September, 1976
Vol. 39, No. 9
Pages 589-664
JMFTA 39(9):589-664 (1976)

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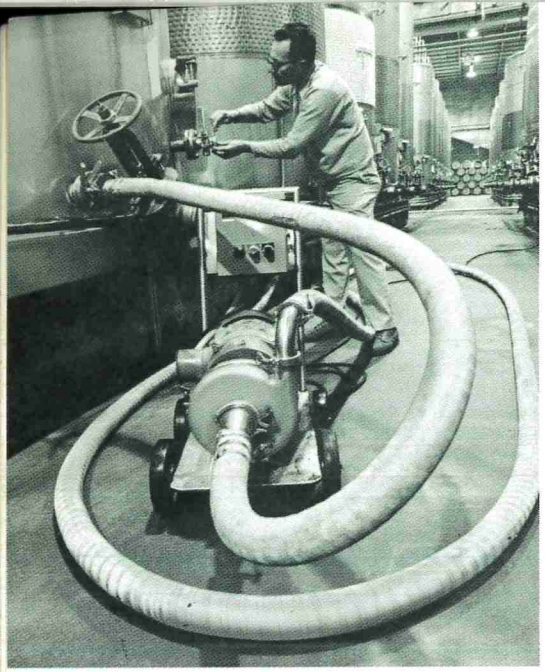

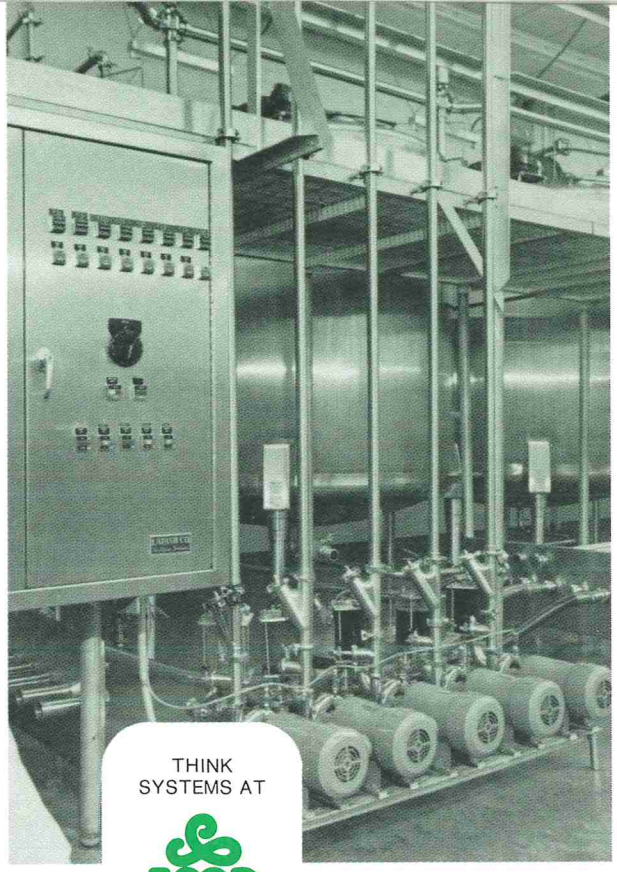

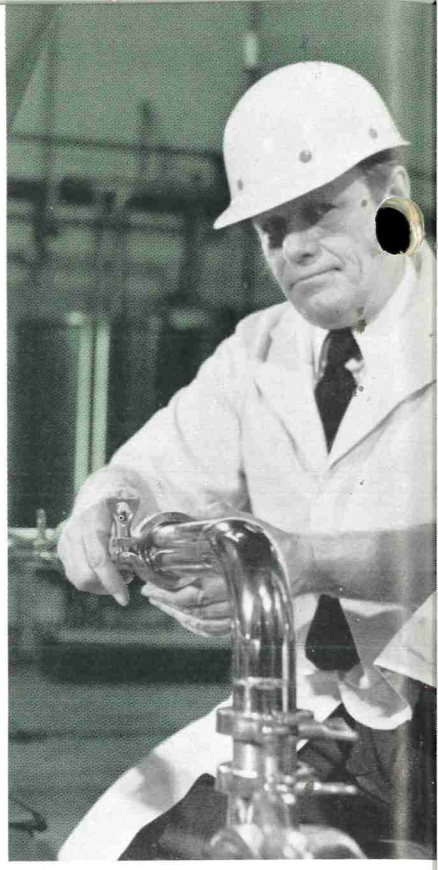

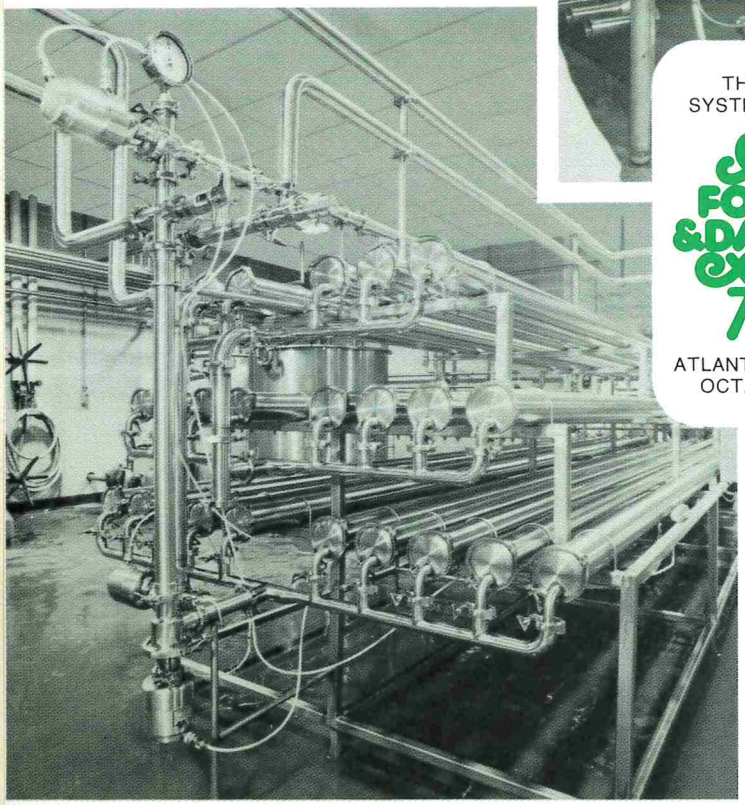

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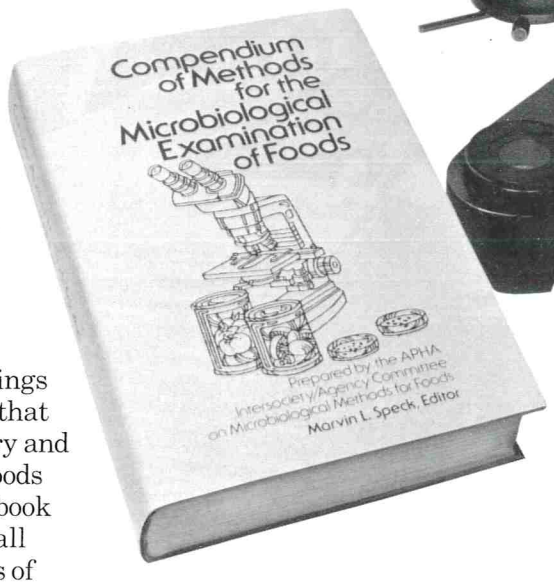

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Journal of Milk and Food Technology

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Official Publication.
International Association of Milk, Food and Environmental Sanitarians, Inc., Reg. U.S. Pat. Off.

Vol. 39 September 1976 No. 9

The Journal of Milk and Food Technology is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association, 413 Kellogg Ave., P.O. Box 701, Ames, Ia. 50010. Printed by Heuss Printing and Signs, Inc., 204 N. Oak, Ames, Iowa 50010.

2nd Class postage paid at Ames, Ia. 50010.

Editorial Offices: Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 53706. Earl O. Wright, P.O. Box 701, Ames, Ia. 50010.

Manuscripts: Correspondence regarding manuscripts and other reading material should be addressed to Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 53706.

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J. Milk Food Technol. Vol. 39, No. 9, Pages 592-599 (September, 1976)

Quality Characteristics of Vacuum Packaged Beef as Affected by Postmortem Chill, Storage Temperature, and Storage Interval

Z. L. CARPENTER, S. D. BEEBE[1], G. C. SMITH, K. E. HOKE[2], and C. VANDERZANT

Department of Animal Science, Texas Agricultural Experiment Station
Texas A&M University, College Station, Texas 77843

(Received for publication February 9, 1976)

ABSTRACT

Sirloin butts from beef carcasses chilled to 1 or 7.2 C were vacuum packaged and stored at either 0 or 5.5 C for intervals of 7, 14, 21, 28, or 35 days. Following storage, steaks were obtained for retail caselife studies and taste panel evaluations. Evaluation of primal cuts revealed significant differences in total desirability (primarily visual appearance) among storage intervals and between storage temperatures. Initial carcass temperature at fabrication influenced quality of cuts less than did storage temperature over extended storage intervals. Storage of vacuum packaged cuts at 0 C consistently enhanced shelf-life compared to storage at 5.5 C.

Fresh meat packaging systems utilize films which vary in oxygen permeability, moisture vapor transmission rate, heat seal or shrinkage properties, tearing strength, tackiness, brittleness, clarity, and numerous other properties. Plastic films for meat have been used principally as a retail wrap, with oxygen permeability which allows optimal "bloom" yet prevents excess shrinkage. Current technology allows for almost total oxygen exclusion from packaged primal, sub-primal, or retail cuts. Vacuum packaging of fresh meats allows for maintenance of ultimate desirable color, reduces moisture loss during storage (*7*), inhibits aerobic microbial spoilage (*2, 25*), and prolongs product acceptance (*24, 26, 27*). According to previous work (*17*), fluid loss from meat can be minimized by quick chilling of hot carcasses.

Consumer studies (*9, 11, 12*) indicated that physical appearance of retail cuts in a display case is the most important factor determining selection. Meat cuts are selected primarily for leanness and then for appearance and freshness. Foods with relatively high microbial populations could be stored for a considerable period without quality loss, if they were held at relatively low temperatures (*19*). In commercial practice, consumer cuts of fresh beef such as steaks and roasts individually packaged in gas-permeable plastic film frequently have a shelflife of only 2 to 4 days due to development of undesirable odor and/or color (*15, 22*). The main cause of these undesirable changes is growth of common psychrotrophic bacteria that can produce off-odors through breakdown of meat proteins and lipids, and can indirectly favor metmyoglobin formation (brown color) by depleting the dissolved oxygen concentration at the meat surface (*5, 13, 15, 22, 23*). Bacterial growth reduces salable shelflife of beef because of discoloration, off-odor, and slime formation (*6*). Bacterial contamination during handling and processing involved in fabrication, trimming, and packaging of retail cuts is closely associated with ultimate caselife (*4*). Storage life of packaged meat could be extended (*18*) by reducing microorganisms, especially *Pseudomonas,* often a predominant organism found on fresh meat (*1*). Bacterial flora of vacuum-packaged lamb cuts changed from a predominance of *Pseudomonas,* before packaging and storage, to a predominance of *Lactobacillus* after 21 days of storage (*31*) which is similar to findings for beef (*21, 25*). Vacuum packaging inhibits surface dehydration, reduces oxygen availability, and can affect bacterial action (*20*).

Surface discoloration limited storage life of lamb cuts (*16*). Four parameters that influenced meat color were classified (*29*) as surface dehydration, temperature, oxygen requirements of the meat, and bacterial contamination. Numerous studies have shown that vacuum packaging extends storage life of meat because of the stress placed on microorganisms during storage. Also, meat cuts stored at lower temperatures exhibit longer retail caselife and lower bacterial counts. Because of the increased acceptance of the "boxed beef" distribution system, whereby carcasses are prefabricated into primal or subprimal cuts, vacuum packaged, and shipped to retail outlets and because vacuum packaging is expected to double in the future, it is imperative that

[1]*Present address: Standard Meat Company, Ft. Worth, Texas.*
[2]*Transportation and Packaging Research Laboratory, Agricultural Marketing Research Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.*

temperatures at which this vacuum-packaged product is stored be investigated. Accordingly, the objective of this study was to determine the effect of initial chill and storage temperature upon purge loss and keeping quality of vacuum-packaged beef stored for extended periods of time.

MATERIAL AND METHODS

One hundred twenty top sirloin butts (IMPS 184) were obtained from beef carcasses weighing 272 to 318 kg and grading U.S. Good and U.S. Choice, Yield Grade 2 (*30*). Cuts were removed from the carcasses at 24-h postmortem for the partially chilled sirloin butts (7.2 C) and 72-h postmortem for fully chilled cuts (1 C). Cuts were vacuum packaged at 29.5 inches of Hg via a chamber type, heat seal system (Conofresh B film, Conofresh 6000 chamber) and shipped by refrigerated truck to the Texas A&M Meat Laboratory (48-h post-packaging). Upon arrival the 60 cuts of each chill condition were weighed and assigned to storage coolers with temperatures of either 0 or 5.5 C for storage intervals of 7, 14, 21, 28, or 35 days, which resulted in six cuts per chill condition/storage temperature/storage interval group. All cuts were weighed upon temination of the storage interval and again after removal of the packaging material for determination of purge loss.

Upon completion of each storage treatment, a two-member experienced panel evaluated each primal cut (30 min after removal of package) for appearance of subcutaneous fat cover according to a six-point scale (6 = very fresh, 4 = normal, 1 = severe discoloration), for muscle color using a nine-point scale (9 = very light cherry red, 6 = cherry red, 2 = very dark red), for surface discoloration of lean on a seven-point scale (7 = no surface discoloration, 4 = 25-50% discoloration, 1 = total surface discoloration), for odor on a four-point scale (4 = no detectable off-odor, 1 = extreme off-odor), and for total desirability according to an eight-point scale (8 = extremely desirable, 5 = slightly desirable, 1 = extremely undesirable).

Microbiological assays for evaluation of the effects of chill condition, storage temperatures and storage intervals are reported separately (*3*).

At completion of primal cut evaluation and bacterial sampling each primal cut was trimmed of surface discoloration. Two steaks, 2.5 cm thick, were obtained from each primal cut. One steak from each pair was designated for retail caselife evaluation and the other for taste panel evaluation. Steaks for retail caselife evaluation studies were placed in styrofoam trays, overwrapped with PVC film, and placed under retail condtions (1 to 3 C under 82 footcandles of incandescent light). Each retail steak was evaluated initially for muscle color and at 24-h intervals for 4 days for surface discoloration, peripherial discoloration (five-point scale: 5 = no peripheral discoloration, 1 = extreme peripheral discoloration) and total desirability.

The remaining steaks were wrapped in freezer paper and frozen at −35 C. After thawing, steaks were oven-broiled in a 177-C electric oven to an internal temperature of 75 C and evaluated by an eight-member trained sensory panel. Each steak was evaluated for tenderness, juiciness, flavor, and overall satisfaction by use of eight-point rating scales (*24*). In addition, objective tenderness measurements were made using 1.3-cm diameter cores and a Warner-Bratzler shear device.

Data were analyzed by use of standard analysis of variance procedures. When significant differences in main effects were identified, mean separation analyses were conducted (*10*).

RESULTS AND DISCUSSION

Appearance of fat cover, muscle color, odor, and surface discoloration of each primal cut were evaluated individually and then assessed collectively as total desirability (Tables 1-5). At 7 and 14 days, cuts fabricated at a higher internal temperature (7.2 C) had more desirable ratings for appearance of fat cover than cuts properly chilled (1 C) before fabrication (Table 1). After 14 days of storage there were no differences ($P > .05$) between treatments. The difference in the time postmortem at packaging may have influenced the fat cover appearance ratings, a variation which cannot be separated from the internal temperature treatment. At

TABLE 1. *Means for ratings of fat cover appearance[e] for primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled Stored / 1 C 0 C	B: Chilled Stored / 1 C 5.5 C	C: Chilled Stored / 7.2 C 0 C	D: Chilled Stored / 7.2 C 5.5 C	Rank order[d] (highest to lowest) Across treatment
7	3.9[a]	3.5[b]	5.2[a]	5.5[a]	DCAB
14	3.0[a]	3.7[a]	4.5[ab]	5.0[a]	DCBA
21	3.2[a]	4.3[a]	3.8[bc]	3.9[b]	BDCA
28	3.8[a]	2.8[c]	3.4[c]	3.3[bc]	ACDB
35	3.5[a]	3.6[ab]	3.8[bc]	2.8[c]	CBAD

[abc]Means in the same column bearing a common superscript do not differ ($P > .05$).
[d]Mean values within the same day of storage, underscored by a common line, do not differ ($P > .05$). Letters A, B, C, D represent treatments in columns A, B, C, D, respectively.
[e]6-point scale: 6 = very fresh; 5 = fresh; 4 = normal; 3 = some discoloration; 2 = moderate discoloration; 1 = severe or extreme discoloration.

TABLE 2. *Means for muscle color scores[d] for primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled Stored / 1 C 0 C	B: Chilled Stored / 1 C 5.5 C	C: Chilled Stored / 7.2 C 0 C	D: Chilled Stored / 7.2 C 5.5 C	Rank order[c] (highest to lowest) Across treatment
7	6.3[b]	5.8[b]	6.2[a]	6.2[a]	ACDB
14	5.9[b]	5.9[b]	6.3[a]	6.3[a]	DCAB
21	6.1[b]	6.7[a]	6.3[a]	5.7[a]	BCAD
28	6.9[a]	6.8[a]	6.3[a]	6.2[a]	ABCD
35	6.3[b]	6.3[ab]	6.4[a]	5.9[a]	CBAD

[ab]Means in the same column bearing a common superscript do not differ ($P > .05$).
[c]Means within the same day of storage, underscored by a common line, do not differ ($P > .05$). Letters A, B, C, D represent treatments in columns A, B, C, D, respectively.
[d]9-point scale: 9 = very light cherry red; 8 = moderately light cherry red; 7 = slightly light cherry red; 6 = cherry red; 5 = slightly dark red; 4 = moderately dark red; 3 = dark red; 2 = very dark red; 1 = black.

each storage interval, primal cuts fabricated at a higher internal temperature (7.2 C) and stored at both 0 and 5.5 C generally appeared to have more desirable fat cover appearance ratings than cuts fabricated at a lower internal temperature (1 C).

At 14 days of storage, cuts fabricated at a higher internal temperature and stored at 5.5 C maintained a significantly ($P < .05$) more desirable muscle color (Table 2) than when cuts were fabricated from carcasses at a lower internal temperature, however, this may be confounded with postmortem time (*16*). At 28 days of storage, cuts fabricated at a lower internal temperature (1 C) and stored at 0 and 5.5 C maintained higher ($P < .05$) muscle color scores than did cuts fabricated at a higher internal temperature (7.2 C) and stored at 5.5 C. There were no significant differences between treatments after 7, 21, and 35 days of storage. Muscle color became lighter from 14 to 28 days for those cuts fabricated at 1 C and stored at 0 and 5.5 C. Generally, cuts stored at 0 C maintained a more desirable color than those stored at 5.5 C, but the cuts were darker after 35 days of storage.

At 21 days of storage, cuts stored at 5.5 C (Treatments B and D) had significantly more pronounced off-odor (Table 3) than did cuts stored at 0 C (Treatments A and C). At 28 days of storage, cuts fabricated at a higher internal temperature (7.2 C) and stored at 0 C (Treatment C) had higher ($P < .05$) scores, indicating less objectionable odor than the other treatments. The difference in temperature at time of fabrication had no consistent effect on the odor scores, and differences in odor ratings were influenced primarily by storage temperatures. Off-odor is usually not encountered in vacuum-packaged beef because of inhibition of putrefactive bacteria, which cause off-odors (*14*). It is assumed that off-odor was not encountered during extended storage because of the excellent vacuum maintained in the packages.

Surface discoloration ratings were based on the percentage of the surface of the exposed muscle which was discolored (Table 4). At 7 and 14 days of storage all treatments were similar in surface discoloration, and although nonsignificant, there was a slight advantage for Treatments C and D (cuts fabricated at 7.2 C). However, at 21 and 28 days of storage, cuts fabricated at a lower internal temperature tended to have a lower, though not significant, incidence of surface discoloration. Surface discoloration is believed to be caused by a combination of residual oxygen in the bag and surface microbial population. Similar results have been previously reported (*21*). There were no significant differences in surface discoloration among storage intervals within Treatments A and B. The major difference among cuts assigned to Treatment C was that cuts observed at 7 days had less ($P < .05$) surface discoloration than those observed at 21 days. In treatment D the only advantage was for cuts stored 7 days over those stored 28 days.

Total desirability takes into consideration all characteristics of the primal cuts, i.e., appearance of fat cover, muscle color, odor, and surface discoloration. At 7

TABLE 3. *Means for odor ratings*[f] *for primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled Stored / 1 C 0 C	Treatment B: Chilled Stored / 1 C 5.5 C	Treatment C: Chilled Stored / 7.2 C 0 C	Treatment D: Chilled Stored / 7.2 C 5.5 C	Rank order[e] (highest to lowest) Across treatment
7	3.9^{b}	4.0^{a}	3.9^{a}	3.9^{a}	BACD
14	4.0^{a}	4.0^{a}	4.0^{a}	3.8^{ab}	ABCD
21	3.5^{bc}	2.6^{c}	3.7^{a}	2.2^{d}	CABD
28	3.2^{c}	3.1^{b}	3.8^{a}	3.3^{bc}	CDAB
35	3.5^{bc}	3.2^{b}	3.8^{a}	3.1^{c}	CABD

[abcd]Means in the same column bearing a common superscript do not differ ($P > .05$).

[e]Means within the same day of storage, underscored by a common line, do not differ ($P > .05$). Letters A, B, C, D represent treatments in columns A, B, C, D, respectively.

[f]4-point scale: 4 = no detectable off-odor; 3 = slight off-odor; 2 = moderate off-odor; 1 = extreme off-odor.

TABLE 4. *Means for surface discoloration*[e] *for primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled Stored / 1 C 0 C	Treatment B: Chilled Stored / 1 C 5.5 C	Treatment C: Chilled Stored / 7.2 C 0 C	Treatment D: Chilled Stored / 7.2 C 5.5 C	Rank order[d] (highest to lowest) Across treatment
7	6.4^{a}	6.5^{a}	6.5^{a}	6.6^{a}	DBCA
14	5.5^{a}	5.6^{a}	5.5^{abc}	5.7^{ab}	DBAC
21	5.8^{a}	5.4^{a}	4.5^{c}	5.7^{ab}	ADBC
28	6.1^{a}	5.6^{a}	4.9^{ab}	4.7^{b}	ABCD
35	5.4^{a}	5.8^{a}	5.8^{ab}	5.7^{ab}	BCDA

[abc]Means in the same column bearing a common superscript do not differ ($P > .05$).

[d]Means within the same day of storage, underscored by a common line, do not differ ($P > .05$). Letters A, B, C, D represent treatments in columns A, B, C, D, respectively.

[e]7-point scale: 7 = no surface discoloration; 6 = < 10% surface discoloration; 5 = 10-25%; 4 = 25-50%; 3 = 50-75%; 2 = 75-99%; 1 = total discoloration.

days, primal cuts in Treatments A, C, and D had higher ($P < .05$) desirability ratings (Table 5) than did those in Treatment B. At 14 days of storage, cuts in Treatments C and D, which had a higher internal temperature (7.2 C) at fabrication, were superior ($P < .05$) to those fabricated at 1 C. The latter result may reflect the difference in postmortem chilling (72 h for Treatments A and B, 24 h for Treatments C and D) before packaging, or perhaps the higher temperature was associated with a greater metmyoglobin reducing activity at the earlier storage intervals. In analyzing total desirability as related to storage intervals, cuts in Treatments A, C, and D had lower ($P < .05$) desirability ratings at 21 days of storage and Treatment B had lower ratings at 35 days. At 28 days of storage, all treatments tended to have higher desirability ratings over the 21-day values, but the ratings were significantly higher only in Treatments A and C.

Reduction of weight due to purge or trim loss in extended storage is a major concern of the meat industry. Weight loss is of great economic importance during shipment and storage of beef; therefore, weight loss evaluations (purge and trim) were made in this study. Generally, there was no difference in percentage purge and trim loss among storage treatments at the various storage intervals (Table 6). Primal cuts removed at each storage interval were evaluated and trimmed if necessary, with no more product removed than absolutely necessary. It is possible that more severe trimming might occur in normal retailing operations. As days of storage increased, the number of cuts requiring trim increased.

TABLE 5. *Means for total desirability ratings[e] for primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled Stored, 1 C 0 C	Treatment B: Chilled Stored, 1 C 5.5 C	Treatment C: Chilled Stored, 7.2 C 0 C	Treatment D: Chilled Stored, 7.2 C 5.5 C	Rank order[d] (highest to lowest) Across treatment
7	6.8[a]	6.2[a]	7.0[a]	7.1[a]	DCAB
14	5.8[b]	5.8[a]	6.9[a]	6.4[b]	CDBA
21	5.0[c]	5.8[a]	5.2[c]	5.3[c]	BDCA
28	6.0[b]	5.8[a]	5.8[b]	5.5[c]	ABCD
35	5.5[bc]	5.3[b]	5.9[b]	5.5[c]	CADB

[abc]Means in the same column bearing a common superscript do not differ ($P > .05$).
[d]Means within the same day of storage, underscored by a common line, do not differ ($P > .05$). Letters A, B, C, D represent treatments in columns A, B, C, D, respectively.
[e]8-point scale: 8 = extremely desirable; 7 = very desirable; 6 = desirable; 5 = slightly desirable; 4 = slightly undesirable; 3 = undesirable; 2 = very undesirable; 1 = extremely undesirable.

TABLE 6. *Means for purge and trim loss for primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	A: Chilled Stored, 1 C 0 C		B: Chilled Stored, 1 C 5.5 C		C: Chilled Stored, 7.2 C 0 C		D: Chilled Stored, 7.2 C 5.5 C	
	Purge (%)	Trim[c] (%)	Purge (%)	Trim (%)	Purge (%)	Trim (%)	Purge (%)	Trim (%)
7	.81[a]	0	.55[a]	0	.32[a]	0	.34[b]	0
14	.80[a]	.51	.97[a]	0	.93[a]	.40	1.11[a]	0
21	.93[a]	0	.84[a]	.62	.80[a]	.62	.86[a]	0
28	1.21[a]	0	1.02[a]	0	.48[a]	.80	.87[a]	1.55
35	.79[a]	.50	.84[a]	.51	.53[a]	.48	1.12[a]	.65

[ab]Means for purge loss in the same column bearing a common superscript do not differ ($P > .05$).
[c]Trim losses were not statistically analyzed.

TABLE 7. *Means for muscle color ratings[e] at day 1 for retail steaks from primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled Stored, 1 C 0 C	Treatment B: Chilled Stored, 1 C 5.5 C	Treatment C: Chilled Stored, 7.2 C 0 C	Treatment D: Chilled Stored, 7.2 C 5.5 C	Rank order[d] (highest to lowest) Across treatment
7	5.9[c]	5.8[b]	5.9[a]	5.8[a]	ACBD
14	6.3[bc]	6.1[b]	5.9[a]	6.4[a]	DABC
21	6.8[ab]	7.1[a]	6.7[a]	6.8[a]	BADC
28	7.1[a]	6.4[ab]	6.4[a]	6.5[a]	ADBC
35	6.8[ab]	6.5[ab]	6.5[a]	6.3[a]	ABCD

[abc]Means in the same column bearing a common superscript do not differ ($P > .05$).
[d]Means within the same day of storage, underscored by a common line, do not differ ($P > .05$). Letters A, B, C, D represent treatments in columns A, B, C, D, respectively.
[e]9-point scale: 9 = very light cherry red; 1 = black.

Cuts with a higher initial temperature (7.2 C) at fabrication and stored for 28 or 35 days sustained greater discoloration and required more extensive trimming than did cuts with lower (1 C) initial temperatures. These findings agree with earlier results (*28*).

Upon termination of each storage treatment, one steak was removed from each primal cut for subsequent retail caselife evaluations. The retail cuts were scored for four consecutive days for surface discoloration, peripheral discoloration, and total desirability (Tables 8-10). In addition, muscle color scores were obtained on day 1 (Table 7).

Cuts in Treatments A and B increased ($P < .05$) in lightness of color after 28 and 21 days of storage, respectively (Table 7). Cuts fabricated at a lower internal temperature and stored at 0 C were slightly brighter than comparable cuts fabricated at 7.2 C and stored at 0 C. Surface discoloration scores for each treatment generally increased as the storage interval increased from 7 to 35 days (Table 8). This indicates less surface discoloration after extended storage than at 7 days. After 21 days of storage, cuts in all treatments discolored less rapidly than did those stored for 7 days.

The only differences ($P < .05$) for peripheral discoloration (Table 9) occurred at day 4 for cuts fabricated at the lower internal temperature (1 C) and stored at 0 C and for both treatments stored at 5.5 C with lower ratings for cuts stored for 35 days. Steaks derived from primal cuts stored longer than 14 days in vacuum packages had more desirable retail caselife ratings at day 4 than those from cuts stored for only 7 and 14 days (Table 10). This may have occurred because of a shift in microflora of the vacuum-packaged cuts from a predominance of *Pseudomonas* at 7 and 14 days of storage to a predominance of lactobacilli after 21 days. Lactobacilli reproduce in the presence of small concentrations of oxygen and can be advantageous because of the lower pH and the apparent antagonistic effects on other bacteria (*3, 7, 25*). In every treatment (Table 10) cuts stored from 21 to 35 days had an "acceptable" rating on an eight-point scale, and thus had a mean caselife of 4 days. Cuts stored for 7 days had only a 1-day caselife, and those stored 14 days had a 3-day caselife. At first, one is tempted to consider evaluation panel drift in scoring to be a causative factor in the lower scores for 7-day versus 14- and 21-day

TABLE 8. *Mean values for surface discoloration ratings[e] for retail steaks from primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Initial internal temperature of 1 C							
	Stored at 0 C				Stored at 5.5 C			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
7	6.2^{a}	5.0^{c}	3.8^{b}	3.5^{b}	5.2^{b}	4.2^{b}	4.0^{b}	3.3^{c}
14	6.6^{a}	5.7^{b}	5.8^{a}	4.2^{b}	6.4^{a}	5.9^{a}	5.9^{a}	4.2^{b}
21	6.8^{a}	5.6^{b}	5.3^{a}	5.2^{a}	6.4^{a}	6.1^{a}	5.6^{a}	5.1^{a}
28	6.3^{a}	6.3^{a}	6.0^{a}	5.7^{a}	6.2^{a}	6.1^{a}	6.0^{a}	5.6^{a}
35	6.5^{a}	6.1^{ab}	5.7^{a}	5.3^{a}	6.5^{a}	6.3^{a}	5.9^{a}	5.5^{a}
Storage interval (days)	Initial internal temperature of 7.2 C							
	Stored at 0 C				Stored at 5.5 C			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
7	5.2^{c}	4.2^{b}	3.7^{b}	3.0^{b}	5.5^{c}	3.7^{c}	3.2^{b}	2.5^{d}
14	6.8^{a}	5.9^{a}	5.4^{a}	4.8^{a}	6.6^{ab}	5.7^{b}	5.4^{a}	4.5^{c}
21	6.0^{b}	5.7^{a}	5.2^{a}	4.9^{a}	6.1^{b}	5.8^{ab}	5.3^{a}	4.9^{bc}
28	6.4^{ab}	6.3^{a}	6.0^{a}	5.3^{a}	6.7^{a}	6.3^{a}	5.7^{a}	5.5^{ab}
35	6.2^{b}	6.0^{a}	5.8^{a}	5.2^{a}	6.7^{a}	6.1^{ab}	5.8^{a}	5.8^{a}

[abcd]Means in the same column bearing a common superscript do not differ ($P > .05$).
[e]7-point scale: 7 = no surface discoloration; 1 = total surface discoloration.

TABLE 9. *Mean values for peripheral discoloration ratings[d] for retail steaks from primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Initial internal temperature of 1 C							
	Stored at 0 C				Stored at 5.5 C			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
7	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}
14	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}
21	5.0^{a}	5.0^{a}	5.0^{a}	4.7^{a}	5.0^{a}	5.0^{a}	5.0^{a}	4.5^{bc}
28	5.0^{a}	5.0^{a}	4.8^{a}	4.8^{a}	5.0^{a}	5.0^{a}	5.0^{a}	4.8^{ab}
35	5.0^{a}	5.0^{a}	4.8^{a}	4.0^{b}	5.0^{a}	5.0^{a}	4.7^{a}	4.3^{c}
Storage interval (days)	Initial internal temperature of 7.2 C							
	Stored at 0 C				Stored at 5.5 C			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
7	5.0^{a}	5.0^{a}	4.8^{a}	4.8^{a}	5.0^{a}	5.0^{a}	4.8^{a}	4.8^{a}
14	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}
21	4.8^{a}	4.8^{a}	4.8^{a}	4.7^{a}	4.4^{a}	5.0^{a}	4.9^{a}	4.8^{a}
28	5.0^{a}	5.0^{a}	4.8^{a}	4.7^{a}	5.0^{a}	5.0^{a}	4.8^{a}	4.5^{ab}
35	4.7^{a}	4.7^{a}	4.7^{a}	4.3^{a}	4.9^{a}	4.8^{a}	4.7^{a}	4.3^{b}

[abc]Means in the same column bearing a common superscript do not differ ($P > .05$).
[d]5-point scale: 5 = no peripheral discoloration; 1 = extreme peripheral discoloration.

storage. This possibility cannot be completely discarded in the present study, since the system is subjective. However, use of a panel system increases confidence in the ratings assigned.

