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Methodology for Enumeration of Coliphages in Foods†

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The effects of eluent composition, pH, and chaotropic agents on the recovery of T2, MS2, and indigenous coliphages from various foods were investigated. Additionally, methods of sample suspension and clarification were evaluated for coliphage recovery and application to various foods. Clarified sample suspensions were assayed for coliphages with a modified agar layer technique and appropriate *Escherichia coli* hosts. Centrifugation and polypropylene mesh filtration were more rapid and effective than glass wool filtration for clarification of sample suspensions and subsequent recovery of coliphages. Blending, stomaching, and shaking procedures were generally comparable for sample liquefaction and release of coliphages from foods. Complex basal eluents, EC medium and 1% casein, were generally more effective than a less complex eluent, phosphate buffer, for elution of coliphages from foods. For most foods, incorporation of sodium chloride or chaotropic agents, i.e., sodium trichloroacetate, urea, Tween 80, Triton X-100, and sodium nitrate, into basal eluents did not enhance recovery of coliphages. Indigenous coliphage recovery was not affected by sample suspension pH over a range of 6.0 to 9.0. With an optimal procedure, i.e., EC medium eluent, blending, and centrifugation, the recovery of T2 and MS2 ranged from 48 to 81% and from 58 to 100%, respectively, depending on the food type.

Coliphages have received increased attention and support in recent years as rapid and inexpensive indicators of fecal pollution and enteric pathogens in water and wastewater (15, 19, 26, 30, 31, 35, 37). Likewise, methods for quantitative recovery of coliphages and other viruses from the environment have been extensively investigated over the past several years (2–4, 26, 33). Although numerous methods for recovering animal viruses from various foods have been developed (5, 6), few attempts have been made to enumerate indigenous bacteriophages in foods, and the procedures reported were not evaluated or refined in relation to recovery efficiency or applicability to various types of food (17, 18, 36). Development of coliphage enumeration methods which are rapid, simple, economical, and efficient as well as broadly applicable to different foods is necessary to investigate the distribution of coliphages and their possible role as indicator organisms in foods.

Techniques used for elution or desorption of viruses and phages from solid materials, e.g., membrane filters, soil, sludge, and aquatic sediments, generally involve lowering the ionic strength of the eluent or sample suspension, adding soluble proteins or other organic compounds to the suspension, raising the pH of the suspension, adding chaotropic agents to the suspension, or various combinations of these techniques (2–4, 7–12, 27, 29, 33, 37). Similar approaches have been used for desorption and recovery of animal viruses from foods with eluents or suspension media containing various concentrations of salts, acids or bases, buffers, proteins, and various combinations of these components (5, 6). Little information is available on the effects of different eluent compositions and suspension medium pHs on the elution of viruses from foods (13), and no information is available on the effects of chaotropic agents.

A number of methods, including shaking, stirring and blending, and homogenization, have been used for physical suspension or liquefaction of food samples to release animal viruses (5, 6, 22), but these methods have not been standardized or extensively evaluated for broad application to various foods. Several techniques for clarifying food sample suspensions prior to virological assay, e.g., centrifugation, filtration, chemical phase separation, and adsorption-elution, have been reported (5, 6). Tierney et al. (32) found the glass wool or woven fiber glass filtration technique to be more efficient than other techniques, including centrifugation, for clarification of ground beef suspensions and subsequent recovery of polioviruses. A modification of the glass wool filtration technique (19, 32) was used to clarify food suspensions for subsequent assay of coliphages, but the technique was time-consuming for most samples (17).

The objective of this study was to develop and evaluate broadly applicable procedures for efficient recovery of coliphages from various types of foods. The effects of eluent composition, eluent and sample suspension pH, and chaotropic agents on the recovery of T2, MS2, and indigenous coliphages from various foods was investigated. Methods of sample suspension and clarification were also evaluated for recovery efficiency and ease of use.

