Bacteriology of Dehydrated Space Foods

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The initial bacteriological requirement established in 1964 for space foods by the U.S. Army Natick Laboratories are: a total aerobic plate count (≤10,000 per g), a total coliform count (≤10 per g), fecal coliforms (negative per gram), fecal streptococci (≤20 per g), coagulase-positive staphylococci (negative in 5 g) and salmonellae (negative in 10 g). Of the space foods and prototypes tested during 1968 and 1969, 93% complied with the total aerobic plate count, 98% had less than 1 coliform per g, and 99% were negative for fecal coliforms; 88% complied with the streptococci requirement; 100 and 98% were negative for staphylococci and salmonellae, respectively. Nineteen food samples which did not comply (as indicated parenthetically by actual counts per gram) with the requirements were (i) total aerobic plate count: beef soup and gravy base (18,000), chicken soup and gravy base (57,000), spaghetti with meat sauce (12,100 and 14,000), sugared coffee (>300,000), chocolate ice cream cubes (20,000), and each of four samples of chocolate candy (12,000 to 61,000); (ii) coliforms: two out of three vanilla milk drinks (16 and 127) and one beef hash bar (14); (iii) fecal coliforms: one sample of chicken soup and gravy base positive; (iv) fecal streptococci: two samples of peanut cubes (40 and 108), coconut cubes (75), chicken soup and gravy base (2,650), beef soup and gravy base (33), and five out of six flavored milk drinks (23 to 300); (v) salmonellae: one each of chicken and beef soup and gravy base were positive.

The microbiological requirements for dehydrated space foods were established in 1964 in an effort to protect the astronauts from food poisoning (7). The stress factor was an important consideration in the establishment of these requirements since stress is known to alter resistance to infection. Simulation of stresses encountered during space flight such as high altitude (3–6), irradiation (10), emotional strain (14), cold (12, 13), heat (12, 15), and sonic stress (M. A. Jensen and A. F. Rasmussen, Bacteriol. Proc., p. 149, 1962) have been shown in the laboratory to alter the resistance of the animal host to microbial infections and intoxication.

This paper presents production experience accumulated over a 2-year period with dehydrated (freeze-dried) space foods and demonstrates the attainability of the microbiological requirements established for these foods.

MATERIALS AND METHODS

Rehydratable space foods. Typical rehydratable products which are used for the Apollo feeding systems are shown in Fig. 1. Eight classes of foods are shown packaged in the original zero-gravity feeder. A newly designed spoon and bowl feeder is now being used in Apollo missions.

Bite-size space foods. In addition to the rehydratables, dehydrated bite-size foods (Fig. 2) are used on the Apollo missions. These products are eaten by depositing them directly into the mouth.

Preparation of slurry. A 25-g amount of the dehydrated space food sample was aseptically transferred to a sterile blender cup and blended in 225 ml of Butterfield's (8) chilled sterile buffered water (SBW) for 2 min. This slurry constituted a 1:10 dilution and contained the equivalent of 0.1 g of food sample per ml. Hereafter this dilution shall be termed extract A. Extracts were maintained at no greater than 5 C and were used within 1 hr as prescribed in the following tests.

Media. All media were purchased from Difco Laboratories, Detroit, Mich.

Total aerobic plate count. Ten milliliters of extract A was transferred into 90 ml of SBW, giving a final dilution of 1:100. One ml of the 1:100 dilution was transferred into each of five petri plates and poured with plate count agar. Plates were incubated at 35 C and counted after 48 hr. The total number of colonies on the five plates should not exceed 500.

Total coliform count. A 1-ml amount of extract A was transferred into each of 10 petri plates and poured with Violet Red Bile (VRB) agar. Solidified plates were overlaid with 5 ml of VRB agar. Plates were incubated at 35 C, and typical coliform colonies (dark red, 0.5 mm or more in diameter) were counted after 18 to 24 hr. The total number of typical colonies on all 10 plates constitutes the total coliform count per 1 g of food and should not exceed 10.

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Fecal coliform count. Each typical VRB colony was transferred into phenol red-lactose broth fermentation tubes. Tubes were incubated at 35 C for 18 to 24 hr. Two loopfuls (3 mm diameter) of broth from each positive tube (displaying acid and gas) were transferred into an EC broth fermentation tube, which was incubated at 45.5 ± 0.2 C for 24 hr. Both temperature and time are critical for this differential test. Hence, incubation was carried out in a constant-temperature bath and monitored with a certified Bureau of Standards thermometer or equivalent. EC tubes displaying gas production were considered positive for fecal coliforms. A single EC-positive culture constituted rejection.

Fecal streptococci count. A 1-ml amount of extract A was transferred into each of 10 petri plates and poured with KF Streptococcus Agar. Plates were incubated at 35 C for 48 hr, and all red or pink colonies were counted. The total number of typical colonies on all 10 KF plates constitutes the fecal streptococci count per 1 g of food and should not exceed 20.