Upon completion of all storage intervals, frozen steaks obtained when primal cuts were removed from the storage coolers were randomly assigned to a cooking sequence. Sensory evaluations for flavor desirability (Table 11) indicated that the only differences ($P < .05$) occurring as a function of storage intervals were with cuts fabricated at 1 C and stored at 5.5 C. In this treatment, steaks stored for 28 and 35 days were significantly less desirable with regard to flavor than steaks stored for 7, 14, and 21 days, a finding that agrees with odor evaluations of the primal cuts. Steaks stored at 5.5 C (Treatments B and D) differed ($P < .05$) at the 28-day interval and steaks in Treatment A were preferred over those at higher storage temperatures after 35 days of storage.

Data for juiciness and tenderness evaluations are not

TABLE 10. *Mean values for total desirability ratings*[f] *for retail steaks from primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Initial internal temperature of 1 C							
	Stored at 0 C				Stored at 5.5 C			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
7	6.3[a]	5.0[b]	4.4[b]	3.7[b]	5.9[e]	4.9[b]	3.8[b]	4.1[b]
14	6.8[a]	6.1[a]	5.7[a]	4.0[b]	7.4[a]	6.5[a]	6.0[a]	4.2[b]
21	6.9[a]	6.1[a]	5.7[a]	5.2[a]	7.2[b]	6.3[a]	6.0[a]	5.1[a]
28	6.8[a]	6.5[a]	6.1[a]	5.7[a]	6.3[d]	6.1[a]	6.0[a]	5.4[a]
35	6.6[a]	6.2[a]	5.7[a]	5.1[a]	6.6[c]	6.4[a]	5.9[a]	5.2[a]
	Initial internal temperature of 7.2 C							
Storage interval (days)	Stored at 0 C				Stored at 5.5 C			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
7	5.5[b]	3.9[b]	3.9[b]	3.3[b]	5.6[c]	3.9[b]	3.8[b]	2.8[c]
14	6.5[a]	6.4[a]	5.6[a]	4.3[a]	5.9[bc]	6.2[a]	5.2[a]	4.2[b]
21	6.8[a]	6.1[a]	5.6[a]	5.1[a]	6.9[a]	6.1[a]	5.6[a]	5.0[a]
28	6.9[a]	6.6[a]	5.9[a]	5.3[a]	6.7[ab]	6.5[a]	5.8[a]	5.3[a]
35	6.3[a]	6.0[a]	5.8[a]	5.0[a]	6.7[ab]	6.3[a]	5.8[a]	5.6[a]

[abcde]Means in the same column bearing a common superscript do not differ ($P > .05$).
[f]8-point scale: 8 = extremely desirable; 1 = extremely undesirable.

TABLE 11. *Mean values for flavor desirability ratings*[d] *for retail steaks from primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment								Rank order[c] (highest to lowest) Across treatment
	A		B		C		D		
	Chilled Stored	1 C 0 C	Chilled Stored	1 C 5.5 C	Chilled Stored	7.2 C 0 C	Chilled Stored	7.2 C 5.5 C	
7	5.8[a]		5.9[a]		5.8[a]		5.6[a]		BACD
14	5.8[a]		6.1[a]		6.0[a]		5.9[a]		BCDA
21	6.0[a]		6.2[a]		5.7[a]		6.3[a]		DBAC
28	6.2[a]		4.9[b]		6.2[a]		5.7[a]		ACDB
35	6.2[a]		4.9[b]		5.8[a]		5.2[a]		ACDB

[ab]Means in the same column bearing a common superscript do not differ ($P > .05$).
[c]Means within the same day of storage, underscored by a common line, do not differ ($P > .05$). Letters A, B, C, D represent treatments in columns A, B, C, D, respectively.
[d]8-point scale: 8 = like extremely; 7 = like very much; 6 = like moderately; 5 = like slightly; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely.

TABLE 12. *Mean values for overall satisfaction ratings*[e] *for retail steaks from primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment								Rank order[d] (highest to lowest) Across treatment
	A		B		C		D		
	Chilled Stored	1 C 0 C	Chilled Stored	1 C 5.5 C	Chilled Stored	7.2 C 0 C	Chilled Stored	7.2 C 5.5 C	
7	5.6[a]		5.6[ab]		5.5[a]		5.4[b]		ABCD
14	5.4[a]		5.8[a]		5.9[a]		5.7[ab]		CBDA
21	5.9[a]		5.9[a]		5.5[a]		6.2[a]		DABC
28	5.8[a]		4.8[c]		5.9[a]		5.3[b]		CADB
35	5.7[a]		5.0[bc]		5.5[a]		5.0[b]		ACBD

[abc]Means in the same column bearing a common superscript do not differ ($P > .05$).
[d]Means within the same day of storage, underscored by a common line, do not differ ($P > .05$). Letters A, B, C, D represent treatments in columns A, B, C, D, respectively.
[e]8-point scale: 8 = like extremely; 5 = like slightly; 4 = dislike slightly; 1 = dislike extremely.

presented in tabular form. There were no significant differences in juiciness ratings among the storage intervals. Taste panel tenderness ratings were not significantly affected by the treatments or storage intervals. This generally agrees with previous research (*8*) in which substantial increases in tenderness did not occur beyond 8 to 11 days of aging. Few differences occurred in shear values as a function of storage time (not presented in tabular form). There was a decrease ($P < .05$) in shear value with an increase in storage interval in cuts of Treatment D. Also, the trend for shear value, although nonsignificant, was that steaks in both Treatments C and D had lower shear values than did steaks in Treatments A and B, indicating that higher temperatures at the time of fabrication accelerated aging and increased tenderness. The two treatments with primal cuts stored at 5.5 C had lower ($P < .05$) overall satisfaction ratings at 28 days of storage than at 21 days (Table 12). The only difference between treatments occurred at 28 days; overall satisfaction scores for steaks from primal cuts fabricated at a lower internal temperature (1 C) and stored at 5.5 C had significantly lower ratings than those steaks from primal cuts stored at 0 C.

It was concluded that mean scores for the quality characteristics of primal cuts generally decreased between the 7th and 14th days of storage and then increased at subsequent storage intervals which was possibly due to microbial activities (*3*) and changes in the biochemical properties of the meat and/or the environment. Cuts with a higher temperature at the time of fabrication and stored for 28 or 35 days sustained greater discoloration and required more extensive trimming than did cuts with lower initial temperatures. Cuts stored at 5.5 C, regardless of packaging temperatures, sustained more purge loss than cuts stored at 0 C.

Few limiting factors can be associated with palatability data as related to either initial internal temperature or storage temperature of vacuum-packaged beef in storage for up to 35 days. However, since flavor desirability decreased after extended storage, it is suggested that metabolic products of the microbial population likely caused flavor deterioration (*28*). It is evident that the most favorable results for extended storage of beef cuts were obtained by the use of low initial internal meat temperatures and low storage temperatures when cuts were packaged in a high vacuum system.

ACKNOWLEDGMENT

T. A. 12411, Texas Agricultural Experiment Station. This research was supported by the Flexible Packaging Division, Continental Can Co. Inc. and Agricultural Marketing Research Institute, Agricultural Research Service, U.S. Department of Agriculture (Contract 12-14-1001-407). This project is a contribution to Western Regional Project WM-62. Reference to a company or product name is for specific information only and does not imply approval or recommendation of the product by the U.S. Department of Agriculture or the Texas Agricultural Experiment Station to the exclusion of others that may be suitable.

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J. Milk Food Technol. Vol. 39, No. 9, Pages 600-605 (September, 1976)

Effect of Initial Internal Temperature and Storage Temperature on the Microbial Flora of Vacuum Packaged Beef

S. D. BEEBE[1], C. VANDERZANT, M. O. HANNA, Z. L. CARPENTER, and G. C. SMITH

Department of Animal Science
Texas Agricultural Experiment Station, College Station, Texas 77840

(Received for publication February 9, 1976)

ABSTRACT

Large increases in bacterial counts of vacuum-packaged top sirloin butts occurred between 7-14 days of storage at 5.5 C and between 14-21 days of storage at 0 C. Differences in post-mortem chill temperature (1 C vs 7.2 C) of the cuts did not significantly influence the counts of the primal cuts during refrigerated storage for up to 35 days. Counts of steaks from primal cuts stored for 21-35 days at 5.5 C usually were somewhat higher than those from cuts held at 0 C. *Lactobacillus* species and members of the *Enterobacteriaceae* dominated the psychrotrophic microbial flora of cuts stored for 35 days. Psychrotrophic and mesophilic counts of cuts from defective bags (leakers) increased rapidly and consisted primarily of *Pseudomonas species.*

During the last ten years, major changes have occurred in the handling and distribution of meat in the United States. Shaw (*14*) estimated that more than half of all fresh beef arrived at the retail outlet as prefabricated primal and subprimal cuts, of which approximately 43% were received in vacuum packages. Several reports (*2, 8-11*) have shown that oxygen-impermeable films are effective in maintaining the quality of fresh beef stored for extended periods under refrigeration. From the microbiological viewpoint, vacuum packaging of fresh meat has certain advantages over meats exposed to air. During refrigerated storage of meats for extended periods in oxygen-impermeable films, bacterial populations increase slower and hence usually do not reach the levels present on meats packaged in oxygen-permeable films (*2*). In addition, microbial types, such as lactic acid bacteria, that become dominant on vacuum-packaged beef do not cause as extensive damage to the quality attributes of meat as typical aerobic spoilage bacteria such as *Pseudomonas* species (*2*).

Packaging in oxygen-impermeable films together with extensive evacuation of air from the package and an effective seal prevents re-entry of oxygen into the package. The oxygen remaining in the package after closure will be converted to carbon dioxide by respiration of the meat tissue and microbial activity (*6, 8*). The CO_2 which accumulates in the package is responsible for inhibition of pseudomonads, which are frequently involved in quality deterioration and subsequent spoilage of refrigerated meats (*2, 8*). Accumulations of CO_2 in vacuum packages of above 20% normally will suppress gram-negative spoilage bacteria (*16*). The rate of accumulation of CO_2 will depend upon the degree of air evacuation, effectiveness of the seal and configuration of the cut.

Several reports (*6, 7, 10*) indicate that lactic acid bacteria become dominant on refrigerated vacuum-packaged beef. However, others (*1, 13*) have shown that members of the family *Enterobacteriaceae* may constitute a significant part of the microbial flora of vacuum-packaged beef or poultry.

Temperature of meat during packaging and storage directly influences the rate of microbial development whether stored in oxygen-permeable or -impermeable films. Jaye et al. (*7*) reported that the rate of growth of lactic acid bacteria in vacuum-packaged meat stored at -1 C was much lower than at 3 C. Typical psychrotrophic food spoilage bacteria develop faster at marginal refrigeration temperatures (7-10 C) than at 1-3 C.

Until recently (*12, 13, 16*) there has been little published research on the changes in level and type of microbial flora of wholesale cuts of vacuum-packaged beef. The objectives of the present study were to determine the effects of differences in post-mortem chill temperature and storage temperature after vacuum packaging upon the quality attributes of vacuum-packaged top sirloin butts stored for up to 35 days. The first paper (*3*) describes the evaluation of primal cuts for vacuum level, evaporative and purge losses, appearance of subcutaneous fat cover, muscle color, surface discoloration, odor, and total desirability. Retail cuts were evaluated for muscle color, surface discoloration, peripheral discoloration, total desirability, organoleptic characteristics (after oven-broiling), and tenderness. This paper describes the effects of initial chill temperature

[1]*Present address: Standard Meat Company, Ft. Worth, Texas.*

and storage temperature on changes in microbial counts of primal and retail cuts. In addition, information is presented on the distribution of the psychrotrophic microbial flora of primal cuts stored for 35 days.

MATERIALS AND METHODS

Samples

One hundred twenty top sirloin butts were selected from carcasses weighing 272.4 to 317.8 kg, from Yield Grade 2, within the U.S. Good and Choice quality grades. The cuts were removed from the carcasses 24-h postmortem in the case of the partially chilled sirloin butts (7.2 C) and 72-h postmortem in the case of the fully chilled butts (1 C). All were fabricated according to Institutional Meat Purchase Specifications (IMPS 184). All cuts were vacuum packaged at 29.5 inches of Hg in a chamber-type heat seal system (Conofresh B film, Continental Can Co., Inc.), boxed for distribution accordingly, and identified relative to initial chill conditions (1 C or 7.2 C). After removal from the shrink tunnel (232 C) a random selection of cuts (4 of each of the initial chill conditions) was sampled to determine initial microbial counts. The vacuum-packaged cuts were then shipped by refrigerated truck (48 h) to the Texas A&M University Meat Laboratory. Upon arrival the packaged cuts were assigned to storage treatments as shown in Table 1. At the completion of the specified storage periods, cuts were evaluated for various quality characteristics. Two steaks (2.5 cm thick) were obtained from each primal cut. One steak from each pair was designated for retail caselife evaluation and the other for organoleptic evaluation. Steaks for retail caselife evaluation studies were placed in styrofoam trays, overwrapped with PVC film, and placed under retail conditions (1 to 3 C under 82 footcandles of incandescent light). Upon completion of a 4-day evaluation for muscle color, surface discoloration, peripheral discoloration, and total desirability, a total of 12 steaks (3 from each treatment) were sampled for enumeration of microbial populations. These steaks were representative of each storage temperature and the two initial temperatures. Bacterial counts also were done on cuts in defective packages (leakers). Ten cuts (initial temperature 1 C) were assigned to storage intervals from 7 to 35 days and held at 0 and 5.5 C (2 cuts per storage period and one at each temperature).

TABLE 1. *Experimental design for packaging study*

Storage interval (days)	Meat temperature at time of packaging: 1 C, Storage temperature 0 C	1 C, Storage temperature 5.5 C	7.2 C, Storage temperature 0 C	7.2 C, Storage temperature 5.5 C
7	6 Top butts	6 Top butts	6 Top butts	6 Top butts
14	6 Top butts	6 Top butts	6 Top butts	6 Top butts
21	6 Top butts	6 Top butts	6 Top butts	6 Top butts
28	6 Top butts	6 Top butts	6 Top butts	6 Top butts
35	6 Top butts	6 Top butts	6 Top butts	6 Top butts

Microbiological

Bacteriological evaluation of the primal cuts was carried out by swabbing the surface with a sterile cellulose sponge (5 × 5 × 1.3 cm) wetted in sterile 0.1% peptone broth (*15*). Each cut was sampled in the same manner, first a 64.5 cm^2 area of the lean surface with one side of the sponge and then 64.5 cm^2 of the subcutaneous fat with the other side. The sponge then was placed in 100 ml of sterile 0.1% peptone broth (in glass jars) and squeezed five times. The sample jar then was shaken 25 times and appropriate dilutions were prepared with sterile 0.1% peptone broth. Psychrotrophic bacterial counts were made on plate count agar (Difco) with plate incubation at 7 C for 10 days. Mesophilic bacterial counts were also made on plate count agar but with plate incubation at 32 C for 3 days. Enumeration of lactobacilli was made utilizing Lactobacillus MRS broth (Difco) with 1.5% agar added. Incubation of plates was at 32 C for 5 days. Anaerobes were determined by plating on trypticase soy agar (TSA, BBL). Plates were incubated for 5 days at 32 C in anaerobic jars with Gas-Paks (BBL). Details of the above procedures are given in a previous paper (*12*).

The distribution of the psychrotrophic microbial flora of cuts stored for 35 days was determined by randomly picking 30-40 colonies from countable plates and placing them on TSA slants for 2-3 days at 25 C. Diagnostic schemes and procedures employed are those published by Vanderzant and Nickelson (*18*). Bacteriological count data were analyzed by standard analysis of variance of log counts (log_{10} per in^2) of individual samples. When significant differences in mean counts were identified, mean separation analyses were conducted according to Duncan (*4*).

RESULTS

Psychrotophic counts of cuts (Table 2) were low at the initial sampling (0-day) and after 7 days of storage. After 0 and 7 days of storage there were no significant differences in counts with respect to initial internal temperature and storage temperature. Significant increases in psychrotrophic counts occurred after 14 days, particularly on the cuts stored at 5.5 C. Mean increases in counts of cuts stored for 14 days at 0 C were 1.07-1.57 logs, for those stored at 5.5 C, 3.36-3.50 logs. From 14 to 35 days of storage, cuts stored at 5.5 C usually had significantly higher counts than those stored at 0 C. Differences in counts due to variation in initial internal temperature were not statistically significant. Largest increases in counts for cuts stored at 5.5 C occurred between 7 and 14 days, and between 14 and 21 days for those stored at 0 C. Cuts with an initial internal temperature of 1 C and stored at 0 C had lower counts after 14 to 35 days than those subjected to the other treatments.

The pattern of mesophilic counts during storage (Table 3) was similar to that of psychrotrophic counts. Significant increases in mesophilic count occurred between 7 and 14 days for cuts stored at 5.5 C, and between 14 and 21 days for samples stored at 0 C.

TABLE 2. *Log values for psychrotrophic bacterial counts of primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled Stored / 1 C 0 C	B: Chilled Stored / 1 C 5.5 C	C: Chilled Stored / 7.2 C 0 C	D: Chilled Stored / 7.2 C 5.5 C	Rank order[e] (highest to lowest) Across treatment
0	1.69[d]	1.69[d]	1.69[d]	1.69[d]	ABCD
7	2.20[cd]	1.85[d]	2.05[d]	1.69[d]	ACDB
14	2.76[c]	5.05[c]	3.26[c]	5.19[c]	DBCA
21	5.71[b]	7.03[b]	5.72[b]	6.94[b]	BDCA
28	6.70[a]	7.33[b]	6.80[a]	7.00[ab]	BDCA
35	6.83[a]	8.05[a]	7.20[a]	7.88[a]	BDCA

[abcd]Means in the same columns bearing different superscripts differ significantly ($P < .05$).

[e]Mean values within the same day of storage, underscored by a common line, do not differ significantly ($P > .05$). Letters A, B, C, D represent the treatments in columns A, B, C, D, respectively.

Between 14 and 35 days of storage, cuts stored at 5.5 C had significantly higher counts than those stored at 0 C. Differences in initial internal temperature of the cuts did not significantly affect the mesophilic counts. After 35 days, mesophilic counts were similar to psychrotrophic counts when comparing cuts with identical temperature treatment. Both psychrotrophic and mesophilic counts exceeded 10^6 per in^2 after 21 days for cuts stored at 5.5 C and after 28 days for cuts stored at 0 C. After 35 days of storage, differences in psychrotrophic counts because of storage temperature were 1.22 (initial temp. 1 C) and 0.68 log (initial temp. 7.2 C). Similar figures for mesophilic counts were 1.28 and 0.70 log, respectively. Initial lactobacillus counts and counts after 7 days of storage were low (Table 4). Large increases in lactobacilli occurred between 7 and 14 days on cuts stored at 5.5 C (1.89-2.08 logs) and between 14 and 21 days on cuts stored at 0 C (2.92-2.93 logs). With one exception (14-day storage), differences in initial internal temperature and storage temperature did not significantly affect the lactobacillus count. The lactobacillus count exceeded 10^6 per in^2 after 21 days of storage and continued to increase particularly in the samples stored at 5.5 C.

Initially and after storage for 7 days, anaerobic plate counts were low (Table 5). Marked increases in anaerobic counts occurred between 7 and 14 days for cuts stored at 5.5 C and between 14 and 21 days for cuts stored at 0 C. Analysis between treatments revealed that after 14, 28, and 35 days, cuts stored at 5.5 C had significantly higher counts than those stored at 0 C. Although anaerobic counts and lactobacillus counts of cuts after 21 days of storage were similar, anaerobic counts after 35 days were somewhat larger than lactobacillus counts of comparable samples.

Psychrotrophic counts of the retail steaks from cuts stored for 7 and 14 days were low (Table 6). Large increases in counts were observed in steaks from cuts stored for 21 days. Counts of steaks exceeded 10^6 per in^2

TABLE 3. *Log values for mesophilic bacterial counts of primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled Stored	A: 1 C 0 C	B: Chilled Stored	B: 1 C 5.5 C	C: Chilled Stored	C: 7.2 C 0 C	D: Chilled Stored	D: 7.2 C 5.5 C	Rank order[e] (highest to lowest) Across treatment
0	2.17^{c}		2.17^{d}		1.77^{d}		1.77^{c}		ABCD
7	2.97^{c}		2.73^{d}		4.85^{b}		2.33^{c}		CABD
14	2.94^{c}		5.11^{c}		3.57^{c}		5.00^{b}		BDCA
21	5.77^{b}		6.93^{b}		5.76^{b}		7.15^{a}		DBAC
28	6.78^{a}		7.23^{b}		6.91^{a}		7.24^{a}		DBCA
35	6.94^{a}		8.22^{a}		7.27^{a}		7.97^{a}		BDCA

[abcd]Means in the same columns bearing different superscripts differ significantly ($P < .05$).
[e]Mean values within the same day of storage, underscored by a common line, do not differ significantly ($P > .05$). Letters A, B, C, D represent the treatments in columnus A, B, C, D, respectively.

TABLE 4. *Log values for Lactobacilli counts of primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled Stored	A: 1 C 0 C	B: Chilled Stored	B: 1 C 5.5 C	C: Chilled Stored	C: 7.2 C 0 C	D: Chilled Stored	D: 7.2 C 5.5 C	Rank order[f] (highest to lowest) Across treatment
0	2.09^{c}		2.09^{e}		1.69^{d}		1.69^{c}		ABCD
7	2.78^{bc}		2.89^{d}		2.29^{d}		2.84^{c}		BDAC
14	3.24^{b}		4.97^{c}		3.24^{c}		4.73^{b}		BDAC
21	6.17^{a}		6.20^{b}		6.16^{ab}		6.20^{a}		BDAC
28	6.32^{a}		7.05^{a}		6.89^{a}		7.12^{a}		DBCA
35	6.29^{a}		7.35^{a}		6.07^{b}		7.31^{a}		BDAC

[abcde]Means in the same columns bearing different superscripts differ significantly ($P < .05$).
[f]Mean values within the same day of storage, underscored by a common line, do not differ significantly ($P > .05$). Letters A, B, C, D represent the treatments in columns A, B, C, D, respectively.

TABLE 5. *Log values for anaerobic bacterial counts of primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled Stored	A: 1 C 0 C	B: Chilled Stored	B: 1 C 5.5 C	C: Chilled Stored	C: 7.2 C 0 C	D: Chilled Stored	D: 7.2 C 5.5 C	Rank order[g] (highest to lowest) Across treatment
0	1.69^{c}		1.69^{f}		1.69^{d}		1.69^{d}		ABCD
7	2.84^{b}		2.39^{e}		2.38^{cd}		2.04^{d}		ABCD
14	3.09^{b}		5.85^{d}		3.20^{c}		5.65^{c}		BDCA
21	6.03^{a}		6.20^{c}		6.16^{b}		6.21^{bc}		DBCA
28	6.89^{a}		7.37^{b}		6.81^{ab}		7.23^{ab}		BDAC
35	6.92^{a}		8.01^{a}		7.00^{a}		7.76^{a}		BDCA

[abcdef]Means in the same columns bearing different superscripts differ significantly ($P < .05$).
[g]Mean values within the same day of storage, underscored by a common line, do not differ significantly ($P > .05$). Letters A, B, C, D represent the treatments in columns A, B, C, D, respectively.

TABLE 6. *Log values for psychrotrophic bacterial counts of retail cuts from primal cuts stratified according to initial internal temperature and storage temperature*

Storage interval (days)	Treatment A: Chilled 1 C / Stored 0 C	Treatment B: Chilled 1 C / Stored 5.5 C	Treatment C: Chilled 7.2 C / Stored 0 C	Treatment D: Chilled 7.2 C / Stored 5.5 C	Rank order[d] (highest to lowest) Across treatment
7	2.45[b]	1.69[c]	2.05[b]	2.37[c]	ADCB
14	1.69[b]	2.48[bc]	2.68[b]	2.32[c]	CBDA
21	4.30[a]	4.61[abc]	4.89[a]	5.94[ab]	DCBA
28	5.92[a]	5.69[ab]	5.45[a]	5.63[b]	ABDC
35	6.02[a]	6.19[a]	6.01[a]	6.62[a]	DBAC

[abc]Means in the same columns bearing different superscripts differ significantly ($P < .05$).
[d]Mean values within the same day of storage, underscored by a common line, do not differ significantly ($P > .05$). Letters A, B, C, D represent the treatments in columns A, B, C, D, respectively.

when prepared from cuts stored for 35 days. No significant differences in psychrotrophic counts of steaks were associated with initial internal temperature or storage temperature of the primal cuts. Psychrotrophic counts of steaks were in most cases lower than those of the cuts from which they were prepared (Tables 2, 6). Irrespective of the initial chill temperature (1 vs. 7.2 C), differences in counts between cuts and steaks were greater for cuts stored at 5.5 C than for those stored at 0 C.

Data on distribution of microbial types on cuts stored for 35 days (Table 7) show little difference in microbial types as related to initial internal temperature or storage temperature. In most samples, *Lactobacillus* species and *Enterobacteriaceae* predominated. However, the microbial flora on meat from defective packages (leakers) consisted primarily of *Pseudomonas* species (Table 8). A comparison of the psychrotrophic, mesophilic, lactobacilli and anaerobic counts of cuts from defective packages (Table 9) with those of intact packages show

TABLE 7. *Percentage distribution of the psychrotrophic microbial flora of cuts stored for 35 days at different temperatures*[a]

Microbial type	Meat temperature at time of packaging: 1 C, Storage temperature 0 C	1 C, Storage temperature 5.5 C	7.2 C, Storage temperature 0 C	7.2 C, Storage temperature 5.5 C
Lactobacillus	84.1-100	36-75.9	74.4-100	80.5-95.7
Microbacterium	—	0-25	0-1.3	—
Bacillus	—	—	—	0-0.4
Pseudomonas	0-2.9	—	2-8.5	—
Aeromonas	—	—	—	0-1.6
Enterobacteriaceae	13.0	24.1-41.0	17.1	4.3-17.9

[a]Four cuts were sampled at each storage temperature.

TABLE 8. *Distribution of the psychrotrophic microbial flora of cuts from defective packages (leakers) held for 35 days at 0 and 5.5 C*[a]

Microbial type	Percentage distribution
Lactobacillus	0-8.5
Microbacterium	1.2-2.8
Coryneform	1.2-2.8
Moraxella-Acinetobacter	0-8.6
Pseudomonas	85.9-89

[a]Four cuts were examined, two from each temperature treatment.

that the psychrotrophic and mesophilic counts of leakers stored for 35 days exceeded those stored in intact bags. Differences were 2.16 and 2.27 logs for the cuts stored at 0 C and 1.38 to >1.15 logs for those stored at 5.5 C. Lactobacillus counts of leakers after 35 days were slightly lower than those of cuts stored in intact bags.

DISCUSSION

Bacterial counts of primal cuts initially and after 7 days of storage were low. Destruction and/or sublethal injury of microorganisms on the surface of the cuts during passage through the shrink tunnel (9 sec at 232 C) may have contibuted to these low counts. Extensive thermal injury and/or destruction of *Pseudomonas* species for example have been reported (*5, 17*) following mild thermal stress. Large increases in counts of cuts occurred somewhat sooner (between 7-14 days) at the higher storage temperature (5.5 C) than at 0 C (between 14-21 days). These increases during the early phases of the storage period probably represent both continued growth of aerobic bacteria such as *Pseudomonas* and a rapid development of lactic acid bacteria (*9, 13*). The extent of the continued growth of the aerobic microflora will depend upon the rate of increase of CO_2 concentration in the package (*16*). This in turn will

TABLE 9. *Log bacterial counts of cuts from defective bags (leakers)*

Storage interval (days)	Temp. (C) Initial	Temp. (C) Storage	Bacterial counts: Psychrotrophic	Mesophilic	Lactobacilli	Anaerobes
7	1	0	1.70	3.45	3.79	3.93
		5.5	1.70	2.48	3.79	1.70
14	1	0	4.23	3.11	2.92	2.60
		5.5	4.62	4.69	3.79	3.65
21	1	0	6.86	7.08	4.88	>6.18
		5.5	>7.18	>7.18	>6.18	>6.18
28	1	0	>7.18	>7.18	7.20	>7.18
		5.5	>7.18	>7.18	6.00	>7.18
35	1	0	9.08	9.15	6.04	7.65
		5.5	>9.18	9.62	7.11	8.71

depend upon the degree of air evacuation from the package, effectiveness of the seal, permeability of the film to O_2 and CO_2, and numbers and types of bacteria on the meat. In packages with residual air pockets, increases in CO_2 concentration will be less rapid and inhibition of gram-negative spoilage bacteria less effective. The rapid increase of lactobacilli was also evident in this study where counts after 21 days exceeded 10^6 per in^2 (Table 4). In the final phases (28-35 days) of the storage period lactobacilli frequently dominate the psychrotrophic microbial flora (*6, 7, 10*) of vacuum packaged meat. More rapid increases in counts at 5.5 C than at 0 C are expected because pseudomonads and lactobacilli grow more rapidly at marginal refrigeration temperatures (*6, 7*). Hence, counts of cuts stored for 14-35 days at 5.5 C were higher than those held at 0 C. Psychrotrophic and mesophilic counts reached levels ($>10^6/in^2$) where defects can be expected after 21 days at 5.5 C and after 28 days at 0 C. Lactobacilli and anaerobes exceeded 10^6 per in^2 after 21 days irrespective of storage temperature. Details of the changes in quality characteristics of the primal cuts and retail steaks were presented in a companion paper (*3*). In general, the mean values for the quality characteristic ratings for the primal cuts tended to decrease from 7 to 14 days of storage and then increased from 21 to 35 days. This may reflect a shift in the composition of the microbial flora from a dominance of aerobic gram-negative bacteria to lactic acid bacteria. The decrease in flavor desirability ratings after extended storage is most likely associated with development of large numbers of lactic acid bacteria. Little information is available on the species of lactic acid bacteria on vacuum packaged meat and the presence of metabolic products other than lactic acid and their effect on the organoleptic characteristics of the meat. Within the limits of this experiment differences in initial chill temperature (1 C vs. 7.2 C) of the carcasses and cuts did not significantly influence the counts of the primal cuts after subsequent refrigerated storage. To evaluate true temperature changes in the cuts, it would be useful in future experiments to determine temperature-time profiles of (*a*) partially chilled cuts placed at lower storage temperatures and (*b*) fully chilled cuts placed at more elevated storage temperatures. After storage for 35 days, counts of cuts with the lower initial and storage temperature (0 C) were lowest.

Psychrotrophic and mesophilic counts of cuts from defective packages (leakers) increased more rapidly and to higher levels than those of comparable cuts from intact packages (Tables 2, 3, 9). This reflects the inhibitory effect of the absence of air and presence of CO_2 on the development of common aerobic gram-negative bacteria (*2, 8*).

Some degree of similarity existed between the bacterial counts of the primal cuts and steaks and the temperature treatment of the primal cut. Counts of steaks from primal cuts stored for 21-35 days at 5.5 C usually were numerically somewhat higher than those from cuts which were held at 0 C. No relationship could be established between the counts of steaks and the internal temperature of the primal cut from which they were prepared.

Lactobacilli and *Enterobacteriaceae* were dominant among the psychrotrophic microbial population of cuts stored for 35 days. Others (*1, 6, 7, 10, 12, 13*) have reported similar observations for vacuum-packaged meats. Members of the family *Enterobacteriaceae* were somewhat more prevalent on cuts stored at 5.5 C than at 0 C. This is expected since many species of this family are marginal psychrotrophs. The psychrotrophic microbial population of cuts from defective bags was dominated by *Pseudomonas*. These species are known to be predominant on refrigerated meats stored under aerobic conditions (*6, 9, 10*).

ACKNOWLEDGMENTS

T. A. 12319, Texas Agricultural Experiment Station. This research was supported by the Flexible Packaging Division, Continental Can Co., Inc. and Agricultural Marketing Research Institute, Agricultural Research Service, U.S. Department of Agriculture (Contract 12-14-1001-407). This project is a contribution to Western Regional Project WM-62. Reference to a company or product name is for specific information only and does not imply approval or recommendation of the product by the U.S. Department of Agriculture or the Texas Agricultural Experiment Station to the exclusion of others that may be suitable.

