HAZARD ANALYSIS OF *CLOSTRIDIUM PERFRINGENS* IN THE SKYLAB FOOD SYSTEM

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ABSTRACT

The Skylab Food System presented unique microbiological problems because food was warmed in null-gravity (diminished convection) and potentially diminished conduction due to poor surface contact), and because the heat source was limited to 69.4 C (to prevent boiling in null-gravity in the approximately one-third atmosphere total pressure). For these reasons, the foods were manufactured using critical control point techniques of quality control coupled with appropriate hazard analyses. One of these hazard analyses evaluated the threat from *Clostridium perfringens*. Samples of food were inoculated with *C. perfringens* and incubated for 2 h at temperatures ranging from 25 to 55 C. Generation times were determined for the foods at various temperatures. Results of these tests were evaluated taking into consideration: food-borne disease epidemiology, the Skylab food manufacturing procedures, and the performance requirements of the Skylab Food System. Based on this hazard analysis, a limit for *C. perfringens* of 100/g was established for Skylab foods.

The Skylab manned space flight program presented unique problems involving the microbiological safety of foods. The unmanned Skylab spacecraft was launched into earth orbit in May of 1973. This unmanned vehicle contained most of the foods for life support of the nine astronauts who later rendezvoused with it and lived in it for over 500 man-days during the next 10 months. The Skylab food supply had to have long-term stability and safety and yet accurately and adequately provide nutrition for the astronauts during their epic expeditionary voyages.

All the Skylab foods, other than beverages, were packed in drawn-aluminum cans fitted with full-panel pullout lids. At meal time, cans were assembled into meals and inserted into a food warmer-serving tray (Fig. 1). In this way, the astronauts warmed their food and consumed it (by use of conventional tableware) directly from the opened cans that were held in the warmer-serving tray. This tray provided the first capability to heat foods during a U.S. space flight. The heaters were electrical resistance wires designed to heat to a maximum of 69.4 C. Higher temperatures had to be avoided to prevent food from boiling and expelling particles in null gravity. Boiling would have occurred near 72.2 C in the Skylab that was approximately one-third atmosphere total pressure.

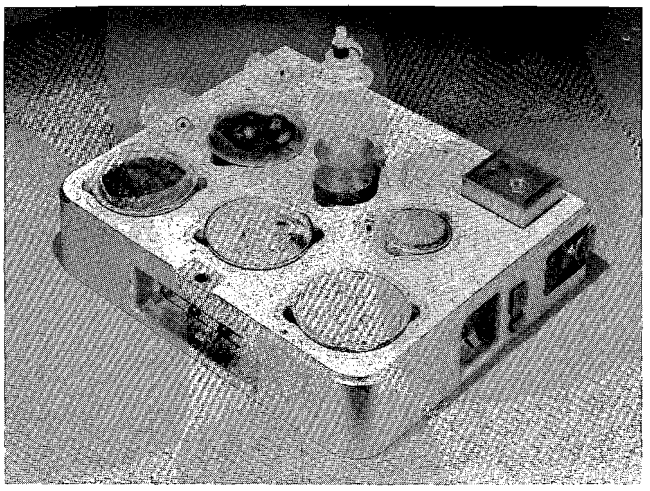

Figure 1. The Skylab food warmer-serving tray.

The food warmer-serving tray was designed so that frozen food (−23.2 ± 5.5 C) could be heated to 65 ± 3.3 C within 2 h under null gravity conditions. The design also provided for holding the food at 65 ± 3.3 C until consumed. These design criteria were established to prevent multiplication of potential pathogenic microorganisms. The watt density design calculations of the food tray assumed heat transfer only by conduction because convection currents were expected to be minimal in null gravity (radiant-heat transfer was ignored as being insignificant). The groundbased time temperature and percent heater-on-time relationships for the Skylab heating-serving tray are presented graphically in Fig. 2. Groundbased testing with the complete absence of convection currents in foods is impossible. The possibility always remained, therefore, that heating of food in null gravity would be slower than that indicated by groundbased testing. Such a condition could result from poor contact between the food and its container during weightless flight. Therefore, before flight there was considerable uncertainty as to the actual heating times in null gravity, and