Coagulase-positive staphylococci. Fifty milliliters of extract A (equivalent to 5 g of food) was transferred into 200 ml of cooked meat medium with NaCl. The medium was prepared overstrength by adding 31.25 g of cooked meat and 24.5 g of NaCl to 200 ml of distilled water. The final concentration of cooked meat and NaCl in 250 ml was 12.5 and 10%, respectively.
The cooked meat medium was incubated at 35 C for 24 hr. A 0.1-ml amount of cooked meat medium was spread on each of two plates of Vogel and Johnson (VJ) agar. VJ plates were incubated at 35 C and examined after 24 and 48 hr. Two or more representative colonies which reduced tellurite were transferred to Brain Heart Infusion (BHI) tubes and incubated at 35 C for 24 hr. The remainder of each colony was emulsified in 0.2 ml of BHI. Then 0.5 ml of coagulase plasma was added, mixed, and incubated in a 35 C water bath for 4 hr. If these tubes were negative, the coagulase test was then repeated with the 24-hr-old culture. A single coagulase-positive colony constituted rejection.

Salmonella. One hundred milliliters of extract A (equivalent to 10 g of food) was transferred into 100 ml of double-strength lactose broth and incubated at 35 C for 24 hr. A 25-ml amount of the lactose broth culture was transferred to 225 ml of each of selenite-cystine broth and TT broth base [modified tetrathionate broth containing Brilliant Green (1:100,000)] and incubated at 35 C for 18 to 24 hr. Each enrichment culture was streaked on one plate each of three selective media: Brilliant Green Sulfadiazine (BGS) agar, Bismuth Sulfitie (BS) agar, and Salmonella-Shigella (SS) agar. BGS and SS plates were incubated for 24 hr and BS plates for 48 hr at 35 C. Two typical colonies were picked from each plate and inoculated on Triple Sugar-Iron (TSI) agar and Christensen’s urea (CU) agar slant. All slants were incubated at 35 C for 24 hr. CU slants were observed periodically for 4 to 6 hr. If the culture showed a urease-positive reaction, the respective colony was Salmonella-negative, and the test was ended. Positive TSI agar tubes associated with a urease-negative reaction constituted a presumptively positive Salmonella culture. Transfers from positive TSI slants were typed against Salmonella O and H polyvalent antisera. Positive reactions constituted confirmed presumptively positive Salmonella in the test sample. Presumptively positive TSI cultures were further confirmed through reactions in the following: dulcitol (+), malonate (−) broths, lysine decarboxylase broth (+), KCN broth (−), and indole broth (−). A single confirmed positive Salmonella culture constituted rejection.

RESULTS

The microbiological requirements (Table 1) established for dehydrated space foods were attainable as evidenced by the microbiological data presented in Table 2. Of the foods analyzed in 1968 and 1969, 93% had total aerobic plate counts of less than 10,000 per gram, 98% had less than 1 coliform per gram, and 99% were negative for fecal coliforms per gram; 88% had less than 20 fecal streptococci per gram; 100% were negative for coagulase-positive staphylococci; and 98% were negative for salmonella.

The microbiology of 19 foods which did not comply with the microbiological requirements for dehydrated space foods during 1968 and 1969 is presented in Table 3. With the exception of some of the chocolate cubes and the peanut cubes, these items were prototype foods which were being tested for space flight. There was no correlation between total aerobic plate counts and other bacterial indicators, except in the case of chicken and beef flavored soup and gravy base. Both soups, in addition to having relatively high total aerobic plate counts, were positive for Salmonella and exceeded the fecal streptococci requirement. The chicken soup was also positive for fecal coliforms.

### Table 1. Microbiological requirements for dehydrated space foods

| Organism                     | Requirement     |
|------------------------------|-----------------|
| Total aerobic plate count    | Not greater than 10,000/g |
| Total coliforms              | Not greater than 10/g |
| Fecal coliforms              | Negative in 1 g  |
| Fecal streptococci           | Not greater than 20/g |
| Coagulase positive staphylococci | Negative in 5 g |
| Salmonella                   | Negative in 10 g |

### Table 2. Microbiological analysis of dehydrated space food during 1968 and 1969

| Organism           | No. of samples | Per cent samples containing (per gram) | Per cent positive | Per cent negative |
|--------------------|----------------|----------------------------------------|-------------------|-------------------|
|                    |                | <1 | <20 | <50 | <100 | <1,000 | <10,000 | <50,000 | <100,000 | >100,000 |            |            |            |
| Total plate count  | 129            | 5  | 11  | 39  | 67   | 93    | 98     | 99     | 1        |          |            |            |            |
| Total coliforms    | 129            | 98 | 99  | 100 |      |       |        |        |          |            |            |            |            |
| Fecal streptococci | 102            | 87 | 88  | 91  | 93   | 99    | 100    |        |          |            |            |            |            |
| Fecal coliforms    | 129            | 87 | 88  | 91  | 93   | 99    | 100    |        |          |            |            |            |            |
| Coagulase-positive staphylococci | 104 | 90 | 99  | 100 |      |       |        |        |          |            |            |            |            |
| Salmonella         | 104            | 90 | 99  | 100 |      |       |        |        |          |            |            |            |            |