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J. Milk Food Technol. Vol. 39, No. 9, Pages 606-613 (September, 1976)

Microorganisms and Flavor Development Associated with the Wild Rice Fermentation

J. F. FRANK, E. H. MARTH, R. C. LINDSAY, and D. B. LUND

Department of Food Science
University of Wisconsin-Madison, Madison, Wisconsin 53706

(Received for publication February 18, 1976)

ABSTRACT

Predominant microorganisms on fresh and fermenting wild rice were isolated, characterized, and then used as inoculum so their effects on processing and flavor characteristics of wild rice could be determined. During storage of wild rice at 4 C for 14 weeks, *Achromobacter* spp., *Flavobacterium* spp., coryneforms, and coliforms, and *Achromobacter* spp. predominated in succession. In rice stored at 21 C for 2 weeks, *Pseudomonas* spp. predominated. Microorganisms isolated from fresh wild rice included pseudomonads, micrococci, coliforms, *Achromobacter* spp., and *Flavobacterium* spp. Wild rice inoculated with some isolates from fermenting rice developed a variety of odors including fecal-putrid, earthy, and rotting vegetation types at 30 C, and the previous odors plus ammonia, fatty acid, and sweet-aromatic types at 7 C. Processing characteristics of wild rice inoculated with selected isolates, were not affected, but there were changes in flavor of the rice. Intensities of both tea-like and earthy flavors in wild rice were increased by inoculation of rice with *Achromobacter, Pseudomonas, Flavobacterium,* and *Micrococcus* isolates.

Although there is some information on the bacterial content of freshly harvested grains (*23*), virtually none is available concerning the ability of these bacteria to influence the final flavor and quality of the product. The ability of bacteria to cause changes in flavor of other foods is well known (*11, 17, 22*), but only recently has the role of bacteria in producing flavor changes in grain been investigated (*12, 13*).

Wild rice (*Zizania aquatica*) provides a unique opportunity to study the natural microflora and its effect on quality of a grain product. This is because wild rice is normally stored for up to several weeks after harvesting before it is dried to a moisture content suitable for extended storage. During this initial storage the rice undergoes a wild fermentation prompted by the large amount (35-50%) of moisture in the grain when it is harvested. The bacterial content of freshly harvested wild rice exceeds 10^9/g and may increase during fermentation (*8*). The mold content of freshly harvested wild rice ranges from 10^5-10^6/g and also increases slightly during fermentation (*8*). Thus, the microflora of fermenting wild rice is composed of about 99.9% bacteria. For other freshly harvested cereal grains the microflora consists of 90-99% bacteria (*23*).

Storage of most grains when they contain sufficient moisture to allow bacterial growth results in rapid deterioration. Wild rice, however, benefits from such storage, provided it is limited. The benefits include (*a*) continued ripening of the seed resulting in a darkening of many green kernels thus giving a more uniformly dark colored finished product (*24*), (*b*) easier removal of the hull after drying, and (*c*) development of tea-like and earthy flavors associated with wild rice. These latter two changes may be caused by microbial interactions with the grain.

The quality changes attributed to fermentation may occur in a relatively short time (2-10 days) depending on the ambient temperature. However, many processors must store their unprocessed rice for longer times because of economics related to a short harvest season and the expense of processing equipment. To delay deterioration of rice it can be refrigerated. Extended refrigerated storage will select for psychrotrophic bacteria which may affect the final quality of the product. In addition to using refrigeration to retard fermentation of rice, the grain also is kept moist and mixed regularly to disperse heat from respiration and to prevent apparent undesirable mold growth (*6*).

Previous research has been concerned with the microbiological quality of raw and processed wild rice (*7*), changes in the microflora during fermentation (*8*), and the possibility of aflatoxin production during the fermentation (*6*). Work reported in this paper was done to extend observations on the microbiology of fermenting rice and to determine if certain bacteria in fermenting rice contribute to flavor of the finished product.

MATERIALS AND METHODS

Storage treatments

Wild rice (Johnson strain) used in this study was obtained from Clearwater Wild Rice Co. in Minnesota. Treatments during fermentation were: (*a*) storage at 21 C with daily mixing and addition of water to keep the rice moist, (*b*) storage at 4 C with daily mixing and addition of water, (*c*) same as (*a*) for one week then as in (*b*), (*d*) storage at 4 C in a vertical bin with water saturated air forced through the rice from the top (this was an attempt to reduce labor by avoiding mixing and adding water). Metal cylinders with open bottoms were used to hold the rice during storage. Rice in 20- to 50-lb lots was stored for 14 weeks with the exception of treatment (*a*) where rice became unacceptable after 2 weeks of fermentation.

Samples for microbiological analysis were taken after the rice was mixed (if required by treatment) by combining small samples from at least three areas of the rice bed. The analyses were done within 1 h of sample collection.

Microbiological analysis

The method of Goel et al. (*7*) was used to prepare and dilute samples for plate counts. Samples were tested for (*a*) total aerobic plate count: Plate Count Agar (Difco), incubation at 30 C for 48 h (when rice was

stored at 4 C for over 2 weeks the incubation was at 21 C for 72 h, at 2 weeks the 30- and 21-C counts were equivalent); (*b*) spore counts: for aerobic spore counts the sample was heat shocked at 80 C for 20 min, Plate Count Agar was used, and incubation was at 30 C for 72 h; for counts of facultative and anaerobic spores, heat shocked samples, liver veal agar, the "GasPak" disposable anaerobic system (BBL,) and incubation at 30 C for 72 h were used; (*c*) mold count: acidified potato dextrose agar (Difco) and incubation at 25 C for 3 days; (*d*) psychrotrophic plate count: Plate Count Agar and incubation at 7 C for 10 days. All counts were done in duplicate and average values are reported.

Characterization of predominant microflora

The predominant microflora of wild rice was determined by picking colonies from uncrowded plates of Plate Count Agar that had been used to obtain the total aerobic count. Fifteen colonies were randomly picked from each of 10 different samples of rice stored at 4 and 21 C for different times, and were transferred to nutrient broth. After 1 to 3 days in nutrient broth at either 30 or 21 C, cultures that grew were examined microscopically for purity and motility (hanging drop), and were transferred to nutrient agar and other media for analysis. The following tests were done on each viable isolate according to methods described in *The Genera of Bacteria* (*20*) and *Manual of Microbiological Methods* (21): reaction in oxidative-fermentative (o-f) medium of Hugh and Liefson, liquefaction of gelatin, reduction of nitrate to nitrite, growth in litmus milk, Kovac's oxidase test, pigment production on nutrient agar and King's agar A, production of indole (Gore), utilization of citrate as a sole carbon source (Koser's citrate medium), and hydrolysis of starch (iodine reaction in nutrient agar). The gram stain and morophology of each isolate were observed using young cultures from nutrient agar. A flagella stain of motile cultures was done by the method of Rhodes (*19*). All fermentative gram-positive cocci were tested for growth on mannitol salt agar (Difco) and production of coagulase (Difco coagulase plasma). All gram-negative gas-forming rods were tested for their IMViC pattern.

TABLE 1. *Classification scheme for miroorganisms isolated from wild rice*

Gram positive	
Rods	
catalase positive, no spores	Coryneforms
Cocci	
catalase positive	
oxidative[a]	*Micrococcus* spp.
fermentative	*Staphylococcus* spp.
Gram negative rods	
Oxidative	
oxidase positive	
polar flagella	*Pseudomonas* spp.
Fermentative with gas	
growth on EMB agar	Coliforms
Fermentative with no gas, or no acid from glucose (oxidative)	
produces non-water soluble pigments	*Flavobacterium* spp.
no pigments produced	*Achromobacter* spp.
Hydrolysis filter paper in 0.5% peptone, rods, fermentative with no gas	*Cellulomonas* spp.

[a]Oxidative-fermentative test was done on the medium of Hugh and Liefson using glucose.

Table 1 gives an abbreviated form of the scheme used to classify the isolates. The *Pseudomonas* spp. were further grouped according to the method proposed by Ayres (*1*). Isolates in the *Achromobacter, Flavobacterium,* and *Cellulomonas* genera were classified according to *Bergey's Manual of Determinative Bacteriology* (*2*).

Procedure to screen isolates for aroma production

A method was devised for quickly determining the gross effects caused by growth of each isolate on raw unfermented wild rice. Each isolate was grown in 5 ml of sterile wild rice broth. This broth was prepared by mixing equal amounts of frozen, unfermented wild rice and distilled water, and incubating the mixture at 4 C for 24 h. The resulting liquid was filtered through cheese cloth and autoclaved for 15 min. After an isolate was grown in this medium (2 days at 30 C or 3 days at 21 C, depending on temperature used to incubate the plate from which the culture was isolated), it was added to 5 g of unfermented wild rice, and incubated at 7 C for 1 week if the isolate was obtained from refrigerated rice or at 30 C for 2 days if the isolate was obtained from unfermented rice or rice held at 21 C. Incubation was done in 50-ml Erlenmeyer flasks sealed with parafilm. After incubation, flasks were allowed to equilibrate to room temperature, parafilm was removed, and head space gases of the flask were sniffed by two experienced judges and compared to those of an uninoculated control. This method resulted in production of a variety of odors when different inocula were used and permitted rapid identification of potentially beneficial cultures.

Inoculation of wild rice with isolates

Ten isolates for subsequent inoculation and fermentation of wild rice at 21 C were obtained from either unfermented wild rice or wild rice fermented at 21 C. In addition, 10 isolates for inoculation and fermentation of rice at 4 C were obtained from wild rice fermented at 4 C. Wild rice to be used for the fermentations had been stored by freezing and was allowed to warm to fermentation temperature before inoculation. Frozen unfermented wild rice was divided into 10-lb lots and placed in 5-gal plastic buckets with loosely fitting lids. The inoculum was prepared by growing each culture in 1200 ml of wild rice broth (previously described). Cultures to inoculate rice for fermentation at 4 C were prepared with 5% inoculum and incubated at 21 C for 72 h; those to inoculate rice for fermentation at 21 C were incubated at 30 C for 48 h. The inoculum was then mixed with the 10 lb of rice and the mixture was incubated either at 4 C for 14 days or 21 C for 7 days. During fermentation, wild rice was mixed daily, temperature and pH of the rice were monitored, and enough water was added to keep the rice saturated. Uninoculated rice (control) was also allowed to ferment at each temperature. Halfway through the fermentation, about 5 lb of each lot was processed (processing is described later). After completion of the fermentation, the remainder of each lot was processed.

Measurement of pH

The pH of fermenting whole wild rice was measured by adding 20 g of wild rice to 100 ml of distilled water, and letting the mixture stand for 30 min with occasional stirring. The pH of the fluid was measured with a Corning pH meter (model 10) equipped with a glass-reference electrode.

Processing of inoculated wild rice

Parching (drying) was done with forced air (15-20 ft/sec) at 250 F (121 C) until the rice (in a 1-ft^3 screened cage) contained 7-10% moisture (less than 20 min). The weight and moisture content of this rice were used to calculate the percent solids that was recovered after the fermentation and parching steps. This value, recorded as "% solids recovered," was the combined yield from both samplings. Wild rice was hulled by passing once through a Kyowa rice huller equipped with 6-inch double rubber rolls (Kyowa Agricultural Machinery, Ltd., Kochi, Japan), and hulls and chaff were removed by aspiration. Weight of this cleaned rice was subtracted from weight of parched rice and the difference was recorded as "wt of hulls and chaff." Cleaned rice was passed through a sample divider (Seedburo Equipment Co., Chicago, Ill.) to get a representative sample one-eighth the size of the original. The divided sample was shaken on a screen to remove cracked rice, leaving whole and unhulled rice. Unhulled rice was separated from whole rice by hand. Weights of whole, cracked, and unhulled rice in the original sample were calculated. The percent unhulled rice was calculated from the weight of parched rice that remained unhulled. The whole, cracked, and hulls and chaff fractions which could be obtained from unhulled rice with additional hulling were calculated using proportions derived from the original sample. Weights of the whole and cracked fractions of rice were combined and used to calculate the "% edible solids" figure, which represents the highest percent of whole and cracked rice that would be left from a sample of parched rice after complete hulling. The percent of edible rice that was whole was also calculated. This percentage may be an indicator of kernel fragility.

Flavor profiles of inocualted wild rice

Three experienced flavor judges evaluated the overall flavor characteristics of each sample of processed, fermented wild rice. To prepare for testing, 50 g of processed rice were added to 250 ml of boiling distilled water and boiled for 35 min. Excess water was poured from the samples before evaluation. Samples from the two periods of fermentation for each inoculated lot were tasted sequentially.

RESULTS AND DISCUSSION

Microbiological analysis of rice subject to different storage treatments

During the first week of fermentation the major changes in wild rice stored at 21 C were (*a*) an increase in pH from 6.3 to 7.5, (*b*) a decrease in number of psychrotrophs (Fig. 1), and (*c*) an increase in number of

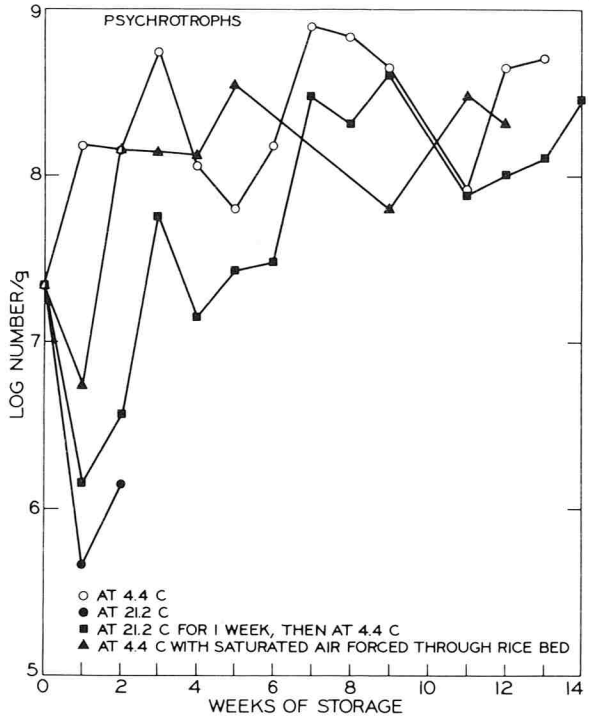

Figure 1. *Number of psychrotrophic bacteria in fermenting wild rice.*

molds to 5×10^6/g (Fig. 2). Similar changes were reported by Goel et al. (*8*). During the second week of storage rice developed a putrid odor. This decrease in quality was accompanied by (*a*) a rise in pH from 7.5 to 7.9, (*b*) a decrease in rice temperature from 39 to 31 C, and (*c*) a 10-fold increase in total count to 2×10^{10}/g (Fig. 3). The increase in pH was a continuation of a trend that began during the first week of fermentation, perhaps resulting from proteolysis. The decrease in temperature of the rice pile probably was the result of decreased respiratory activity of the rice. The decrease in respiration and increase in total aerobic count may be related to the decrease in quality of rice.

Wild rice held at 4 C remained organoleptically acceptable throughout 14 weeks of storage. In this rice, the total count did not increase above the original count (Fig. 3). Also, the pH remained between 6.4 and 7.2

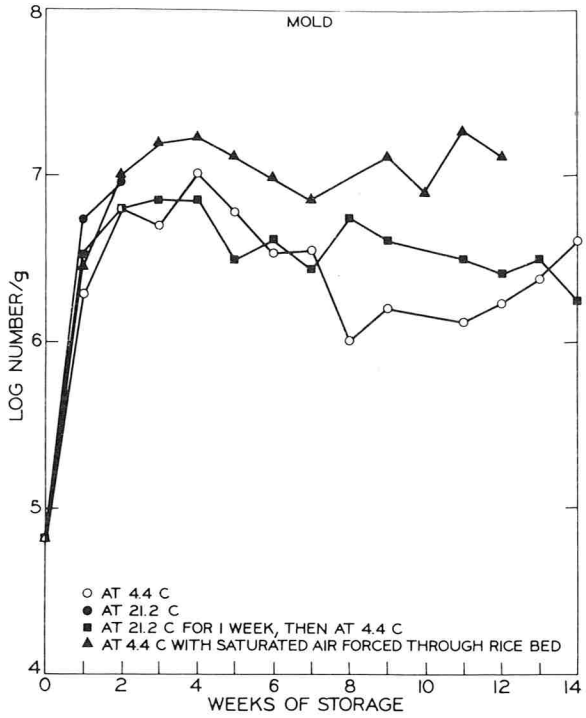

Figure 2. *Number of molds in fermenting wild rice.*

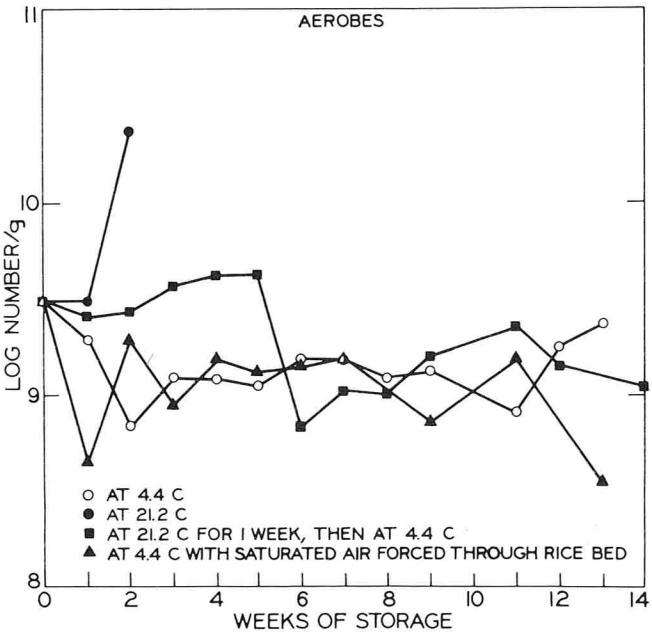

Figure 3. *Aerobic plate count of fermenting wild rice.*

throughout the fermentation. However, the psychrotophs increased to within one log of the total count (Fig. 1 and 3). Goel et al. (*8*) found a similar increase in psychrotrophs in wild rice stored at 10 C. Refrigerated rice exhibited a sharp increase in the mold count during the first two weeks of storage (Fig. 2). This was similar to the mold count observed when fermentation was at 21 C. Aerobic and anaerobic spore (included facultative types) counts were lower in refrigerated than in unrefrigerated rice (Fig. 4 and 5). After 11 weeks of storage, the aerobic

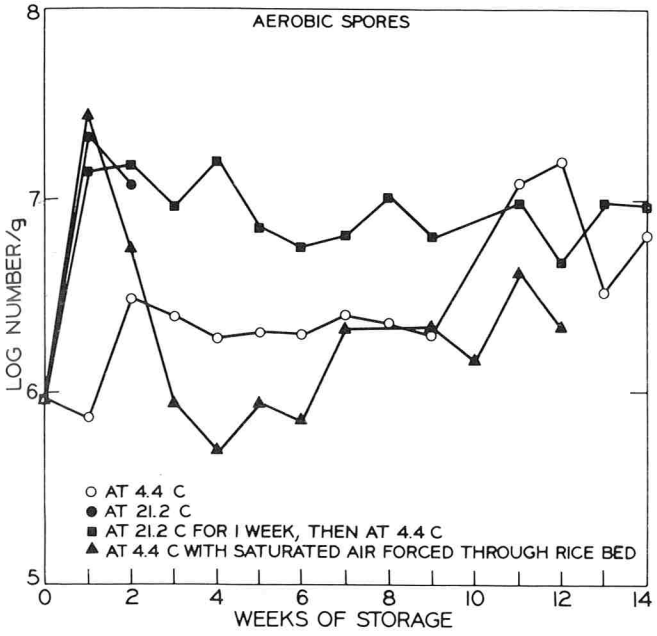

Figure 4. *Number of aerobic spores in fermenting wild rice.*

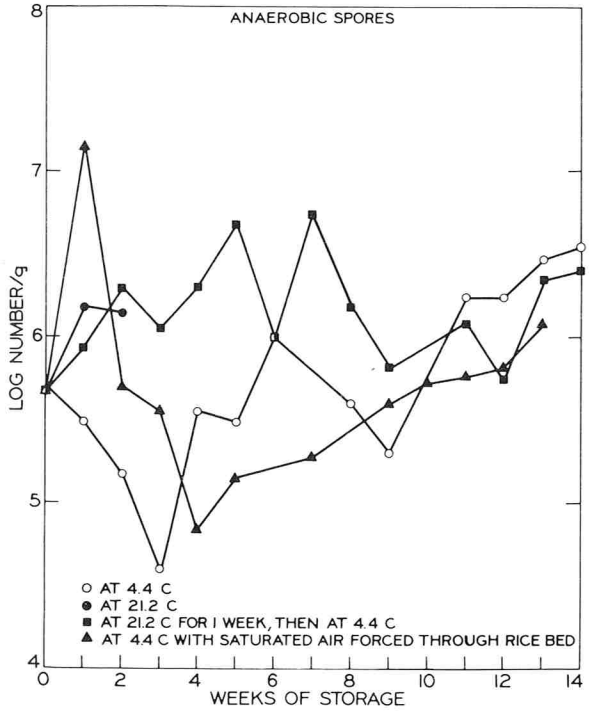

Figure 5. *Number of facultative and anaerobic spores in fermenting wild rice.*

spores in refrigerated rice had reached approximately the same number as did spores in rice at 21 C after 1 week of fermentation.

Rice stored at 21 C for 1 week and then at 4 C for 13 weeks did not show an increase in total aerobic count; after 5 weeks at 4 C the total count became similar to that of rice stored only at 4 C. This rice also had mold and psychrotroph growth similar to that in rice that was immediately refrigerated. Wild rice stored in the water-saturated forced air system at 4 C was microbiologically similar to the other refrigerated rice with two exceptions. First, spore counts after 1 week were much higher than would be expected (Fig. 4 and 5). This probably resulted because the rice was cooled inadequately before it was put into the storage system. Second, mold counts on this rice were higher than those of the other refrigerated samples (Fig. 2). This could have resulted from constant aeration.

Groups of bacteria isolated from wild rice

Gram negative rods were the predominant microorganisns isolated from unfermented and fermented wild rice (Tables 2 and 3). This agrees with the

TABLE 2. *Bacteria isolated from wild rice fermented at 21 C (70 F)*

Type of bacteria	Unfermented wild rice	7 Days of fermentation	14 Days of fermentation
	(No. specific type/total isolates)		
Gram negative rods	9/14	6/8	7/10
Gram positive rods	0/14	1/8	2/10
Gram positive cocci	5/14	1/8	0/10
Micrococcus spp.	3/14	1/8	0/10
Pseudomonas spp.	3/14	4/8	4/10
Achromobacter spp.	4/14	1/8	1/10
Coliforms	1/14	0/8	0/10
Flavobacterium spp.	1/14	0/8	0/10
Coryneforms	0/14	2/8	3/10
Cellulolytic spp.	0/14	1/8	1/10
Streptomycetes spp.	0/14	0/8	1/10
Staphylococcus spp. (coagulase negative)	1/14	0/8	0/10

TABLE 3. *Bacteria isolated from wild rice fermented at 4.4 C (40 F)*

Type of bacteria	Weeks of fermentation						
	1	2	3	4	6	8	12
	(No. of specific type/total isolated)						
Gram negative rods	8/10	6/8	7/10	5/10	5/12	6/7	13/15
Gram positive rods	0/10	1/8	2/10	3/10	5/12	1/7	2/15
Gram positive cocci	2/10	1/8	1/10	2/10	2/12	0/7	0/15
Micrococcus spp.	0/10	0/8	1/10	0/10	0/12	0/7	0/15
Pseudomonas spp.	3/10	1/8	2/10	2/10	0/12	0/7	1/15
Achromobacter spp.	4/10	3/8	0/10	0/10	2/12	2/7	7/15
Coliforms	0/10	1/8	1/10	1/10	3/12	4/7	0/15
Flavobacterium spp.	1/10	1/8	4/10	2/10	0/12	0/7	2/15
Coryneforms	0/10	1/8	2/10	3/10	5/12	1/7	4/15
Cellulolytic spp.	0/10	0/8	2/10	2/10	0/12	0/7	2/15
Staphylococcus spp. (coagulase negative)	2/10	1/8	0/10	1/10	0/12	0/7	0/15
Enterobacter aerogenes	0/10	1/8	1/8	0/10	1/12	3/7	0/15

results of Goel et al. (*8*). When unfermented wild rice was examined, *Pseudomonas* spp., *Achromobacter* spp., and *Micrococcus* spp. were isolated most often. Coliform bacteria, *Flavobacterium* spp., and *Staphylococcus* spp. were also isolated. In rice fermented at 21 C, *Pseudomonas* spp. predominated with coryneforms, *Achromobacter* spp. and *Micrococcus* spp. also being isolated (Table 2). Two of the coryneforms isolated were cellulolytic. Isolation of celluloytic bacteria lends support to the theory that microorganisms contribute to degradation of wild rice hulls, thus making them easier to remove. After 2 weeks of fermentation at 21 C a *Streptomyces* sp. was isolated. These microorganisms are known for their production of compounds with potent odors (*4,5*), and since they are present in fermenting wild

rice, they could be important in development of the earthy flavor commonly associated with the finished product.

Four different groups of bacteria, in succession (Table 3), appeared in wild rice fermented at 4 C for 12 weeks. *Achromobacter* spp. predominated during the first 2 weeks, *Flavobacterium* spp. during the third week, and then *Achromobacter* spp. again after 12 weeks. Cellulolytic species were isolated at the third, fourth, and twelfth weeks of fermentation. Coagulase-negative *Staphylococcus aureus* appeared at the first, second, and fourth weeks of fermentation. Although only a small number of isolates was examined at each sampling period, the total picture obtained from the data (Tables 2 and 3) suggests that the predominating microflora in fermenting wild rice is a complicated mixture of species. Thus the effects of the presence of these bacteria on the flavor and processing characteristics of the wild rice could be the result of complicated interactions. In assessing the significance these bacteria may have on processing characteristics and final quality of the wild rice it should be remembered that the total plate counts from which these bacteria were isolated exceeded 10^9 per gram of rice, indicating the presence of the isolates in extremely large numbers.

The seventh (*2*) rather than the eighth edition of *Bergey's Manual of Determinative Bacteriology* served as the basis for classifying bacteria that were isolated from wild rice. This was done because the eighth edition was not available when this work was completed. Many isolates designated as *Achromobacter* sp. in this paper would probably be called *Acinetobacter* sp. or *Alcaligenes* sp. if the eighth edition of *Bergey's Manual* had been used. The entire genus *Achromobacter* has been eliminated from the eighth edition of the manual.

Description of bacteria isolated from wild rice

The *Pseudomonas* spp. isolated were mainly in the *Pseudomonas putida* and *Pseudomonas ambigua* groups, although *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* types were found. Six of the 20 pseudomonads isolated were not pigmented and seven were proteolytic in litmus milk. Only four liquefied gelatin and two hydrolyzed starch. *Flavobacterium* species were isolated from both unfermented rice and rice fermented at 4 C. Five different types were isolated from fermenting wild rice inculding *Flavobacterium dormitator, Flavobacterium solare, Flavobacterium arborescens,* and two that could not be identified. Eleven coliforms were isolated from wild rice. Of these, six were *Enterobacter* spp. and the remainder were unidentified intermediate forms. Goel et al. (*8*) found that coliforms isolated with Violet Red Bile Agar from wild rice were mainly intermediate types with some *Enterobacter aerogenes* and fewer *Escherichia coli.*

Eight cellulolytic bacteria were isolated from fermenting wild rice. These isolates all hydrolyzed cellulose in a medium containing cellulose as the sole carbon source. Thus it appears that celluloytic bacteria from wild rice were active under a variety of cultural conditions. However when these bacteria were cultured on wild rice hulls, no measurable degradation occurred at 30 C during 5 days of incubation as measured by weight loss of the hulls. Two other cellulolytic bacteria were isolated using an enrichment procedure similar to that of Han and Srinivasan (*9*). These isolates, in the genus *Cellulomonas,* utilized cellulose as a sole carbon source, but were not active in nutrient broth. They also caused no noticeable degradation of wild rice hulls.

Odors caused by bacteria used to ferment wild rice

All isolates were divided into two groups to test them for odor production in wild rice. The first group included isolates from unfermented wild rice and rice fermented at 21 C. These were grown in wild rice incubated at 30 C for 2 days. The second group of isolates were those from rice fermented at 4 C. These were grown in rice incubated at 7 C for 1 week. Isolates grown at 30 C produced odors which were grouped into four general categories listed in Table 4. Fecal, earthy, and fermented odors were most commonly produced by these isolates. An odor was labeled "typical fermented" if it was similar to that produced in the uninoculated rice. The isolates from the refrigerated rice produced a more interesting variety of odors. These were put into the seven general groups listed in Table 5. Production of ammonia- and fatty acid-like odors could be the result of oxidative deamination and decarboxylation of amino acids with release of ammonia and fatty acids (*18*). The fatty acid-like odors also could be the result of transamination reactions producing fatty acids from amino acids. The "sweet, aromatic" odors were the miscellaneous pleasant odors which included ester-like aromas. Bacterial metabolites able to cause such odors have been described (*3, 10*). Since these odors were the result of a mixed fermentation (inoculum plus natural microflora), microbial growth other than the

TABLE 4. *Odors caused by bacteria added to wild rice and incubated at 30 C*

Type of odor	Week of isolation		
	0	1	2
	(No. producing odor/total no.)		
Fecal, putrid	4/14	4/10	4/10
Earthy	4/14	0/10	3/10
Rotting vegetation	0/14	0/10	2/10
Typical fermented	6/14	6/10	1/10

TABLE 5. *Odors caused by bacteria added to wild rice and incubated at 7 C*

Type of odor	Week of isolation						
	1	2	3	4	6	8	12
	(No. producing odor/total no.)						
Fecal, putrid	5/10	4/8	2/10	0/10	7/10	3/11	1/14
Ammonia	0/10	2/8	0/10	0/10	0/10	0/11	0/14
Earthy	3/10	0/8	0/10	3/10	0/10	3/11	2/14
Fatty acid	0/10	0/8	5/10	2/10	0/10	0/11	4/14
Rotting vegetation	1/10	0/8	3/10	3/10	2/10	3/11	0/14
Sweet, aromatic	0/10	1/8	0/10	1/10	0/10	0/11	5/14
Typical fermented	1/10	1/8	0/10	1/10	1/10	2/11	2/14

inoculum may have occurred and contributed to the odors.

When incubation was at 7 C, fewer "typical fermented" odors were produced. However, the fecal-putrid, earthy, and rotting vegetation odors predominated through most of the fermentation, the same as at 30 C. After 12 weeks of refrigerated fermentation, isolates produced mainly fatty acid and sweet-aromatic types of odors (Table 5). Since at 12 weeks the rice had a putrid, earthy odor, it was not expected that the isolates would produce these other odors. Even though only a minority of the isolates produced putrid, earthy odors, during fermentation they could be intense enough to overshadow odors that were formed by the other microbes. Kaminski and Stawicki (*12*) reported results somewhat similar to those we observed when they grew bacterial isolates on moistened wheat meal. They described the odors produced by their isolates as musty, malty, sour, fruity, "granary," herbal, and medicinal. They also detected fatty acid production by their isolates; included were acetic, propionic, isobutyric, butyric, isovaleric, and capric acid.

Processing characteristics of inoculated wild rice

Inoculation of unfermented wild rice with bacterial isolates able to hydrolyze starch or cellulose in laboratory media did not affect processing characteristics of rice (data not shown). This was true of rice fermented at both 21 and 4 C using the bacterial isolates listed in Tables 6 and 7. Processing characteristics measured included total solids recovered, percent of whole rice in final product, and hulling efficiency. Inability of these isolates to affect yield could have resulted from either failure of the bacteria to produce the necessary enzymes when growing on the rice or protection of the kernel by its bran layer. The normal reduction of yield which occurs during fermentation probably results from seed respiration.

Flavor changes in wild rice caused by fermentation with bacteria

Cultures used to inoculate wild rice for fermentation at 4 C were able to cause noticeable changes in the flavor of the rice both after 7 and 14 days of growth (Table 6). Differences were most dramatic after 14 days. Several of the cultures caused development of black tea-like and earthy flavors. The uninoculated rice at 14 days had a tea-like flavor, but this was a green or grassy type of tea flavor. It is not known how microorganisms could promote development of black tea-like flavors, however Miller et al. (*15, 16, 17*) using bacteria that were taxonomically similar to ours, observed that their organisms produced volatile compounds in sterile fish muscle.

After only 7 days of fermentation, two of the inoculated samples of rice had developed an earthy flavor. In rice fermented with culture 257 (Table 6) the earthy flavor had an undesirable coarseness and this continued to appear in the 14-day old sample. A black tea flavor developed along with the earthiness in this sample. In rice fermented with culture 314, an excellent tea-like and earthy flavor had developed after 7 days. This sample had a flavor which normally would be found in rice fermented for a longer time at a refrigeration temperature. After 14 days, flavor of this rice had become too strong. Since at this point, the uninoculated rice had barely begun to develop an earthy flavor, it appears that *Achromobacter* 314 greatly increased the rate of flavor development. After 14 days of fermentation, many of the other inoculated samples also had developed more intense earthy flavors than did the control. Cultures that were judged to give rice a more desirable flavor than was found in the control were: *Pseudomonas fluorescens* 264, *Flavobacterium solare* 266, and *Achromobacter* 307. Pseudomonads have been shown to produce earthy, potato-like odors in other foods (*17, 22*).

After 7 days of fermentation at 21 C, eight of the nine inoculated samples and the uninoculated sample had developed unacceptable putrid flavors (Table 7). This flavor was probably the result of rapid growth of bacteria (from 1.5×10^9 to 3×10^{10}) in rice at this temperature.