MATERIALS AND METHODS

Media and chemicals. The chemicals used in these studies and their sources were as follows: sodium trichloroacetate (NTCA), sodium chloride, sodium nitrate, sodium hydroxide, potassium phosphate monobasic, hydrochloric acid, and Tween 80 were obtained from Fisher Scientific Co., Springfield, N.J.; lysine, casein, and urea were obtained from Sigma Chemical Co., St. Louis, Mo.; and Triton X-100 was obtained from Eastman Kodak Co., Rochester, N.Y. All bacteriological media and peptone and bile salts mixture number 3 were obtained from Difco Laboratories, Detroit, Mich.

Samples. Samples of fresh chicken breasts, fresh ground

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beef, fresh pork sausage, canned corned beef, and frozen mixed vegetables were obtained at retail markets in the Gainesville area. Samples were transported to the laboratory in an insulated container with ice and held at 1 to 2°C in the laboratory until analysis 18 to 36 h later. For a given experimental trial, a homogenous sample lot (1 to 2 kg) of a particular food was prepared by mixing or finely chopping it by aseptic techniques. A sample lot was divided into an appropriate number (16 to 30) of subsamples (50 to 100 g) of uniform composition for triplicate treatment comparisons. Frozen mixed vegetables were thawed at 5°C for 4 to 8 h before being mixed and divided into subsamples. Subsamples were held in sterile Whirlpak bags (Fisher Scientific) at 1 to 2°C until analysis.

For some comparisons, freshly prepared subsamples were inoculated with approximately 10^5 T2 and 10^7 MS2 phage particles per 100 g of sample. After inoculation, each subsample was aseptically mixed by hand and held in sterile Whirlpak bags at 1 to 2°C for 4 to 6 h before analysis.

Coliphage assays. *Escherichia coli* B (ATCC 11303), *E. coli* C-3000 (ATCC 15597), and *E. coli* C (ATCC 13706) were used as host strains for the assay of T2, MS2, and indigenous coliphages, respectively. *E. coli* C was used for indigenous coliphages because these phage form comparatively high numbers of PFU in this host in water and wastewater (14) and in various foods (data not presented).

Coliphages were assayed by a modification of the agar layer technique, with EC medium added to the assay media as previously described (16, 17). For indigenous coliphage assays, clarified sample suspensions were assayed directly, and for T2 and MS2 assays, suspensions were serially diluted in sterile EC medium before assay. Indigenous coliphages were assayed by mixing 2.0-ml portions of sample suspensions with 0.3 ml of *E. coli* C (6-h culture, 35°C, in EC medium) and 3.0 ml of molten (45°C) overlay agar (EC medium, 0.75% agar); this mixture was overlaid on prepoured plates of EC medium (1.5% agar). Five plates were prepared in this manner for each sample examined. For assay of T2 and MS2 phages in the inoculated subsamples, 1.0-ml portions of appropriate dilutions of sample suspensions were likewise prepared in triplicate with the indicated host strains. For each experiment involving T2 and MS2, coliphage suspensions used for sample inoculations were assayed as indicated to derive percentage recovery values; background levels of indigenous coliphages were also monitored in corresponding uninoculated subsamples.

Preparation of sample suspensions. Subsamples (50 or 100 g) were diluted 1:2 (sample-eluent, wt/wt) in a given eluent and liquified or suspended by one of the following general techniques: (i) blending at 2,000 or 4,500 rpm in sterile glass containers with a Waring blender (Waring Products Division, New Hartford, Conn.) attached to a Variable Autotransformer (Superior Electric Co., Bristol, Conn.) to control blending speed, (ii) stomaching in sterile plastic bags with a Coleworth Stomacher 400 (Dynatech Laboratories, Inc., Alexandria, Va.), or (iii) shaking in sterile plastic bags on a model G2 rotary shaker (New Brunswick Scientific Co., Inc., New Brunswick, N.J.) at 300 rpm. Samples suspended by shaking generally required some kneading to break up clumps before shaking; all tissue samples were finely chopped as mentioned above.

For each suspension technique, various sample processing times were compared for recovery of coliphages. Additionally, relatively slow (ca. 2,000 to 2,500 rpm) and fast (ca. 4,000 to 4,500 rpm) blending speeds were compared for their effect on the release or elution of coliphages. The effect of sonication for 2 min at 125 W (model B-220 ultrasonic cleaner; Branson Cleaning Equipment Co., Shelton, Conn.) on the release of indigenous coliphages from suspensions of fresh chicken prepared by blending (2,000 rpm, 10 min), shaking (300 rpm, 30 min), and stomaching (10 min) was also examined. For studies comparing different methods or times of sample processing, EC medium was used as the eluent.

