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A PROCEDURE  
FOR THE BACTERIOLOGICAL EXAMINATION  
OF POTATO FLAKES

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## ABSTRACT

A method for determining the bacteria colony count of potato flakes is described. Included with the description of the testing method is a listing of the culture medium, dilution water and equipment required for carrying out this procedure in the Laboratory.

# A PROCEDURE FOR THE BACTERIOLOGICAL EXAMINATION OF POTATO FLAKES

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This paper describes a simple, economical, and reproducible procedure developed at the Eastern Utilization Research and Development Division, U. S. Department of Agriculture, for microbiological testing of dehydrated mashed potato flakes. The method, which tests the dried flakes for bacteria colony count, is conducted as follows:

## DESCRIPTION OF PROCEDURE

### Equipment Required

1. Milk dilution bottles, A. P. H. A. 99 ml. size.
2. Bacteriological can opener<sup>1</sup> or scissors, depending upon the type of package used for the product.
3. Pipettes - 11.0 ml. milk pipettes A. P. H. A.
4. Waring<sup>2</sup> blender, single speed, 15,000 r.p.m.
5. Waring blender cups with screw-cap lids or Mason jars modified by fitting Waring blender knives into the jar lid.
6. Petri dishes - 100 mm. x 15 mm.
7. Metal cans for pipettes and Petri dishes.
8. Erlenmeyer flasks - 750 ml. and 1500 ml. for media.

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<sup>1</sup> This instrument is also known as the "special can opener" used for can break-down studies in the canning industry.

<sup>2</sup> Reference to certain products or companies does not imply an endorsement by the U. S. Department of Agriculture over others not mentioned.

## Dilution Water and Culture Medium

### Phosphate Buffered Dilution Water<sup>1</sup>

1. Stock Solution
  - a. Dissolve 34 gm. of  $\text{KH}_2\text{PO}_4$  in 500 ml. of distilled water.
  - b. Adjust to pH 7.2 with 1N NaOH.
  - c. Make up volume to 1 liter with distilled water.
2. Add 1.25 ml. of the above solution to distilled water and make up to 1 liter for dispensing in dilution bottles.

### Tryptone Glucose Extract Agar<sup>2</sup>

- |                    |           |
|--------------------|-----------|
| 1. Beef Extract    | - 3 gm.   |
| 2. Tryptone        | - 5 gm.   |
| 3. Dextrose        | - 1 gm.   |
| 4. Agar            | - 15 gm.  |
| 5. Distilled water | - 1 liter |

### Testing Method

1. Prepare sterile dilution blanks with phosphate buffered dilution water. Sufficient water should be placed in the Waring blender cups to permit thorough blending of a 1:15 dilution of the product, e.g., 20 gm. of product + 280 ml. of dilution water. Subsequent dilutions should be made in the 99 ml. A.P.H.A. milk dilution bottles.
2. Using aseptic precautions, weigh out a sufficient amount of product to prepare a 1:15 dilution directly into the Waring blender cup.
3. Permit the product to rehydrate for 5 minutes with occasional agitation to prevent localized caking.
4. Blend for 2 minutes at 15,000 r.p.m.

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<sup>1</sup> American Public Health Association, Incorporated, 1953. Standard methods for the examination of dairy products. 10th edition, p. 95.

<sup>2</sup> Difco Laboratories Incorporated, 1953. Difco manual, 9th edition p. 57.

5. Using aseptic precautions immediately pipette 1 ml. portions of the 1:15 dilution into each of three Petri dishes.
6. Make a further dilution of 1:150 in a sterile phosphate-buffered water blank by adding 11 ml. of the 1:15 dilution to the blank containing 99 ml. of dilution water.
7. Shake the 1:150 dilution bottle vigorously to ensure good dispersion of the inoculum and immediately pipette 1 ml. aliquots aseptically into each of three Petri dishes.
8. Add approximately 15 ml. of tryptone glucose extract agar that has been melted and cooled to approximately 45° C. to each Petri dish and disperse the inoculum by gentle rotation of the dish.
9. After the agar has solidified, pour an overlay of tryptone glucose extract agar on each Petri dish.
10. Incubate Petri dishes at 32° C. for 48 hours to secure the colony count for mesophilic organisms. If the colony count for thermophilic organisms is desired, incubate a duplicate series of plates at 55° C. for 48 hours.
11. Control plates should be prepared for each incubation temperature.

## DISCUSSION

Use of the standard, commercial type of blender cup is recommended; but it is possible to substitute Mason jars with blender knives fitted into the caps for blender cups. The knives must be fixed securely in the center of the jar lid so that no leakage will occur. The knife blades should be bent slightly upward to permit easy, rapid opening and closing of the container during aseptic operations. If a large sampling program is anticipated, it would be advisable to construct a holder which would keep the blender jar securely in position during blending. A ring stand equipped with a large, adjustable clamp or a spring clamp works well for this purpose.

