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# Surfactants for the Effective Recovery of *Salmonella* in Fatty Foods

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**ABSTRACT**

Inhibitory concentrations of 8 surfactants were determined for *Salmonella typhimurium* and *Salmonella enteritidis*. Pure culture work resulted in the exclusion of Tween 20, Teepol 610 and Brij 35 and retention of Tergitol-7 (T-7), Tween 80 (TW 80), Triton X-100 (TX), Myrj 52S (M), and Arlacel 80 + Tween 60 (AT) for a study on the quantitative recovery of *Salmonella* in 45 naturally contaminated fatty foods. Replicate food samples (100 g) were preenriched overnight at 35 C in nutrient broth supplemented with 3% (w/v) surfactant except AT (10%). Serial dilutions of preenrichment cultures were selectively enriched overnight in tetrathionate brilliant green (43 C) and selenite cystine (35 C) broths and streaked on bismuth sulfite and brilliant green sulfa agar media. Recovery with all test surfactants was comparable to that obtained with nutrient broth controls; of 270 preenrichment cultures tested, only 7 false-negative results attributable to TX (3), AT (2), M (1), and nutrient broth control (1) were obtained. None of the surfactants consistently yielded greater populations of *Salmonella* for given foods or food categories; median counts for preenrichment cultures were $10^4$-$10^5$ salmonellae/ml for low and high moisture foods and $10^6$-$10^7$ salmonellae/ml for animal feeds. These results suggest that use of surfactants to facilitate detection of *Salmonella* in fatty foods is not warranted.

Surfactants are widely used for the detection of *Salmonella* in fatty foods because isolation and identification of the salmonellae usually present in low numbers in food samples reportedly depend on their release from the lipid phase and proliferation to detectable levels in non-selective and selective broth media. Tergitol-7 at concentrations of 0.5 to 1.0% (w/v) is used extensively in standard cultural procedures (*1,9,14,24*) whereas other surfactants, including Triton X-100 (*24*), Teepol 515 (*13*) and Tween 80 (*2,15,16*), have found limited application. Although Tergitol-7 improves isolation of *Salmonella* from pork sausages, it is ineffective for dried milk products (*20,21*); increasing evidence further suggests that surfactants are not determinants in the isolation of indicator organisms or salmonellae from food and environmental samples and that they may be toxic under selected cultural conditions (*2,3,6,13,19,20*).

This study evaluates the toxicity of selected surfactants and compares their efficacy for the detection of *Salmonella* in naturally contaminated fatty foods.

## MATERIALS AND METHODS

### *Toxicity of selected surfactants*

*Salmonella typhimurium* and *Salmonella enteritidis* were used to determine the toxicity of Tween 20 (TW 20), Tween 80 (TW 80), Triton X-100 (TX), anionic Tergitol-7 (T-7), Arlacel 80 plus Tween 60 (AT), Myrj 52S (M), Teepol 610 (Teepol) and Brij 35 (Brij) at concentrations of 0.5-10% (w/v), except AT (3-20%), prepared with equal amounts of each component. Surfactants were obtained from J. T. Baker Chemical Company, except Teepol (British Drug House) and Brij, AT and M (Atlas Chemical Industries). Portions (0.1 ml) of overnight nutrient broth cultures of each test organism were transferred into 100 ml of nutrient broth with added surfactant and grown 18-24 h at 35 C. Serial dilutions of the resulting broth cultures were plated quantitatively on tryptic soy agar plates and enumerated following overnight incubation at 35 C.

### *Salmonella analyses*

Naturally contaminated food and animal feed products were obtained from retail outlets or as a result of monitoring activities of Canadian federal agencies (Table 1). For surfactant evaluation, each food sample was homogenized in a blender and replicate 100-g samples of the homogenate were preenriched overnight at 35 C in 9 volumes of nutrient broth and nutrient broth supplemented with 3% (w/v) surfactant except AT (10%). Poultry carcasses were thoroughly rinsed in 1 liter of nutrient broth and replicate 100-ml rinse samples were preenriched as described above. Animal feeds were mixed in a plastic bag and replicate 100-g samples were added directly to the preenrichment media. Following overnight incubation at 35 C, each preenrichment culture was serially diluted in peptone water and 1-ml portions from each dilution was selectively enriched in 9 ml each of tetrathionate brilliant green (TBG) and selenite cystine (SC) incubated at 43 C and 35 C, respectively. Selective enrichment cultures were streaked on bismuth sulfite (BiS) and brilliant green sulfa (BGS) agar media and incubated overnight at 35 C; presumptive isolates were screened biochemically and serologically.

