentration of 25 p.p.m. for 1 min.

Streptomyces albus was the most common actinomycete found in wheat and flour (4). Approximately 0.5% of the spores from four of six isolates survived a chlorine concentration of 25 p.p.m. for 1 min.; but at 50 p.p.m. for 1 min., a few (0.1%) spores from only one isolate survived.

TABLE I. EFFECT OF CHLORINE CONCENTRATION ON THE SURVIVAL OF BACILLUS CEREUS AND BACILLUS PUMILIS[a]

Isolate	NRRL No.	Source of Isolate	Treatment Time and Average No. of Surviving Cells		
			5 p.p.m. Cl 1 min.	25 p.p.m. Cl 1 min.	50 p.p.m. Cl 1 min.
B. cereus	B-3476	Flour	1×10^6	5×10^5	1×10^5
B. cereus	B-3477	Flour	1×10^6	1×10^6	5×10^5
B. cereus	B-3478	Wheat	1×10^6	5×10^5	2×10^5
B. cereus	B-3479	Wheat	1×10^6	6×10^5	5×10^4
B. pumilis	B-3480	Flour	5×10^4	5×10^2	24
B. pumilis	B-3481	Flour	3×10^4	4×10^2	1×10^2
			100 p.p.m. Cl 1 min.	200 p.p.m. Cl 1 min.	200 p.p.m. Cl 5 min.
B. cereus	B-3476	Flour	1×10^3	5×10^2	23
B. cereus	B-3477	Flour	2×10^3	1×10^2	0
B. cereus	B-3478	Wheat	1×10^5	1×10^3	50
B. cereus	B-3479	Wheat	8×10^2	1×10^2	28
B. pumilis	B-3480	Flour	1	0	0
B. pumilis	B-3481	Flour	6	0	0

[a] 1×10^6 Cells per treatment. B. cereus culture, 95% spores; B. pumilis culture, 10% spores.

Of the bacteria common to wheat and flour, only isolates of *Bacillus cereus* and *B. pumilis* survived relatively high concentrations of chlorine. As shown in Table I, a few cells of *B. pumilis* were still viable after exposure to 100 p.p.m. of chlorine for 1 min., and cells of *B. cereus* survived after exposure to 200 p.p.m. for 5 min.

DISCUSSION

The results of this study indicate that the presence of microorganisms in flour does not relate to their ability to survive the chlorinated water customarily used in washing and tempering wheat. In nearly all cases the concentration of chlorine required to kill microorganisms present in wheat and flour was substantially below that used to treat wheat. The only possible exception was the species of *Bacillus* tested, which had a high chlorine tolerance.

Carry-over of microorganisms from wheat to flour apparently results from their escaping the action of chlorine. Certain of the microorganisms are protected by virtue of being within the kernel. This situation is particularly true of such storage molds as the Aspergilli and Penicillia, which penetrate well into the kernel. In other cases where the microorganisms are not within the kernel, protection undoubtedly comes about as a result of the chlorine's reacting with other organic material such as the wheat itself. Thus, the concentration of chlorine at the surface of the wheat would be negligible.

Literature Cited

1. FINCH, R. M., and HARRIS, M. C. Chlorination of wheat wash and tempering waters. Am. Miller Processor 83: 25 (1955).
2. VOJNOVICH, C., PFEIFER, V. F., and GRIFFIN, E. L., Jr. Reducing the microbial count of flour. Cereal Sci. Today 11: 16 (1966).
3. GRAVES, R. R., and HESSELTINE, C. W. Fungi in flour and refrigerated dough products. Mycopathol. Mycol. Appl. 29: 277 (1966).
4. GRAVES, R. R., ROGERS, RUTH F., LYONS, A. J., Jr., and HESSELTINE, C. W. Bacterial and actinomycete flora of Kansas-Nebraska and Pacific Northwest wheat and wheat flour. Cereal Chem. 44: 288 (1967).
5. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. AACC Approved methods (formerly Cereal laboratory methods, 7th ed.). The Association: St. Paul, Minn. (1962).

[Received March 10, 1969. Accepted November 10, 1969]