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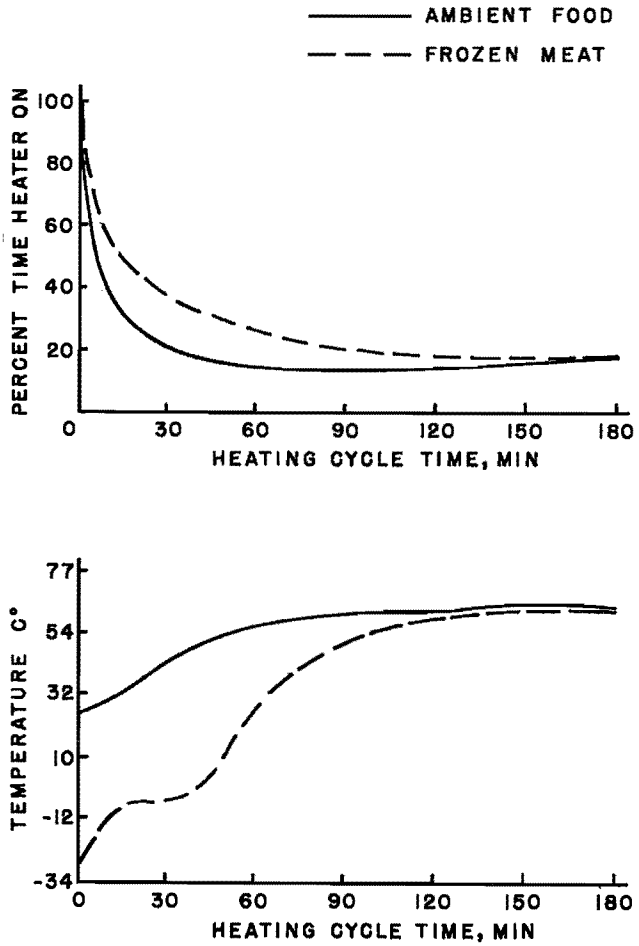

Figure 2. Time-Temperature relationships in Skylab food heating-serving tray.

there was no definitive means to test these heating characteristics before flight. Skylab food was, therefore, manufactured under a uniquely rigid quality control program that incorporated a critical control point system of production and test standards established after appropriate hazard analyses *(2, 6, 8)*. One of the hazard analyses conducted involved evaluation of the potential for multiplication of *Clostridium perfringens* in the foods during food warming times.

C. perfringens was chosen as the test organism for hazard analysis because of its importance in food poisoning, ubiquitous nature, heat tolerance, and known capability to multiply in foods. The growth rates of *C. perfringens* in the Skylab food heating profile was judged critical to the hazard analyses of food handling procedures and crew safety. A test plan was devised to determine the rate of growth of *C. perfringens* using foods selected to represent three categories – "all" meat items, half meat-half vegetable items, and "all" vegetable items. Growth rates were measured over a range of potential temperature profiles that foods could have been subjected to during the Skylab flight while meals were in preparation. The tests measured the spore germination, outgrowth, and vegetative cell proliferation at various temperatures for periods up to a maximum of 2 h. This was the maximum time for heating in Skylab because of automatic timing of heater operations which was implemented to reduce power consumption.

MATERIALS AND METHODS

Skylab foods used for this study were freeze-dehydrated turkey rice soup and mashed potatoes. Crew preparation procedures for these foods required addition of hot water (55 C), mixing, and heating in the Skylab food warmer-serving tray for periods up to 2 h. Turkey rice soup and mashed potatoes were flight food items produced in accordance with Skylab specifications *(7)*. Turkey rice soup was composed of freeze-dried diced turkey meat, 45.58%; freeze-dried rice, 17.09%; parsley flakes, 0.29%; and soup base, 37.04%. The soup base was composed of dried chicken broth, 49.04%; waxy maize starch, 50.36%; and white pepper, 0.60%. The turkey meat, rice, parsley flakes, and soup base were individually weighed into portions, packaged in rehydratable packages and flushed with nitrogen three times before vacuum sealing. Mashed potatoes were composed of potatoes, 75.54%; whole milk, 19.59%; butter, 4.55%; salt, 0.31%; and antioxidant (20% BHA, 20% BHT, 60% corn oil) 0.01%. Potatoes and other ingredients were mashed and then freeze-dried. The freeze-dried mashed potatoes were packaged in the same manner as the turkey rice soup. The third "food" used was cooked meat medium (Difco). Sterile cooked meat was tempered for 2 h and other foods 1/2 h before inoculation. Sterile cooked meat was reconstituted according to manufacturer's directions. Other foods were diluted 1:10 with sterile deionized water, mixed, and transferred to a sterile 500 ml Erlenmeyer flask. Flasks were plugged with cotton, covered with aluminum foil, and placed in a water bath that had been preset at the incubation temperature.