TABLE 6. *Changes in wild rice caused by fermentation with bacteria at 4 C*[a]

Culture	Head space odor[b]	Flavor after 7 days	Flavor after 14 days
Fermented 14 days no inoculum[c]	Grassy, slight fatty acid	—[d]	Tea-like, grainy, no earthiness
F. solare 255	Fatty acid	Astringent, grassy, tea-like	Astringent, toasted, strong, unbalanced
Coliform 257	Pleasant, aromatic	Tea-like with course earthiness	Black tea with course earthiness
P. fluorescens 264	Fermented grass	Grassy, bland	Good full tea-like with slight earthiness
F. solare 266	Clean, earthy, slight fatty acid	Grassy, grainy	Well-balance black tea and earthy
F. arborescens 304	Fermented	Grassy, tea-like	Black tea and earthy
C. uda 306	Rotting manure[e]	Bland, grassy	Mild tea-like
Achromobacter sp. 307	Fatty acid, sulfury[c]	Bland, grassy	Good full well-balanced tea-like and earthy
Achromobacter sp. 308	Slightly fruity	Grassy, tea-like	Strong earthy
Achromobacter sp. 314	Ester-like background[f]	Balanced tea-like and earthy	Strong black tea, undersirable
Cellulomonas sp. CMC	Fermented[f]	Bland, astringent	Astringent, earthy, undesirable

[a]The highest temperature reached by wild rice during fermentation was from 5-6 C. The highest pH reached was from 8.5-8.7.
[b]From Table 5.
[c]The highest pH reached was 7.5 afer 2 weeks.
[d]Dash = No data.
[e]This culture gave a fishy, amine odor on nutrient agar.
[f]Gave a fruity odor on nutrient agar.

TABLE 4. *Changes in wild rice caused by fermentation with bacteria at 21 C*[a]

Culture	Head space odor[b]	Flavor after 4 days	Flavor after 7 days
Fermented 7 days no inoculum	Slighty putrid	—[c]	Strong putrid, unacceptable
P. ambigua 2	Pleasant earthy	Strong tea-like, slightly earthy	Strong earthy and tea-like, acceptable
P. reptilivora 10	Putrid	Well-balanced tea-like and earthy	Putrid and earthy, unacceptable
P. aeruginosa 28	Pungent, earthy	Slightly putrid, unacceptable	Earthy and putrid, unacceptable
Micrococcus sp. 30	Slightly putrid	Earthy and tea-like, slightly putrid	Intense earthiness, slightly putrid, unacceptable
C. acidula 107	Slightly putrid[d]	Earthy, tea-like astrigent	Earthy, slightly putrid, unacceptable
Micrococcus sp. 113	Fecal	Full earthy and tea-like	Putrid, earthy, unacceptable
P. putida 120	Pleasant earthy	Grassy, tea-like, slight earthy	Putrid, earthy, unacceptable
C. gelida 131	Slightly putrid	Slight tea-like, slight earthy	Slightly putrid, unacceptable
C. flavigena C-5	Slightly putrid	Slight tea-like	Putrid, unacceptable

[a]The highest temperature reached by wild rice during fermentation ranged from 27-30.5 C. The highest pH reached ranged from 8.5-8.7, except for the control and no. 129 which reached 7.9 and 8.1, respectively.
[b]From Table 4.
[c]Dash = No data.
[d]Gave an ethyl acetate-like odor on nutrient agar.

This rice may have been made more susceptible to spoilage by having been frozen. After only 4 days at 21 C, rice fermented with cultures 10, 113, 120, and 131 (Table 7) had developed the best flavors. Both *Pseudomonas reptilivora* 10 and *Micrococcus* sp. 113, although producing rice with a satisfactory flavor after a short fermentation, gave undesirable head space odors when grown on wild rice according to screening procedures outlined previously. This is not unusual because in comparing the head space odor and the flavor produced by each isolate listed in Table 6 and 7, it appears that an undesirable head space odor is not always associated with development of an undesirable flavor. The odor of raw (unparched) wild rice will not necessarily reflect its final taste because of chemical changes which may occur during the drying which is usually done at relatively high temperatures.

ACKNOWLEDGMENTS

This research was supported by the College of Agricultural and Life Sciences and through a cooperative research agreement with the United States Department of Agriculture. Presented in part at the 62nd Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Toronto, Ontario, August 11-13, 1975.

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J. Milk Food Technol. Vol. 39, No. 9, Pages 614-618 (September, 1976)

Some Factors Involved in the Clarification of Whey Wine [1,2]

PETER K. LARSON and H. Y. YANG

Department of Food Science and Technology
Oregon State University, Corvallis, Oregon 97331

(Received for publication January 26, 1976)

ABSTRACT

A naturally cloudy wine of approximately 10.5% alcohol content was produced from Cheddar whey with added dextrose. Addition of 0.20-0.50% KWK bentonite, followed by polishing filtration, was found to be the most feasible clarification procedure. Flavor panel results indicated no significant difference in taste or odor between unclarified and clarified wine. Tests for Brix, pH, titratable acidity, and alcohol content; and determinations for lactose, protein, fat, ash, and total solids were done on whey, wine, and wine sediments to monitor the effects of fermentation, aging, and clarification.

Development of whey-based beverages, a subject reviewed by Holsinger et al. (*8*), has been an area of whey utilization research. This particular study was a facet of a research effort by Yang and co-workers (*17*) to develop a commercially salable wine from sweet whey, notably Cheddar whey, that could be produced with the existing facilities of wineries. The wine making procedure in this study allows for utilization of whole whey and requires minimal inputs of energy and processing equipment. Whey wine has a potentially good economic value and yields nutritious sediments as by-products.

The appearance of wine is a critical attribute, and preliminary testing showed clarified (clear) wine to be preferable to unclarified (cloudy) wine. Experimentation was undertaken to find an acceptable clarification method and was based on the assumption that the wine cloud was comprised largely of proteins and yeast materials. The experimental criteria were simplicity, legality, reasonable cost, minimal usage of energy and equipment, and maintenance of the wine character and quality.

After deciding upon a clarification procedure, the differences in cloudy and clear wines were assessed by sensory evaluation. The changes that took place during the wine making steps of fermentation, aging, and clarification were also monitored by several tests and analyses.

MATERIALS AND METHODS

Wine making procedure

A 10-gal can of fresh Cheddar cheese whey served as the starting material. Sufficient dextrose, 22% of the whey by weight, was mixed into the whey to give a Brix reading of approximately 29.0. Ten numbered 1-gal glass jugs were each filled with 3600 ml of sweetened whey and inoculated. One gram of yeast, *Saccharomyces cerevisiae* sub. sp. *elipsoideus,* Montrachet strain, was dissolved in 10 ml of water at 43.4 C and added to each container without mixing. The jugs were fitted with fermentation locks, and the fermentation was allowed to proceed at room temperature. The fermentation was judged as finished when the Brix reading remained unchanged for 3 consecutive days. At this point the wine was racked into new glass jugs, and the sediment from each jug was frozen separately for subsequent analysis. The new jugs were purged with carbon dioxide and sealed with screw caps. The wine was racked a second time after 17 additional days and a third time after another 33 additional days, following the same procedures. At this point the wine was considered cloudy or unclarified wine. In some instances small amounts of material of a lipid nature were observed floating on the surface of the wine and were skimmed off before racking.

Clarification procedures

Attempts were made to clarify the wine by heating, filtration, centrifugation, pH adjustment, addition of a precipitating agent, and fining agents.

(*a*) Heating.--Heating consisted of placing a test tube with 50 ml of wine in boiling water with stirring for various times up to 90 sec.

(*b*) Filtration.--The filtration system consisted of an Ertel ESP model self-priming stainless steel pump and an Ertel E-1 model stainless steel filter fitted with a no. 9 grade Ertel asbestos filter pad. The pad was placed in the filter and washed with 1 gal of 1% lactic acid solution. Sufficient water was then pumped through the system until no "paper taste" could be detected. The wine was then filtered at 15-20 psi.

(*c*) Centrifugation.--A model UV centrifuge manufactured by International Equipment Company was used to centrifuge 250-ml portions of cloudy wine at 9,750 × *g* for various times.

(*d*) pH adjustment.--Amounts of a 20% potassium carbonate solution were added to 50-ml portions of unclarified wine using a burette. The addition was monitored with a pH meter to give a continuum from pH 4.5 to pH 5.9. The mixtures were then transferred to test tubes and shaken vigorously for 10 sec.

(*e*) Precipitating agent.--Various amounts of a 5% solution of sodium hexametaphosphate were added to 50-ml portions of cloudy wine in test tubes with 10 sec of vigorous shaking. All test tubes were sealed with corks after shaking and visually observed for clarification after 72 h.

(*f*) Fining agents.--Solutions of 2% casein, 1% gelatin, and 1% tannin were prepared according to Amerine et al. (*1*). Following the manufacturer's directions (Scott Laboratories), 100 ml of cold distilled water and 2.4 g of Cold Mix Sparkolloid were mixed at high speed to yield a smooth slurry ready for use. An 8% solution of KWK bentonite was pre-swelled with steam before addition to the wine in accordance with present industry practice. The amount of water introduced by the steam resulted in a final slurry of 6% bentonite.

Changes during fermentation, aging, and clarification

Measurements of Brix, pH, titratable acidity, and alcohol content; and analyses for lactose, protein, fat, ash, and total solids were done to monitor changes during the wine making process. Samples for these

[1] *Technical Paper No. 4094, Oregon Agricultural Experiment Station.*
[2] *Adapted from the M.S. dissertation of Peter K. Larson, Oregon State University, Corvallis.*

analyses included whey, wine, and sediments.

An ebulliometer was used to determine alcohol content and a saccharometer was used to measure Brix. For the titratable acidity expressed as percent lactic acid, 10 ml of sample were titrated with a standard solution of 0.1 *N* sodium hydroxide to an endpoint of pH 8.3

Official AOAC methods were the basis for the various compositional determinations (*4*). The gravimetric method was used for lactose, a modification of Kjeldahl determination for nitrogen for protein which uses a packet containing potassium sulfate and copper pentahydrate instead of mercury, and a modification of the method for the fat content of raw milk as proposed by Anderson Laboratories (*3*) using the TeSa reagent.

Subjective evaluation

Unclarified and filtered wines were compared in a triangle difference test involving 20 blindfolded tasters. Both wines had 6% added sucrose, and their acidities were adjusted to 1.0% with lactic acid.

The same 20 tasters took part in a similar test of filtered wine and wine fined with 0.30% bentonite and filtered after 7 days. The tasters were seated one at a time at a counter and blindfolded. One-ounce samples at room temperature in 6-oz drinking glasses were presented to the taster in a randomized order. The taster was asked verbally to identify the different sample. Retasting of the samples was permitted.

RESULTS AND DISCUSSION

Effects of clarification procedures

Sensory evaluation tests by Yang et al. (*17*) indicated filtered whey wine was preferable to unclarified whey wine. Tasters gave the former a rating of 6.3 and the latter a rating of 5.2 on a 9-point hedonic scale with the value 1 representing "Dislike Extremely," 5 representing "Neither Like or Dislike," and 9 representing "Like Extremely." The color of the clear wine is a light amber similar to a white grape wine while that of cloudy wine is a dull, opague yellow-gray. The wine cloud was assumed to be composed mostly of protein colloids with some yeast materials. Only a small amount of the colloidal content of new grape wines is due to yeast cells as they tend to settle rapidly after fermentation.

Although heat denaturation is a common method for precipitating whey proteins (*10*) heating the whey wine for 90 sec at 100 C caused only a partial reduction in cloudiness, imparted a cooked flavor and odor, and reduced the alcohol content from 10.6% to 8.7%.

Clarification of the wine by filtration proved to be impractical because a filter pad designed for fine filtration was required to remove the cloud. The operation was characterized by a slow flow rate and frequent replacement of the pad. The filtration efficiency was not significantly increased by employing a filter aid. It was apparent that filtration needed to be preceded by coagulation or precipitation of all or most of the wine cloud.

Centrifugation as means of clarification represented an energy input and the need for expensive equipment at the commercial production level. These reasons together with the fact that promising results were being obtained with bentonite limited experimentation in this area. In one instance whey wine centrifuged for 30 min at 9,750 × *g* exhibited only slight decrease in cloudiness.

The adjustment of the pH of the wine to the isoelectric point of the major whey proteins was investigated. The results of mixing 0.65 ml to 1.30 ml of 20% potassium carbonate solution into 50 ml of whey wine to produce a continuum from pH 4.5 to pH 5.9 are given in Table 1.

TABLE 1. *Effect of pH adjustment on whey wine clarification*

Sample number	20% Potassium carbonate added (ml)	(%)	pH	Appearance (after 24 h)
1	0.65	0.26[a]	4.5	Slightly cloudy
2	0.80	0.32	4.7	Slightly cloudy
3	0.95	0.38	4.9	Clear
4	1.10	0.44	5.1	Clear
5	1.20	0.48	5.3	Very clear
6	1.25	0.50	5.5	Very clear
7	1.30	0.52	5.7	Clear
8	1.35	0.54	5.9	Clear

[a]Dry weight basis.

Observations were made after 24 h. A range of pH 4.9-5.9 resulted in wines with good clarity and a white coagulum. Maximum clarity was observed at pH 5.3-5.5. This pH is slightly higher than the isoelectric points for beta-lactoglobulin and alpha-lactalbumin, but ionic concentration can shift the isoelectric point of proteins. The precipitation may also have been due to a combination of the hydrogen ion concentration and the alcohol level.

Since the wine tasted flat as a result of acid neutralization, each sample was racked and its titratable acidity increased to 0.7% with lactic acid. No change in clarity was seen, but an unacceptable salt taste, presumably carbonate, was detectable.

Sodium hexametaphosphate (*7*) was added to the cloudy wine at concentrations of 0.0025-0.15%. Good clarity was observed at levels of 0.10% and higher, but the wine possessed an off-flavor. The ability of the trivalent phosphate anion to form aggregates of the positively charged protein molecules is the probable cause of precipation.

TABLE 2. *Effect of fining agents on whey wine clarification*

Fining agent and percentage added Dry weight basis	Clarifying action[a] Poor	Fair	Good	Excellent
Casein				
0.01-0.20	x			
Cold Mix				
Sparkolloid				
0.01-0.20	x			
Gelatin				
0.01-0.16	x			
Tannin, gelatin				
0.04, 0.01-0.16	x			
0.08, 0.01-0.16		x		
0.16, 0.01-0.16		x		
Tannin				
0.04	x			
0.08		x		
0.16			x	
Bentonite				
0.10	x			
0.20		x		
0.30			x	
0.50				x

[a]Based on visual observation 72 h after addition of fining agent.

The negligible effect of fining agents on the character of grape wine is a desirable attribute of these agents. However, their effect on colloidal suspensions of animal origin seems to be unknown. The efficacy of casein, gelatin, tannin, Cold Mix Sparkolloid, and bentonite to fine whey wine when added in various concentrations on a percent dry weight basis is shown in Table 2.

Casein exhibited no fining action when incorporated at levels of 0.01-0.20%. Since the acidities of grape and whey wine are somewhat similar, it is reasonable to expect casein to precipitate with a clarifying action in whey wine as it does in grape wine. Furthermore, the ethanol present would tend to decrease the charge on the casein particles and their solubility by reducing the dielectric constant.

Casein exists in milk as part of a complex system, so its behavior upon addition to whey wine is speculative. Above its isoelectric point of pH 4.7, casein tends to bind divalent cations, particularly calcium and magnesium, which favors its aggregation and precipitation.

Cold Mix Sparkolloid was introduced into the wine at concentrations of 0.01-0.20% with no observable change in the wine cloud. It solubilizes as a negatively charged macromolecule in grape wine and would be expected to flocculate with the protein colloids of whey wine. This failure to flocculatae may involve an inability of the polysaccharide to disperse properly.

The degree of cloudiness was not reduced when gelatin was mixed with the wine at concentrations of 0.01-0.16%. This was somewhat expected since gelatin would be positively charged at the pH of whey wine. In the fining of grape wines tannin, whether added or present naturally in the wine, combines with gelatin to form a precipitate. Several combinations of tannin and gelatin in the respective ranges of 0.04-0.16% and 0.01-0.16% were added to whey wine with the tannin being introduced 4 h before the gelatin. Fining action was observed to be correlated with the higher amounts of tannin and resulted in a granular sediment of light violet color. The fined wine had a distinct astringent taste.

Tannin by itself produced acceptable fining at a concentration of 0.16%. The coagulum was dark violet, and the wine again possessed an unacceptable astringent note. The oxidation of phenolic compounds in the tannin to ultimately form colored polymers is the probable explanation for the violet sediment.

Bentonite was introduced at levels of 0.01-0.50% and was found to be the most effective fining agent tested. It is noted for its ability to clarify recalcitrant wines as well as remove proteins (*2*).

At concentrations of 0.30% and 0.50%, respectively, bentonite produced an acceptably clear and a brilliantly clear wine after 72 h, each with a loose, white sediment. Fining continued with time and after 10 days wine containing 0.20% bentonite had a good clarity. Observation after 20 additional days showed 0.20% to be the concentration which gave a clear wine. The amount of protein and the degree of cloudiness decreased with increasing concentrations of bentonite. Only a slight cloud was visible at 0.15% bentonite and, as stated, concentrations of 0.20% or more produced clear wines. From these observations it can be theorized that the cloud is comprised largely of proteins, there is a limit to the amount of proteins that bentonite will remove, over-fining with bentonite will still result in a clear wine, and a significant quantity of proteins soluble under those conditions still exist in the clear wine.

There are two problems apparent with bentonite fining. One problem is the bulkiness of the lees, approximately 8% by volume, which would result in product loss or necessitate additional steps to recover the wine from lees. The other problem is the concentration of the bentonite suspension and the amount of suspension necessary for fining serve to dilute the wine more than federal regulations (*15*) allow in the bentonite fining of grape wines. Whether bentonite usage in the fining of whey wine would be subject to such limitations is not known.

The improved procedure by Yang et al. (*17*) for producing clear whey wine may be relevant. It includes heating the whey for 5 min at 82 C to cause partial protein precipitation followed by pouring the liquid off the precipatate before addition of the dextrose. The cloud in the resulting wine would contain less protein, hence fining would require less bentonite.

Effects of fermentation, aging and clarification

A chronology of the wine making procedure based on determinations of pH, titratable acidity, Brix, and alcohol content is given in Table 3. Determinations on the sampled contents of wine jugs number 1,2, and 3, one determination per jug, were averaged to provide the data. "Bentonite-filtered wine" denotes the addition of 0.30% bentonite to cloudy wine, followed 7 days later by racking and filtration.

TABLE 3. *Changes in pH, titratable acidity, Brix, and alcohol content during wine making*

Day	Material	pH	Titratable acidity[a]	Brix	Alcohol
			(%)	(deg)	(%)
1	Whey	4.7	0.26	6.8	—
1	Whey plus dextrose	—	—	21.9	—
13	First racking	4.3	0.63	3.1	9.7
68	Third racking	4.2	0.53	3.0	10.6
85	Cloudy wine	3.9	0.59	2.9	10.6
89	Filtered wine	3.9	0.50	2.9	10.5
92	Bentonite-filtered wine	4.0	0.48	2.6	9.2

[a]Expressed as lactic acid.

Lactic acid and alcoholic fermentations are the two major processes in the conversion of the whey to wine. The former decreases the Brix by utilizing lactose and increases the titratable acidity by the production of lactic acid. The yeast introduced does not utilize lactose. This accounts for the increase in titratable acidity from 0.26% in the whey to 0.59% in the cloudy wine. Titrations of whey wine aged several months indicated that the acidity stabilized at approximately 0.6%. The relationship between the pH and titratable acidity is influenced by the high buffering capacity of the system due to the presence

of lactate, phosphate, citrate, and proteins.

Alcoholic fermentation affects all of the determinations of Table 3. The decrease in Brix and increase in the alcohol content coincides with the production of ethanol and trace amounts of higher alcohols from dextrose. An alcohol content of 10.6% from 22.0% dextrose represents a yield of 48.2%. This is 94.3% of the theoretical yield of 51.1% which is in the usual range for grape wines. The acidity is affected by the small amounts of lactic, acetic, and succinic acids which are normal by-products of alcoholic fermentation. The final Brix reflects the lactose not fermented to lactic acid. No lactose is utilized in the alcoholic fermentation because the Montrachet yeast does not produce lactase.

The decrease in the acidity by filtration could be caused by the loss of volatile acids. Also, some alcohol would be expected to volatilize. Bentonite fining would have the additional effects of diluting the system and causing the loss of alcohol due to mixing and racking.

The gross compositions of the whey and the wine at various points in the wine making process are presented in Table 4. The values given are the averages of three

TABLE 4. *Changes in gross composition during wine making*

Day	Material	Percent				
		Lactose	Protein	Fat	Ash	Total Solids
1	Whey	4.58	0.88	0.0	0.52	6.33
85	Cloudy wine	4.14	0.69	0.0	0.34	6.33
89	Filtered wine	3.72	0.56	0.0	0.31	5.68
92	Bentonite-filtered wine	3.59	0.47	0.0	0.40	5.40

analyses, either from the can of whey or one each from wine jugs number 1,2, and 3. The data in Tables 3 and 4 lend themselves to joint consideration.

Composition of the whey in Table 4 is typical with the exception of fat which is commonly reported as being 0.1-0.3%. However, the cheese plant which supplied the whey achieved very efficient separation and routinely obtained fat readings of less than 0.005% using the Babcock test.

The decrease in the lactose during fermentation is due to the lactic acid fermentation. As previously stated, the Montrachet yeast produces no lactase so there is no enzymatic breakdown of the lactose.

The reduction of the protein level involves the utilization of nitrogenous materials by the wine yeasts and their removal after settling by racking. Racking also removes the proteins which precipitate during fermentation and aging. Bentonite fining flocculates the proteins and diminishes both the lactose and soluble protein concentrations by dilution.

Bentonite fining increased the concentration of ash, however. This phenomenon has been reported in the literature as occurring with grape wines (*2*). The metabolism of minerals by the wine yeast during fermentation would account for the lessening of the ash level. They require relatively large amounts of potassium, magnesium, and calcium salts plus inorganic phosphate and sulfate for growth.

The percentages of total solids in the wine analyses are significantly more than the totals for lactose, protein, and ash. The probable explanation is that yeast and bacterial metabolism produced by-products such as cellular materials and fixed acids which would contribute to total solids but not to the other categories.

Table 5 presents the gross compositions of two sediments, the lees from the first racking and the coagulum from bentonite fining. Both sediments show a concentration of the non-soluble solids, protein and ash, and a dilution of the soluble lactose. The large increase in the ash of the bentonite coagulum is no doubt due to the fining agent itself.

On a dry weight basis the lees contain roughly 35% lactose, 31% protein, and 5% ash. All three significant for their nutritional potential, but the proteins are of singular importance because of their high nutritional quality (*5, 16*).

TABLE 5. *Gross composition of wine sediments*

Sediment	Lactose	Protein	Fat	Ash	Total solids
			(%)		
Lees from first racking	3.87	3.43	0.0	0.54	10.95
Coagulum from bentonite fining	3.37	1.25	0.0	1.25	6.84

The functional properties of whey proteins in the lees would be very important in food applications. Morr comments on the solibility and whipability of whey protein concentrates in one study (*12*). Jaynes and Asan (*9*) have succeeded in preparing fibrous proteins from cheese whey, an accomplishment with very real significance.

Protein is also present in the yeast portion of the lees. Various studies indicate that *S. cerevisiae* contains approximately 20% nitrogen of which half is protein. Although media and growth conditions are influencing factors, most yeasts, *S. cerevisiae* included, have an amino acid composition closely resembling that of soy protein (*6, 13*).

The bentonite coagulum probably contains a higher ration of whey to yeast proteins than the lees. The presence of bentonite in this sediment would prohibit its use as a human food. Bentonite in feed rations, however, can be desirable. Rundsig et al. (*14*) studied the effects of incorporating bentonite into high-grain dairy rations. Whereas a grain-containing diet normally depresses the fat level of the milk, addition of 5-10% bentonite increased both the fat content and milk yield significantly. Lotif (*11*) concluded that 5% bentonite added to the diets of commercial laying hens increased their body weight, egg production, and fed efficiency.

Both sediments could be processed into foods and feeds with present whey processing technology. Drying, condensing, ultrafiltration, and various fermentations are all possibilities.

Sensory differences in cloudy and clear wines

The basis for the relative preference of the clarified wine by taste panelists was investigated to determine

whether the appearance of clarified wine or a taste difference was the more significant factor in its preference relative to cloudy wine. Of the 20 blindfolded tasters who compared unclarified and filtered wines, only eight (a statistically insignificant number) chose the different sample which indicated appearance to be the major reason for clear wine preference. Only seven of the same 20 tasters selected the different sample in the comparison of filtered wine and wine fined with 0.30% bentonite and filtered 7 days later. This indicates that bentonite fining does not significantly alter the taste and odor of the wine.

ACKNOWLEDGMENTS

This investigation was supported by finds from the Environmental Protection Agency, under Grant No. R-803301.

Presented at the 36th Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 6-9, 1976.

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J. Milk Food Technol. Vol. 39, No. 9, Pages 619-623 (September, 1976)

Prediction of Microbial Death During Drying of a Macaroni Product

FU-HUNG HSIEH, KAREN ACOTT, and THEODORE P. LABUZA

Department of Food Science and Nutrition
University of Minnesota, St. Paul, Minnesota 55108

(Received for publication November 3, 1975)

ABSTRACT

Death of *Staphylococcus aureus* 196E in a semolina-egg dough was studied during a variable temperature simulated drying test. Data for the death rate constant of the organism collected under steady state conditions of constant temperature and water activity were used to predict the amount of death occurring in the unsteady state test. Very good agreement was found. Utilizing the steady state information it was predicted that over 5-log cycles of kill would occur for *S. aureus* 196E and *Salmonellae anatum* NF_3 during the pasta drying process. This indicates that kill caused by the process itself may not be enough if high levels of these pathogens occur initially in the dough.

Recently it was found that amoung 89 pasta plants manufacturing noodles and macaroni products, only 46% were judged sanitary, 26% needed voluntary clean-up action, and 28% had serious violations [FDA memorandum, July 19, 1974 (AP)]. Lee et al. (*8*) also reported that routine surveillance by the Food and Drug Administration showed *Staphylococcus aureus* contamination of some pasta products manufactured in the United States. This pathogen and salmonellae pose a danger because of the nature of flour (*4*), and eggs (*1*). More importantly, macaroni products are usually not subjected to heating during manufacturing and the drying temperatures are not high. Because of this, some microorganisms will be found in even the most carefully processed macaroni products (*10*).

The presence of 10^3-10^6 of *S. aureus* per gram in pasta products has been reported by Lee et al. (*8*). The widespread consumption of these products and the increased number of seizures and recalls which involved microbiologically contaminated products, suggest that extensive investigation is needed to determine the effect of pasta processing on the viability of microorganisms (*10*).

Extrusion and drying are the major operations in the macaroni industry. In spaghetti extrusion, Walsh et al. (*10*) found that less than two log cycles death of *Salmonella typhimurium* resulted at 35 C. At higher temperature (up to 55 C), however, survival was greater probably because of a faster flow rate in the extruder. Less than one log cycle reduction in *S. aureus* at 35 C extrusion temperature was also reported (*11*). Only a slight decrease in population was observed when the temperature was increased to 55 C which was the upper temperature limit for extruding spaghetti (*10*). This shows that microbial death during the extrusion process of pasta making is not significant. Using an experimental dryer in which the temperature was kept at 35 C and the relative humidity was lowered from 95 to 61% in a linear manner over an 18 h period, Walsh et al. (*10*) found 95% of *S. typhimurium* were destroyed. It should be noted that growth of microorganisms during the early stages of drying was possible because of the optimal temperature (35 C) and the high initial relative humidity (95%) in the drying cabinet. Walsh and Funke (*11*) recently confirmed that a sevenfold increase in *S. aureus* resulted during a pasta drying operation under the same conditions. Although no enterotoxin was detected, the hazard potential is apparent. Lee et al. (*8*) showed the potential for growth and enterotoxin formation by *S. aureus* in pasta dough, as well as their persistence in the dried finished products.

The purpose of this study was to predict the death of *S. aureus* for the conditions of an actual drying operation used in a local pasta company. Since it is not feasible to inoculate food pathogens in the large commercial dryer, a simulated process was developed and the experimental results were used to compare with the theoretical prediction.

MATERIALS AND METHODS

Theoretical considerations

Generally, in real food processes one or more of the physical-chemical characteristics, such as temperature and a_w of foods will undergo continuous changes. If temperature and a_w are the only two parameters that change with time during processing and if the heat resistance or death rate as a function of a_w and temperature is known for a certain microorganism, then prediction of death for this organism during processing is possible for food processes in which changes in both temperature and a_w are known.

Prediction of the microbial death is based on the assumption that the microbial death follows a first order reaction, i.e.

$$\frac{dN}{dt} = -kN \quad [1]$$

or

$$\frac{d\ln N}{dt} = -k \quad [2]$$

where

t = time of heating
N = number of survivors at time t
k = death rate constant

The death rate constant k, can be determined experimentally at certain levels of a_w and temperature, which are of interest, as was shown by Hsieh et al. (*3*). For other a_w values and temperatures incurred in real food processes, k must be estimated from these experimental data. One way to do it through the use of the Arrhenius equation,

$$\frac{d\ln k}{dT} = \frac{E_a}{RT^2} \quad [3]$$

where

T = absolute temperature
E_a = activation energy of microbial death
R = universal gas constant

$$\frac{d\ln k(a_w, T)}{dT} = \frac{E_a(a_w)}{RT^2} \quad [4]$$

Equation [4] stresses the fact that k is a function of both a_w and temperature while E_a is a function of a_w which is true only within a narrow range of temperature. Upon integration, equation [4] gives,

$$K(a_w, T) = k_o(a_w) \exp [-E_a(a_w)/RT] \quad [5]$$

The constrants k_o and E_a for a certain a_w can be calculated when the death rate constant k, is known for more than two different temperatures. This has been assessed for *Staphylococcus aureus* 196E and *Salmonella anatum* NF[3] in semolina-egg medium (*5*). These constants are shown in Table 1 and Table 2 for *S. aureus* 196E and *S. anatum* NF_3, respectively. Substituting equation [5] into equation [2] leads to:

$$\frac{d\ln N}{dt} = -k_o(a_w) \exp [-E_a(a_w)/RT] \quad [6]$$

An integration of equation [6] gives,

$$\log (N_t/N_o) = \frac{-1}{2.3} \int_o^t k_o(a_w) \exp [-E_a(a_w)/RT]\, dt \quad [7]$$

N_o = initial number of cells
Nt = number of survivors at time t

This is a general equation in which the amount of organisms surviving a process can be predicted if the following information is available (*a*) a_w of food versus time and (*b*) product-temperature versus time.

Cultures

S. aureus 196E was obtained from Sita R. Tatini, Tepartment of Food Science and Nutrition, University of Minnesota. *S. anatum* NF_3 was selected from the Culture Collection of the same Department. They were grown in 100 ml of TSYB [Trypticase Soy Broth (BBL) in which 0.5% of Yeast Extract (BBL) was added], with glass beads and incubated at 37 c for 24 h with shaking.

Solid media

The solid media was composed of semolina (Como No. 1 Semolina, Capitol Durum Division, International Multifoods Corp., Minneapolis, MN), whole egg solids (A. J. Pietrus & Sons Co., Sleepy Eye, MN 56085) and distilled water. Semolina and whole egg solids were mixed first in the mixing bowl of a Brabender Farinograph (C. W. Brabender Instruments, Inc., South Hackensack, NJ) and various amounts of water were added such that a_w values of the solid medium varied from 0.5 to 0.92. The a_w was measured by the Vapor Pressure Manometer technique as described by Labuza (*7*). The solid medium was not sterilized so that its characteristics would not be changed. Counts made on the TSYB agar used in the actual study showed less than 10 organisms per gram. Since a large inoculum was used for the death study, it was felt that interference would not be a problem.

Simulated food process

Because of the difficulty in predicting the a_w during drying, a simulated process was developed in which temperature was the only variable. This was done through use of the mixer bowl of a Brabender Farinograph heated by a Haake FK2 Constant Temperature Circulator (Haake Instruments, Inc., Saddle Brook, NJ). The solid medium of a certain a_w value was prepared and placed into the mixer bowl. The temperature of the medium was monitored with thermocouple probes spaced throughout the bowl and was continuously recorded. The mixer was started at high speed and water at 40 C was circulated through the jacket of the mixer bowl. One ml of organisms from TSYB was then inoculated into 99 g of solid medium to give an initial population of 10^6 to 10^7 CFU/g. When 5 to 6 min had elapsed which allowed uniform distribution of cells to about 10^5 per gram, two steps were done simultaneously: (*a*) Approximately 1 g of the sample was taken out to estimate the initial population of organisms of TSYB agar. All counts were done at 37 C after 24 h. (*b*) The setting of temperature of the water bath circulator was changed to 75 C so that the product would now heat up linearly. At appropriate time intervals, about 1 to 2 g of sample was removed with a sterile tongue depressor and weighed into a blender jar which contained 99 ml of sterile 0.1% peptone water (DIFCO). The exact weight of the sample was recorded. The sample was then blended well and plated immediately by the surface spread technique on TSYB agar. The survivors were estimated by enumerating these plates after 24 h incubation at 37 C. It should be noted that prolonged incubation up to 48 h did not affect the number of survivors estimated.