Clarification of sample suspensions. The following techniques for clarification of sample suspensions were evaluated for speed, application, and subsequent recovery of coliphages from the suspensions. (i) Filtration: sample suspensions were filtered through 5 g of sterile glass wool, which was fitted into a large polypropylene funnel (top diameter, 150 mm; stem outer diameter, 28 mm) and pretreated with 30 ml of sterile EC medium to minimize possible adsorption of coliphages to the filter itself; these methods were similar to those previously described (17, 20). (ii) Rapid filtration: portions of the sample suspension were drawn through a wide-mouthed 10-ml pipette fitted with a sterile disposable filter tip capped with 500-μm polypropylene mesh (Spectramesh; Spectrum Medical Industries, Los Angeles, Calif.); the filter tips were constructed generally as described by Peterkin and Sharpe (23). (iii) Centrifugation: sample suspensions were centrifuged at 3,000 × *g* for 5 min.

Eluent studies. For studies evaluating the effects of basal eluents, eluent composition, and pH on the recovery of coliphages from foods, sample suspensions were prepared by blending and clarified by polypropylene mesh (500 μm) or centrifugation (3,000 × *g*, 5 min) as indicated above. Elution media compared for desorption and recovery of indigenous coliphages from fresh chicken included Butterfield phosphate buffer (1), 1% casein, 0.1 M lysine, and EC medium. In addition to these eluents, tryptose phosphate (TP) broth was also used in various comparisons. Eluents were sterilized by autoclaving at 121°C for 15 min. The pH of various eluents was adjusted in some studies with 1.0 N NaOH or HCl to achieve a particular pH in the resulting sample suspension.

Various concentrations of chaotropic salts of high potency, e.g., NTCA, or low potency, e.g., NaCl and $NaNO_3$, as well as other chaotropic agents such as urea, Tween 80, and Triton X-100 were incorporated into various eluents to investigate their effect on the desorption and recovery of coliphages. Control phage assay plates containing corresponding concentrations of these compounds were prepared with sample suspensions of basal eluents to determine their effect on the growth of the host bacteria and the ability of the coliphages to produce plaques on resulting host lawns.

Statistical analyses. Indigenous coliphage counts were converted to PFU per 100 grams of sample, and T2 and MS2 counts were converted to percent recovery for subsequent statistical comparisons. Data were analyzed by several procedures, including the paired *t* test, a one-way analysis of variance, and a factorial design, as deemed appropriate for a given experiment or comparison (23). Means of coliphage counts were compared with Duncan's multiple range test when indicated. A determination of $P < 0.05$ was considered significant for all analyses.

RESULTS

Sample clarification. Previous observations in this laboratory (17) indicated that the glass wool filtration technique for clarifying blended sample suspensions was tedious for routine analysis. Rapid clarification of 20 to 25 ml of many sample suspensions could be achieved with 500-μm polypropylene mesh, although sample suspensions prepared by blending at speeds above ca. 5,000 rpm could not be clarified

TABLE 1. Comparison of methods for clarifying sample suspensions with EC medium as an eluent[a]

Clarification method	Mean[b] coliphage recovery (10^3 PFU/100 g)	
	Chicken	Pork
Glass wool	3.6*	2.7†
Polypropylene mesh	4.0†	2.4†
Centrifugation	4.0†	3.1†

[a] Sample suspensions were prepared by blending (4,500 rpm, 10 min). For other methods, see the text.

[b] Mean of three subsamples (100 g each). Within each column, means having the same superscripts were not significantly different ($P > 0.05$).

by this technique due to the viscosity of the suspension. Larger volumes of clarified sample suspension could readily be obtained by centrifugation at relatively low speeds (3,000 × *g*) for 5 min. Excluding material preparation, the total time required to clarify a chicken or pork sausage sample suspension was approximately 2, 10, and 30 min for the polypropylene mesh, centrifugation, and glass wool clarification methods, respectively.

