to make intelligent price comparisons. Of course, the detailed standardization of tags and components which the Institute has assisted in developing tends to make more serviceable the information reported to the individual under the Tag Industry Agreement and by him collated and disseminated among the Subscribers. But if the reporting agreement is otherwise lawful, such enhanced usefulness of the agreement as results from standardization would hardly infect it with illegality."

It is undeniably true that standardization may, under certain circumstances, have a tendency to produce similarity of product, and that similarity of product may have a tendency to produce similar prices. However, these similar prices are a result of the necessity of meeting or approximating the lowest price for a similar product, and hence are a result of free competitive forces. Since standardization accompanied by price reporting is not unlawful, how much less so is standardization standing alone. The *Tag Institute* case, however, demonstrates that even a perfectly lawful activity can be given a very bad time when a regulatory agency gains the wrong impression. "Legal aspects" can easily be construed as illegal.

STUDIES ON THE BACTERIOLOGICAL QUALITY OF FROZEN MEAT PIES

II. A COMPARISON OF THE METHODS FOR THE ENUMERATION OF COLIFORMS

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The detection of the presence and enumeration of the numbers of coliform bacteria in frozen foods has become an important part of the bacteriological examination of frozen foods. Breed and Norton (*3*) suggested that the term "coliform" be used to designate the gram negative, lactose-fermenting, aerobic bacteria as a measure of pollution of water. Since its introduction, the coliform group bacteria have been extensively studied in milk, food, and water as indicators of sanitation or contamination. Now they have been carried over into the frozen food field as one of the measurements of bacteriological quality.

From a study of 6,500 strains of the coliform group isolated from various sources, Griffen and Stuart (*7*) stated that the *Escherichia* strains were normal inhabitants of fecal material, while *Aerobacter* strains were typical of nonfecal material. However, they admitted that the latter might, at times, be found in fecal materials but they considered this occurrence to be adventitious.

Elrod (*5*) has demonstrated that a genus of plant pathogens, *Erwinia*, is closely related to the coliform bacteria. He stated that, since the *Erwinia* have the ability to ferment lactose and have an IMVIC (indole, methyl red, Voges-Proskauer, and citrate reactions) pattern similar to that of the *Escherichia-Aerobacter* group, results of some previous investigations were misleading and fecal contamination was not necessarily indicated.

In examining 376 samples of commercially frozen vegetables and cantaloupe for fecal contamination, Burton (*4*) used a presumptive coliform and interococcus test. The coliform bacteria were found to be more dependable than the entercocci for indicating contamination in foods prior to freezing.

Berry (*2*) expressed the need for standardization of methods in bacteriological examination of frozen foods. He considered the use of *Escherichia coli* as a test organism of doubtful value because the organism died during low temperature storage.

Zaborowski *et al.* (*10*) evaluated some of the microbiological methods used for the examination of precooked frozen foods; however, they did not evaluate any of the methods used in the enumerating of coliforms.

In bacteriological surveys of commercially frozen precooked frozen foods, (*6, 8, 9*), several methods were used to determine the number of coliform organisms present: a plating method using violet red bile agar (*9*), desoxycholate lactose agar (*10*), and a most probable number method (*8*) using lactose broth for the presumptive and brilliant green bile broth for confirmatory tests.

The present investigation was undertaken to compare a plating method using desoxycholate lactose agar and the most probable number method in the enumeration of coliforms in frozen meat pies.

EXPERIMENTAL METHODS

The frozen meat pies used in this investigation were purchased at retail markets in the city of Omaha. Samples of the frozen meat pies for bacteriological analysis were obtained by using a stainless steel cheese trier which was previously sterilized by dip-

ping into alcohol and flaming in a Bunsen burner. The sample was placed into a sterile Waring blender and sterile 2 per cent peptone water was added to give a 1:10 dilution by weight. The samples were blended for three minutes and serial dilutions were made from this suspension.

The most probable number (MPN) of coliform bacteria was obtained by adding 10, 1, 0.1 and 0.01 ml. portions of the diluted sample to replicate sets of five lactose broth tubes each. Transfers were made into brilliant green bile broth from all tubes in which gas had formed within 48 hours. Streaks were also made on eosin-methylene blue agar plates from the gas positive lactose broth tubes.

Total counts were determined by plating serial dilutions of the blended suspension with desoxycholate lactose agar (Difco). The plates were overlayed with desoxycholate lactose agar. Characteristic red opaque colonies surrounded by a zone of precipitated bile were counted after a 24-hour incubation period at 35° C.

Colonies were selected and transferred to triple sugar iron agar slants (Difco) from eosin-methylene agar plates and from the highest positive dilution of brilliant green bile broth tubes. After incubation at 37° C. for 48 hours the slants were read. The cultures were tested for indol production, methyl red reaction, Voges-Proskauer and citrate utilization (IMVIC patterns) urease production and motility.

RESULTS AND DISCUSSION

A total of 93 commercially produced frozen meat pies from various manufacturers were examined for the presence of coliform bacteria. In this study an evaluation was made of the most probable number method (MPN) and a pour plate method. These two methods were selected because of their extensive use in the enumeration of coliform bacteria in food materials.

The results of this investigation are tabulated in Table 1. The examination of the results in Table 1 indicate that the MPN method may recover a slightly larger number of organisms than the pour plate method. The confirmatory technique of making streaks on eosin-mehtylene blue agar plates or inoculating brilliant green bile tubes from positive lactose broth tubes gave compariable results.

