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A RADIOMETRIC METHOD FOR RAPID SCREENING OF COOKED FOODS FOR MICROBIAL ACCEPTABILITY

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ABSTRACT

Radiometry shows promise as a rapid screening method for determining if cooked, frozen foods meet microbial limits indicating adequate processing conditions (aerobic plate count, APC $< 1.0 \times 10^5$ /g). In this method bacteria metabolize ^{14}C -labeled substrates to $^{14}\text{CO}_2$. The time to detect labeled CO_2 (detection time) is inversely related to the bacterial concentration in the food. Using radiometry, about 75% of a wide variety of cooked and frozen foods were correctly classified as acceptable ($< 1.0 \times 10^5$) or unacceptable ($> 1.0 \times 10^5$ bacteria/g) within 6 hr. A minimum of 1 and a maximum of 5 of the 404 tested foods were incorrectly classified. Twenty-three percent of tested samples were classified as suspect and required confirmation of acceptability by the standard plate count (24–48 hr).

INTRODUCTION

MICROBIOLOGICAL CRITERIA are often established as one means of assuring that processed foods have been prepared under good sanitary conditions and proper time-temperature profiles. If these conditions are unsatisfactory, microorganisms may multiply in the food and present a health hazard to the consumer. The total aerobic plate count (APC) and that of coliforms and fecal coliforms are widely used criteria for detecting inadequate processing, poor sanitation, and post-processing contamination. The APC may be performed by pour plate, drop plate and surface plate methods (Thatcher and Clark, 1968). A spiral plating technique may require less material and time than the pour plate method (Gilchrist et al., 1973; Campbell and Gilchrist, 1973). All APC methods require an incubation period of 1–2 days to permit formation of countable colonies. However, if poor conditions have existed during food processing one should initiate corrective action prior to a 24–48 hr wait.

The need for more rapid screening methods to determine the probable range of indicator bacteria in foods is evident. Radiometry (measurement of $^{14}\text{CO}_2$ produced by bacterial conversion of ^{14}C -labeled substrates) may be used to detect a wide variety of bacteria (Levin et al., 1956; DeBlanc et al., 1971; Previte, 1972; Waters, 1972; Bachrach and Bachrach, 1974; Evancho et al., 1974) and to estimate clostridial numbers (Evancho et al., 1974). The purpose of this report is to evaluate the use of radiometry as a rapid screening method to determine whether cooked foods meet specified microbial limits.

MATERIALS & METHODS

Detection of $^{14}\text{CO}_2$

Septum-stoppered 50-ml serum vials, containing 10 ml of a TYT broth medium (5.0% Thiotone, 0.5% yeast extract, 0.5% Trypticase, 0.25% K_2HPO_4 , 0.5% NaCl, 0.1% NaHCO_3 , pH 7.2) with ^{14}C -labeled substrates (Table 1) and about 1g of food were incubated at 37°C on a rotating shaker at 200 rpm. Radioactive substrates were purchased from the New England Nuclear Corp., Boston, MA. At hourly intervals, the headspace of each vial was flushed with a sterile mixture of air and CO_2 (90:10) or with sterile room air. The evolution of $^{14}\text{CO}_2$ was detected with the BACTEC Model 301 (Johnston Laboratories, Cockeysville,

Table 1—Medium for radiometric screening of microbial quality of cooked, frozen foods

Constituents of medium ^a	Percent w/v	Radioactivity $\mu\text{Ci/ml}$
Thiotone (BBL)	5.00	—
Yeast extract (BBL)	0.50	—
Trypticase (BBL)	0.50	—
K_2HPO_4	0.25	—
NaCl	0.50	—
NaHCO_3	0.10	—
DL-(5- ^{14}C) Glutamic acid ^b	0.00035	0.100
(^{14}C) Sodium formate ^b	0.00023	0.100
D-(UL- ^{14}C) Glucose ^b	0.00067	0.175

^a Broth medium (pH 7.2), without labeled substrates, was designated TYT.

^b The specific activities (mCi/g) of the glutamic acid, sodium formate and glucose were 28.41, 43.86 and 26.31, respectively.

MD). A reading of $\geq 20\%$ (≥ 5.0 nCi) of full scale on the BACTEC instrument was considered as positive evidence for $^{14}\text{CO}_2$ production by bacteria.

Cooked frozen foods

All foods were prepared in the central preparation facility at Francis E. Warren Air Force Base, Cheyenne, WY. Strategic Air Command (SAC) Regulation 146-1 specifies that these foods must meet the following microbiological criteria to be acceptable: APC $< 1.0 \times 10^5$ bacteria/g; coliforms < 100 /g; fecal coliforms, negative/g.