The suggested dilutions and blend times were worked out in the Laboratory. In general, the counts on commercial potato flakes can be derived easily from a 1:15 dilution of the product. Samples of commercial flakes examined at the Eastern Utilization Research and Development Division have usually shown counts of less than 2,000 colonies per gram. A bacterial population of this magnitude can be counted easily by plating the suggested dilutions. The 2 minute blend

time is most important in this procedure. Blending a 1:15 dilution of potato flakes for less than 2 minutes tends to produce plates that are difficult to count, because large numbers of poorly blended flake particles make colony counting confusing. It was found that the 2 minute blend time was optimum because the plates made from 2 minute blend material had a minimum of large particles and showed the highest counts. While blending for less than 2 minutes led to difficulties in counting the plates, blending for longer periods produced lower counts, because heat generated during blending was sufficient to partially pasteurize the blend. In the course of establishing the 2 minute blend time, commercial potato flakes and flakes produced in the pilot plant were blended for time intervals of 1.0, 1.5, 2.0, 3.0, 5.0, and 7.0 minutes. None of the plates made from blends of less than 2 minutes duration were satisfactory, because they were clouded by poorly macerated flake particles. Blending for 2 minutes or longer gave a slurry which contained few large flake fragments, and plates made from these blends were much easier to read. However, blending for more than 2 minutes produced counts that were lower than the count derived from the 2 minute blend. Hence, 2 minutes was selected as the optimum blend time which would produce the highest colony counts on plates which were not partially obscured by flake fragments. While considerable variation was found in the decrease in colony count of various commercial samples with increased blending time, the addition of each blend time increment above 2 minutes always showed an attendant decline in colony count. The average percentage decrease in colony count with the increase of blend time noted in this work is shown in Figure 1.

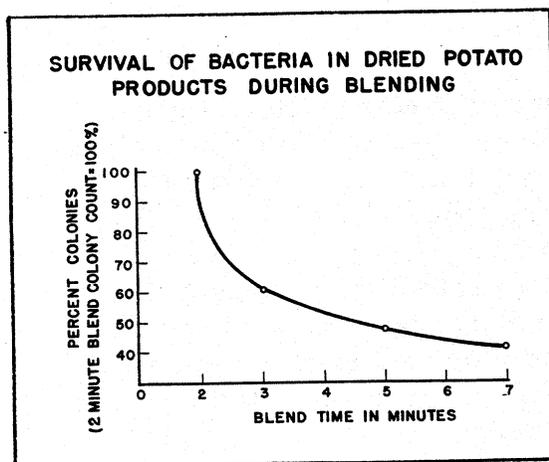


Figure 1.

These figures were secured mainly from commercial samples of undetermined age. An industrial laboratory working solely on fresh material might find some variation in rate of decline in count from this pattern due to the presence of more vegetative cells; but work done on flakes produced in the Eastern Utilization Research and Development Division pilot plant indicates that the 2 minute blend time at 15,000 r.p.m. is the optimum for flake samples regardless of age.

Care must be exercised to make certain that the inoculum is evenly dispersed across the surface of the Petri dish. Inadequate dispersal of the inoculum will produce plates which are crowded with colonies in the areas occupied by the inoculum. Plates of this type are difficult to read and lead to erroneous results.

The application of an agar overlay on all plates is most important. Many of the organisms found in the products examined produced rapidly growing "spreader" colonies. These colonies frequently cover the entire surface of a Petri dish in the course of incubation, rendering the plate useless for colony counting. The use of the agar overlay acts as a deterrent to the activity of these organisms. In addition to this, the use of triplicate plates for any dilution decreases the possibility of losing a dilution series for counting purposes due to "spreader" organisms. One, or even two, plates in a series may be lost due to these organisms; but it is seldom that all three plates would be obscured by "spreaders."

Experience in this Laboratory indicates that commercial flakes usually have a count of 2,000 colonies per gram or less. Some exceptional samples have had counts below 500 colonies per gram. No bacteriological standards are available for this product, at present; but it is felt that the use of the bacteria colony count can serve as a valuable tool in a plant sanitation and quality control program. The aforementioned count could serve as a reference point for starting such a program.

#### SUMMARY

A procedure for the bacteriological evaluation of potato flakes is presented in this paper. Equipment, culture medium, diluent, and a step-by-step testing procedure are given. In addition, equipment modifications, selection of processing time, and precautions required for the successful performance of the test are discussed.