Quantitation of *Salmonella* in original sample material was determined by the 3-tube Most Probable Number technique using the homogenate prepared for surfactant evaluation.

Analysis of variance (ANOVA) and multiple comparison tests were used to identify significant differences at a 5% level of significance between the selectivity of four enrichment-plating conditions and the effect of surfactants on the incidence of competitive flora. Selective conditions for isolation included A = $TBG_{43}$ + BiS; B = $TBG_{43}$ + BGS; C = $SC_{35}$ + BiS; D = $SC_{35}$ + BGS. Growth of non-salmonellae on BiS and BGS was scored using the following scale: 1 = 0-25% incidence; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%. These arbitrary values were averaged to obtain the mean score for competitive flora for each enrichment-plating condition (Table 5).

## RESULTS AND DISCUSSION

The sensitivity of *S. typhimurium* (Fig. 1) to increasing concentrations of surfactant was similar to that observed with *S. enteritidis* (data not shown). Growth of both serovars in nutrient broth supplemented with 0.5-10% surfactant except T-7 and Teepol produced cell populations comparable to that obtained in nutrient broth controls (1.5-1.6 × $10^8$ salmonellae/ml). Strong inhibition was observed at low concentrations of Teepol and at T-7 concentrations of 5% or greater. Teepol was excluded from further study with naturally contaminated foods because of toxicity (Fig. 1) and TW 20 and Brij because of responses comparable to other test surfactants with similar hydrophilic - lipophilic characteristics. T-7 and TX were retained because of their use in standard methods (*1,23,24*) and M, TW 80 and AT for their detergency or use in bacteriological analyses of food products (*18,22*).

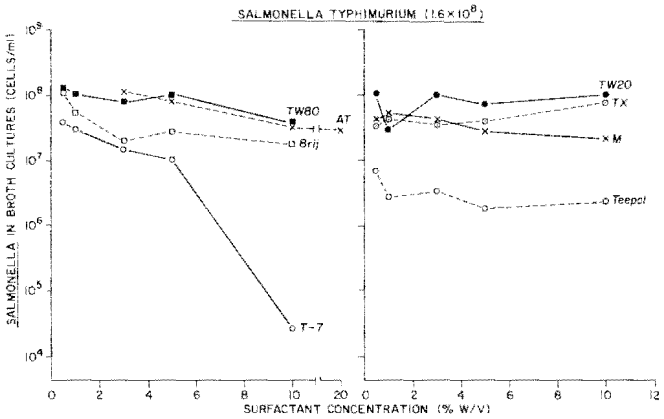


Figure 1. *Inhibitory concentrations of surfactants for* Salmonella typhimurium. *Surfactants include Tween 80 (TW 80), Arlacel 80 + Tween 60 (AT), Brij 35 (Brij), Tergitol-7 (T-7), Tween 20 (TW 20), Triton X-100 (TX), Myrj 52S (M) and Teepol 610 (Teepol).*

Of 118 foods tested, 45 were retained for evaluation of selected surfactants (Table 1). A total of 17 serovars were isolated from high moisture foods, 4 from low moisture foods and 7 from animal feeds (Table 2); levels of contamination varied widely within each food category. Results with selected surfactants were comparable to those obtained with nutrient broth controls, and no single surfactant consistently produced greater recoveries of *Salmonella* in any food or food category (Table 3). These findings concur with earlier reports on the inability of Tween 80 and Tergitol-7 to increase aerobic plate counts or recovery of coliforms and *Salmonella* in cocoa powder and other fatty foods through improved dispersion of lipids (*2,3,19,21*). Of 25 positive high moisture foods tested (Table 3), one sample each of raw chicken and pork sausage could not be identified from AT-supplemented preenrichment cultures and one sample of pork sausage from a nutrient broth culture. One of the 4 contaminated coconut samples yielded false-negative results in M-supplemented preenrichment medium. TX-mediated inhibition of salmonellae in 2 meat meal and 1 finished feed sample appears to be product-dependent because recovery of *Salmonella* from other feed samples using the same lot of surfactant compared favorably with control conditions; data further