Standard curves

Foods were thawed for 16–18 hr at 5°C. Twenty-gram samples were incubated at 37°C for periods up to 12 hr to allow an increase in the normal flora. The samples were blended at high speed for 2 min in 160 ml of TYT in a Waring Blendor. APC's were made from the blended samples using the pour plate method with Plate Count Agar (Difco) and 48-hr incubation at 37°C. With a 10-ml syringe and a 16-gauge needle, 9 ml of the blended sample, containing approximately 1g of the test food, were delivered into a sterile capped vaccine vial containing 1.75 μCi of glucose, 1.0 μCi of glutamic acid and 1.0 μCi of sodium formate in a 1 ml volume. The vials were incubated at 37°C on a rotating shaker at 200 rpm, and the headspace of each vial was checked periodically for $^{14}\text{CO}_2$. Fifty to sixty samples per food from a minimum of three production lots were analyzed. The bacterial concentration in a specific food, as determined by the pour plate APC method was plotted against the time in hr required for the first detection of labeled $^{14}\text{CO}_2$. The curve of best fit and 95% confidence limits were determined by the least squares polynomial curve fitting method (Univac, 1970). The quadratic fit provided data points closer to the arithmetic mean of the \log_{10} number of bacteria/g than did the linear fit.

Radiometric assessment of the microbiological quality of cooked, frozen foods at Warren Air Force Base

To determine APCs, 50g of frozen sample, added to 200 ml of Butterfield's buffered phosphate was blended for 1 min, diluted, and 1.0 ml of the 10^{-2} and 10^{-3} dilutions were pipetted into duplicate plates, mixed with tryptone glucose extract agar (Difco) and incubated at 35°C for 48 hr. For radiometry, 30 ml of the initial 1:5 dilution were mixed with 24 ml of double strength TYT medium. Nine ml of the mixed sample, containing approximately 1g of the test food, were delivered into a sterile capped vaccine vial containing 1 ml of the labeled substrates. The vials were incubated at 35°C on a rotating shaker at 200 rpm and the headspace was checked for $^{14}\text{CO}_2$ at hourly intervals. Detection time was used as the basis for determining the accepta-

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bility ($<1.0 \times 10^5$ bacteria/g) of the foods. Samples tested included roast turkey, fried chicken, chicken pot pie, barbecued (BBQ) chicken, roast beef with gravy, country steak with gravy, beef pot pie, Swedish meat balls, baked macaroni with cheese, sliced pork, pork chop suey, BBQ franks, spaghetti, corn, peas, green beans, peas and carrots, mixed vegetables, and chocolate cake.

RESULTS & DISCUSSION

SOME FOODBORNE BACTERIA, such as strains of pseudomonads, do not readily produce $^{14}\text{CO}_2$ from D- $\{^{14}\text{C}\}$ glucose. However, by the incorporation of $\{^{14}\text{C}\}$ glutamate and $\{^{14}\text{C}\}$ formate into a broth medium, Previte et al. (1975) radiometrically detected *Alcaligenes faecalis* and three strains of *Pseudomonas* within 4–6 hr. This medium also allowed the rapid detection of many foodborne pathogens (*Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium botulinum* and *Salmonella typhimurium*).

A well defined relation exists between the number of bacteria and the time to detect $^{14}\text{CO}_2$, indicating that the radiometric technique might be used as a rapid screening procedure (Rowley et al., 1976). With both pure and mixed cultures, as the initial cell concentration increased, the time to detect $^{14}\text{CO}_2$ decreased (Previte, 1972; Waters, 1972; Rowley et al., 1974; 1976). Standard curves were prepared for cooked, frozen meat loaf, roast beef, and mixed vegetables. The \log_{10} APC/g of food was plotted against the time in hr required for the first detection of labeled $^{14}\text{CO}_2$ (Fig. 1). The exact shape of the curve was affected slightly by the food. With an APC $\leq 1.0 \times 10^5$ bacteria/g as the criterion of good microbiological quality, a radiometric detection time ≥ 5 hr for cooked frozen meat loaf indicated acceptability. If labeled CO_2 were not detected in 4 hr the probability of the number of bacteria per gram being $\leq 1.0 \times 10^5$ would be greater than 0.975. A detection time of 1 hr indicated an unacceptably high count ($>1.0 \times 10^5$ /g). Samples with detection times of 2, 3 and 4 hr were classified as suspect and required confirmation by a standard plate count.

Radiometric detection times ≥ 6 hr and ≥ 7 hr for roast beef and mixed vegetables, respectively, suggested acceptability. Detection times of 1–2 hr indicated that both foods were unacceptable.