The polypropylene mesh, centrifugation, and glass wool clarification techniques were compared for recovery of indigenous coliphages from fresh chicken and pork sausage (Table 1). The mean recovery of coliphage from suspensions of chicken was significantly higher when they were clarified by mesh filtration or centrifugation than by glass wool filtration; differences were not significant with pork sausage suspensions. Since no significant difference was noted between the mesh filtration and centrifugation technique in terms of coliphage recovery, either of these techniques was used to clarify sample suspensions in other comparisons.

Sample suspension. Using methods reported for sample liquefaction for recovery of coliphages (17) and animal viruses (5, 6) from foods and the types of equipment generally available for microbiological analysis of food (1), various procedures involving blending, shaking, and stomaching were evaluated for recovery of coliphages from foods (Table 2). The sample suspension times in Table 2 represent minimal times for optimal coliphage recovery with each method as determined in preliminary experiments (data not shown). Methods of sample suspension were compared for recovery of indigenous coliphages from fresh chicken and of T2 and MS2 coliphages from chicken, ground beef, corned beef, and mixed vegetables (Table 2). Since faster blending speeds may be needed to disaggregate meat tissues and prepared foods, a blending speed of approximately 4,500 rpm was also used in these comparisons.

Blending at 4,500 rpm resulted in better recovery of indigenous coliphages than blending at 2,000 rpm, but this difference was not significant (Table 2). It is important to qualify these results in relation to the shaking procedure used in that the chicken sample lots were finely chopped before analysis. Chopping was necessary to obtain relatively homogeneous sample lots but may have enhanced the recovery of phages over the shaking technique because of better disaggregation of sample tissue.

There were few consistent or significant differences between various suspension techniques for recovery of T2 and MS2 coliphages from inoculated chicken, ground beef, corned beef, and mixed vegetables (Table 2). Mean recoveries of T2 were generally higher with shaking or slow-blending techniques than with fast-blending or stomaching techniques. However, significant differences were noted for ground beef, with shaking and slow blending resulting in significantly higher recoveries of T2 than fast blending or stomaching. In contrast, higher recoveries of MS2 from chicken and ground beef were observed with fast-blending than with shaking or slow-blending methods, although the differences were not significant. Moreover, there were no significant differences between suspension techniques for recovery of MS2 from any food tested. Trends for recovery of MS2 from chicken and ground beef were similar to those for indigenous coliphages from chicken with regard to method of processing. Recoveries of T2 or MS2 were generally higher from inoculated chicken, ground beef, and mixed vegetables than from corned beef.

Since sonication has been found to be effective in enhancing the release of enteroviruses from suspensions of shellfish and sewage sludge (34), the effect of sonication on the elution of indigenous coliphages from chicken suspensions prepared by blending, shaking, and stomaching was also investigated (data not shown). Sonicating sample suspensions for 2 min at 125 W had no significant effect on the recovery of indigenous coliphages from chicken suspensions compared with that from nonsonicated control suspensions. Longer sonication times were not evaluated due to excessive heating of sample suspensions and increased overall time of analysis.

Eluent composition and pH. Several eluents differing in content of protein or protein-type compounds, ionic strength, buffering capacity, and selective detergents, e.g., bile salts, were compared for elution and recovery of T2, MS2, and indigenous coliphages from fresh chicken (Table 3). Recovery of indigenous coliphages increased with increasing eluent protein content. For example, the EC medium and 1% casein eluents resulted in significantly higher recoveries of indigenous coliphages from chicken than did Butterfield phosphate buffer. Butterfield buffer was evaluated because of its common use in bacteriological analyses of food (1). The highest mean recovery of T2 from fresh

TABLE 2. Effect of sample processing methods on coliphage recovery[a]

Method	Indigenous coliphages recovery from chicken (10^3 PFU/100 g)	% Recovery							
		Chicken		Ground beef		Corned beef		Mixed vegetables	
		T2	MS2	T2	MS2	T2	MS2	T2	MS2
Blending									
2,000 rpm, 10 min	4.5†	80†	71†	89†	76†	51†	56†	88†	89†
4,500 rpm, 10 min	5.4†	63†	78†	60*	86†	47†	57†	81†	78†
Shaking (300 rpm, 10 min)	5.3†	83†	74†	93†	76†	65†	59†	81†	77†
Stomaching (10 min)	5.2†	70†	70†	72*‡	87†	49†	57†	78†	81†

[a] Sample suspensions were prepared with EC medium eluent and clarified by centrifugation (3,000 × *g*, 5 min). Values represent means of three subsamples. Within each column, means having the same superscript(s) were not significantly different ($P > 0.05$).