TABLE 1. *Incidence of* Salmonella *in fatty foods.*

| Food | *Salmonella* isolation | |
|---|---|---|
| | Number tested | Number positive |
| High moisture | | |
| Raw poultry and giblets | 20 | 13 |
| Pork and organ meats | 41 | 8 |
| Bovine organ meats | 14 | 0 |
| Shellfish | 10 | 3 |
| Others[a] | 5 | 1 |
| *Sub total:* | 90 | 25 |
| Low moisture | | |
| Coconut | 7 | 4 |
| Fermented sausages | 4 | 2 |
| Others[b] | 4 | 2 |
| *Sub total:* | 15 | 8 |
| Animal feeds | 13 | 12 |
| *Total:* | 118 | 45 |

[a]Lamb, liquid whole eggs and blood pudding.
[b]Milk chocolate, cocoa beans and chili powder.

TABLE 2. *Distribution of* Salmonella *serovars in fatty foods.*

| Food | Number of positive samples | Serovars isolated | Level of contamination (salmonellae/100 g) |
|---|---|---|---|
| High moisture | | | |
| Raw poultry and giblets | 13 | schwarzengrund (3); heidelberg (2); niedstedten (1); albany (1); bredeney (1); manila (1); saint-paul (1); lille (1); thompson (1); infantis (1) | 2.3->110[a] |
| Pork and organ meats | 8 | brandenburg (3); agona (2); london (2); haardt (1) | 0.91->110 |
| Shellfish | 3 | abaetetuba (1); miami (1); sundsvall (1) | 15-110 |
| Others | 1 | thompson | 460 |
| Low moisture | | | |
| Coconut | 4 | senftenberg (4) | 1.4-9.3 |
| Fermented sausages | 2 | indiana (1); typhimurium (1) | 15->110 |
| Others | 2 | chandans (1); senftenberg (1) | 2.3-230 |
| Animal feeds | 12 | montevideo (4); drypool (2); thomasville (2); binza (1); havana (1); senftenberg (1); tennessee (1) | 2.3-460 |

[a]Number of cells per 100 g of product or 100 ml of whole carcass rinse.

TABLE 3. *Quantitative recovery of* Salmonella *with selected surfactants.*

| Surfactant | Number of positive samples | Counts of *Salmonella* in preenrichment culture ($log_{10}$/ml) | |
|---|---|---|---|
| | | Range | Median |
| High moisture (25)[a] | | | |
| AT | 23 | 0-7 | 4.0 |
| M | 25 | 1-7 | 5.0 |
| T-7 | 25 | 0-7 | 4.0 |
| TX | 25 | 1-7 | 5.0 |
| TW80 | 25 | 2-7 | 4.0 |
| Control | 24 | 0-7 | 4.0 |
| Low moisture (8)[a] | | | |
| AT | 8 | 2-7 | 4.5 |
| M | 7 | 0-7 | 5.5 |
| T-7 | 8 | 4-6 | 5.0 |
| TX | 8 | 3-7 | 5.5 |
| TW80 | 8 | 1-7 | 4.5 |
| Control | 8 | 0-6 | 5.0 |
| Animal feeds (12)[a] | | | |
| AT | 12 | 2-8 | 7.0 |
| M | 12 | 2-8 | 6.0 |
| T-7 | 12 | 2-8 | 6.5 |
| TX | 9 | 6-8 | 7.0 |
| TW80 | 12 | 4-8 | 6.5 |
| Control | 12 | 3-8 | 7.0 |

[a]Total number of *Salmonella*-contaminated foods.

suggest that levels of salmonellae in the original sample material were not a determinant for detection.

Enrichment in TBG (43 C) was markedly superior to SC (35 C) for the detection of salmonellae in high but not in low moisture foods and animal feeds (Table 4). These findings support earlier reports on temperature-dependent recovery of *Salmonella* from high moisture foods (*5,10,11,17*). The superiority of BiS agar medium is well established (Table 4 and *4,12*) and rests on its greater selectivity against competing microflora including *Citrobacter* and *Proteus* spp. (*7,8*). Notwithstanding its reliability, BiS in combination with one or more agar media is widely used for the isolation of *Salmonella* in foods (*14,23,24*).