The reliability of the radiometric standard curve as a means of classifying cooked, frozen meat loaf as microbiologically acceptable ($\leq 1.0 \times 10^5$) or unacceptable ($>1.0 \times 10^5$ bacteria/g) was evaluated. One hundred three samples from 10 new production lots, not previously used to establish the standard curve, were preincubated at 37°C for various times to obtain samples of meat loaf with different concentrations of bacteria. The microbial acceptability of each sample was estimated by standard pour plates and radiometrically by reference to the standard curve. The time to first detect $^{14}\text{CO}_2$ was plotted against the \log_{10} APC/g (Fig. 2). The detection time ranged from 1 hr in a product containing $>10^5$ bacteria/g to 8 hr with ca 10^2 /g. Any point above the horizontal line at 10^5 bacteria/g represents unacceptable samples as determined by the APC; acceptable samples are represented by points on or below the horizontal line. Using the radiometric standard curve, 64 of the samples were given a classification, within 4 hr, identical to that using the pour plate method. In the case of four samples, with detection times of 5 hr, the classification by radiometry disagreed with that by APC (1.2 – 1.7×10^5 bacteria/g). This may not be a real defect. Hardy et al. (1977) showed that the mean standard deviation of standard plate counts for frozen vegetables was 0.3 log or, that 68% of the time, APCs of samples containing 1.0×10^5 bacteria/g could be counted as having from 5.0×10^4 to 2.0×10^5 bacteria/g. Thirty-five of the 103 samples, with detection times of 2–4 hr, could only be classified as suspect and required the conventional 24–48 hr for confirmation. The confirmatory aerobic

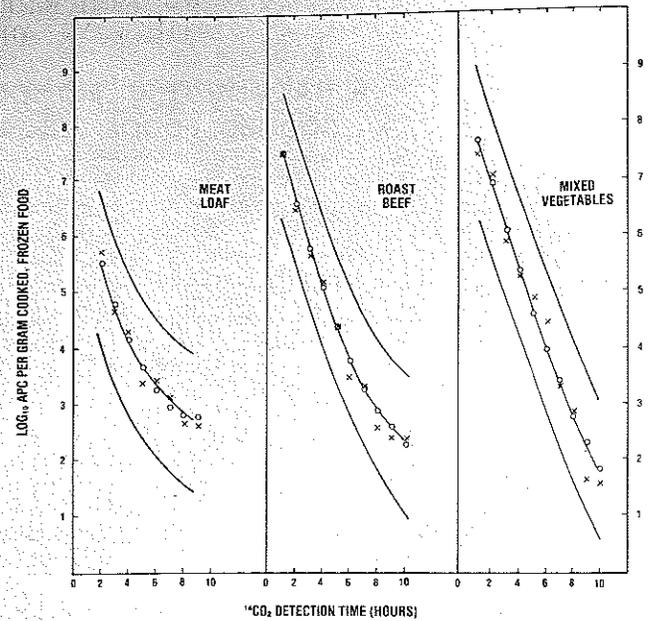


Fig. 1—Standard curves for rapidly screening the microbial acceptability ($\text{APC} \leq 1.0 \times 10^5$ bacteria/g) of cooked, frozen foods. The medium and labeled substrates were as shown in Table 1. Mean \log_{10} APC/g (X), quadratic fit (O), and 95% confidence limits as indicated.

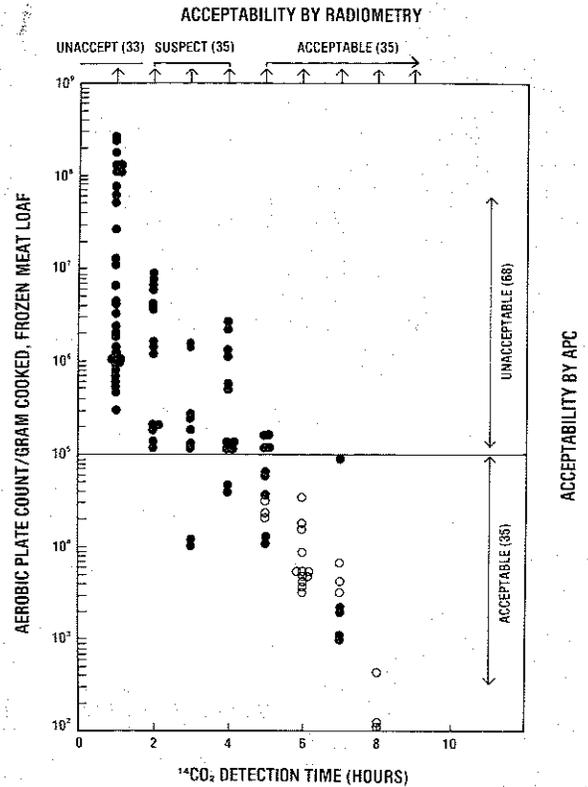


Fig. 2—The microbiological acceptability of cooked, frozen meat loaf as determined by radiometry and the standard plate count. Samples were tested either immediately (\circ) after thawing or after incubation at 37°C for 8 to 12 hr (\bullet). Numbers in parentheses represent those samples which were categorized as unacceptable, suspect or acceptable.

plate counts revealed that 31 of the 35 suspect samples were unacceptable and 4 were acceptable.