RESULTS AND DISCUSSION

Simulated food process

A solid medium (semolina-egg) of a_w 0.87 was chosen. Figure 1 shows how the temperature of this medium in the mixer bowl of the Brabender Farinograph increased with time when the temperature regulator of the circulating water bath was changed from 40 to 75 C. A linear increase in temperature was observed after about 3 min for the center or 2 min for the wall of the mixer bowl. After 21 min, the temperature rise was no longer linear. The increase in temperature for the center and the wall from 3 to 21 min can be approximated by,

$$T_c = (73 + 2.5 \times (t\text{-}3)) \times 5/9 \quad [8]$$

$$T_w = (77 + 2.5 \times (t\text{-}3)) \times 5/9 \quad [9]$$

where

T_c = center temperature in C
T_w = wall temperature in C
t = time of heating in minutes

It should be noted that the mean average temperature of the solid medium is not uniform but falls between T_c and T_w. Thus, both temperatures were used to calculate the upper and lower limits of death. To predict death,

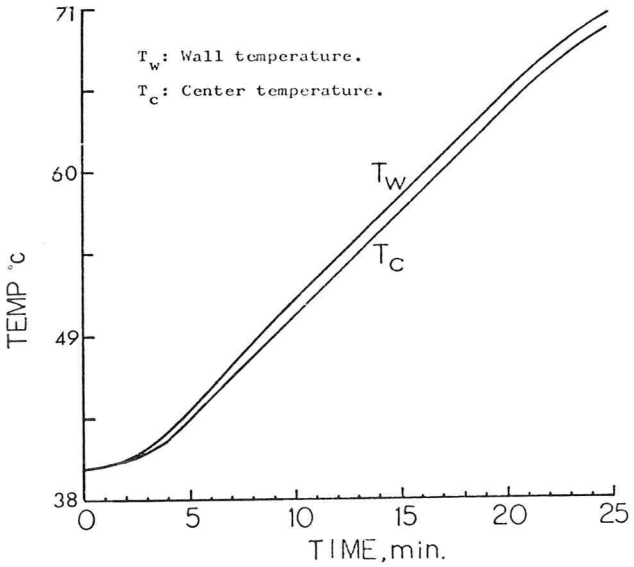

Figure 1. *Center and wall temperature of semolina-egg dough mix as a function of time during heating and mixing.*

equation [7] was integrated numerically using k_o from Table 1 in the case of *S. aureus* 196E or $k_o$from Table 2 for *S. anatum* NF_3. Figure 2 shows the prediction of the

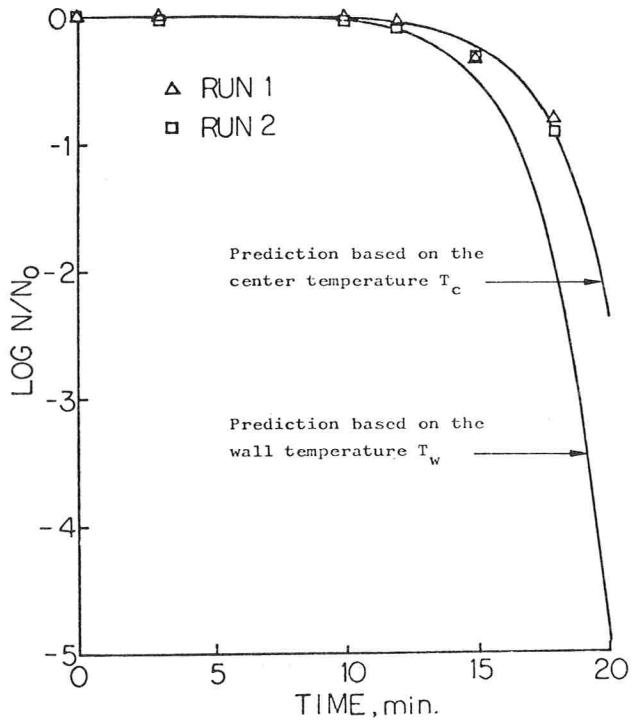

Figure 2. *Predicted (lines) and actual death (△, □) of* S. aureus *196E during unsteady state heating process in semolina-egg dough.*

death of *S. aureus* 196E in a solid medium of a_w 0.87. The upper curve was estimated from the lowest temperature T_c. Experimental results are also shown in this figure. In general, they are within the upper and lower limits as expected. Similar results for *S. anatum* NF_3 are shown in Fig. 3. It should be noted that most of the destruction

TABLE 1. *Kinetic constants for death rates of* S. aureus *196E in semolina-egg at various a_w values (3)*

$$\frac{2.3}{D} = k = k_o e^{E_a/RT}$$

a_w	Death rate absolute constant k_o min^{-1}	Activation energy E_a kcal/mole
0.61	7.56×10^{30}	48.5
0.76	3.74×10^{31}	49.9
9.80	2.67×10^{34}	54.6
0.83	3.72×10^{33}	52.2
0.85	8.26×10^{42}	66.1
0.87	6.04×10^{47}	73.2
0.92	1.52×10^{50}	76.6

TABLE 2. *Kinetic constants for death rates of* S. anatum *NF_3 in semolina-egg at various a_w values (3)*

$$\frac{2.3}{D} = k = k_o e^{-E_a/RT}$$

a_w	Death rate absolute constant k_o min^{-1}	Activation energy E_a kcal/mole
0.61	4.52×10^{42}	65.3
0.76	3.69×10^{44}	68.2
0.80	9.04×10^{43}	67.4
0.83	7.30×10^{51}	78.9
0.85	5.97×10^{46}	71.2
0.87	1.07×10^{48}	72.9
0.92	7.50×10^{56}	85.6

k = death rate constant
D = min for 90% destruction
T = temperature in °K
R = 1.986 cal/mole °K

occurred during the final stage of heating. This is not surprising since a temperature at which death starts to occur is not reached until 10 to 12 min have elapsed as shown in Fig. 1. An examination of Fig. 2 and Fig. 3 also reveals that the experimental results are better fitted by the prediction based on the center temperature of the medium. This is probably because the center temperature is a better representation of the median temperature of the medium. Analysis was not done beyond 1 to 2 log cycles since at that point death was so rapid, counts could not be determined accurately with the amount of sample available.

Microbial death during macaroni drying process

Table 3 shows the drying conditions utilized for various pasta prodcuts produced at the Creamette Company, Minneapolis, Minnesota. The change in a_w of the product can be determined by direct measurement of the product, before and after each drying stage. The first product, G.M. Macaroni, listed in Table 3, was chosen for prediction. Samples were secured and the a_w and moisture content were measured by the Vapor Pressure Manometer and vacuum oven method, respectively. It was assumed that the decrease in a_w during drying is linear. The temperature history of the product during drying, however, is very difficult to assess. Because of the thinness of the elbow macaroni, it was assumed that

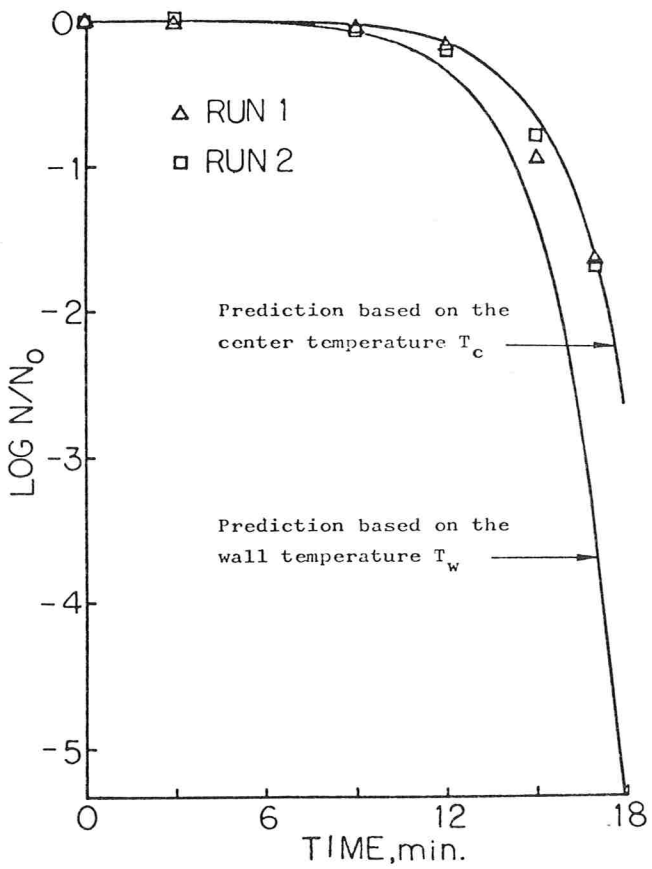

Figure 3. *Predicted (lines) and actual death* (△, □) *of* S. anatum NF_3 *during unsteady state heating process in semolina-egg dough.*

there is no temperature gradient within the product itself. In the beginning of preliminary drying, Charm (*2*) suggested that the surface of the product reaches the wet-bulb temperature of the drying air immediately. The temperature of the product will be maintained at this level till the end of the constant drying period (*9*). The product temperature will then increase when it enters into the falling rate drying period and gradually reaches the dry-bulb air temperature (*2, 6, 9*). It was assumed that the increase in temperature is linear and that the temperature reaches the dry-bulb air temperature at the end of preliminary drying. It was further assumed that the product will be maintained at the lower dry-bulb air temperature throughout the finishing drying stage. Both dry-bulb and wet-bulb measurements were taken for each stage.

Husain et al. (*6*) observed that for the entire drying process of potato tuber slices, 5 cm in diameter and 0.25 cm thick, only the falling rate period occurred with no constant rate period. Earle and Rogers (*3*) reported, however, that a constant rate period took place when the free moisture content of sheet macaroni was above 15%. With respect to elbow macaroni, they found that the falling rate prevailed when the moisture content of the product was lower than 32% (dry basis). The moisture content of our product was decreased from 42.0% (dry basis) to 18.9% in the preliminary drying stage. This was further lowered to 11.3% after finishing drying. It was assumed that the decrease in moisture was also linear with drying time and the constant drying rate period

TABLE 3. *Drying conditions for various macaroni products in a typical finishing dryer*

Creamettes Company product names®	Preliminary dryer (0.5 h) Air temperature, C			Finishing dryer (5 h) Air temperature, C		
	Dry bulb	Wet bulb	%RH[a]	Dry bulb	Wet bulb	%RH
G. M. Macaroni	60.6	53.9	70	55.7	51.1	79
G. M. Spaghetti	63.9	49.4	49	54.4	50.8	80
Creamettes	63.3	55.8	70	55.7	51.4	80
R. C. Spaghetti	62.8	52.2	60	54.4	50.8	80

[a] % relative humidity = $a_w \times 100$

TABLE 4. *Prediction of the destruction of* S. aureus *196E in G. M. Macaroni during drying from the projected* a_w *temperature profile*

Time t min.	Water activity of product a_w	Temperature of product T C	Death rate constant k min^{-1}	$k\Delta t$	$\Sigma k\Delta t$	Log cycles death $\log_{10}\frac{N_t}{N_0}$
0	0.93	53.9	0.11	0	0	0
6	0.91	53.9	0.096	0.576	0.576	-0.250
12	0.88	53.9	0.083	0.498	1.074	-0.467
15	0.87	54.7	0.095	0.285	1.359	-0.591
18	0.86	55.9	0.125	0.375	1.734	-0.754
21	0.85	57.0	0.148	0.444	2.178	-0.947
24	0.84	58.2	0.165	0.495	2.673	-1.162
27	0.83	59.4	0.170	0.510	3.183	-1.384
30	0.82	60.6	0.135	0.405	3.588	-1.560
45	0.805	55.7	0.020	0.30	3.888	-1.690
60	0.79	55.7	0.016	0.24	4.128	-1.795
90	0.765	55.7	0.023	0.69	4.818	-2.095
120	0.74	55.7	0.026	0.78	5.598	-2.434
180	0.68	55.7	0.031	1.86	7.458	-3.243
240	0.63	55.7	0.036	2.16	9.618	-4.182
300	0.58	55.7	0.040	2.40	12.018	-5.225
330	0.55	55.7	0.043	1.29	13.308	-5.786

takes place when the mositure content of the product decreases from 42% to 32%. After that, the falling rate prevails. The projected changes based on initial and final values in a_W moisture content and temperature of the product are summarized in Fig. 4.

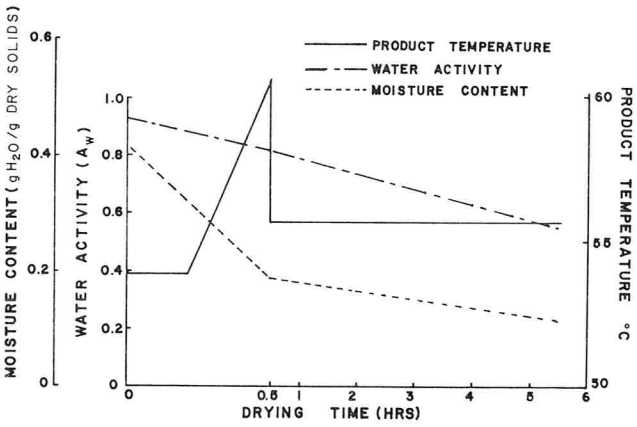

Figure 4. *Conditions of water activity, moisture content, and temperature of a typical pasta product during drying based on predicted and actual measurements.*

To predict the decrease in viable cells occurring, the procedure was as follows:

(*a*) The a_W and temperature of the product at increments of the drying time Δt is found from Figure 4.

(*b*) The death constant k corresponding to the particular a_W and temperature was derived from Table 1 or 2 (or a plot of k vs. a_W and T).

(*c*) Equation [7] was solved with this data for the small time interval Δt and the population decrease was found.

(*d*) The procedure was repeated for the next Δt and the death was summed.

The results are given in Table 4 for *S. aureus* 196E. As seen, about one and a half lograithmic cycles of death will occur within the first half hour of preliminary drying. Four more log cycles of death will result from the subsequent finishing drying. Based on the constants for *S. anatum* NF_3 there would be about six log cycles decrease in this same time period. This suggests that if initial populations are high enough pathogens can survive the process and might result in product seizure. Although these calculations are based on the measured a_W and temperature at the start and finish of each stage, assuming that the engineering parameters hold as discussed above the death predictions should be valid and would indicate the safety of the process. Thus if counts are initially high normal pasta drying will not be sufficient to make the product safe.

ACKNOWLEDGMENTS

This research was supported in part by the University of Minnesota Agr. Expt. Station Project 18-52m and contract #NAS 9-12560, Lyndon Johnson Space Center, Houston, TX. Scientific series, paper no. 9298 from the U.M. Agr. Expt. Station.

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J. Milk Food Technol. Vol. 39, No. 9, Pages 624-627 (September, 1976)

Counting Somatic Cells in Milk with a Rapid Flow-Through Cytophotometer

C. BREER[1], H. LUTZ[1], and B. S. SUPER[2]

Veterinär bakteriologisches Institut der Universität Zürich, Zürich, Switzerland, and Bio/Physics Systems, Inc., Baldwin Place Road, Mahopac, New York 10541

(Received for publication October 23, 1975)

ABSTRACT

Somatic cells are differentially counted in the presence of fat globules by detecting fluorescence of nuclear DNA in somatic cells after staining with acridine orange. Milk is diluted and stained in one step and passed through a new instrument, the Cytofluorograf, a rapid flow-through cytophotometer, which illuminates each particle with 4880Å light from an argon ion laser. A pulse of green fluorescence is emitted by each somatic cell nucleus. These pulses are used by the instrument to tally the cells. Damaged cells are clearly distinguished from fat globules. There is no difficulty in setting the threshold for the differential count, even in samples with damaged cells, and additional information of potential diagnostic value is available.

The prime criterion for the health of a cow's udder and hence for the quality of its milk is the somatic cell count (*1*). The study reported here is part of a search for a reliable, reproducible method, that is easy to automate. Determination of the somatic cell count by direct microscopic observation was introduced by Prescott and Breed (*8*). Later, a number of workers applied to this problem electronic cell counters of the type which give a measure of cell volume by detecting changes of electrical conductivity as the cells pass through an orifice. This method requires elimination of fat globules, either by centrifugation (*2, 7, 9*) or emulsification, (*10, 11*) because of the overlap in size between the fat globules and the somatic cells—primarily leukocytes. The following method, using a new instrument (Cytofluorograf Model 4800A, Bio/Physics Systems, Inc., New York), discriminates between somatic cells and fat globules by nuclear DNA uptake of a fluorescent dye (*6*) and subsequent electronic counting.

MATERIAL AND METHODS

Equipment

(*a*) Electrical conductivity cell counter, Digicell 100, Contraves A. G., Zürich. (*b*) Rapid flow-through cytophotometer, Cytofluorograf Model 4800A. Bio/Physics Systems, Inc., Mahopac, New York 10541. (*c*) Rack of test tubes for dilution of the milk samples in the reagent containing the fluorescent dye which binds to nucleic acids.

[1]*Universität Zürich.*
[2]*Bio/Physics Systems, Inc.*

Operation of the Cytofluorograf Model 4800A has been described in detail elsewhere (*3, 4*) and will be indicated only briefly here (Fig. 1). In

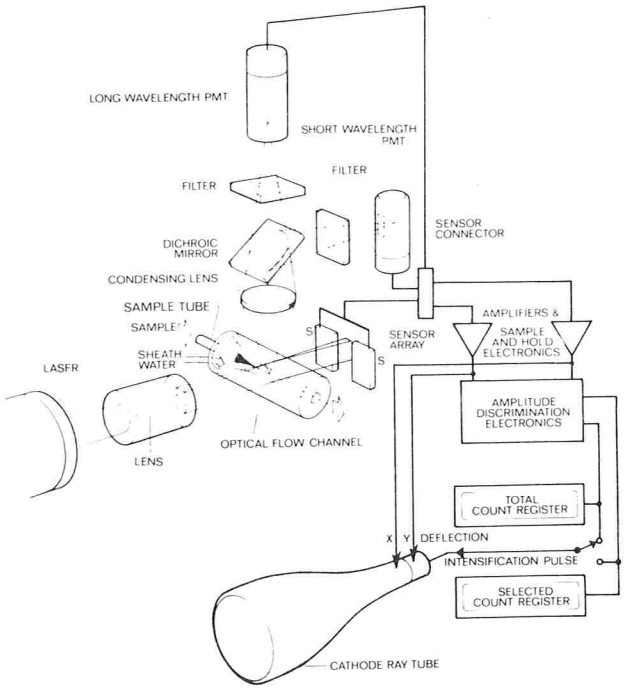


Figure 1. *Schematic diagram of Cytofluorograf.*

this instrument cells in liquid suspension are passed in single file through a focused argon ion laser beam emitting at 4880Å (blue light). Cells that have taken up a fluorochrome that is excited by the 4880Å light fluoresce and are distinguished by the instrument from non-fluorescing particles. Particles are processed by the instrument at a rate of approximately 1,000/sec. In this study the fluorochrome acridine orange, which binds to double stranded nuclear DNA to give a green fluorescence (*6*), was used to label the nucleated somatic cells. The non-fluorescing fat globules could then be distinguished from the fluorescing somatic cells, even though the two groups overlap in size. The method to which the above was compared is the cell colume technique, using an electronic cell counter of the conductivity type (in this study the Digicell 100), with removal of fat globule interference by emulsification (*10, 11*).

Reagents

(*a*) Fluorochrome-containing diluent, consisted of isotonic phosphate buffered saline, pH 7.4, with pure acridine orange dissolved in a concentration of 1 ppm (1 μg per ml). In what follows this reagent will be designated PBS-AO. (*b*) Reagents for fat emulsification technique for use with the electrical conductivity counter were prepared according to Tolle et al. (*10, 11*).

Measurement procedure

(*a*) The Cytofluorograf was set to display Scatter (measure of particle size) (*3*) on the Y axis and Green Fluorescence (measure DNA content per cell) (*6*) on the X axis. Scatter gain was set to Medium; green fluorescence gain was set at 8.00 (not a critical setting).

(*b*) A stained test sample was passed through the instrument and the Selected Count Area controls set to differentially count the somatic (fluorescing) cells.

(*c*) The milk specimens to be tested were mixed by inversion and diluted 400:1 in the PBS-AO reagent (e.g., 50 μl of milk in 20 ml of PBS-AO). After incubation for 5 min, samples were inverted and then introduced ony by one into the Cytofluorograf for counting. The instrument counts 0.1 ml of the diluted milk; hence, the result displayed on the Selected Count register must be multiplied by 4,000 to give the somatic cell count per ml.

RESULTS

In tests conducted according to the above methods on a series of 20 milk samples, the following results were obtained: (*a*) Figure 2 shows a regression line indicating

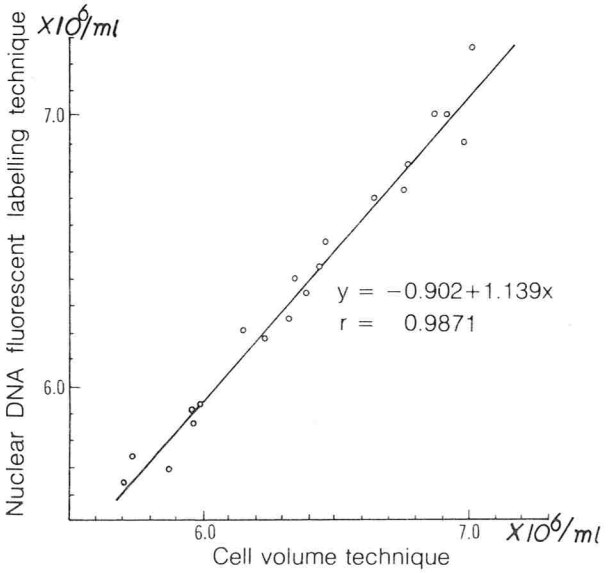


Figure 2. *Regression line showing the correlation between somatic cell counts by the fluorescence (Cytrofluorograf 4800A) and cell volume (Digicell 100) methods on a twenty sample series of milk from cows with mastitis.*

the degree of correlation between the two methods of counting somatic cells in milk samples of cows with mastitis. The two methods were (i) the nuclear DNA fluorescent labelling technique (using a Cytofluorograf 4800A) and (ii) the cell volume technique (using a Digicell 100). The correlation coefficient was r = 0.987.

(*b*) To assess the ability of the Cytofluorograf to count damaged cells in milk, the following experiment was done. Twenty mastitis milk samples were sonicated in a Vortex-Genie Sonicator (Scientific Industries, Inc., Springfield, Massachusetts) for 30 sec at setting #10, and then one half of each sample was frozen at —18 C for 24 h. The other half was stored for the same period of time at + 2 C. After thawing, the frozen and unfrozen samples were stained as described above, and simultaneously processed on the Cytofluorograf 4800A and the Digicell 100.

The average decrease in the somatic cell count in the samples after freezing and thawing as above was 11.9% in the fluorescence based method compared to 69.7% in the cell volume method. It seems that freezing and thawing reduces the cell size (Fig. 3b, 4, 5), so that the cell volume technique cannot differentiate between the cell fragments and the background.

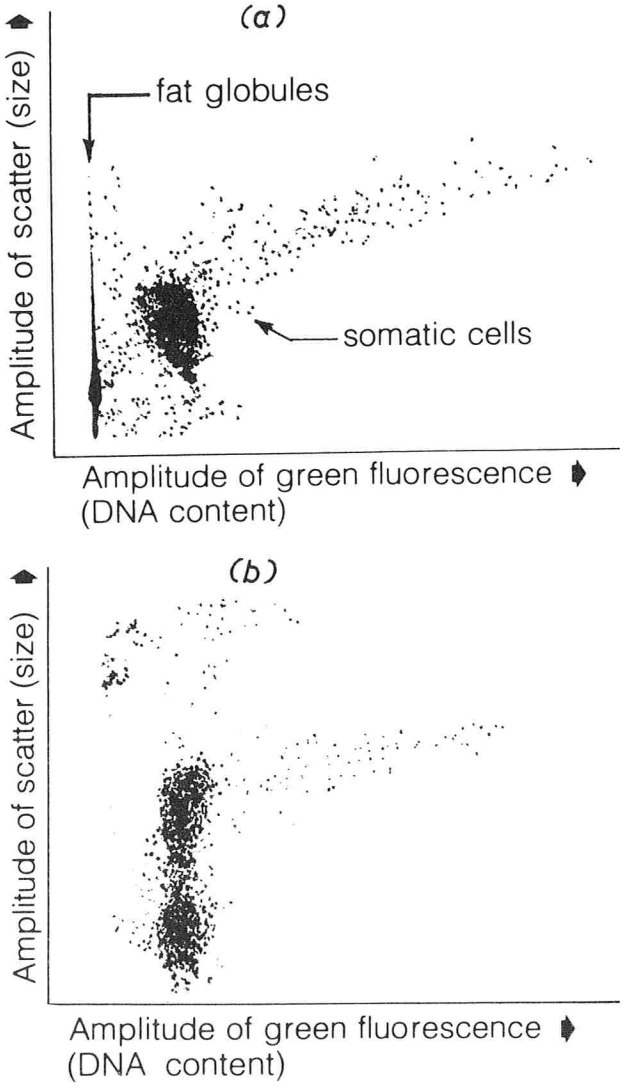


Figure 3. *Two-dimensional scatter diagram of size vs DNA content for all particles (fat and somatic cells) in a typical mastitis milk sample. (a) Fat globules are distributed along vertical axis. The nucleated somatic cells fluoresce and appear as the population to the right. In (b) the size vs DNA scatter diagram of a mastitis milk sample that has undergone freezing and thawing is shown for comparision. The fat flobules have been eliminated by the electronic threshold of the instrument. The nucleated cells are seen to divide into two sub-populations, presumably representing cells with intact cytoplasms (upper cluster, larger size) and cells stripped of their cytoplasm (lower cluster, smaller size).*

Figures 3a and b show the size vs. DNA content distributions of a mastitis milk sample, of which one half had not been frozen (Fig. 3a) and the other half had been frozen and thawed before measurement (Fig. 3b). The lower population in Fig. 3b (smaller size) presumably represents damaged cells which still retain an intact nucleus as evidenced by the fact that the DNA content is the same as in the upper (larger size) population. The lower population probably represents the fraction that would be lost in the cell volume method. Figures 4a and b show the cell volume distributions for an unfrozen and a frozen sample respectively, illustrating the severe decrease in resolution following freezing and thawing treatment. Figures 5a and c give the DNA per cell distribution for somatic cells, as obtained with the Cytofluorograf in an untreated and a treated sample, showing little difference in resolution between the two.

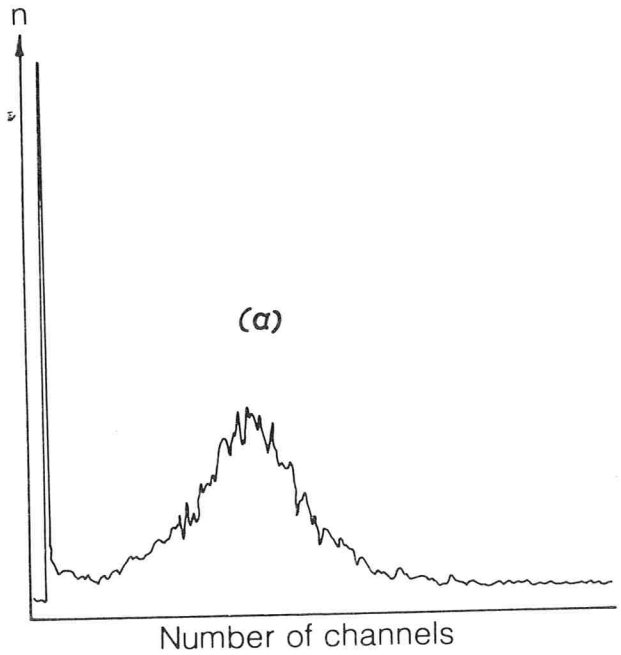


Figure 4. *(a) Typical size distribution of somatic cells of an unfrozen milk sample as obtained by the cell volume technique. (b) Size distribution of somatic cells of frozen and thawed sample obtained by the cell volume technique on the same scale. Resolution of somatic cells is poor in this instance.*

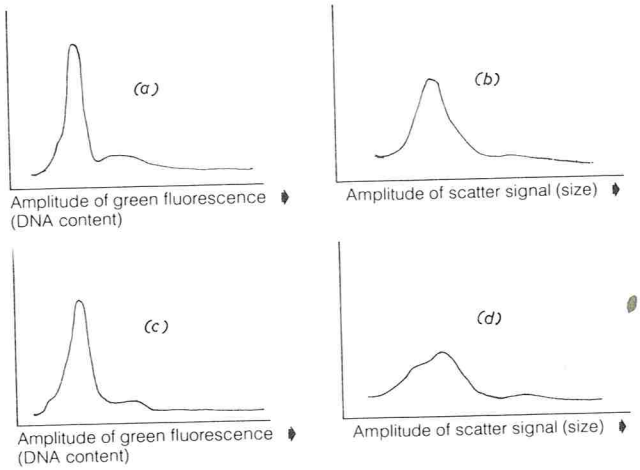
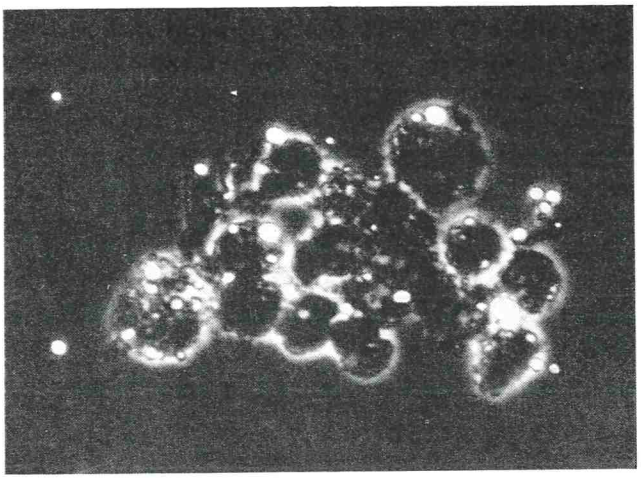


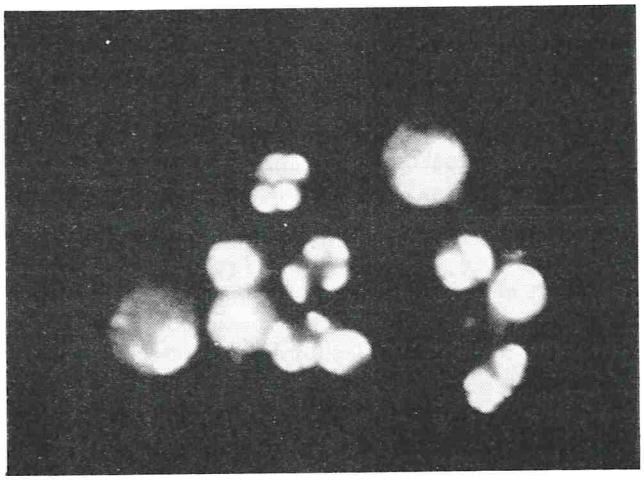
Figure 5. *(a) (b) Respectively, DNA content distribution and size distribution of somatic cells in the unfrozen milk sample shown in Fig. 3 (a).*
(c) (d) DNA content distribution and size distribution of somatic cells of the frozen and thawed sample in Fig. 3(b). Note that DNA distributions of frozen and unfrozen samples are the same, while the size distributions differ considerably.

DISCUSSION

Figures 6a and b (originally taken in color) show how the acridine orange-stained nuclei fluoresce while fat and protein elements do not. Figure 7 shows the distribution of size on the electrical conductivity cell counting instrument after emulsification of the fat, compared to the distribution of DNA per cell (intensity of green fluorescence) obtained with the Cytofluorograf. Figure 3a shows the distribution of size versus DNA content for all the cells in a typical mastitis sample as obtained by means of the Cytofluorograf. Each dot on the diagram represents a cell that has passed through the laser beam. DNA content (measured by green fluorescence of acridine orange bound to DNA (*6*) forms the X coordinate and size (measured by amplitude of forward angle scatter (*3*) forms the Y coordinate. The fat globules are clustered along the Y (size) axis with no fluorescence, while the somatic cells are distributed horizontally across the cytogram since they have both a size and a DNA distribution. The small fraction of particles to the far right represent clumps of two or more somatic cells or somatic cells in various stages of DNA



(*a*) *Non-fluorescent illumination*



(*b*) *Fluorescent illumination*

Figure 6. (*a*) (*b*) *Photos with fluorescent and non-fluorescent illumination to show uptake of A.O. by somatic cell nuclei.*

synthesis. For counting the somatic cells a vertical threshold provided by the Cytofluorograf electronics is set between the fat globule and the somatic cell clusters. This setting is not critical in that any setting in the gap between the two clusters will give the same reading within a fraction of a percent.

CONCLUSION

The method described here is a simple and more rapid procedure for counting somatic cells in milk. By its nature, the method would appear to be very specific for determination of nucleated cells. The present work was undertaken to evaluate the feasibility of the method before committing resources to a more extensive study.