Variance analysis of data on selectivity of enrichment-plating conditions showed that preenrichment, selective enrichment and plating media significantly affected the incidence of competitive flora for raw meat and feed samples ($P < 0.005$); only plating media were significant for dry foods ($P<0.05$). The generally lower yield of competitive flora with conditions A and B (see Materials and Methods) and high incidence of non-salmonellae with condition D underline the superiority of TBG and limited selectivity of SC (Table 5). Data further indicate that condition B provided highest selectivity for detection of *Salmonella* in meat products whereas both BiS-dependent conditions (A and C), with a single exception, were not significantly different for detection of salmonellae in feed and dry foods. Performance ranking

of the four enrichment-plating conditions by surfactant failed to show consistent trends within a food category or between different food categories.

Our results do not support the use of surfactants for detection of *Salmonella* in fatty foods because of absence of any increase in method sensitivity and occurrence of toxic effects.

TABLE 4. *Isolation of* Salmonella *under selected analytical conditions*[a].

| Food | Total number of positive samples[b] | Selective enrichment | Percent recovery on plating media | |
|---|---|---|---|---|
| | | | BiS | BGS |
| High moisture | 147 | $TBG_{43}$ | 90 (8)[c] | 92 (7) |
| | | $SC_{35}$ | 77 (19) | 67 (10) |
| Low moisture | 47 | $TBG_{43}$ | 100 (2) | 98 (0) |
| | | $SC_{35}$ | 96 (13) | 83 (0) |
| Animal feed | 69 | $TBG_{43}$ | 100 (3) | 97 (0) |
| | | $SC_{35}$ | 99 (6) | 93 (0) |

[a]Data for undiluted preenrichment cultures with or without added surfactant.
[b]See Table 3.
[c]Figure in brackets denotes percent number of samples positive on one but negative on the homologous plating medium for each selective enrichment condition.

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TABLE 5. *Multiple comparisons of selectivity of enrichment-plating conditions against competitive flora.*

| Surfactant | Food | Performance ranking of enrichment-plating conditions[a] | | | |
|---|---|---|---|---|---|
| AT | High moisture | B(2.39)[b] | D(3.43) | A(3.48) | C(3.57) |
| | Low moisture | A(1.50) | C(1.75) | B(2.38) | D(2.50) |
| | Animal feed | A(1.36) | C(1.36) | B(1.73) | D(1.73) |
| M | High moisture | B(2.09) | A(2.52) | D(3.13) | C(3.30) |
| | Low moisture | A(1.88) | C(2.25) | B(2.88) | D(3.13) |
| | Animal feed | A(1.00) | B(1.27) | C(1.36) | D(1.82) |
| T-7 | High moisture | B(1.52) | A(1.96) | C(3.13) | D(3.48) |
| | Low moisture | C(1.63) | A(1.75) | B(2.13) | D(3.50) |
| | Animal feed | A(1.00) | B(1.27) | C(2.45) | D(3.18) |
| TX | High moisture | B(2.39) | D(2.87) | A(2.91) | C(3.00) |
| | Low moisture | C(1.63) | A(1.75) | D(2.38) | B(2.88) |
| | Animal feed | A(1.82) | C(1.91) | B(2.00) | D(3.00) |
| TW 80 | High moisture | B(2.39) | A(3.13) | D(3.22) | C(3.30) |
| | Low moisture | A(1.25) | C(1.38) | B(2.00) | D(2.00) |
| | Animal feed | C(1.00) | A(1.18) | B(1.36) | D(1.55) |
| Control | High moisture | B(2.43) | A(2.52) | D(3.00) | C(3.09) |
| | Low moisture | C(1.38) | A(1.75) | B(2.13) | D(2.13) |
| | Animal feed | C(1.09) | A(1.18) | B(1.55) | D(2.64) |

[a]Enrichment/plating conditions include: A = $TBG_{43}$ + BiS; B = $TBG_{43}$ + BGS; C = $SC_{35}$ + BiS; D = $SC_{35}$ + BGS. Conditions A to D are listed from left to right in decreasing order of efficacy and are scored by the same line if not found to be significantly different.
[b]Number in brackets is mean score for competitive flora.

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