C. perfringens S-89, obtained from the Food and Drug Administration (Food Microbiology Branch, Cincinnatti, Ohio), was used in all of the generation time determinations. Stock cultures were maintained in cooked meat medium and transferred at monthly intervals. Stock cultures were transferred to 10 ml cooked meat medium 24 h before each determination. Two milliliters of the 24-h old culture were inoculated into 198 ml of either sterile cooked meat, turkey rice soup, or mashed potatoes that had been tempered to the test temperature. Incubation temperatures were 25, 30, 35, 40, 45, 50, and 55 C. The liquid level of the flask was submerged 5 cm below the liquid level of the water bath. One milliliter aliquots were removed at 0, 20, 40, 60, 80, 100, and 120 min exposure. Flask contents were mixed by swirling before each sampling. Care was taken to prevent the contents from reaching 5 cm above the liquid level during mixing. One milliliter aliquots were transferred to 9 ml of freshly boiled and cooled (4 C) thioglycolate medium (Difco). These 1:10 dilutions were maintained at 4 C until the end of each experiment (120 min) and then surface plated on duplicate SFP agar *(12)* plates. Plates were placed into GasPak anaerobic jars (BBL) and incubated at 37 C for 24 h. Total vegetative cell counts were done on each sample. The number of generations (n) and generation time (g) was calculated for each incubation time.

TABLE 1. GENERATION TIME[a] (g) OF *Clostridium perfringens* IN DIFFERENT FOODS AT VARIOUS TEMPERATURES

Incubation time (t) (Minutes)	25 C		30 C		35 C		40 C		45 C		50 C		55 C	
	n[b]	g[a]	n	g	n	g	n	g	n	g	n	g	n	g
Cooked meat														
20	0.14	143	—	—	2.07	10	0.86	23	—	—	0.71	28	—	—
40	—[c]	—	—	—	0.50	80	0.26	154	—	—	0.35	114	—	—
60	0.44	182	—	—	1.45	41	—	—	0.29	207	0.35	171	—	—
80	—	—	—	—	2.28	35	0.16	500	0.13	615	—	—	—	—
100	—	—	0.48	208	3.01	33	—	—	—	—	—	—	—	—
120	0.14	857	0.33	364	4.36	28	—	—	—	—	—	—	—	—
Turkey rice soup														
20	1.03	19	—	—	—	—	—	—	—	—	—	—	—	—
40	0.63	63	—	—	—	—	0.02	2000	—	—	—	—	—	—
60	0.29	207	—	—	—	—	0.11	545	—	—	—	—	—	—
80	—	—	0.18	444	—	—	0.36	222	—	—	—	—	—	—
100	0.35	286	—	—	—	—	—	—	—	—	—	—	—	—
120	1.28	94	—	—	—	—	—	—	—	—	—	—	—	—
Mashed potatoes														
20	2.10	10	—	—	2.07	10	0.37	55	1.21	16	—	—	—	—
40	3.56	11	—	—	1.90	21	—	—	2.31	17	—	—	—	—
60	3.07	20	—	—	2.07	29	—	—	2.35	26	—	—	—	—
80	4.00	20	—	—	1.68	48	—	—	2.40	33	—	—	—	—
100	3.59	28	—	—	1.54	65	0.44	227	2.26	44	—	—	—	—
120	2.98	40	—	—	1.42	85	0.47	255	1.86	65	—	—	—	—

[a] g (generation time) $= \frac{t}{n}$, where n is the number of generations at time, t *(9)*.

[b] n (number of generations) $= 3.3 \log_{10} \frac{y}{x}$, where y is the number of organisms present at time, t, and x is the number of organisms present initially *(9)*.

[c] = No growth detected.

RESULTS AND DISCUSSION

Three categories of foods were utilized in this analysis of the hazard from *C. perfringens* in Skylab foods. The three categories represented an "all" meat food, a food composed of approximately half meat and half vegetable, and an "all" vegetable item. Cooked meat medium was selected as the optimal growth support medium and representative of the microbial growth supporting potential of the Skylab foods that were largely composed of meat. Skylab turkey rice soup was used as a characteristic half meat-half vegetable product, and Skylab mashed potatoes represented the non-meat foods.

The media as well as the incubation temperature influenced the growth rate of *C. perfringens* (Table 1). Significant increase in the number of generations was observed in cooked meat only at 35 C and the average time for the population to change 1 log cycle (generation time) over the 2-h period was 28 min. Others have shown that *C. perfringens* does grow at higher temperatures *(3)*. In the turkey rice soup, significant generation was seen only at 25 C (average generation time, 94 min), and in the mashed potatoes at 25, 35, and 45 C (at which average generation times were 40, 85, and 65 min, respectively). Decreases in the number of organisms occurred at 55 C in the cooked meat, at 45, 50, and 55 C in the turkey rice soup and at 50 and 55 C in the mashed potatoes. Brown and Twedt *(3)* reported greater than 99% reduction of *C. perfringens* on roast beef in less than 6 h at 53.3 C.

In a single test, *C. perfringens* was incubated in the turkey rice soup at 35 C for 28 h. A total of 8.7 generations were produced with a generation time of 193 min. This observation indicates that, although the organism did not multiply in the 2 h hazard time tested, it cannot be concluded that longer times will not allow growth to occur. The hazard analysis conducted here was limited to the 2 h time range because of the limits of heating foods in the Skylab mission. Most of the cultures were probably in a temporary lag or phase of slow development during the 2 h incubation period. Brown and Twedt *(3)* reported growth of *C. perfringens* in roast beef chucks up to 12 h at 51.1 C followed by a reduction in viable cells at 18 h.