ACKNOWLEDGMENT

We wish to thank Sandoz AG, Basel, for the use of their equipment and facilities.

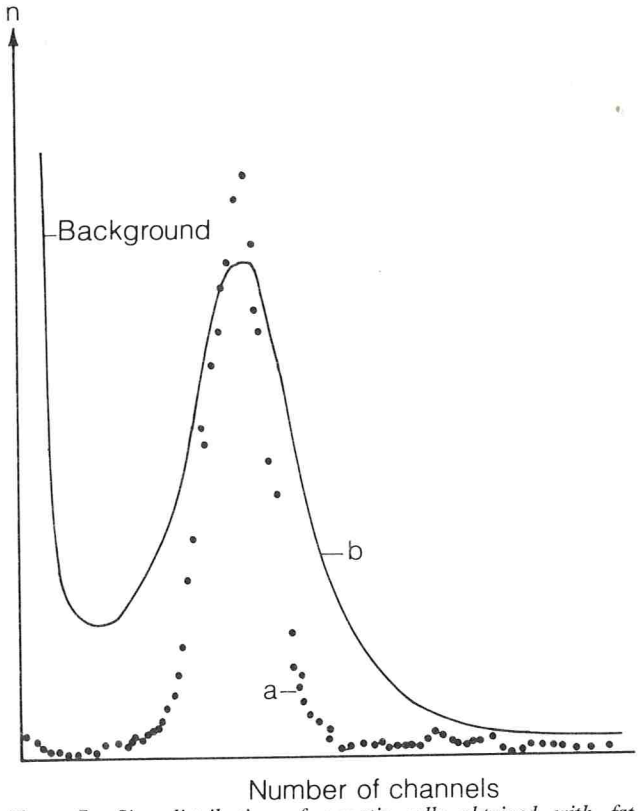


Figure 7. *Size distribution of somatic cells obtained with fat emulsification and measured on the Digicell 100 (b), super-imposed on the cellular DNA content distribution as obtained with the Cytofluorograf (a).*

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J. Milk Food Technol. Vol. 39, No. 9, Pages 628-646 (September, 1976)

Enrichment and Plating Methodology for Salmonella Detection in Food. A Review[1]

DIANE J. FAGERBERG and JOHN S. AVENS

Department of Animal Sciences
Colorado State University, Fort Collins, Colorado 80523

(Received for publication March 18, 1976)

ABSTRACT

Much research concerned with enrichment and plating methodology to detect salmonella contamination in foods has been reported by many scientists. This review brings reported findings of major proponents together into one text for greater understanding and appreciation of the complexity of the problem. Discussed in this review are reported applications and mechanisms of 11 enrichment media and eight plating media, incubation temperatures and times, and serotype specificity involving enrichment and plating media. Also, enrichment emulsifying agents, agitation during incubation, sample type, and level and proportion of salmonellae and competitors as related to salmonella enrichment are discussed. Other factors related to salmonella recovery, such as multiple media methods, preparation methods, storage of media, and media brand are included in this review, as well as a discussion of the methodology dilemma and some general recommendations for future direction.

The genus *Salmonella,* within the family *Enterobacteriaceae,* is composed of more than 1400 serotypes and bioserotypes. These bacteria are gram-negative, non-spore-forming, aerobic rods, most of which are peritrichously flagellated and naturally inhabit the intestinal tract of humans and many other animals and have worldwide distribution.

All species are potentially pathogenic for humans, animals, or both (*28*). A few species are host specific; *Salmonella typhi* causes typhoid fever in man, *Salmonella gallinarum* causes fowl typhoid in chickens and turkeys and *Salmonella pullorum* causes pullorum disease in poultry. Most serotypes show little host specificity and can cause the gastrointestinal disease, salmonellosis, when ingested by man. Symptoms associated with the illness are frequently mild and self-limiting with recovery within 3 or 4 days. Despite usual rapid recovery from the disease, more severe complications often occur among very young, elderly, and persons with underlying disease problems as the infection spreads from the intestine to other systems of the body, resulting in serious morbidity and even mortality. The number of human salmonellosis cases per year in the United States is conservatively estimated to be two million (*38, 90, 99*).

[1]*Published with approval of the Director of the Colorado State University Experiment Station as Scientific Series paper number 2136.*

Salmonellae are widespread in the environment and appear in a wide variety of foods and food ingredients, posing a great problem to the food industry. Salmonella organisms are transmitted primarily via the fecal-oral route since the primary reservoir is the intestinal tract of man and animals. From this reservoir, organisms are spread to air, food, feed, soil and water from where they may invade other hosts. These organisms can contaminate our food supply by many direct and indirect routes.

Salmonella organisms have been recovered from domestic and wild animals, specifically poultry and other livestock, rodents, reptiles, birds, and insects and other arthropods (*99*). Transmission of salmonellae from animals to humans via food is a serious public health problem. Foods implicated in salmonellosis cases include: poultry meat, eggs, dairy products, red meat and meat products, sausage, meat pies, fish and fishery products, desiccated coconut, cake mixes, custard-filled bakery products, chocolate and other confections, cereals and grain products, carmine dye, and other products which are cooked at a low temperature and are subject to much handling. Frequency of salmonella isolation from domestic poultry indicates they may be the largest single reservoir of these organisms among animals (*29, 99*). More than two-thirds of the salmonellae isolated from animals involved poultry. Foster (*39*) indicated three-fourths of the salmonella outbreaks in which the vehicle could be identified were attributable to meat, poultry, and egg products.

Food processors are not allowed to sell products containing salmonellae. Such food products, according to the Food, Drug and Cosmetics Act (*61*) are adulterated because they contain harmful or pathogenic micro-organisms. Most food processors make reasonable effort to achieve a high degree of processing plant sanitation and, thereby, control salmonella contamination; many have incorporated testing and control programs and have improved and remodeled facilities and equipment. Reflecting the economic impact and importance of the salmonella problem, a National Academy of Sciences report (*90*) estimates total cost to the American economy to be at least $300 million annually, including victim and

industry costs.

Public health agencies as well as food processors are searching for solutions to the salmonella problem, including determination of sources of salmonella contamination, and most important, remedial measures. The ultimate goal in food processing plants is to prevent salmonella contamination of food products, which depends on a control program requiring adequate sanitation and monitoring of the environment and all processing steps.

Assessment that a salmonella control program is effective depends on the reliability of bacteriological analytical procedures employed. As Lewis and Hall (*99*) state, laboratory detection of salmonalle is not an end in itself toward salmonella control, but it is an important and necessary tool for directing and monitoring control efforts. It is with this phase of the complex salmonella control effort, laboratory detection of salmonella contamination, that the present review is concerned. The large amount of research reported concerning this topic necessitates a literature review to bring the reported findings of many proponents together into one text for greater understanding and appreciation of the complexity of the problem.

GENERAL DESCRIPTION AND DISCUSSION OF ANALYTICAL METHODS

Identification of sources of food product contamination and/or recontamination, and determination that food samples are below detectable levels of salmonellae--major problems confronting food industries, especially poultry, today--can be provided only by bacteriological laboratory examination. The validity of results is directly dependent on laboratory techniques and media employed in the analyses.

Numerous salmonella analytical procedures have been proposed for food, but not one method has been proven better than all others for all applications. Most accepted methods are cumbersome, time-consuming and laborious, and basically involve pre-enrichment, enrichment, selective plating, and biochemical and serological tests. Raw foods and finished products that have not been heat processed do not require the pre-enrichment step and are usually examined by direct enrichment in selective broths.

Methods for isolation of salmonellae were first developed for clinical specimens because of widespread human and animal diseases (*42*). Later, when food became apparent as an important factor in the chain of salmonellosis infections, microbiologists naturally applied methods shown satisfactory for clinical samples to food samples. It was found these methods did not always effectively detect salmonellae in foods and, in fact, the media were somewhat toxic to the organisms (*53*). No ideal procedure has yet been devised for recovery of all samonella serotypes from all types of food. Also, there is no single recommended procedure for some specific food products, for example, poultry meat which is one of the most frequently incriminated food products.

The Subcommittee on Food Microbiology of the Food Protection Committee of the National Research Council, National Academy of Sciences, has published a booklet entitled, *Reference Methods for the Microbiological Examination of Foods* (*95*). They stated if microbiological criteria (maximum acceptable numbers of microorganisms) for classes of foods are to be set and enforced, methodology for sampling and analysis must be agreed upon. They also said such methods have not been standardized. At least nine different agencies have published, or are considering publishing microbiological methods and criteria for foods. These methods are not in complete agreement as to analytical procedure. Standard methods have not yet been agreed upon for most food commodities.

The Subcommittee on Food Microbiology (*95*) has proposed "reference methods" to serve as guides to the development of uniform standard methods. They state that "...a reference method should be the best available provided it is practicable. It should not represent compromise between an excellent method and one of lesser excellence because of expediency. Such limitations as the reference method has should be recognized and stated." They recommended where a single reference procedure has not been agreed upon, a study should be set up in each of several laboratories to compare variations in procedure using specific foods. Acceptable methods evolved should then be subjected to collaborative study.

The search for more sensitive and rapid salmonella analytical techniques has led to a proliferation of methods which propose new and modified media, both in the clinical microbiology field and in food and feed analysis. Research on methodology has been aimed toward improvements in specificity, sensitivity, simplicity, and rapidity. Although there have been indications one method may be superior to another, Banwart and Ayres (*8*) stated, in some instances results obtained from certain samples or from a given quantity of sample were basis for stating the superiority of a method for any sample of any size. Conclusions derived from such comparisons do not necessarily mean one method is better for all types of food or for all salmonella serotypes. Another inadequacy of comparative methodology studies was revealed by Taylor and Schelhart (*123*), who warned of an inherent lack of correlation between results noted in pure culture studies and observations resulting from empiric practice. They claimed, "nowhere in microbiology is that lack of corroboration any more apparent than in isolation of enteric pathogens from clinical specimens or food products, animal feeds, and similar substances." Observations from researcher to researcher, or even analyses by the same person done on the same premises at different times often depict lack of consistency (*123*).

Many variables have been implicated as important to salmonella analyses; some are controllable, others are not. These variables, including types of enrichment and plating media, technical factors causing variations in en-

richment and plating media, number of pathogens versus competitors, salmonella serotypes, sample types, and length and temperature of incubation will be discussed in the following literature review. References are made throughout the review to sample types other than foods; but it must be realized that findings with other sample types, i.e. clinical specimens, are not necessarily exclusive for that sample type, but may be quite applicable to food products and should not be ignored.

MECHANISMS OF ENRICHMENT MEDIA AND REPORTED APPLICATIONS

Usually if salmonella organisms are present, their relative numbers in almost every kind of food product examined are small compared to greater numbers of non-pathogenic organisms of similar cultural characteristics. Proof of the presence of salmonellae requires isolation of the organism in pure culture from among the more numerous associated microorganisms. This may be achieved by utilization of enrichment media that through various mechanisms may favor and enhance growth and multiplication of salmonellae while other gram-negative organisms such as coliforms, *Proteus,* and *Pseudomonas* are suppressed or inhibited. Gram-positive organisms are easily inhibited since they are, in general, quite susceptible to the toxic effects of substances in enrichment media such as dyes, metals and other selective agents. Galton et al. (*42*) mentioned it is difficult to recover salmonellae without selective enrichment if the ratio of normal coliforms to salmonellae is as low as 10:1 It is generally agreed that enrichment of food samples in either selective or non-selective broths is superior to direct plating, especially when very small numbers of salmonellae are anticipated (*42*). An ideal enrichment broth medium, then, should not be too inhibitory to support multiplication of salmonellae to detectable levels yet should be selective enough to prevent overgrowth by non-pathogens. An enrichment broth, to be acceptable, must also meet other criteria. Provided the medium is prepared and used properly, it must not lose selectivity due to addition of a sample, and should allow recovery of all salmonella serotypes. Type of samples inoculated, proportion of inoculum to broth, and time and temperature of incubation must also be considered since such factors may substantially influence selectivity of enrichment media (*42*).

It is apparent from the many modifications in enrichment medium and methodology proposed throughout the years that no one enrichment medium or methodology is perfectly satisfactory (*87*). No single enrichment broth can be depended upon to give consistent isolation of salmonellae under all conditions encountered. The literature is filled with various claims for superiority of one or another medium, with obvious diversifications, contradictions, and ambiguity of opinions.

Tetrathionate brilliant green and selenite-F media

Many enrichment media have been proposed for isolation of salmonellae. Muller's (*94*) tetrathionate broth as modified by Kauffman (*75, 76*) and Leifson's (*84*) selenite-F (SF) broth have been used widely to recover salmonellae from fecal specimens as well as food products. They have been widely used to isolate salmonellae from egg products, dried milk, poultry products, fish meal and other animal feeds, fertilizers, mesenteric glands, sewage, and fecal specimens (*70*). Galton (*40*) acknowledged that tetrathionate brilliant green (TETBG), Kauffman's modification of Muller's tetrathionate broth, has been successfully used to isolate salmonellae from a variety of human and animal food products. Edwards and Ewing (*27*) endorsed the usefulness of tetrathionate broth for isolating salmonellae from clinical specimens. Selenite and TETBG were deemed greatly superior to other enrichment media by Smith (*113*). Guinee and Kampelmacher (*47*) found TETBG equal to a modification of selenite, selenite brilliant green, in isolating salmonellae from porcine feces and skin scrapings. Heidrich (*56*) observed no appreciable difference between TETBG and SF in isolating salmonellae from fecal and organ samples, whereas Sharma and Packer (*111*) experimented with artificially contaminated cow and pig feces and found that SF yielded fewer salmonella isolations than did TETBG. Contrary to these observations, Smyser and Snoeyenbos (*116*) observed quite poor TETBG performance with poultry litter and animal feeds, though an earlier publication by Smyser et al. (*115*) indicated tetrathionate and TETBG to be better than SF for detecting *S. typhimurium* in artifically contaminated poultry feed and animal by-products. Carlson and Snoeyenbos (*13*) found tetrathionate to be generally better than the selenites for recovery of salmonellae artificially introduced into chick starter, used poultry litter, and mixed in a test system with fecal microflora. McCullough and Byrne (*87*) considered TETBG better than SF for salmonella isolation from artificially infected human volunteers, Studies with egg meat, turkey rolls, and chicken feces reported by Cox et al. (*22*) revealed TETBG inferior to other enrichment media tested for salmonella isolation.

TETBG is one of the enrichment media recommended in the Food and Drug Administration's (FDA's) *Bacteriological Analytical Manual* (*37*) for analysis of raw or highly contaminated meats, animal substances, glandular products, and fish meal. The National Academy of Sciences (NAS) (*95*) recommended TETBG as one of the enrichments for detection of salmonellae in foods. Galton et al. (*42*), in procedures for meats, other non-processed food, rendered animal by-products, and feeds suggested TETBG enrichment. The Association of Official Analytical Chemists (AOAC) (*5*) microbiological methods for analysis of egg and milk products also recommended TETBG as one of the acceptable enrichments.

As stated in the USDA *Microbiology Laboratory Guidebook* (*19*), TETBG is a peptone medium containing bile salts for inhibiting gram-positive organisms, brilliant green for inhibiting gram-positive

and gram-negative lactose fermenters and tetrathionate for toxicity to enterics besides salmonellae. It is highly buffered with calcium carbonate so that pH is of no concern. In their 1970 publication, Palumbo and Alford (*98*) stated the mechanisms of tetrathionate inhibition were not yet fully known. They stated that the iodides probably have no lethal effect. Lethality to bacteria was directly related to the concentration of thiosulfate and tetrathionate--only the combination of the two being toxic. They stated the lethal effect was a growth-related phenomenon--since no killing occurs with non-growing cells. They related it is known that tetrathionate reacts with free sulfhydryl groups of enzymes and causes their inactivation and that thiosulfate can also react with sulfhydrl groups. They suggested tetrathionate interferes with synthesis, activity, or both of sulfur-containing enzymes or cell wall and membrane components.

Selenite-F is a peptone base medium containing sodium selenite as a selective agent against enterics besides salmonellae. The mechanism of selenite toxicity is also not known, but Weiss et al. (*128*) proposed two different general mechanisms, (*a*) selenite reacts with sulfhydryl groups of cellular components and (*b*) selenium is incorporated into analogues of sulfur compounds (seleno-amino acids may possibly be formed because of metabolic similarity of selenium with sulfur). They found during early incubation, a sharply increased uptake of selenium coincided with high susceptibility to selenite broth. They suggested that possibly the relative ratios of seleno-amino acids to their sulfur analogues caused the cell protein to become partially or completely inactive--resulting in less total growth, a reduced growth rate or complete cessation of growth. They stated there was also evidence of a non-metabolic reaction by which inorganic selenium compunds exert their toxic effects on organisms.

In Leifson's report (*84*) on his SF medium, he stated lactose in the medium served to maintain uniform pH. He reported that when selenite was reduced by growth of bacteria, the pH increased and could lessen the toxicity of the selenite and result in overgrowth of competitors if the acid produced by fermentation of lactose by enterococci and some colon bacilli was not present to maintain neutral or slightly lowered pH.

TT and selenite cystine media

Two important modifications of the two broths, Muller-Kauffman's tetrathionate and Leifson's SF were introduced in the 1950's. Hajna and Damon (*51*) noted considerable variation in productivity when tetrathionate broth was prepared according to many proposed modifications and therefore they made an effort to develop a better tetrathionate broth which would include the best features of the broths. They introduced a new tetrathionate enrichment, TT broth, that was selective for only the salmonella and *Arizona* group of bacteria. Almost twice as many positive salmonella isolations from stool specimens were achieved with TT compared to SF (*51*). Reviewed literature indicates TT broth has not been studied as extensively as many other enrichment broths, but United States Department of Agriculture (USDA) methods (*19*) recommended TT as the sole enrichment broth for raw meat, subsequent to lactose pre-enrichment. In a study to determine a preferred enrichment method for egg and diverse food specimens, Montford and Thatcher (*92*) found TT yielded many coliforms, but failed to provide a single colony of Salmonellae.

North and Bartram (*96*) modified selenite media by adjustment of types and amounts of phosphates and peptones to optimal levels to facilitate isolation of salmonellae from food products. They also added cystine to enhance growth of salmonellae in the presence of large amounts of organic material, thus termed selenite cystine (SC) enrichment broth. SC has been cited in many studies and is recommended by the FDA (*37*) in conjuction with TETBG for raw meats, by the NAS (*95*) in conjunction with TETBG for isolation of salmonellae from foods, also by the AOAC (*5*) in conjunction with TETBG for egg and milk products. When SC and TETBG were compared during salmonella analysis of meat and bone meal, Huhtanen and Naghski (*62*) found no difference in total number of positive samples between the two enrichments. SC was found to be one of the useful broths for detecting *S. typhimurium* from artificially contaminated poultry feed and animal by-products (*115*). SC was found to be the preferred enrichment for egg specimens and diverse foods (*92*), allowing the most favorable multiplication of lactose-negative organisms and inhibition of coliforms. Of the various media tested for selective enrichment of *S. typhimurium* from infected poultry tissues, Yamamoto et al. (*134*) found SC gave the most favorable results. Taylor and Silliker (*126*) remarked that in their years of experience comparing SC and TETBG, superiority of one or the other could not be claimed because usually one would be superior in one experiment and the other would prove superior in the next.

The Baltimore Biological Laboratory (BBL) *Manual of Products and Laboratory Procedures* (*6*) states TT is an improved form of TETBG with better defined composition than TETBG. TT is greatly enriched with additional nutriment by addition of yeast extract. Brilliant green and sodium desoxycholate serve as inhibitors of gram-positive organisms. Fermentation of dextrose and mannitol, according to Difco (*24*), aids the selective property of tetrathionate decomposition in the medium.

Selenite brilliant green and selenite brilliant green sulfa media

Significant modifications to the selenite group of media, particularly adapted to the search for salmonellae in foods, were introduced by Osborne and Stokes (*97, 120*) in 1955. The first modification described, selenite brilliant green (SBG), was claimed to prevent development of *Escherichi* and *Proteus,* yet support luxuriant growth of salmonellae even when the inoculum consisted only of one salmonella cell per ml medium

(*120*). Their data were derived from studies with pure cultures having a ratio of 1 salmonella to 100 other organisms; however, they conceded different results might be obtained when isolation of salmonellae from natural materials was attempted. In fact, they found addition of whole egg or egg yolk considerably reduced the selective properties of SBG, and subsequently found the neutralizing effect of egg products on SBG was eliminated by addition of sulfapyridine (*97*). Their selenite brilliant green sulfa (SBGS) enrichment medium also claimed successful recovery of salmonellae when only 1 salmonella cell and 100 *Proteus* or *Escherichia* were present per ml of medium. SBGS compared to SBG and SF, provided best recovery with the widest variety of salmonella serotypes from commercial egg whites in tests conducted by Wells et al (*129*). Some researchers have also tested SBG and SBGS enrichment broths for recovery of salmonellae from clinical specimens. Guinee et al. (*48*) determined SBG provided a better medium for examination of lymph nodes than did TETBG, whereas no difference between the two were noted from examination of feces. Kumar et al. (*81, 82*) had successful results employing SBGS for cloacal swab monitoring of salmonella-infected breeding flocks, and contaminated flock environments and feed. Carlson and Snoeyenbos (*13*) reported SBGS to be distinctly unsatisfactory for some salmonella strains and allowed major die-off of salmonellae between 24 and 48 h of incubation, especially at 43 C. Their research involved artificial mixtures of salmonellae and fecal microflora and artificial contamination of chick starter and used poultry litter. Montford and Thatcher (*92*) declared negative results with SBG and SBGS enrichment of frozen whole egg melange. Both enrichments provided many coliforms, but no salmonellae on subsequent plating, possibly due to the high proportion of coliforms to salmonellae, 39,000:0.15 cells per gram. Yamamoto et al. (*134*) considered SBGS too selective and less effective than SC and TETBG for salmonella isolation from turkey tissues and fecal samples. Fagerberg (*32*) and Fagerberg and Avens (*33, 34*) found SBGS to be superior, to 11 other enrichments (SC, SF, TETBG, TT, SBG, Rappaport's, GN, strontium selenite, strontium chloride, brilliant green MacConkey's, and neutral red-lysine-iron-cystine) for recovering salmonellae from contaminated turkey carcasses.

Stokes and Osborne (*120*), originators of SBG and SBGS, stated that inhibitory properties of the media against many gram-positive and gram-negative organisms were the sum of the activities of selenite, brilliant green, and sodium taurocholate in combination. They stated the balanced mixture of brilliant green and taurocholate made the medium more selective than SF. Yeast extract was added for additional nutriment and phosphate was added at a level to meet salmonella requirements. They used mannitol instead of lactose to help maintain proper pH because salmonellae can ferment mannitol.

Rappaport's medium

In 1956 Rappaport et al. (*106*) devised an enrichment broth claimed to permit unrestricted development of salmonellae yet inhibit growth of coliforms due to 4% magnesium chloride and 0.012% malachite green. Magnesium chloride counteracted the toxic effect of malachite green on salmonellae without affecting inhibition of gram-negative contaminants (*42*). The originators of Rappaport's (RAP) medium found it detected almost twice as many cases of salmonellosis as selenite or tetrathionate broths when inoculated with a dilute, 1:1,000, suspension of feces (*106*). Several researchers confirmed the superiority of Rappaport's magnesium chloride-malachite green medium. Collard and Unwin (*18*), Hooper and Jenkins (*60*), Taylor and Schelhart (*124*) Galton et al. (*42*), and Iveson and Kovacs (*69*), in comparative studies with different enrichment broths, maintained RAP was superior for isolation of salmonellae from fecal specimens. Since neither SF nor tetrathionate broths were deemed as effective as RAP for detection of salmonellae, Taylor and Schelhart (*124*) asserted either or both should be replaced by RAP in clinical microbiological laboratories. Taylor and Schelhart also mentioned RAP had become widely used in European laboratories, and in food industries even though it was designed for stool cultures. Iveson et al. (*70*), sampling contaminated desiccated coconut, and Anderson and Kennedy (*3*), working and pure cultures, also showed superiority of RAP. Burman (*10*) claimed RAP was superior to selenite broth because it did not react with selective plating media to produce inhibitory products or combinations of products which produce a growth inhibition zone at the site of inoculation. Other researcher's results differ: Jacobs et al. (*73*) compared RAP with tetrathionate, TETBG, and SBG in the examination of fish meal, and observed no differences in recovery of salmonellae among the four enrichments. Sen (*109*) obtained higher salmonellae yield in selenite broth, and Zajc-Satler and Banic (*135*) found their preparation of tetrathionate broth better than RAP for fecal isolations.

Rappaport et al. (*106*) devised the peptone-based RAP medium with selectivity system of magnesium chloride and malachite green to inhibit coliforms yet permit development of salmonellae. They stated that hypertonic salt solutions may dehydrate and kill bacteria and with this basis determined ions from magnesium chloride at the 4% level were selective against many organisms besides salmonellae. They found malachite green (0.012%) was better than other dyes tested for coliform inhibition.

Gram-negative broth

Hajna (*50*) in 1955 devised gram-negative (GN) broth for use as an enrichment medium for stool specimens and reported increased numbers of salmonellae and *Shigella* were isolated. Taylor and Schelhart (*122, 123, 124*) conducted several comparative studies which included GN broth. In 1967 they found 97.2% efficacy of

GN broth in detection of salmonellae from clinical specimens (*122*). The following year they found GN comparable to selenite for recovery of salmonellae from clinical specimens (*123*). In a later report they considered GN broth the best all-purpose broth for use with stool cultures, but when specifically concerned with salmonellae, found RAP was better than GN (*124*). Rollender et al. (*108*) achieved 98% salmonella recovery from fecal and urine samples using GN broth. Cox et al. (*22*) tested egg meat, chicken feces and turkey rolls for salmonellae and reported only slightly lower recovery with GN compared to RAP and SC. Edwards and Ewing (*27*) suggested a combination of media such as selenite and GN should be used in clinical laboratories when possible.

The tryptose in GN is nutriment, the phosphates are for buffering, and selectivity is attained by incorporation of carbohydrates and salts. The sodium citrate and desoxycholate are bactericial to gram-positive organisms and inhibit growth of coliforms. The greater concentration of mannitol over dextrose is to limit *Proteus* growth and accelerate growth of salmonellae (*24*).

Strontium selenite and strontium chloride media

Iveson and Mackay-Scollay (*71*) introduced two media, strontium selenite (SrSe) and strontium chloride (SrCl) M, modifications of which have replaced enrichment procedures previously used in their Australian Salmonella Diagnostic and Reference Laboratory. In 1969 they found SrCl M (with malachite green) comparable to RAP for recovery of a wide range of salmonella serotypes from human feces, sewage, pig feces, and glands (*71*). In 1971, Iveson (*67*) introduced a less selective SrClB (without malachite green) for improved isolation of *Edwardsiella* as well as salmonellae and *Arizona.* SrSe, also introduced in 1969, was determined superior to other enrichment media for recovery of *S. typhi* from human fecal matter (*71*) and useful for a variety of other products (*72*). Chau and Huang (*16*) and Chau and Forrest (*15*) found SrSe superior to SF for isolation of *S. Typhi* from clinical specimens. Iveson (*68*) modified SrSe and reported improved results with the SrSe A modification. He concluded SrCl B and SrSe A enrichments allowed improved isolation of salmonellae, *Arizona, Edwardsiella,* and *Shigella* from human, animal and environmental samples (*68*).

Iveson and Mackay-Scollay (*71*) utilized essentially the same basis for development of SrSe and SrCl as Rappaport et al. (*106*) did in development of RAP-that of incorporating specific ions into an enrichment capable of recovering salmonellae and inhibiting non-pathogenic gram-negative organisms. They found the strontium ion in the form of a chloride and also in the form of a selenite salt to be favorable. Iveson (*68*) found it unnecessary to include fermentable carbohydrates, dyes, antibiotics, or accessory growth factors in either SrSe or SrCl. Di-sodium hydrogen phosphate was added to maintain optimum pH. Iveson stated the reduction of selenite to selenium in the SrSe media was similar to that in SF, but slightly less intense.

Neutral red-lysine-iron-cystine broth

Hargrove et al. (*53*), in 1971, developed a medium, neutral red-lysine-iron-cystine broth, to differentiate salmonellae from other *Enterobacteriaceae* and organisms commonly found in dairy products and to shorten the time required for identification. A positive presumptive test for salmonellae in dairy products was indicated by a color change from red to yellow and/or production of massive black precipitate of iron sulfide after 24 h of incubation. Absence of salmonellae was indicated by no color change or no medium blackening. Results from several dairy products tests indicated its usefulness in rapid screening of these foods. Hoben et al. (*59*) evaluated the procedure of Hargrove et al. and modified the technique for routine testing of foods, food ingredients, and feed materials for the presence of viable salmonellae. Samples were first enriched in tetrathionate broth, then transferred to a broth they termed lysine-iron-cystine-neutral red, for further enrichment and detection of salmonellae by color change. They established lysine-iron-cystine-neutral red was useful as a screening technique to rapidly eliminate salmonella-negative samples and presumptively identify salmonella-positive samples.

MAJOR VARIABLE FACTORS ASSOCIATED WITH ENRICHMENT METHODOLOGY

The course of fluid enrichment, as in many biological phenomena, can be determined by studying the complex interactions of variables. Manipulations of variables, conducive to attaining the most efficient and accurate methodology, must be made for each specific situation encountered for salmonella enrichment. Researchers have reported influence of enrichment media performance by elevated temperatures of incubation, addition of detergent emulsifiers, and agitation or aeration of incubating enrichments. Incubation time, numbers of salmonellae versus competitors, sample material, and salmonella serotypes also affect the enriching ability of fluid media for salmonellae.

Incubation temperature

Harvey and Thomson in 1953 (*55*) first reported selenite broth incubated at 43 C was superior to 37 C incubation for isolating salmonellae from human fecal samples. They tested 42, 43, and 44 C and mentioned 43 C was optimum with the proviso that 43 C might be safer, as it possibly represented the upper end of the useful temperature range. Alteration of incubation temperatures is a well known aid in purifying bacterial cultures, but elevated temperature techniques have mainly been used to isolate thermophiles (*54*). Since the report of Harvey and Thomson (*55*), other investigators have endorsed the advantage of elevated temperatures (41 to 43 C) for recovering salmonellae from mixed pure cultures; human, poultry, pig, and reptile fecal specimens; stream, river, sewage, and abattoir waters; minced meat, sausage, milk, and other foodstuffs; meat and bone meal, animal by-products,

pathological tissues, and various other specimens. The advantage most often observed has been suppression of competing contaminants without inhibition of salmonellae; the salmonellae multiplying more rapidly and profusely in an environment of reduced competition, thus producing a relatively pure culture and providing an advantage for isolation and identification (*12, 14, 93, 116, 119*).

Some researchers, to ascertain intrinsic principles responsible for increased salmonella recovery at elevated temperatures, studied population dynamics of salmonellae and common competitor contaminants in selective and non-selective media at higher temperatures. Georgala and Boothroyd (*43*) tested three salmonella serotypes, two paracolon organisms, *Proteus,* and *Escherichia freundii* in SF enrichment broth. Growth curves showed at 37 C salmonellae reached the greatest population and *E. freundii* and *Proteus* developed two logarithms lower than salmonellae. At 43 C salmonella growth still remained greater than the other organisms, but less than at 37 C. They stated incubating SF at 43 instead of 37 C appeared to improve selectivity of the medium for salmonellae likely to be found in foods. Carlson et al. (*14*) studied pure growth of *S. montivideo, Proteus mirabilis,* and *E.coli* in SBGS broth and non-selective trypticase soy broth incubated at 37 and 43 C. Growth of all organisms in trypticase soy broth incubated at 43 C was equal to 37 C growth. SBGS at both temperatures was inhibitory to all three organisms, but much more to *Proteus* and *Escherichia.* Salmonellae optimally developed at 37 C in SBGS, compared to 10^4 fewer salmonella organisms from 43 C incubation. In SBGS enrichment there was no difference in *Proteus* population between 37 and 43 C, but the *Escherichia* population was less at 43 than 37 C. SBGS broth appeared to be sufficiently selective at 37 C, 10^7 to 10^8 more salmonella cells than either competitor. An elevated temperature of 43 C was unnecessary and actually not an improvement in selectivity since at 43 C there were only 10^3 to 10^5 more salmonellae than competitors. Carlson and Snoeyenbos later devoted an entire study to population kinetics (*12*). Their study revealed pure culture populations of *S. typhimurium*, in trypticase soy broth, TETBG, and SBGS, were greater at 37 than 43 C during the stationary growth phase. Increased temperature lengthened the generation time of salmonellae in both TETBG and SBGS. Nearly the same observations were noted with simulated natural contamination in meat and bone meal, but with samples of naturally contaminated poultry litter they demonstrated the advantageous effects of elevated temperature incubation. With the latter samples, competitor organisms reached a peak after 16 h then decreased in SBGS and TETBG at 43 C, whereas at 37 C the competitor population in TETBG was equal to the salmonella population, and greater than the salmonella population in SBGS. The rapid die-off of competitor organisms during a period of relative stability of the salmonella population demonstrated elevated temperature had a significant effect with naturally contaminated poultry litter. Erdman (*30*) found 43 C better than 37 C for incubation of SBGS, SC, and TETBG to recover salmonellae from artificially contaminated ground beef. Carlson and Snoeyenbos (*12*) remarked that the improved rate of salmonella isolation after higher enrichment incubation temperatures compared to 35-37 C enrichment incubation is probably explained by greater ease of selecting salmonella colonies from plates showing fewer coliforms but not salmonellae. With pure culture growth curves derived from salmonella and *E. coli* in SC, Alford and Knight (*2*) found 42 C incubation shortened the lag phase of *E. coli* and lengthened the lag of *S. blockley* compared to 37 C, both lag phases lasting 5 h at 42 C. Because there was a better lag differential, S. *blockley* 4 h and *E. coli* 8 h at 37 C, Alford and Knight chose not to use elevated temperatures in their further studies.