TABLE 3. Effect of various eluents on the recovery of T2, MS2 and indigenous coliphages from fresh chicken[a]

Eluent	Indigenous coliphage recovery (10^3 PFU/100 g)		% Recovery	
	Expt 1	Expt 2	T2	MS2
Butterfield buffer	0.7*	3.4*	32*	91†
0.1 M Lysine	1.3†	8.0†	41*	89†
1% Casein	1.5†	10.0†	53†*	85†
EC medium	1.7†	9.8†	78†	89†

[a] Sample suspensions were prepared by blending (4,500 rpm, 10 min) and clarified by centrifugation (3,000 × g, 5 min). The pH of eluents was preadjusted to obtain the same pH in suspensions as in suspensions with EC medium (pH 6.3 ± 0.2). Values represent means of three subsamples. Within each column, means having the same superscript(s) were not significantly different ($P > 0.05$).

chicken was obtained with EC medium; recoveries of T2 were significantly higher with EC medium than with Butterfield buffer or 0.1 M lysine. There were no significant differences between these eluents for elution of MS2 from chicken, in contrast to other comparisons with MS2.

The effect of various concentrations of chaotropic agents and detergents incorporated into 0.1 M lysine on the recovery of indigenous coliphages from fresh chicken was examined (Table 4). Similar experiments were also conducted with TP broth eluent (data not shown). The pH of the 0.1 M lysine eluent was 7.2, and the pH of resulting sample suspensions was ca. 6.0. The possible interactive effect of chaotropic agents and pH on coliphage elution from chicken was examined in a subsequent experiment. All concentrations of chaotropic agents used in these comparisons allowed the *E. coli* host to grow in the overlay medium as well as it did in a basal medium control. The recovery of indigenous coliphages from chicken was not increased by NTCA concentrations of 0.02 to 0.20 M in 0.1 M lysine or TP broth; the recovery of coliphages with 0.4 M NTCA in either eluent was significantly lower than at any other concentration or with the basal eluent (Table 4). A concentration of NTCA in overlay media corresponding to 0.4 MNTCA in the eluents inhibited plaque production by indigenous coliphages in basal eluent suspensions (data not shown). The recovery of indigenous coliphages also was not increased by the addition of various concentrations of urea (0.4 to 2.0 M) to the basal eluents. $NaNO_3$ and NaCl at concentrations of 0.1, 0.5, and 1.0 M generally did not enhance the recovery of indigenous coliphages from chicken. Although the recovery of coliphages with 0.1 M lysine plus 0.1 M $NaNO_3$ was significantly higher than with 0.1 M lysine alone, this difference was not considered to have a practical significance since this effect was not seen with TP broth or in subsequent studies (Table 5). Recoveries with 0.5 or 1.0 M $NaNO_3$ plus basal eluent were significantly lower than those with the basal eluent alone. Concentrations of $NaNO_3$ in overlay medium corresponding to 0.5 or 1.0 M $NaNO_3$ in the eluents also inhibited plaque production by indigenous coliphages from basal eluent suspensions (data not shown). The recovery of coliphages generally decreased with increasing concentrations of NaCl in basal eluents, although no significant differences were noted with 0.1 M lysine eluent. The addition of detergents such as Tween 80 and Triton X-100 to basal eluents at concentrations of 0.1 to 1.0% had no significant effect on the recovery of indigenous coliphages from fresh chicken (Table 4).