Standard curves (data not shown) were constructed for a variety of cooked frozen foods and used to assess their microbiological quality (Table 2). One of the 91 samples of roast beef contained 1.2×10^4 bacteria/g and was rejected by the radiometric method. All of the acceptability ratings which were assigned to swiss steak, salisbury steak, fried chicken, roast pork, and mixed vegetables on the basis of radiometry were proven correct by APC. Of 372 food samples tested 72.8% were correctly classified as acceptable or unacceptable within a maximum of 6 hr by the radiometric assay. Suspect samples, which included 25.8% of the samples, required the conventional technique and 24–48 hr for correct classification. The classification by radiometry and APC disagreed with only 1.3% of the samples. Using impedance as a rapid system for monitoring the microbial quality of frozen vegetables Hardy et al. (1977), incorrectly classified 7% of the tested samples.

It is evident that the 95% confidence limits of the radiometric standard curves are too broad to allow radiometry to be used for precise estimation of actual numbers of bacteria per g of food. However, the technique shows promise as a rapid screening technique for estimating the microbiological quality of cooked, frozen foods. The test time is dependent on the food under study (Table 2). For convenience, this test time could be increased for some foods so as to have a uniform cut-off time. A uniform cut-off time of 6 hr would decrease the potential number of samples of meat loaf, roast beef, swiss steak, salisbury steak, fried chicken and roast pork incorrectly accepted. However, the number of suspect samples would be increased as would the necessity for use of the confirmatory APC method.

A field study was conducted at Warren Air Force Base to assess the microbiological quality of a variety of cooked, frozen foods by the standard plate count and radiometry with a uniform cut-off time of 6 hr. If ^{14}C were not detected by 6 hr the samples were classified as acceptable ($\leq 1.0 \times 10^5$ bacteria/g). It is important to note that most of the cooked, frozen foods produced at Warren have $< 1.0 \times 10^5$ bacteria/g. In 1976 only 1 of 8,125 food items tested was rejected because of a high APC. The aerobic plate counts of the 32 food items tested in this study ranged from $< 1.0 \times 10^3$ to 7.0×10^3 bacteria/g and the earliest radiometric detection time was 7 hr. In most cases the detection times were more than 8 hr. Thus all 32 items tested were correctly classified as acceptable by radiometry within 6 hr. The same determination by conventional methods required about 2 days, making for a significant savings in time. The time saved with acceptable or unacceptable samples is due to the greater sensitivity in detecting growth by radiometry. As mentioned previously there would be no reduction in time with "suspect" samples.

Radiometry is a simple technique which is adaptable to foods as a rapid screening method and allows necessary corrective action to be initiated with minimum delay when unacceptable samples are encountered. It is less laborious and time consuming than the APC method. Sample size may be varied. However, in these studies the sample size was ca 1 g/vial. Like the APC method the sample may be incubated at any desirable temperature. A comparison of this assay with micro-calorimetry, turbidity and impedance methods was discussed by Cady (1977). He reported that the high cost of substrate was a disadvantage of radiometry. The cost of one impedance module is 37.5¢ per test and comparable to the cost of three plates with medium and other equipment used in the pour plate method (Hardy et al., 1977). A mixture of labeled substrates such as used in this study costs ca \$1.35/vial or test. If ^{14}C -labeled sodium formate could be used in place of the mixture, the cost would be reduced to about 35¢ per test.

Table 2—Assessing the microbiological quality^a of cooked frozen foods by radiometry

Product	No. samples tested	^{14}C test time ^b (hr)	% incorrectly	
			Accepted	Rejected
Meat loaf	103	4	3.9	0.0
Roast beef	91	5	0.0	1.1
Swiss steak	60	5	0.0	0.0
Salisbury steak	39	3	0.0	0.0
Fried chicken	24	4	0.0	0.0
Roast pork	15	5	0.0	0.0
Mixed vegetables	40	6	0.0	0.0

^a Products having an aerobic, mesophilic count $\leq 1.0 \times 10^5$ and $> 1.0 \times 10^5$ bacteria/g (pour plate method) were considered acceptable and unacceptable, respectively.

^b Detection of ^{14}C in periods $>$ than the number of hours listed signified counts $\leq 10^5$ /g.

Rowley et al. (1977) showed that ^{14}C -labeled formate worked as well as a mixture of labeled glucose, formate and glutamate in a radiometric screening method for determining rapidly if cooked, frozen mixed vegetables met the microbial criterion of no more than 1.0×10^5 total aerobic mesophiles/g.

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