Incubation temperature comparisons by Morris and Dunn (*93*) using TETBG inoculated with sausage samples, Smyser et al. (*117*) testing animal by-products and poultry litter in SBGS and TETBG, Radan et al. (*103*) isolating salmonellae from food and feeds of animal origin in tetrathionate and selenite broths, Greenfield and Bankier (*45*) employing SF inoculated from pathological tissues, foodstuffs and various materials submitted to their veterinary laboratory, Dixon (*25*) employing selenite for enrichment of human feces, and Iveson and Mackay-Scollay (*72*) using SrCl M and SF with effluent samples, have all demonstrated improved salmonella isolation at 43 C incubation temperature compared to 37 C. Spino (*119*) utilized a temperature of 41.5 C with SBGS and tetrathionate for isolating salmonellae from stream waters and described consistent recovery of salmonellae at the elevated temperature, whereas no salmonellae were recovered at 37 C. He, as well as Smyser and Snoeyenbos (*116*), noted indirectly, from growth on selective plating media, growth of competing organisms was markedly reduced in enrichments incubated at elevated temperatures compared to 37 C incubation. Smyser and Snoeyenbos (*116*) analyzing poultry litter in SBGS at 43 C and in TETBG at 37 C, found that of 73 positive samples only two plates showed *Proteus* from SBGS at 43 C, whereas from TETBG at 37 C all plates were so overgrown with *Proteus* they did not even attempt selection of colonies for further analysis. Carlson et al. (*14*) enriching meat and bone meal and poultry litter samples in SBGS found the percent positive samples were higher and there was a decrease in number of *Proteus, Citrobacter,* and coliforms on plating media when 43 C enrichment incubation was used compared to 37 C incubation. Enumeration from their samples indicated only about 100 salmonella organisms per gram. They noted the improved isolation rate at 43 C would probably not have been apparent if samples had been heavily contaminated with salmonellae, since the elevated temperature probably facilitated isolation when the organism was present in low numbers by preventing

overgrowth by competitors. Wells et al. (*130*) reported more salmonella recoveries from raw milk were made at 43 C incubation of TETBG at salmonella inoculum levels of 1000, 100, and 10 cells per liter of milk, than at 37 C. Also, at 43 C frequent recoveries were made from samples containing one salmonella organism per liter, whereas no recoveries were made at this level at 37 C. Burman (*10*) reported the Metropolitan Water Board found for samples such as sewage effluent containing large numbers of interfering organisms, incubation of selenite cultures at 43 C gave better recovery of salmonellae than at 37 C, whereas with cleaner samples such as river water 37 C was better. Banffer (*7*) showed SF enrichment at 43 C was significantly better than at 35 C and seemed appropriate for isolation of salmonellae from human convalescent excretors and carriers having few salmonellae. Banffer (*7*) and Carlson et al. (*14*) found more salmonella serotypes were isolated from convalescent excretors and carriers more frequently at 43 C. Edel and Kampelmacher (*26*) found 43 C enrichment improved salmonella isolation from pig feces and minced meat, but also indicated other factors such as the nature of the medium played an important role in addition to temperature. Analyzing reptile feces, Koopman and Janssen (*79*) found tetrathionate incubated at 43 C better than tetrathionate or selenite at 37 C, but in analyzing dog and cat feces found 37 C better.

McCoy (*86*) in 1962 found tetrathionate incubated at 43 C was lethal to salmonellae and most organisms, and isolation of salmonellae from selenite incubated at 43 C was significantly less than at 37 C incubation. Aleksic et al. (*1*), in 1973, reported distinctly better salmonellae isolation from carriers when selenite was incubated at 37 C compared to 43 C. Harvey and Price (*54*) suggested lack of success with the elevated temperature of 43 C in some laboratories probably could be explained on the basis of difference in sample types or techniques. They mentioned specimens yielding salmonellae easily at 37 C enrichment incubation were not likely to yield more at 43 C and samples containing minimal numbers of salmonellae, most in need of resuscitation, may be better examined at 37 C. They also stated media preparation was a relevant factor to utilizing 43 C incubation. They never heat their SF, but rather filter-sterilize it.

Fagerberg (*32*) and Fagerberg and Avens (*33*) found effects of 35-37 C versus 41-43 C enrichment broth incubation were enrichment media dependent for SC, SBGS, TETBG, TT, Rappaport's, and strontium selenite. SC and TETBG, compared to the other broths, showed the least differences between the two temperatures and lent themselves to incubation at either temperature better than any of the other broths, yet neither performed as well at 41-43 C.

Methods of analysis of the AOAC, FDA, and USDA (*5, 19, 37*), do not currently recommend elevated temperature incubation of enrichments in their procedures for salmonella analysis. The USDA *Microbiology Laboratory Guidebook* (*19*) states they are evaluating the alternative of enriching at 43 C and the "analyst may, at his discretion, incubate enrichment media at 43 C."

Emulsifying agents

When Galton (*40*) enriched sausage for salmonella isolation, after incubation she found the heavy layer of fat on the surface made it difficult to obtain a satisfactory loopful of material for streaking plates. To overcome the problem, she added a wetting agent, Tergitol 7 (T-7) to a level in the enrichment broth, 0.6%, determined by titration to provide emulsification of the fat and give a distinct head of foam. No evidence of salmonella inhibition was noted with concentrations up to 0.7%, rather T-7 appeared to enhance salmonella growth. Similar findings were noted with Tween 80. T-7 is now commonly used in salmonella enrichments for products of animal origin and other high fat samples. Galton et al. (*42*), in their recommended procedures for isolation of salmonellae from foods and feeds, the FDA (*37*), USDA (*19*), and NAS (*95*) recommend use of T-7 in enrichments for fatty samples. Fagerberg and Avens (*31, 33, 35*) and Chen (*17*) have found Tween 80 emulsifier better than T-7 or no emulsification for enriching turkey skin samples. Morris and Dunn (*93*), using T-7 at 0.6% in TETBG incubated at 37 C for sausage samples, found significantly more salmonella isolations compared to not adding T-7. However, no advantage was derived from adding T-7 when enrichments were incubated at 43 C. At the higher temperature the fat problem was not so troublesome, but results indicated there may have even been a disadvantage to adding T-7 and incubating the enrichments at 43 C. Some authors reported negative results on adding T-7. Carlson et al. (*14*) found the additon of T-7 at 0.6% in SBGS inoculated with meat and bone meal, incubated at both 37 and 43 C, did not increase the number of salmonella isolations at either temperature. They also indicated it was slightly better not to add T-7 and incubate the enrichment at 43 C. Montford and Thatcher (*92*), analyzing Cheddar cheese (48% milkfat) in SC, found T-7 had no effect.

Agitation during incubation

Agitation of enrichment cultures during incubation to facilitate aeration and/or nutrient availability or other advantageous physical conditions has not received much attention in studies of salmonella enrichment methodology. Acceleration of aerobic growth by aeration has been widely used in the fermentation industry and in growth studies, but its potential for speeding up isolation of salmonellae is reported only minimally (*2*). Fagerberg and Avens (*33, 35*) found stationary, compared to 150 rpm agitation, enrichment was generally better for 13 different enrichment broths tested for efficacy in salmonella detection from turkey skin. Chen (*17*) found agitating SBGS enrichments at 150 rpm yielded significantly fewer salmonella recoveries than stationary incubation. Insalata and Sunga (*65*) indicated salmonella detection by fluorescent microscopy was accelerated by aeration of enrichment broths. With the objective of

determining whether aeration would alter the sensitivity of either *E. coli* or salmonellae, Alford and Knight (*2*) incubated pure cultures of the organisms in SC at 200 rpm on a rotary shaker incubator. Though their report does not precisely indicate enhancement of salmonella isolation resultant from the agitation, they stated the normal lag differential between *E. coli* and salmonellae in selenite medium was not affected by their aeration procedure. They maintained though, since toxic compounds may be formed from some foods when aerated, specifically chocolate as reported by Busta and Speck (*11*), agitation should be explored for each different sample material.

Incubation time

Recommended incubation times for salmonella enrichment broths range from 16 to 96 h. The National Academy of Science's booklet (*95*) summarized incubation periods recommended by various food protection agencies. Association of Food and Drug Officials of the United States (AFDOUS) recommends 24 h for SC and tetrathionate, FDA recommends 24 ± 2 h for SC and TETBG, USDA suggests 18-24 h for TT, and the American Public Health Association (APHA) recommends 16-24 h for SC or tetrathionate. The APHA recommends when small numbers of salmonellae are present, or when initial plates are negative, enrichment should be incubated another 24 h and streaked again on selective media. They recommend streaking from enrichment media up to 3 consecutive days. The National Academy of Science's reference method (*95*) suggest 18-24 h of incubation for SC and TETBG and add that although it is not required or recommended, restreaking after an additional 24 h of incubation may produce additional positive samples. Galton et al. (*42*) stated that subculture after at least two different incubation periods, 24 and 48 h or 24 and 72 h, usually resulted in isolation of salmonellae from more samples. Galton et al. (*42*) endorsed 24- and 48-h incubation of TETBG in their recommended procedure for meats, other raw non-processed foods, rendered animal by-products, and feeds.

Morris and Dunn (*93*), testing sausage samples in TETBG for 24- and 48-h incubation periods, found best results and recommended plating both after 24 and 48 h because 15.4% of the positive samples would have been missed if only 24-h streaking had been employed. Carlson et al. (*14*) reported plating from SBGS inoculated with meat and bone meal samples after 48 h yielded greatest number of positives. Guniee et al. (*48*) obtained more positive results by subculturing SBGS and TETBG to plates after 24 and 72 h, but found plating from two jars of duplicate enrichments at the same time resulted in approximately the same increase in number of positive findings as did plating from the same single jar at two different times. Huhtanen and Naghski (*62*), in tests with meat and bone meal, did not show any advantage of 48-h SBGS incubation as far as detecting positive samples, but found fewer false positives after 48-h incubation compared to 24-h incubation. Incubation periods of 48 h were found unfavorable for most salmonella strains in SBGS inoculated with egg products by Osborne and Stokes (*97*). SBGS cultures incubated 24 h yielded a billion salmonella cells per ml, but after 48 h only a few million viable salmonella cells were detected, and greater growth of competitor organisms was noted. The salmonella population had started to die off by 48 h after full growth. They found the only advantage of 48-h incubation was greatly increased growth of *S. pullorum.* Sharma and Packer (*111*) incubated an inoculum of 15 salmonella organisms in 12 ml of liquid cow feces in TETBG and SF. The optimum length of TETBG incubation was 24-30 h, after which a decrease in salmonella isolation efficiency was noted. SF was optimally incubated 24 through 48 h with no noticeable falling off in efficiency at 36 or 48 h. Carlson and Snoeyenbos (*12*) studied times for plating after TETBG and SBGS were incubated at 43 C. They stated although *S. typhimurium* populations resulting from inoculums used were at or near peak in 16-24 h, the subsequent decrease of coliform organisms indicated that it would be advantageous to delay plating until the sample had been incubated 32 to 48 h. Chen (*17*) found no significant differences in recovery of salmonellae among 20-, 24-, 30-, and 48-h SBGS enrichment times of 15 different serotypes used to artificially contaminate turkey tails. However, she found increased numbers of recoveries as time increased even though the amount of competitor development was greatest at 30 and 48 h.

Few researchers report testing enrichment incubation periods of less than 16 or 18 h. Sharma and Packer (*111*) incubated TETBG and SF, initially containing less than one salmonella organism per ml, at 37 C for 0, 6, 9, 12, 15, 18, 24, 30, 36, and 48 h. Salmonellae were not recovered from any broth before 12 h of incubation. Incubation periods, 12, 15, and 18 h, of enrichments initially containing very few salmonella organisms, revealed recoveries, but not as many positives as were found from incubation periods of 24 h or longer. A rapid method for detecting salmonellae, including decreased time for enrichment incubation, would be very important, but as Sperber and Deibel (*118*) maintain, to be acceptable, any improved salmonella detection method must not only facilitate a decreased time factor, but it must also be as sensitive and accurate as possible.

Level and proportion of salmonella and competitor-organism contamination

Another important factor that must also be considered in enrichment methodology is the level and proportion of salmonella and competitor-organism contamination. Prost and Riemann (*102*) remarked it is a well established fact a large number of salmonella organisms is necessary to produce food poisoning. Experiments with human volunteers have demonstrated that to produce illness or lesions, the quantity of organisms necessary varies between hundreds of thousands and millions, dependent on the strain of salmonella (*102*).

Although the level of salmonella contamination in foods is frequently extremely low, even a low level may present a potential hazard if the food is mishandled, for example time-temperature abuse before consumption (*9*). According to the Food, Drug, and Cosmetics Act, no salmonella organisms are allowed in food products; there is zero tolerance (*61*). Lewis and Hall (*99*) admit it is most attractive to think in terms of salmonellae-free products, but the term "zero tolerance" has little actual meaning and is a "nightmare" to the laboratory. It is impossible to be 100% certain that salmonellae are absent since the entirety of the food products would have to be tested, so a compromise at some lower level of assurance is a practical necessity (*39*). When there is the pressure of marketing a "salmonellae-free" product, the microbiologists' problem becomes frustrating and the laboratory is faced with the important consideration of sensitivity (*99*). Lewis and Hall (*99*) cite that some salmonella detection methods will detect less than 10 salmonellae per 100 g of specimen, while others are reported to recover 10 per gram. They assert that with variations such as these, the term "salmonellae-free" obviously can mean quite different things in different situations.

Though they recognize or mention the difficulty, not many researchers have devoted efforts toward or reported enrichment methodology for very low numbers of salmonellae in samples. When salmonellae are present in large numbers they are readily recoverable by various procedures, but when they are present in small numbers, efforts often fail (*92, 116*). If two persons examine a food containing low numbers of salmonellae, it is not unusual that one will detect the organisms while the other will not (*95*). Edel and Kampelmacher (*27*) sent, for evaluation purposes, identically contaminated samples to nine different European laboratories. When there was 1 salmonella cell per sample, most labs were able to obtain high percentages of positives when working with "clean" material, but when there were many competitors and few salmonellae, the results became divergent. Isolation using a particular enrichment medium is not governed solely by number of salmonella organisms present; undoubtedly other factors such as degree of contamination with extraneous bacteria affect results obtained (*134*). Taylor and Schelhart (*123*), in a discussion of the effect of microbiological numbers on variation of results, said detection of salmonellae is influenced by numbers in two basic ways. One, there must be sufficient "absolute" numbers of salmonellae in the sample to initiate growth in enrichment broth. Two, the ratio of salmonellae to competitors must not be so disparate that competitors overgrow the salmonellae. The absolute number of salmonellae necessary to initiate growth and multiplication in inhibitory broths is higher than in non-inhibitory broths. Taylor and Schelhart (*123*) specified that sensitivity correlates with small absolute numbers and selectivity is a major factor if both salmonellae and competitors occur in high absolute numbers or if disparity between relative numbers of salmonellae and competitors constitutes a great disadvantage to the salmonellae. Jameson (*74*) and McCoy (*86*) found optimum length of enrichment incubation is a function of the number of salmonellae present in the sample being examined. McCoy (*86*) found 96 h was necessary for all possible positive recoveries if there were one or two salmonellae per 100 ml of water, 24 and 48 h were necessary to find all positives when there were three to nine per 100 ml. When the salmonella count reached 12 per 100 ml, a single plating after 24 h was adequate. Various enrichment broths were tested by Montford and Thatcher (*92*) for recovering salmonellae from specimens of frozen whole egg melange with a concentration of 0.15 to 2 salmonellae cells per gram in the presence of diverse contaminants including up to 40,000 coliforms per gram. Only SC allowed recovery of salmonellae at that contamination level. Chen (*17*) found that when only 1 to 25 salmonella cells per cm^2 of turkey skin were present, even over 1 million competitors per cm^2 did not prevent salmonella recovery. Wells et al. (*130*) reported TETBG incubated at 43 C was capable of allowing recovery at a level of 10 salmonella organisms per liter of raw milk and frequent recovery at the level of 1 salmonella cell per liter. Osborne and Stokes (*97*) developed SBGS that was determined capable of recovering 1 salmonella organism per ml from egg products in the presence of 100 *Proteus* or *Escherichia* per ml. The ideal enrichment technique would detect 1 salmonella organism if it were present in the sample being analyzed.

Serotype specificity

Tendency for some enrichment broths to inhibit certain salmonella serotypes noticeably enough to cause concern has been described by several researchers. *Salmonella cholerasuis, typhi,* and *paratyphi* occupy many of the reports of suppression or toxicity of enrichment media. Smith in 1952 (*113*) found tetrathionate broth toxic for *S. cholerasuis* and in 1959 (*114*) reported selenite toxicity also, but found MacConkey's broth with a 1:5,000 solution of brilliant green added was acceptable for its recovery. Leifson (*84*) noted high toxicity of selenite for *S. cholerasuis* when he first described the SF medium. Greenfield and Bigland (*46*) also observed unacceptability of SF for *S. cholerasuis* and Sharma and Packer (*111*) confirmed these findings that the organism could not be recovered from SF or TETBG, but could be with brilliant green MacConkey's broth. Edwards and Ewing (*27*) stated tetrathionate with added brilliant green and SC were apparently of value only in isolation of salmonellae other than *S. typhi.* Chau and Forrest (*15*) observed marked inhibition of *S. typhi* by RAP and often to some degree by tetrationate, especially if brilliant green was added. They mentioned SF was generally accepted as most suitable for *S. typhi,* but found SrSe-A better than SF. Hajna and Perry (*52*) reported SF allowed recovery of *S. typhi,* but Greenfield and Bigland (*46*) reported the serotype did not do well in SF. Hobbs and Allison (*58*) and Cook et al. (*22*) found

selenite media superior ot tetrathionate for isolation of *S. paratyphi* B. Rappaport and Konforti (*105*) and Iveson et al. (*70*) determined RAP supported growth of *S. paratyphi* B. Banwart and Ayres (*8*), studying *S. paratyphi* A in pure culture, found definite inhibition in tetrathionate broth; fewer cells were detected after incubation than immediately after inoculation. Smith (*113*) noted tetrathionate toxicity to *S. abortus-ovis,* Greenfield and Bigland (*46*) found *S. gallinarum* and *S. pullorum* did not do well in SF, Smyser and Snoeyenbos (*116*) determined SBGS was better for *S. senftenberg* than TETBG, and Osborne and Stokes (*97*) indicated SBGS did not support luxuriant growth of *S. pullorum* in 24 h, but allowed sufficient growth to permit isolation. Galton et al. (*42*) mentioned SF and tetrathionate enrichments were toxic for certain serotypes. Erdman (*30*) stated the serotype involved influenced choice of enrichment broth, since in comparisons of SBGS, SC, and TETBG for enrichment of artificially contaminated ground beef SBGS was found best for *S. schwartzengrund, S. typhimurium,* and *S. dublin;* SC best for *S. senftenberg;* and TETBG and SBGS equally effective of *S. newport* and *S. worthington.* Significant differences in recovery of serotypes, dependent on whether SC or tetrathionate was used, where shown in a study by Huhtanen and Naghski (*62*). They found significantly higher isolation of serogroup C_1 from tetrationate and of G, 35, and poly D from selenite. Fagerberg and Avens (*36*) artificially contaminated turkey skin with less than 50 organisms per ml of 61 different serotypes, used SBGS enrichment plated to BGS, and were able to recover all serotypes tested except *S. paratyphi* C. Low numbers of positive recoveries were noted with *S. cholerasuis, S. gallinarum, S. missouri,* and *S. typhi.* Banwart and Ayres (*8*) studied pure culture growth cures of *S. paratyphi* A, *S. bredeny, S. typhimurium, S. oranienburg, S. pullorum, S. anatum, S. give,* and *S. worthington,* in SF and found a decrease in number of viable cells during initial incubation with all organisms; the decrease was significant with *S. anatum. S. give* was inhibited approximately 3 h in tetrathionate during initial incubation. The SF inhibition of all the organisms was deemed by Banwart and Ayres (*8*) undesirable in the case of samples with low counts of salmonellae since destruction during the lag growth phase may result in failure to isolate the organisms. Carlson and Snoeyenbos (*13*) reported that host-adapted serotypes grew poorly or not at all in TETBG, tetrathionate, SC, and SBGS. Certain media may function better than others in certain aspects of salmonella recovery, but literature reports indicated no one medium functions maximally for all serotypes.

Sample type

The deleterious impact of addition of certain food materials to enrichment broths was demonstrated by Silliker and Taylor (*112*). They studied effects on salmonella isolation of adding various food products to selenite and tetrathionate enrichments and found the diminution of salmonella numbers was affected more by the kind of food than by either strain of salmonellae inoculated or enrichment employed. Neither an overwhelming number of coliforms nor unfavorable balance in ratio of coliforms to salmonellae affected selectivity of tetrathionate or selenite broths as much as the addition of foods including gelatin, albumen, egg yolk, or dried beef. Galton et al. (*42*) indicated addition of relatively large amounts of organic matter, such as food samples, may have an adverse effect on selectivity and enrichment quality of certain media, depending on the type of food. Lewis and Hall (*99*) stated methods for salmonella isolation must be modified slightly or markedly, depending on the product being analyzed. According to experience of Prost and Riemann (*102*), technique for recovery of salmonellae must be adapted to material that is being examined. Hurley and Ayres (*63*) reported addition of egg reduced selectivity of SF, tetrathionate, and TETBG. SBGS broth was developed by Osborne and Stokes (*97*) because additon of egg markedly reduced effectiveness of SBG. Diversity of sample materials relative to acidity, salinity, sugar, fat, moisture, etc., causing various alterations in enrichment medium pH, nutritional composition, or other physical factors that selectivity and/or sensitivity of the medium relies upon, can result in variable success in subsequent isolation of salmonellae. Galton et al. (*42*), USDA (*19*), and FDA (*37*) all recognize different samples require different methodology for isolation of salmonellae and list recommended procedures for specific products.

MECHANISMS OF PLATING MEDIA AND REPORTED APPLICATIONS

Plating media employed for salmonella isolation should, as with enrichment media, exert minimum inhibition toward salmonellae and maximum bacteriostatic action toward competitor organisms. While enrichment media allow salmonellae an opportunity to grow and multiply, the resulting culture usually includes a mixed flora and thus selective plating media have been devised to allow isolation of salmonellae from other organisms (*19*). Many such media have been described and numerous modifications have been proposed. These generally consist, as outlined in the USDA *Microbiology Laboratory Guidebook* (*19*), of a basic nutritional medium with dyes, antibiotics, bile salts, and/or other chemicals to inhibit growth of undesirable microorganisms and an indicator system to reveal by characteristic color, colonies likely to be salmonellae. Differentiation of salmonellae is usually by one of two characteristics, hydrogen sulfide production or the ability to ferment a certain carbohydrate such as lactose or sucrose. Media range from only slightly selective, such as MacConkey agar, through moderately selective and differential, including Salmonella-Shigella (SS), Desoxycholate Citrate (DC), Hektoen Enteric (HE), and Xylose Lysine Desoxycholate (XLD) agars, to highly selective and differential, for example Bismuth Sulfite (BS), Brilliant Green (BG), and Brilliant Green Sulfa (BGS)

agars. The highly selective plating media are said by Galton (*42*) to be more effective, in both clinical and food microbiology, in the isolation of salmonellae from enrichment broths since they are designed to inhibit many gram-negative organisms that are frequently present and not sufficiently suppressed on less selective media to prevent overgrowth.

Brilliant green and brilliant green sulfa agars

Brilliant green agar, first developed in 1925 by Kristensen et al. (*80*) and later modified by Kauffman (*76*), and BGS (*41, 97*) have been found highly satisfactory as plating media for salmonellae by many researchers. Banwart and Ayres (*8*) and Georgala and Boothroyd (*43*) with pure salmonella cultures; Goo et al. (*44*), Taylor et al. (*127*), Montford and Thatcher (*92*), and Morris and Dunn (*93*) testing various foods; Yamamoto et al. (*134*) testing turkey tissues; and Smyser et al. (*115*) testing poultry feed and animal by-products described best salmonella recoveries from BG and BGS agars. Guinee and Kampelmacher (*47*) and Galton (*40*) reported excellent suppression of other enteric organisms on BG agar. Galton et al. (*42*) mentioned *Escherichia* and *Enterobacter* types grow on BG, but are often suppressed as much as or more than on other selective agars. Montford and Thatcher (*92*) and Yamamoto et al. (*134*) reported that even though coliform growth was high on BG, the salmonella colonies were readily differentiated from *Proteus, Pseudomonas,* and other extraneous lactose-negative organisms. Sulfadiazine or sulfapyridine added to BG agar reportedly inhibits *Proteus* and pseudomonads (*19, 41, 42, 95, 97*). Yamamoto et al. (*134*) found *Proteus* was inhibited when sulfapyridine was added to BG, though the rate of salmonella recovery was not improved. Montford and Thatcher (*92*) found BGS highly dependable and more advantageous to use than BG because of increased coliform inhibition and high percentage of apparent salmonella colonies picked that were confirmed as salmonellae. Smyser et al. (*117*) noted essentially no difference between BG and BGS for isolating salmonellae. They found growth of competitors was occasionally more restricted on BGS than BG; however, at times salmonella growth was sufficiently restricted on BGS to make colony selection difficult. In another study Smyser et al. (*115*) preferred BG because BGS was too restrictive. Wells et al. (*129*) noticed smaller salmonella colonies on BGS compared to BG. Fagerberg (*32*) and Fagerberg and Avens (*33*) tested seven different plating media and 12 different enrichment broths in all combinations and found BGS was superior to all other plating media regardless of the enrichment type. Despite the excellent record, some problems have been noted in controlling the selectivity of BG and BGS. Hobbs (*57*) noted BG was only slightly selective and Read and Reyes (*107*) found some lots of commercial BG (to which they added sulfadiazine) would not support satisfactory growth of salmonellae. Galton et al. (*42*) claimed maximal yields of salmonellae and inhibition of coliforms when BG is prepared properly. The FDA (*37*), USDA (*19*), NAS (*95*), APHA (*110*), AFDOUS (*4*), AOAC (*5*), and Galton et al. (*42*), in their recommended methods for detection of salmonellae, all include BG or BGS as one, if not the only, plating media.

BG and BGS are basically peptone and yeast extract media with brilliant green dye to suppress gram-positive organisms and coliforms. Sulfapyradine in BGS suppresses *Proteus* multiplication to a greater extent than salmonella. The selectivity and differential system is dependent on two fermentable carbohydrates, lactose and sucrose, and an acid indicator system utilizing phenol red. Salmonellae are unable to ferment lactose or sucrose and their metabolism of peptone results in alkaline end products, therefore, since phenol red produces red color in the alkaline state, salmonellae colonies are red (*18*). Sugar fermenters such as coliforms are yellowish since their production of acid causes phenol red to turn yellow.

Bismuth sulfite agar

Bismuth Sulfite agar (BS), another one of the most widely known and used plating media, was developed by Wilson and Blair in 1927 (*132*). In the early 1930's, Wilson and Blair (*133*), Hajna and Perry (*52*), and Gunther and Tuft (*49*) acknowledged the superiority of BS for salmonella detection. Several recommended procedures for salmonella isolation include BS as one of the plating media: AOAC (*5*), FDA (*37*), NAS (*95*), APHA (*110*), and Galton et al. (*42*). Edwards and Ewing (*27*) stated salmonella usually grow well on BS and it is a good media for their isolation, including isolation of a rare strain of salmonella that ferments lactose rapidly . The indicator system in BS is not based on fermentation of any carbohydrate, rather glucose, fermentable by all *Enterobacteriaceae,* is included. Most salmonellae appear as black colonies on this medium because of hydrogen sulfide production. BS is often recommended for use in conjunction with one of the plating media reliant on the inability of salmonellae to ferment lactose, for example BG agar, so as not to miss the types that ferment lactose rapidly (*19*). There are occasional aberrant salmonella and *Arizona* serotypes that ferment lactose, and as well, serotypes that do not produce hydrogen sulfide. BS allows growth of many gram-negative enteric commensal bacteria, but is an especially effective medium for isolation of *S. typhi* (*42*). Wells et al. (*129*) reported BS was more inhibitory than BG to organisms other than salmonellae. Montford and Thatcher (*92*), on the other hand, found BS allowed development of high numbers of coliforms and yielded high numbers of false positives (apparent salmonella colonies picked that could not be confirmed as salmonellae). Erdman (*30*) found in research with artificially contaminated ground beef that BS was successful when samples were salmonella-positive, but gave large numbers of false-positives when samples were negative—an occurrence not found with brilliant green agars. BS was deemed unsatisfactory by Yamamoto et al.

(*134*) because of frequent formation of ill-defined colonies and rapid decomposition of the medium. Cox et al. (*23*) reported very low positive recovery percentages with BS compared to other media.

The BS medium does not depend on any fermentative action for recognition of salmonellae, but rather utilizes a hydrogen sulfide indicator system. Wilson and Blair (*132*) stated that the principle of BS medium rested in the ability of some salmonellae to reduce sulfite to sulfide in the presence of glucose. For the hydrogen sulfide indicator system to yield black salmonella colonies, they stated that a source of iron and phosphate were necessary. They related that the inhibitory action to coliforms was due to the combined action of the bismuth sulfite precipitate and sodium sulfite solution.

Desoxycholate citrate lactose sucrose, Salmonella-Shigella, and MacConkey agars

Desoxycholate citrate agar (DC) introduced by Leifson (*83*), modified by Hynes (*64*) and Salmonella-Shigella agar (SS), essentially a modification of the DC agar (*6*), two moderately selective and differential plating media, and MacConkey agar (MAC) (*88*) a slightly selective agar, have been used often for isolation of salmonella organisms. MAC, being less inhibitory than most other plating media for salmonella isolation, is not considered useful for streaking from enrichment broths where the flora is likely to be highly mixed and contain *Proteus,* but is useful at particular times when a relatively non-inhibitory medium is desired (*19*). The FDA (*37*), AFDOUS (*4*), APHA (*110*), and AOAC (*5*) include SS as one of the plating media in their recommended procedures. Cox et al. (*22*) and McCullough and Byrne (*87*) found SS superior to other agars tested for isolating salmonellae. Late lactose fermenters or lactose-negative organisms such as *Proteus* and *Pseudomonas* can develop on DC and SS media, and cannot always be differentiated from salmonellae, causing relatively high numbers of false-positives—the major objection most authors express. Pollock and Dahlgren (*101*) found the more inhibitory SS yielded more false-positives than the less inhibitory MAC medium, generating more wasted effort and fewer isolates than any other plating media they tested. Rollender et al. (*108*) and Yamamoto et al. (*134*) reported difficult differentiation between salmonellae, *Proteus,* and other non-lactose fermenting organisms and high incidence of false-positives with SS agar. Montford and Thatcher (*92*) found good coliform inhibition with SS, but high coliform growth on DC agar. Differentially, though, approximately 60% of apparent salmonella colonies picked from either of these media were confirmed as not salmonellae. Testing diluted, pure salmonella enrichment cultures plated onto SS, desoxycholate citrate lactose sucrose agar (DCLS), a modification of the DC formula, BS and BG, Banwart and Ayres (*8*) reported SS and DCLS showed significantly lower numbers of colonies of every salmonella serotype tested.

DCLS and SS are media based on peptone and meat extract. DCLS utilizes sodium desoxycholate for inhibitory action against gram-positive organisms and coliforms, while bile salts, rather than purified chemicals, are incorporated into SS for the same purpose. Sodium desoxycholate has strong solvent action on some gram-positive bacteria similar to bile but more powerful (*83*). The sodium chloride in DCLS, brilliant green in SS, and sodium citrate in both DCLS and SS exert inhibitory action except toward gram-negative pathogenic bacilli. Both media utilize carbohydrates which change the neutral red in the media to a red color when fermented. Salmonellae colonies are colorless on these media. DCLS employs both lactose and sucrose while SS incorporates only lactose. A hydrogen sulfide selective system is also employed in the SS medium by incorporation of ferric and sodium citrates and sodium thiosulfate.

MacConkey's is a simple peptone-based medium which has a small amount of bile salts and crystal violet to inhibit gram-positive organisms. Lactose is incorporated as a fermentable carbohydrate and neutral red as an indicator. Lactose fermenters are red in color due to neutral red reacting in acidic conditions, but since salmonellae do not ferment lactose, colonies are uncolored and transparent.