An upper limit for *C. perfringens* was established for Skylab foods after hazard analysis which included

TABLE 2. SKYLAB FOODS WHICH WERE WARMED PRIOR TO EATING AND IN WHICH *Clostridium perfringens* WAS JUDGED TO BE A POTENTIAL HAZARD

Rehydratable foods
Sausage Patties
Cream of Tomato Soup
Potato Soup
Turkey Rice Soup
Chicken and Rice
Chicken and Gravy
Pork and Scalloped Potatoes
Beef Hash
Veal and Barbecue Sauce
Spaghetti and Meat Sauce
Macaroni and Cheese
Frozen foods
Filet Mignon
Pork Loin and Dressing
Lobster Newburg
Prime Rib of Beef

the evaluation of their generation times and the following factors: epidemiology of food-borne diseases, nature of each Skylab food in regards to its potential to support growth of *C. perfringens*, Skylab food processing procedures, microbiological data of comparable space foods, quality control standards enforced during manufacture *(8)*, recommended limits established by public health organizations *(10, 11)*, problems associated with null gravity heating and refrigeration, and proposed crew handling procedures. The specification limit of not greater than 100 *C. perfringens*/g was established after review of the above parameters and assuming that: *(a)* a dose of 10^6 viable organisms would be required to cause symptoms, *(b)* no lag phase would occur in multiplications of the organisms, *(c)* a generation time would be at least 20 min, *(d)* any one Skylab meal would not contain more than 100 g of contaminated food (foods involved are generally entrees or rehydratables which contain about 30 g per serving, dry weight). Predicted on these assumptions, it would require that food be held 2 h or longer under optimum conditions for growth of *C. perfringens* before a population could be built up sufficient to cause a clinical episode. The limit of 100 *C. perfringens*/g was, therefore, set for the Skylab foods listed in Table 2. These standards proved to be adequate to protect the Skylab astronauts. The Skylab was inhabited for over 500 man-days without any evidence of food-associated illness. Routine testing of Skylab foods utilized the sample plan and method for *C. perfringens* as specified by Heidelbaugh, et al *(6)*. This procedure employed SFP agar *(12)* as used in the hazard analysis tests reported here. This medium is less selective than other media *(5)* for *C. perfringens*. This non-selectivity may have somewhat increased the conservativeness of the test limit. All of the Skylab foods examined for *C. perfringens* (listed in Table 2) proved to have less than 10 *C. perfringens*/g.

Foods are commonly exposed to temperatures that support microbial growth during preparation under a variety of food service operations (including space flight, commercial restaurant, institutions, vending machines, and at home). Bryan and Kilpatrick *(4)* isolated *C. perfringens* from 11 of 36 samples of sliced roast beef, cooked to an internal temperature of 68.4 C. Fortunately, food must either be grossly contaminated and/or grossly mishandled for it to produce a safety hazard from *C. perfringens*. The recommended temperature for holding potentially hazardous foods in food service operations, in vending machines, and aboard aircraft is 60 C *(10, 11)*. Various investigators *(1, 3, 4)* have suggested that the holding temperature of 60 C could be reduced for roast beef without endangering the public health from growth of staphylococci, salmonellae or *C. perfringens*. Results of the hazard analyses conducted for Skylab substantiate Brown and Twedt's *(3)* suggestion relative to *C. perfringens*. However, the same degree of safety is not provided as the serving temperature is lowered to 54 C. Allowance must always be made for inadvertent errors in maintaining time-temperature profiles during preparation and serving.

The findings of these hazard analyses suggested that an upper limit of 100/g for *C. perfringens* was adequate for Skylab foods. This limit was predicted on a sampling plan and enumeration of the organisms by a stipulated procedure *(6)*. This limit merits consideration for any food that is manufactured under quality control procedures comparable to those used for Skylab foods *(7, 8)*. Hazard analysis indicates that this limit provides safety for such foods even when they are exposed to temperatures between 25 and 55 C for up to 2 h.

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AMENDMENT TO 3-A SANITARY STANDARDS FOR MULTIPLE-USE PLASTIC MATERIALS USED AS PRODUCT CONTACT SURFACES FOR DAIRY EQUIPMENT

Serial # 20-06

Formulated by

International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

The "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial # 20-00 are hereby amended as indicated in the following:

Section I. Standards for Acceptability, Sub-paragraph (2):

Add the following material to the list of Generic Classes of Plastics:

Cross-linked polyester resins *0.20* *0.20* *0.20* (vinyl ester-styrene copolymer)

These standards shall become effective Nov. 15, 1974.