TABLE 4. Effect of various chaotropic agents on the recovery of indigenous coliphages from fresh chicken[a]

Agent added	Coliphage recovery (10^3 PFU/100 g)	Agent added	Coliphage recovery (10^3 PFU/100 g)
NTCA		Urea	
None	4.1†	None	2.9†
0.02 M	3.7†	0.4 M	3.1†
0.20 M	3.4†	1.0 M	3.1†
0.40 M	1.8*	2.0 M	3.1†
$NaNO_3$		NaCl	
None	3.5†	None	1.9†
0.1 M	4.2*	0.1 M	2.2†
0.5 M	1.1‡	0.5 M	1.8†
1.0 M	1.0‡	1.0 M	1.6†
Tween 80		Triton X-100	
None	1.3†	None	27.0†
0.1%	1.6†	0.1%	27.0†
0.5%	1.7†	0.5%	24.1†
1.0%	1.4†		

[a] Sample suspensions were prepared by blending (2,000 rpm, 5 min) and clarified by polypropylene mesh filtration. Values represent means of three subsamples. Within each set of data for a given compound, means having the same superscript were not significantly different ($P > 0.05$).

TABLE 5. Effect of pH, eluent composition, and chaotropic agents on the recovery of indigenous coliphages from fresh chicken[a]

Eluents[b]	Coliphage recovery (10^3 PFU/100 g) at pH:					
	6.0	6.5	7.0	8.0	9.0	10.0
Expt 1[c]						
Lysine	1.6*	—[e]	1.7*	1.6*	1.5*	1.5*
TP broth	—	3.1*	—	2.6*	2.6*	0.7†
Expt 2[d]						
Lysine	1.2	—	—	1.4	—	—
Lysine plus $NaNO_3$	1.5	—	—	1.2	—	—
Lysine plus urea	1.2	—	—	1.5	—	—
Lysine plus Tween 80	1.5	—	—	1.5	—	—

[a] Sample suspensions were prepared by blending (2,000 rpm, 5 min) and clarified by polypropylene mesh filtration. Values represent means of three subsamples.

[b] Concentrations: lysine, 0.1 M; $NaNO_3$, 0.1 M; urea, 0.4 M; and Tween 80, 0.5%.

[c] Means within each row having the same superscript were not significantly different ($P > 0.05$).

[d] No significant differences for any values in experiment 2.

[e] —, Not tested.

With 0.1 M lysine as an eluent, the recovery of indigenous coliphages from fresh chicken was not significantly affected by the pH of the sample suspension over a range of ca. 6.0 to 10.0 (Table 5). With TP broth as an eluent, there were no significant differences between sample suspension pHs of 6.5 to 9.0, but significantly fewer coliphages were recovered at pH 10.0 than at other pHs. Although coliphages as a group are susceptible to inactivation at high pH (25), the relative inactivation of indigenous coliphages by pH in these experiments cannot be determined from these data. The effects of representative chaotropic agents in 0.1 M lysine eluent on the recovery of indigenous coliphages from chicken suspensions at pH 6.0 and 8.0 were also studied (Table 5, experiment 2). The recovery of coliphages from sample suspensions at pH 6.0 and 8.0 was not significantly different for any formulation of 0.1 M lysine. Likewise, the recovery of coliphages with any formulation was not significantly higher

TABLE 6. Effect of eluent composition and pH on recovery of coliphages[a]

Eluent (pH)	Coliphage recovery from chicken (10^3 PFU/100 g)	% Recovery							
		Chicken		Ground beef		Corned beef		Mixed vegetables	
		T2	MS2	T2	MS2	T2	MS2	T2	MS2
0.1 M lysine (7.2)	1.6†	38†	82†	25§	81*‡†	57†	63†	61†	71†
0.1 M lysine plus 0.5% Tween 80 (7.2)	1.8†	35†	83†	42*†	70†	49†	56†	64†	78†
0.1 M lysine (8.5)	1.6†	52*	85†	47*	88*‡	59†	61†	55†	75†
EC medium (7.0)	2.3†	66‡	101*	64‡	90*	48†	58†	58†	87†
EC medium plus 0.5% Tween 80 (7.0)	1.9†	55*	90*†	43*†	81*‡†	46†	58†	58†	69†
EC medium (8.5)	2.1†	70‡	92*†	66‡	93*	46†	60†	60†	77†

[a] All sample suspensions were prepared by blending (4,500 rpm, 10 min) and clarified by centrifugation (3,000 × g, 5 min). Values represent means of three subsamples. Within each column, means having the same superscript(s) were not significantly different ($P > 0.05$).

than with 0.1 M lysine alone, regardless of the sample suspension pH. These results supported findings in previous experiments for chaotropic agents (Table 4).