TABLE 3. Microbiology of dehydrated space foods and prototypes which did not comply with microbiological requirements

| Product                          | Total aerobic count (per g) | Total coliforms (per g) | Fecal coliforms (per g) | Fecal streptococci (per g) | Coagulase-positive staphylococci | Salmonella |
|---------------------------------|-----------------------------|-------------------------|-------------------------|-----------------------------|---------------------------------|------------|
| Prototypes                      |                             |                         |                         |                             |                                 |            |
| Beef hash bar                   | 5,000                       | 14<sup>a</sup>          | Neg                     | <1                          | Neg                             | Neg        |
| Spaghetti and meat balls        | 12,100<sup>a</sup>          | <1                      | Neg                     | <1                          | Neg                             | Neg        |
| Spaghetti and meat sauce        | 14,000<sup>a</sup>          | <1                      | Neg                     | <1                          | Neg                             | Neg        |
| Chocolate cubes                 | 32,100<sup>a</sup>          | <1                      | Neg                     | <1                          | Neg                             | Neg        |
| Chocolate oatmeal bar           | 12,300<sup>a</sup>          | <1                      | Neg                     | <1                          | Neg                             | Neg        |
| Chocolate drink                 | 1,500                       | >300,000<sup>a</sup>    | <1                      | <1                          | Neg                             | Neg        |
| Chicken flavored soup and gravy base | 57,000<sup>a</sup>   | 2                       | Pos<sup>a</sup>         | 2650<sup>a</sup>             | Neg                             | Pos<sup>a</sup> |
| Coffee with sugar               | 17,800<sup>a</sup>          | <1                      | Neg                     | 33<sup>a</sup>               | Neg                             | Pos<sup>a</sup> |
| Space foods                     |                             |                         |                         |                             |                                 |            |
| Chocolate cubes                 | 26,000<sup>a</sup>          | <1                      | Neg                     | <1                          | Neg                             | Neg        |
| Peanut cubes                    | 820                         | <1                      | Neg                     | 40<sup>a</sup>               | Neg                             | Neg        |
| Peanut cubes                    | 370                         | <1                      | Neg                     | 108<sup>a</sup>              | Neg                             | Neg        |
| Coconut cubes                   | 1,000                       | <1                      | Neg                     | 75<sup>a</sup>               | Neg                             | Neg        |

<sup>a</sup> Counts which exceeded the microbiological requirements.

There was only a 30% correlation between the presence of coliforms and fecal streptococci in the 19 foods presented in Table 3. For example, of the 10 foods which contained fecal streptococci, only three contained coliforms. The higher recovery rate of fecal streptococci may have been due to the greater resistance of streptococci to drying, heat, and other food manufacturing processes.

**DISCUSSION**

The microbial indexes (Table 1) were selected to measure the sanitary conditions during space food production and to detect the presence of foodborne pathogens and organisms that may indicate the presence of enteric pathogens. The methods employed to recover and enumerate the microorganisms are generally accepted by microbiologists worldwide and are modifications of standard methods recommended by many authorities (1, 2, 11, 16).

All of the microorganisms tested for, with the exception of coagulase-positive staphylococci, were detected in at least one of the space food prototypes (Table 3), indicating that the methods employed were suitable. Although it is possible that staphylococci were not present in the foods tested, it is also possible that 10% sodium chloride (NaCl) was too inhibitory, particularly to injured staphylococci. It has been demonstrated, for example, that thermally injured cells of *Staphylococcus aureus* were salt-sensitive and unable to grow in Trypticase Soy Agar containing 7.5% NaCl (9). Allowing for this possibility and to improve the recovery of staphylococci, the procedure has been modified by incubating the food sample in Tryptic Soy Broth (TSB) for 3 to 4 hr before the addition of 10% NaCl to the medium. Iandolo and Ordal (9) reported that the recovery of thermally injured cells of *Staphylococcus aureus* was completed within 4 hr in Tryptic Soy Broth incubated at 37 C and that thereafter growth in 7.5% salt-agar equaled the untreated controls.

The attainability of the microbiological requirements established for dehydrated space foods is evidenced by the high percentage of foods which complied with these requirements (Table 2). The excellent microbiological quality of these space foods was achieved by purchasing and properly storing good quality raw products, by processing these foods in special laminar airflow and conventional clean rooms (class 100 and 10,000, respectively), and by observing good manufacturing practices and sanitary operating procedures.

The microbiological requirements for space foods are under continual surveillance and will continue to be amended as warranted by new
knowledge and the state of the art. Process- and product-specific studies are still needed to determine clearly the etiological significance of the organisms tested for, as well as organisms such as Clostridium perfringens, Bacillus cereus, and others that are less well known, including viruses, rickettsia, vibrios, and mycoplasma.

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