Though the MPN method recovered a slightly larger number of coliform bacteria than the pour plate method, the pour plate method using either desoxycholate lactose agar or violet red bile agar has its economies in equipment as well as time. The results of the pour plates were obtained in a much shorter period of time. In a preliminary study using desoxycholate lactose agar and violet red bile agar in the pour plates, no significant differences were noted in the total bacterial count. As desoxycholate lactose agar was already being used in this laboratory for the routine isolation and enumeration of coliform bacteria, desoxycholate lactose agar was selected for use in this study. However, it is the author's belief that violet red bile agar gives a more easily discernible coliform colony.

Each frozen meat pie giving a positive test for the coliform group was further investigated in order to determine the most predominant species of coliform bacteria present. Typical coliform colonies were isolated from desoxycholate lactose agar plates and colonies from tryptose glucose extract agar plates streaked from samples of positive tubes of brilliant green bile broth were used for identification studies.

TABLE 1—BACTERIOLOGICAL EXAMINATION OF COMMERCIALLY PRODUCED FROZEN MEAT PIES

Processor	Desoxycholate agar plate count [a]	Most probable number: Brilliant green Bile Broth	Most probable number: Eosin methylene blue agar
Chicken Meat Pies			
A	0	1.5	0
B	0	11	46
C	1,000	16,600	7,200
D	10	62	460
E	66	2.8	46
F	800	2,000	1,100
G	26	700	160
H	10	0	0
I	3	3	0
J	0	4	90
Turkey Meat Pies			
A	10	1.5	4.2
B	10	30	90
C	0	6	7
D	10	60	40
E	4	17	7
F	0	26	30
G	10	97	90
H	0	80	280
Beef Meat Pies			
A	30	45	45
B	7	4	4
C	13	300	127
G	0	7	7
I	7	4	4
J	0	92	126
K	0	17	11
L	3	0	0
Tuna Meat Pies			
B	0	90	60
G	0	7	0
D, L, M	0	0	0

[a] Average of three pies having the same manufacturers code or lot number.

TABLE 2—SPECIES OF COLIFORM BACTERIA ISOLATED FROM FROZEN MEAT PIES

Processor and Samples	Species of coliform bacteria isolated [a]
Turkey Meat Pies	
A1, A2	*Aerobacter aerogenes* var I.
B1	*Aerobacter aerogenes* var I.
B2	*Escherichia coli* var I
D1, D,3	*Escherichia freundii*
E2	*Escherichia freundii*
	Aerobacter aerogenes var I.
F2	*Aerobacter aerogenes* var I.
G1	*Aerobacter aerogenes* var I.
G2	*Aerobacter aerogenes* var I.
G3	*Aerobacter aerogenes* var I.
	Escherichia coli var I
H1	*Escherichia freundii*
H2	*Escherichia coli* var I
Tuna Meat Pies	
A1	*Escherichia freundii*
B1, B2, B3	*Aerobacter aerogenes* var I
Chicken Meat Pies	
B1, B3	*Aerobacter aerogenes* var I
C1, C2, C3	*Aerobacter aerogenes* var I
	Escherichia freundii
D1, D2, D3	*Escherichia freundii*
F1	*Escherichia coli* var I
G2	*Escherichia coli* var I
H2	*Aerobacter aerogenes* var II
I2	*Aerobacter aerogenes* var II
J3	*Aerobacter aerogenes* var II
Beef Meat Pies	
A1	*Aerobacter aerogenes* var I
C1, C2, C3	*Aerobacter aerogenes* var I
	Escherichia coli var II
J1, J2, J3	*Aerobacter aerogenes* var I

[a]The differentiation of the members of the coliform group was based on the table given on page 391 of Standard Methods for the Examination of Water, Sewage, and Industrial Wastes, 10th edition, 1955

The identification of the members of the coliform group was based on the table given on Page 391 of *Standard Methods for the Examination of Water, Sewage, and Industrial Wastes* (*1*). The results are recorded in Table 2. The examination of the results in Table 2 indicates that the predominant source of coliform contamination of frozen meat pies was a nonfecal source. Of 37 frozen meat pies demonstrating identifiable coliform bacteria, 30 pies contained a predominance of *Aerobacter* species. Seven of the pies demonstrated the presence of *Escherichia Freundii* varities, which are also of a probable nonfecal origin. Of the 37 pies, seven showed a predominance of *Escherichia coli* varities. These bacteria are usually associated with fecal contamination. About 18 per cent of the frozen meat pies demonstrated a probable fecal contamination while the remaining 82 per cent had a nonfecal or "soil" type of contamination. Whether or not it is necessary to determine if foods are contaminated with a fecal or nonfecal strains of coliform bacteria, the presence of coliform bacteria in frozen foods might indicate whether the foods had been cooked insufficiently or that they were contaminated after cooking or during processing prior to freezing.

SUMMARY

An evaluation of the most probable number method (MPN) and a pour plate method for the isolation and enumeration of the coliform bacteria was conducted on 93 commercially produced frozen meat pies from various producers. The MPN method recovered a slightly larger number of coliform bacteria than did the pour plate method. The MPN method did not demonstrate a sufficiently higher recovery of coliform bacteria to warrant its use in place of a pour plate method.

Predominant species of coliform bacteria recovered from frozen meat pies were demonstrated to be members of the species *Aerobacter*.

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