Since EC medium resulted in better recovery of T2 and indigenous coliphages from fresh chicken than the other eluents tested, various formulations and pHs of EC medium and a less complex basal eluent, 0.1 M lysine, were compared for recovery of T2 and MS2 from chicken, fresh ground beef, canned corn beef, and thawed mixed vegetables (Table 6). EC medium (pH 7.0) resulted in significantly higher or equivalent recoveries of indigenous coliphages from chicken as well as T2 or MS2 from all foods tested than any formulation of 0.1 M lysine. Few significant differences due to pH were noted in coliphage recovery. Recovery of T2 from fresh chicken and ground beef was significantly higher with 0.1 M lysine at pH 8.5 than at pH 7.2; recovery of T2 from these foods with 0.1 M lysine (pH 8.5) was significantly lower than with ambient EC eluent (pH 7.0). No significant differences were observed for the recovery of MS2 or indigenous coliphages as a result of eluent pH, and recovery of T2 with EC eluent were not significantly affected by eluent pH (7.0 or 8.5). The recovery of T2 from ground beef was significantly higher with 0.1 M lysine plus 0.5% Tween 80 than with 0.1 M lysine alone; recovery of T2 from ground beef was significantly lower with 0.1 M lysine plus 0.5% Tween 80 than with EC medium eluent. The addition of 0.5% Tween 80 to EC medium eluent resulted in significantly lower recoveries of T2 from chicken and ground beef than EC medium alone. With EC medium eluent and a blending technique (4,500 rpm, 10 min) for sample suspension, recovery of T2 ranged from 48% for corned beef to 66% for chicken, whereas that for MS2 ranged from 58% for corned beef to 101% for chicken (Table 6).

DISCUSSION

Clarification of food sample suspensions by centrifugation and polypropylene mesh filtration was superior to that by glass wool filtration in terms of decreased labor and time requirements as well as increased recovery of coliphages. These results differ to some extent from those reported by Tierney et al. (32), who compared several clarification techniques, including glass wool filtration and low-speed centrifugation, for recovery of enteroviruses from ground beef and found that clarification by glass wool resulted in higher recoveries of enteroviruses than centrifugation, although both methods were considered effective. Polypropylene mesh filtration was the most simple and rapid clarification technique for some sample suspensions but was not practical for large volumes of viscous or well-homogenized sample suspensions. Centrifugation was relatively fast, and large volumes of sample suspension could be readily clarified regardless of the food type or degree of homogenization.

Few consistent or significant differences were noted between blending, stomaching, and shaking techniques for recovery of T2, MS2, and indigenous coliphages from the foods examined (Table 2). However, it should be noted that the effectiveness of a shaking technique for sample suspension in releasing coliphages or other viruses from certain foods may depend on adequate chopping or fragmentation of food aggregates before suspension. In this regard, shaking techniques may be considerably more labor intensive than blending or stomaching techniques for suspension of certain types of food samples. Some differences were noted between MS2 and T2 in the optimal suspension technique for a given food (Table 2). These results are difficult to interpret, although the observed differences in the recovery of MS2 and T2 from foods as a function of sample suspension technique may be related to morphological or size differences between these two phages. The relatively large, complex structure of T2 coliphages may have been more subject to damage by shearing forces during sample suspension than the smaller, cubic MS2 coliphages. The use of a chopping-kneading-shaking method for suspension of shellfish samples was much less effective for recovery of enteroviruses than homogenization at 16,000 rpm (21). In contrast, a kneading-shaking method for suspension of ground beef samples was quite effective for recovering enteroviruses (32). Based on the present results, the technique used to suspend samples may be chosen by (i) equipment availability (blender, shaker, stomacher, or centrifuge), (ii) the type of food sample (whole tissue versus ground or surface-contaminated foods); (iii) the type of clarification technique used, and (iv) a combination of these factors.

Higher recoveries of coliphages were consistently obtained with EC medium eluent, which contains 2% soluble protein (tryptose) and 0.15% bile salts, 0.5% lactose, NaCl, and phosphate buffers than with more simple eluents such as Butterfield phosphate buffer or 0.1 M lysine. The increased elution of viruses from surfaces and solids by relatively high concentrations of soluble proteins or other organic compounds is well documented (2). However, there were no significant differences between high-protein eluents and 0.1 M lysine in recovery of coliphages from some foods. The elution of coliphages from a food suspension may be a function of both the soluble proteins in the eluent and the endogenous proteins and other organic compounds in the food itself which are solubilized to some extent in the eluent.

Chaotropic agents have not been evaluated for the elution

of animal viruses or coliphages from foods. The general inability of the chaotropic agents evaluated in this study to improve the elution or recovery of T2, MS2, and indigenous coliphages from various foods was surprising in light of previous findings on the efficacy of these compounds in eluting enteroviruses and coliphages from membrane filters (7, 9, 11), aquatic sediments (4, 33), and sewage sludge (12). Tween 80 has also been used to enhance the recovery of bacteria from fatty foods such as ground beef (28). The improved recovery of T2 from ground beef with 0.1 M lysine plus 0.5% Tween 80 than with 0.1 M lysine may have been related to the high fat content of the ground beef. However, a similar effect was not observed for MS2 with 0.1 M lysine or for either T2 or MS2 with EC medium eluent. For NTCA, urea, $NaNO_3$, and NaCl, concentrations similar to those used in environmental studies could not be used in our phage assay medium because these concentrations inhibited host growth or plaque production by the coliphages. The eluting effects of endogenous dissolved proteins and other organic compounds in the sample suspensions of many foods also may have obscured the eluting effects of chaotropic agents. Finance et al. (13) reported that the conductivity of food sample suspensions affected the elution of polioviruses from some foods but not others over a range from 0.1 to 1.6% NaCl. In the present study, the recovery of indigenous coliphages from chicken was not significantly affected by NaCl concentrations over a range from 0 to 5.8% with 0.1 M lysine eluent.

The elution of coliphages and enteroviruses from various surfaces is generally enhanced by increasing the pH of the suspending medium and thereby minimizing the attractive electrostatic interactions between viruses and solid surfaces (2). However, the elution of polioviruses from estuarine sediments was often variable over a pH range from 5.5 to 9.5 with various eluents (33). The pH of most food suspensions are adjusted to relatively high pH (pH 8.5 or greater) for the elution and recovery of animal viruses from foods (6). Finance et al. (13) reported that the elution of polioviruses from sample suspensions of various foods as a function of pH varied according to food type. In the present study, the recovery of indigenous coliphages from chicken was not affected by the pH of the sample suspension over a range from ca. 6.0 to 9.0 with various eluents. Highest or equivalent recoveries of T2, MS2, and indigenous coliphages were consistently observed with EC medium at ambient pH (pH 6.9) than with any other basal eluent or eluent formulation. These data indicate that use of a complex eluting medium such as EC medium obviates the need for adjusting the pH of sample suspensions.

With EC medium as an eluent, recovery of T2 and MS2 varied according to the method of sample suspension and the food type. With optimal suspension methods, the mean recovery of T2 and MS2 was generally greater than 80% for chicken, ground beef, and mixed vegetables and 10 to 15% lower for corned beef. Differences in coliphage recovery as a function of food type were primarily attributed to occlusion of the phages by fats or other components of the food during sample processing. Based on overall applicability and the results of comparative studies, a procedure involving blending (4,500 rpm, 10 min) with EC eluent and clarification of resulting sample suspensions by centrifugation (3,000 × *g*, 5 min) was considered effective for elution and recovery of indigenous coliphages from the foods tested. With this procedure, mean recovery of T2 ranged from 48 to 81% and recovery of MS2 ranged from 58 to 100%, depending on the food type.

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