Xylose lysine desoxycholate and Hektoen enteric agars

Generally, if a plating media is highly inhibitory to some *Enterobacteriaceae,* i.e. coliforms, there is a concomitant loss of sensitivity for fastidious pathogens such as salmonellae; thus, plating media embodying the extremes of sensitivity and selectivity have been chosen and recommended for standard methodology (*125*). Two recently introduced plating media, xylose lysine desoxycholate (XLD) developed by Taylor (*121*) and Hektoen enteric (HE) developed by King and Metzger (*77*), were formulated to retain necessary sensitivity for less hardy pathogens, yet be selective enough to prevent overgrowth by the coliform majority. Isenberg et al. (*66*) found the performance of XLD and HE very similar, both media readily permitting recovery of salmonellae. Pollock and Dahlgren (*101*) found XLD and HE superior to SS and MAC agar, yielding more salmonella isolations and fewer false-positives. McCarthy (*85*) found XLD equaled the best performances of traditional plating media for salmonella isolation. Taylor and Schelhart in 1967 (*122*) reported XLD was better than BG and SS agars and in 1969 (*124*) found superiority of XLD over SS and BS agars. In 1971 (*125*) they found XLD and HE quite similar, though XLD showed slightly fewer false-positives than HE. Rollender et al. (*108*) claimed superiority of XLD and mentioned salmonella identification was greatly facilitated by the distinctive morphological appearance. King and Metzger (*77*) found good growth of salmonellae and *Shigella,* inhibition of many non-pathogens and good colonial differentiation between major groups on HE agar and reported superiority over SS agar (*78*). Goo et al. (*44*), comparing BG and HE, found BG detected significantly more

salmonella isolations than HE, though about 11% of the total positives would not have been detected without HE and HE was far more selective than BG in inhibiting non-lactose fermenters. The USDA (*19*) recommends employing XLD as one of the plating media for salmonella isolation from foods.

XLD medium differentiates salmonellae from other enterics on the basis of xylose fermentation, lysine decarboxylation, and hydrogen sulfide production (*121*). Three fermentable sugars, xylose, sucrose, and lactose are incorporated. After salmonellae exhaust the xylose, they decarboxylate lysine and cause a reversion to alkaline pH which is indicated by red color with the phenol red indicator. Lysine-positive coliforms do not revert to the red color because fermentation of lactose and sucrose in the medium produces excess acid. Desoxycholate is added to prevent *Proteus* swarming.

HE agar employs a gram-positive and coliform inhibitor system of bile salts and sodium desoxycholate, fairly high amounts of carbohydrates (sucrose, lactose, and salicin) and peptones for growth promotion and a hydrogen sulfide indicator system. King and Metzger (*77*) related that this medium was different from existing media in that the indicator system was of minimal toxicity to enteric pathogens and the amounts of peptones and carbohydrates were greater. Bromothymol blue and Anchade's are used as indicators and the non-lactose fermenting salmonellae appear blue-green to blue. *Arizona* and *Proteus* also appear blue-green to blue, *Shigella* and *Providencia* appear green, *Pseudomonas* appear green or brown, and coliforms appear salmon colored.

SEROTYPE SPECIFICITY INVOLVING PLATING MEDIA

The efficacy of some plating media employed for salmonella isolation, as with some enrichment media, is dependent on the salmonella serotype involved. Banwart and Ayres (*8*) testing *S. paratyphi* A, *S. bredeny, S. typhimurium, S. oranienburg, S. pullorum, S. anatum, S. give,* and *S. worthington,* observed DCLS was somewhat inhibitory to all organisms, SS significantly inhibited all organisms, and all organisms were significantly inhibited on BS, except *S. paratyphi* A. and *S. pullorum.* BG agar was found least inhibitory to all organisms. Galton (*40*) said in her experience, use of BG was indicated for maximum yield of salmonellae other than *S. typhi* following enrichment of clinical and food specimens. Edwards and Ewing (*27*) also maintained *S. typhi* was not expected to be isolated on BG agar. They claimed BS was the most effective medium yet devised for isolating *S. typhi.* In their comparative study, Chau and Forrest (*15*) found XLD was clearly less efficient than SS for isolation of *S. typhi.*

INCUBATION TIME OF PLATING MEDIA

The recommended incubation temperature for any selective plating media does not vary from between 35 and 37 C among most authors or any food protection agency method, but length of incubation varies among type of media and the agency method involved. Generally, 18 to 24 h of incubation is recommended for most plating media, except BS, for which 48 h is advisable. Both FDA (*37*) and AOAC (*5*) suggest if BG, SS, or BS do not have typical or suspicious salmonella colonies or do not have growth within 24 h, they should be incubated an additional 24 h. Yamamoto et al. (*134*) could not readily detect *S. typhimurium* growth on BG until after 48 h of incubation and it is mentioned in the BBL manual (*6*) that BG should be incubated 48 h. MacConkey agar, according to the *Difco Manual* (*23*), should be incubated 16 to 18 h because prolonged incubation may lead to confusion of results due to excessive colony growth.

SECONDARY TECHNICAL FACTORS ASSOCIATED WITH ENRICHMENT AND PLATING MEDIA

Multiple methods and media

Several other factors involved in salmonella enrichment or plating methodology reportedly influence recoverability of salmonellae, i.e. the use of multiple media, media preparation techniques, and storage and tempering of media. In the opinion of McCullough and Byrne (*87*), Jameson (*74*), Malo and Cousineau (*89*), Sen (*109*), Ramadan et al. (*104*), BBL (*6*), and Difco (*23*), the more methods and media employed, the greater the success in isolating salmonellae. Huhtanen and Naghski (*62*) comparing SC and tetrathionate, Taylor and Silliker (*126*) using TETBG and SF, Radan et al. (*103*) considering tetrathionate and selenite broths, Edwards and Ewing (*27*) citing GN and selenite, and Hajna and Damon (*51*) indicating SF and TT, recommended concurrent use of two different enrichment media because of the inability of one alone to detect all positive samples. Huhtanen and Naghski (*62*) felt it important to use two different enrichment broths because of serotype specificity and Taylor and Silliker (*126*) stated to use either TETBG or SF alone must be considered a calculated risk. Koopman and Janssen (*79*) conceded the use of more than one enrichment medium would give further increases in yield, but felt this increase would not counterbalance the additional work and materials. They indicated a single specific standard method should be devised for each different specific specimen to avoid the need for parallel use of different enrichment media.

The situation is similar for plating media; Banwart and Ayres (*8*) and King and Metzger (*78*) conceded to the advantage of plating on multiple media, but alleged that time and materials consumed in such a process were deterrants. Edwards and Ewing (*27*) found it advisable to employ a variety of plating media; at least one plate of each of slightly selective, selective, and highly selective media. The USDA (*19*) maintained the most thorough job of salmonella isolation can be done when two plating media are used, one reliant on hydrogen sulfide production and one employing lactose for a differential

sugar. They mentioned since highly selective media tend to be inhibitory for salmonellae also, usually a less selective medium additionally is employed, the choice being governed by the flora likely to be found in the product being examined. King and Metzger (*78*), attempting to develop an agar, HE, that could singly yet realiably facilitate identification of salmonellae with a minimum of further investigation, found even though HE was superior to SS, without the SS agar some positive samples would have been missed.

The general opinion that multiple media offer greater reliability toward salmonella recovery than single enrichment and plating media, permeates through recommendations of various food protection agencies. The FDA (*37*), AFDOUS (*4*), and NAS (*95*) recommend concurrent use of tetrathionate and SC enrichments. The FDA (*37*) and AOAC (*5*) recommend BG, SS, and BS agars, AFDOUS (*4*) suggests BG and SS, the NAS (*95*) recommends BG and BS and USDA (*19*) recommends XLD and BGS, used simultaneously for recovering salmonellae after specimen enrichment. Taylor and Schelhart (*122*) assert it is axiomatic that maximal isolations of salmonellae result from use of multiple enrichment broths and plating media. They cite two contributing factors: one factor is quantitative in that replication reduces the effect of sampling error and the other is qualitative in that since each specialized medium, enrichment or plating, represents a compromise between desired sensitivity and selectivity, no one is completely adequate. Jameson (*74*) commented on multiple isolation procedures:

> "Since a limit must always be set on the number of different media and procedures which can be used during routine examinations, a corresponding limit has also to be set on the combined efficiency of procedures used. Not even a very laborious combination of methods can be expected to yield quite 100 % of the isolations that are technically possible. When this rather unpalatable conclusion is accpeted, a way is opened up for compromises between endeavor and reward, in terms of salmonella isolations. Judicious application of principles and methods assessed in these papers might be expected sometimes to lead to modifications in techniques which increase their efficiency, and in other cases to modifications which reduce labor without diminishing efficiency."

Preparation methods

Galton et al. (*42*) stated the problem of finding overgrowth and even *Proteus* swarming on BG agar may be due to improper sterilization—a critical factor in the preparation of this medium. They designated 15 min at 15 psi for BG agar. Difco (*23*) and BBL (*6*) recommended BG sterilization at 121 C for 15 min. Poelma (*100*), USDA (*19*), and FDA (*37*) suggest 1—liter portions of BG agar be autoclaved 12 min at 121 C. Galton et al. (*42*) Difco (*23*), and FDA (*37*) mentioned that additional heating tends to decrease selectivity, FDA also mentioned that less heating increased selectivity of the media. Guinee and Kampelmacher (*47*) attributed excellent suppression of coliform organisms and *Proteus* and selection of salmonellae on BG agar in part to adding brilliant green dye solution after sterilization of the other ingredients. Moats and Kinner (*91*) reported commercial BG agar was quite variable in selectivity and mentioned variations in autoclave performance and geometry of containers may alter effective heat treatment the medium receives, which in turn may affect inhibitory properties of BG if brilliant green dye is added before autoclaving.

References to preparation of BS agar are quite ambiguous and contradictory. Cook (*20*) and Hobbs (*57*) observed BS was too inhibitory for salmonella serotypes other than *S. typhi* unless it was refrigerated at 4 C for at least 24 h before streaking. To isolate *S. typhi* they found the medium should be streaked immediately after pouring. The USDA (*19*) stated BS should be poured and used promptly, but could be stored refrigerated up to 3 days. Edwards and Ewing (*28*) stated BS was too inhibitory after refrigeration storage for more than 24 to 36 h. McCoy (*86*) said for *S. typhi* freshly poured BS plates were satisfactory, but they were too inhibitory for other serotypes. He stated many serotypes grow well on the medium stored at 4 C for 4 to 5 days, inhibition lessening with age. He claimed aging was essential for production of characteristic salmonella colonies, adding that salmonella colonies could be easily recognized on properly aged media after 18 h of incubation, but freshly poured or insufficiently aged plates required 48 h. Galton et al. (*42*) did not find it necessary to "age" BS.

Miscellaneous technical factors

Taylor and Schelhart (*123*) discussing factors causing variations in plating media, mentioned variations due to different brands of media in which different peptones and bile salts or dyes, or even variations from one lot number to another, may be great sources of dissimilarities in results. They stressed since plating media attempt an effective compromise between sensitivity for salmonellae and selectivity against competitors, extremely minute chemical changes "wreak havoc" on delicate sensitivity-selectivity balance. When any of the media are allowed to dehydrate, the Eh is changed and inhibitor concentration is increased, but if freshly poured plates are replaced into the mylar bags and resealed, aberrant results can be deterred. SBGS broths, one self-prepared from basic ingredients according to formula and two brands available in dehydrated form, were compared by Fagerberg (unpublished) for effectiveness in salmonella recovery and distinctively different results were achieved from each.

Huhtanen and Naghski (*62*), Smyser et al. (*117*), and Carlson and Snoeyenbos (*12*) recommended tempering enrichment broths to respective incubation temperatures before inoculation on the basis that a major heating time lag may occur if the incubation period is started with media at temperatures much below the desired incubation temperature. Utilizing enrichment media directly from refrigerator storage (3 C) was found comparable by Fagerberg and Avens (*31*) to media tempered to incubation temperatures before inoculation of SF, SC,

TETBG, or TT with turkey skin samples.

The experience of Whitehill and Gardener (*131*) indicated once a dehydrated SF medium bottle is opened, it should be stored over calcium chloride in a dessicator. Their experiments showed broths prepared from exposed SF cannot support growth of small numbers of *S. typhimurium* and media freshly prepared from unopened stock gave best recovery. They emphasized that commercial producers of media warn it should be stored in a cool place. Zajc-Satler et al. (*135*) found tetrathionate broth freshly prepared from infusion broth supported salmonella growth better than dehydrated media. North and Bartram (*96*) felt greater emphasis should be placed on the necessity of determining the efficiency of enrichment broths when using new batches.

The point of concern with these seemingly minor details in laboratory tests can only be as accurate as the techniques employed allow. Not all laboratories are equally proficient at isolating salmonellae, because of differences in attention to technical details (*99*). Frequently, workers neglect to report technical details of their studies, making it difficult to assess the effects or import of various secondary techniques, and possibly causing some of the inconsistency observed among authors attempting to duplicate and confirm reported research results.

CONCLUDING REMARKS

Methodology optimum for salmonella enrichment and plating should be determined for every different food product. For any given food product the best enrichment medium and plating medium combination for the greatest positive recovery of serotypes expected to be encountered should be found. All variables including enrichment emulsification, time and temperature of incubation, agitation during incubation, plating media inoculation methods, and time and temperature of incubation should be tested and decisions made as to the most effective combinations for detecting very low numbers of salmonellae amidst high levels of expected competitors. Determining optimum methodology for individual types of food products is a tedious and laborious task, but such research is indeed an essential part of the complex salmonella control efforts. Once researchers determine optimum methods for any given food, they should submit their results through proper channels so collaborative and/or pilot studies can confirm the proposed method as superior to others. The proven superior method for any specific food should be adopted as a "standard method" and recommended for use by all analytical laboratories. Public health agencies and organizations should work together and support analytical methodology investigators and help them channel results appropriately to ultimately derive standardized methods useful to all food analysts for most effectively protecting public health.

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J. Milk Food Technol. Vol. 39, No. 9, Pages 647-650 (September, 1976)

Regulation, Reason, Responsibility, and Respect

FRITZ C. FRIDAY

Friday Canning Corporation
New Richmond, Wisconsin 54017

(Received for publication April 30, 1976)

ABSTRACT

Reason and responsibilty on the part of business and consumers alike are needed when formulating government regulations. We need a more open and honest relationship with the regulatory community and we need mutual trust. The agencies must understand their responsibility and quit over-reacting to vocal minority positions. Congress must insist upon legislative review for each agency. Legislation creating a new regulatory agency should be for a specified time and not open ended. Canners recognize and encourage consumers' right to know all about the food purchased and eaten. I am spending more than 15% of my time dealing with measures that have a direct relationship to government rule-making and we have added to our office staff by half a person. We aren't necessarily doing a better job, we are simply reporting more information. We have no quarrel with improving our operations and protecting the consumer. If we don't do an adequate job of protecting the consumer, we won't be here tomorrow. The bureaucratic agency will continue, in all likelihood. As we enter America's third century I suggest that our business motto be reason, responsibility, and respect; for if we do our job in good moral consciousness we will develop the strength of our own vocal constituency, the consumer, and we will have the political base to keep regulation within reason.

INDUSTRY'S RESPONSIBILITIES

I would like to outline the concerns of a small canner in dealing with the recent increases in federal and state regulations and also highlight some changes in attitude on the part of the National Canners' Association (NCA) in its work in Washington. Before I go into this, I would like to mention an additional area of increasing personal concern regarding the relationship of business and government.

As food producers we have a fundamental responsibility to deliver a safe, wholesome, and nutritious product to our customers in the most economical manner possible. The canning industry has done this for decades, and can take pride in the part it has played in keeping America's food bill the most reasonable in the world. Generally we have met this responsibility well.

As business men in the United States in 1976 we have an additional obligation that we may or may not be meeting. I speak of our responsibility to the free enterprise system operating in openness. We have a duty to conduct our daily business in a manner that will prove the system is worth preserving. If we do not we will lose it. As we witnessed the enormous expansion of our plants in the past decade, we also watched government grow even faster. But growing along with both of us was an enormourly frustrated population that saw large economic growth without equal economic benefit. We saw a nation rip itself apart in a painful self evaluation of goals. The anguish was first expressed by student activists protesting with vigor their opposition to the war in Vietnam. This soon turned to an expression of disdain for the establishment and the way business and government were being conducted.

Many of us reacted poorly to this protest. We didn't understand or approve of the methods, so we tuned out the message. I think the message was valid. How could the United States defend a blatantly corrupt government and not be corrupted itself? It couldn't and it didn't!

American business suffered the same disease, and only now are the pathological complications being recognized. The lack of moral leadership by the business community was painfully exhibited throughout the Vietnamese war years and haunts us today. The over-building, over-billing, blackmail, theft, and disappearance that were allowed then appear today. Currently we revolt in self-righteousness when multi-national firms reveal the so-called "payments" they have made to foreign sales agencies which in this country would be bribery. Are we certain the same thing doesn't exist here? We can't condone illegal and immoral business dealings abroad without allowing the same disease to infect us at home. Honesty knows no international boundaries.

We also need a more open and honest relationship with the regulatory community in Washington as well as in the several states. We need some mutual trust. The agencies must better understand their responsibility to all consumers and quit over-reacting to a few vocal minority positions. Congress must also recognize this, and take up its responsibility to control the administrative bureaucracy it legislates into existence each session.

CONGRESS' RESPONSIBILITY

Congress must insist upon legislative review of the need for each agency, and consider at appropriate intervals whether or not the job has been done, and should the agency close its doors. Legislation creating a new regulatory agency should be for a specified time and not open-ended, so that a necessary review of need is mandated.

Congress must also consider the position of the judicial system. With the myriad of regulatory rulemakers operating independently of Congress, the only effective review of agency actions falls to the court, and every minor dispute must be settled by a judicial hearing. I feel we are placing an unreal burden on our nation's judges. We are asking men and women of little but legal background to singularly decide important questions of great scientific import. They are very often unqualified to render a scientific decision and the delay and uncertainty so created is apalling.

All of the uncertainty and reevaluation we face still evolves itself into more legislation and more regulation. We in the food industry appear to be affected more by legislative attention than any other industry. In going through a recent copy of NCA's legislative report I wrote down the current "hot" subjects and I list them as a matter of concern and interest: food surveillance, natural gas de-regulation, water and air pollution, pesticides, wage/price controls, food studies, consumer protection, toxic substances, secondary boycott, drained weight labeling, open dating, corporate farming, ingredient labeling, minimum wage, inventory taxes, child labor, bonding, meat imports, food salvage, and solid waste. That's quite a list and obviously I can't, and fortunately won't, go into all of the industry concerns about all of these. I would like to discuss several of these in light of NCA's new direction in legislative matters.

NCA'S NEW DIRECTION

S. 641 is the Senate's food surveillance bill that is a rebirth of a bill passed by the Senate in the last Congress. When this bill was originally proposed as an over-reation to FDA's request for more authority, the Senate went through an inadequate hearing process while adding new provisions with little opportunity for thorough study. The Senate, with almost no one present on the Floor, passed S.2373 in July, 1974. The bill proposed disastrous new powers for FDA. Industry's response was late and un-coordinated. Fortunately the House, confronted with the lateness of the legislation, did not have time to schedule hearings on the measure.

NCA, in viewing the possibility of the re-introduction of a similar proposal in the following session, went to work and adequately analyzed the proposal in a new light. What did the sponsor really request and were the needs valid? What changes in the present law would make necessary, and I emphasize necessary, regulation more effective and efficient?Leading a task force of industry representatives, NCA proposed a model substitute bill. We took the position that some changes were necessary. Some of the old interpretations (absolute criminal liability) were no longer valid, but some new inspection authority was needed.

I think it is significant that many of the proposals in the model bill were adopted by the several committees which had jurisdiction over the legislation and the final bill appears to be one that is much closer to deserving our support. Obviously it does some things we prefer it did not, but it is a more responsible piece of legislation by reason of our having made the decision to enter the process on a positive rather than a negative basis. The industry still has time to continue to work for additional changes when the measure is considered in the House.

WHAT CHANGES IN PRESENT LAW WOULD MAKE NECESSARY REGULATION MORE EFFECTIVE AND EFFICIENT?

Currently FDA is proposing one more change in labeling practice. This time in response to *Consumer Union*, industry is being told it must involve itself with drained weight labeling. First of all, the magnitude of the proposed requirements appear to be an extreme reaction to such a small, but well orchestrated request. The compliance and competitive ramifications of drained weight labeling in the extreme measure proposed presents a most serious economic and safety burden to our industry. This subject is splendid example of a simplified proposed solution to a highly complex problem.

We recognize and encourage the consumer's right to know all about the food purchased and eaten.We question, however, the advisability of adding what we believe to be a high compliance cost to the market basket price. We believe that when presented with the economic facts regarding the cost of compliance the actual consumer will not feel the desire for drained weight labeling worth the investment.

In addition, we are collecting for FDA's inspection great amounts of data regarding actual industry situations relative to fill, drained filled weight, and process drained weight to support what we know to be the practice of this industry; and that is to fill our containers as full as possible given all the variables of product and the mandated safety requirements. An industry as vast and as competitive as this one would not exist competitively for as long as it has if we were playing with short weights.

As before, our response to the request for additional regulation must be reasonable. We ask the respect of the agencies and the consumer groups for technical and economic knowledge we possess. We, in turn, respect the right of the consumer to know...whatever the question. We don't believe, however, that the answer to every question is a new regulation; for each new regulation costs. Let all those involved-the consumer, the regulator, and the producer-respect the needs of each other and work together in an atmosphere of reason-not reaction.

REGULATION COSTS

In any discussion of government regulation it becomes exceedingly difficult to quantify all of the costs that each new request adds to the burden of the businessman.In preparing this paper I sat down and looked at the number of agencies with which we deal or to which we are responsible and tried to put down the costs that we have attributed to new regulations proposed and implemented by the several agencies during the last 3 to 5 years. Being specific is sometimes difficult because the

cost increases are insidious. Just when you had to add the new person to the payroll is a function of time relative to increased rule making. After a while the compliance burden becomes too great for the existing staff, and a new person is added. I simply looked at our staff 5 years ago and where we are today and reflected on the whys and wherefores of the increase. To keep the thing in proper perspective I am going to simply discuss the effect on one plant, because in our case we have expanded several of our plants so significantly in this period as to distort the numbers.

TIME SPENT IN DEALING WITH MEASURES HAVING A DIRECT RELATIONSHIP TO GOVERNMENT RULE-MAKING

First of all, I find that I am spending more than 15% of my time dealing with measures that have a direct relationship to government rulemaking. This includes trade association contacts, legislative contacts, and other direct contact with the agencies. It does not include time taken to discuss regulatory problems with my staff or department heads. This is a continual ongoing situation.

Simply to fill out the forms required by all the census, FTC, and related general business regulatory agencies we have added half a person to our office staff.

Label changes in the past 4 years mandated by the Fair Packaging and Labeling Act and the requirements for nutrition labeling have caused us to completely redesign our labels, throw out an extensive inventory of labels when they became obsolete, and conduct an ongoing testing program to maintain nutrition labeling. The work load to keep track of all our customers' labels, and police the legal requirements, required addition of another person in our labeling department. Incidentally, the proposal for drained weight labeling that is before us in its extreme interpretation today, would alone cost us our current projected net profit. I would suggest that is significant!

Occupational safety and health regulations from the federal level have been a nightmare. Recognizing that this plant is in Wisconsin, which has been a leader in safety and health and whose laws were among the most stringent in the country, this plant before OSHA was a safe place in which to work. Aside from the addition of noise control, most OSHA-mandated requirements to a Wisconsin plant are what we would consider "Mickey Mouse" changes. We used one man's time for a year simply re-doing all of the guards on equipment in the plant. We don't know what the cost increases are for each machine that we now purchase, but we can tell that each new machine has a significant cost increase due to OSHA. We had to add a half-time person in our personnel department for record keeping and all of the additional followup requirements that before were done on a less formal basis. Just this past summer we added a new warehouse addition to the plant. The OSHA-mandated requirements added 20% to the cost of this building without increasing its effectiveness or adding anything to the safety of the employes who work in the building.

INDUSTRY HAS NOT RECOVERED YET FROM THE DISASTROUS WAGE/PRICE CONTROLS OF 1971

Between EPA and the Department of Natural Resources in Wisconsin, we added one-half technologist to the staff due to increased record keeping and technical responsibilities. When you recognize that water pollution control in Wisconsin was the best of almost any state, and this plant was in compliance with the very strict Wisconsin laws before the birth of the EPA, this type of increased cost makes you angry. We aren't necessarily doing a better job; we are simply reporting more information.

Obviously our consciousness level was raised, and along with energy cost-mandated economies, we have been making major capital expenditures in the area of water reuse, part of whose cost must be charged to our fear of the EPA and more stringent regulations.

The changes in the minimum wage laws have added a neat 15% or more to our direct labor costs. Because of the changes in the over-time requirement, the increased surveillance required by the personnel department caused the addition of a half person there.

The rules proposed in final form for the pension reform act are among the most maddening that I deal with. Our employees are covered by an extremely adequate and generous profit-sharing retirement program and through a luxuriously funded program for the bargaining unit employes. We have not come to grips with these final regulations, but our current feeling is that the profit-sharing plan may have to be scrapped, because of the ridiculous inclusion of seasonal workers, who in most instances would not ever collect a retirement benefit. Rules add so much to the administrative cost, and take from the available fund to a degree that an effective plan may now be impossible. Updating of the bargaining unit plan because it is one over which we have absolutely no administrative control could double the cost if it is made to comply with the pension reform act.

This industry has not recovered yet from the disastrous wage price controls of 1971. Disallocation and disruption of historic trends and values in this industry caused immeasurable chaos. Our current economic plight is severe, if not catastrophic, and I lay much of this problem to the inability of the industry to effectively deal with the problems caused by price controls.

One of everbody's favorite whipping boys is the Interstate Commerce Commission and its archaic bureaucracy. As a private carrier our cost of truck transportation is almost doubled because of the lack of back haul and the empty miles we must travel. We maintain a private truck fleet because there is no commercial alternative, and we could do the job for almost half our existing costs if we were operating under intelligent regulations.

Pesticide requirements are difficult to analyze. Aside from the fact that we are unable to use certain chemicals that were highly effective and for which there are no

adequate substitutes, we have added two people on a seasonal basis and had to upgrade the quality of our field supervisory people just to handle the sophisticated requirements of pesticide application and control. How much we lose from bugs and disease that are no longer controllable is impossible to quantify. We know it is significant.

The Food and Drug Administration is the major regulatory agency that we deal with on an unique basis. The plant I have been talking about is an exemplary plant and has long been so. We are proud of the fact that it was the first private label plant to be accepted into the Cooperative Quality Assurance Program. I think this speaks to the level of competence of the supervisory staff and the condition of the plant. It was not necessary to rebuild this plant to comply. However, recent food safety regulations from FDA have required addition of one and one-half people to our permanent staff simply to deal with the technical requirements. Most of the things that have been mandated we have been doing for many years but on a much less formal basis. The need for feeding the bureaucracy with paper required the additional staff. We also spent a fair amount of money to change individual controls and specific pieces of equipment to comply to what heretofore had been accepted as good manufacturing practices.

WE SHOULD PROCEED IN OUR RELATIONSHIPS WITH GOVERNMENT ON A POSITIVE BASIS

Here I speak of stupid instructions such as to move a bleeder petcock 6 inches on a continous cooker because the individual writing the regulation did not understand the process and blindly accepted a specific 12-inch number from a memorandum. Much of our regulated lives are spent dealing with frustrating examples like this.

The cost implications of all of this compliance represented an amount that was about half of our net profit, or put another way, we have had to increase our sales by 36% just to remain even. Had we not, we would be out of business, for it is obvious that in an industry whose growth rate is less than 5%, sales increases of this magnitude simply forced someone else to the wall. Some smaller community lost a major employer; some smaller community lost a major source of revenue for its area farmers.I hope that the members of that community appreciate what Washington has done for them.

We have no quarrel with improving our operations and protecting the consumer. Obviously we understand this more than the agencies because it is our business, not theirs. If we don't do an adequate job of protecting the consumer, we won't be here tomorrow. The bureaucratic agency will continue, in all likelihood.

NEW ATTITUDES NEEDED

If you sense a note of bitterness and frustration in my comments, so be it. I consider my business integrity among the most important things my company possesses, and dealing daily with the overwhelming blanket of regulatory people taxes my patience to the extreme. I sincerely respect the need for product and economic safety in the world, and submit that most businessmen are capable of producing honestly and effectively. I resent highly the implication of the regulatory agencies that all knowledge starts in Washington and that the only protection we have in our daily lives comes through the munificent benefit derived by washing our tax dollars in the Potomac. We need some new attitudes and an alternative government.

NCA is doing this. Mr. Charles Carey has developed some new directions for NCA and has been presenting his ideas to the area canner meetings. Essentially what Carey has been saying is that we should proceed in our relationships with government on a positive basis, and not the defensive stance as is so often the position of trade associations. The Consumer Cost Evaluation Act that was introduced in Congress last session was one such positive step. While it hasn't yet made it through the legislative process, the President adopted many of our ideas in an executive order.

Jerry Goulan is leading the legislative committee of NCA on another positive approach. Our new direction is to present the facts to the legislators in a firm, responsible manner recognizing that some parts of proposed legislation are valid and worthwhile, and to work with Congressional staff to see that the bills as presented do the job the legislator desires, without presenting a whole new world of problems to our businesses. No longer are we going to play the over-react game. This is apparently a popular pastime in Washington. The committee lawyer drafts a bill with language designed specifically to get the ire of industry attorneys, who then lead their clients screaming to the Hill in protest. To borrow a phrase, I sometimes think we "protesteth too much." Let's quit playing one-upmanship and deal with regulators on an adult basis, and expect them to work with us as knowledgeable and experienced adults.

As we enter America's third century I suggest that our business motto be reason, responsibility, and respect; for if we do our job in good moral consciousness we will develop the strength of our own vocal constituency, the consumer, and we will have the political base to keep regulation within reason.

ACKNOWLEDGMENT

This paper was presented as part of the "INDUSTRY SPEAKS" series at the National Canners Association Convention in San Francisco, California on January 20, 1976.

J. Milk Food Technol. Vol. 39, No. 9, Pages 651-653 (September, 1976)

E-3-A Sanitary Standards for Egg Breaking and Separating Machines

Number E-0600

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Department of Agriculture
Poultry & Egg Institute of America
Dairy and Food Industries Supply Association

It is the purpose of the IAMFES, USDA, PEIA, and DFISA in connection with the development of the E-3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Specifications for egg breaking and separating machines which are developed and which so differ in design, material and construction, or otherwise, as not to conform to the following standards but which, in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USDA, PEIA and DFISA at any time.

A.

SCOPE

A.1

These E-3-A Sanitary Standards cover the sanitary aspects of egg breaking and separating machines including processing air sources. They do not cover associated egg washers or conveyors used for the purpose of delivering eggs to the machine nor to equipment used beyond the point the product and the egg shell are discharged from the machine.

A.2

In order to conform to these E-3-A Sanitary Standards, egg breaking and separating machines shall comply with the following design, material and fabrication criteria.

B.

DEFINITIONS

B.1

Product: Shall mean the edible egg content removed from the shell egg delivered to the machine.

B.2

Egg Shells: Shall mean shells from which product has been removed.

B.3

Egg Breaking and Separating Machine: Shall mean equipment which opens and removes egg content from the shell eggs and integral parts used to separate whites and yolks, referred to as the machine throughout these standards.

B.4

Critical Surface (Product Contact): Shall mean all surfaces over which product normally flows, surfaces from which liquids may normally drain or drop into the product or surfaces onto which liquid may splash or be carried and then drop into the product. Examples of such surfaces include product trays and portions of the cracker and receiving cup assemblies and portions of the guiding rails.

B.5

Cracker Assembly: The mechanism which receives the shell egg, cracks and opens the shell egg and retains the shell until ejected.

B 6

Cracker Knife: The part of the cracker assembly which penetrates the shell and assists in retaining the shell when opened.

B.7

Receiving Cup Assembly: The mechanism which receives, for inspection, the shell egg content from the cracker assembly and may also separate whites and yolks.

B.8

Product Tray: Trays which collect egg content and deliver it to the product discharge.

B.9

Parts Washer: A mechanism for washing the critical surfaces of the cracker and/or receiving cup assemblies.

B.10

Semi-Critical Surface (Splash Contact): Shall mean surfaces adjoining critical surfaces on which product does not normally flow but on which product may splash or be carried onto, and then drop onto critical surfaces and/or into the product. Examples of such surfaces include portions of the cracker and receiving cup assemblies as well as portions of their supporting and operating mechanisms, and portions of the guiding rails.

B.11

Non-Critical Surface (Non-Product Contact): Shall mean all other exposed surfaces. Examples of such surfaces include conveyors, cams, empty shell ejecting mechanisms, portions of the supporting and operating mechanisms of the cracker and receiving cup assemblies and portions of the guiding rails.

B.12

Processing Air: Shall mean air prepared by filtration which is intended to be used in contact with the product for such purposes as sensory inspection, aid in separation of the egg components and removal of adhering albumen from the interior of the shell and removal of empty shells. (See D.14).

B.13

Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned by mechanical means.

B.14

Engineering Plating: Shall mean plated to specific dimensions or processed to specified dimensions after plating.[1]

C.

MATERIALS

C.1

Critical and semi-critical surfaces shall be of stainless steel of the AISI 300 series[2] or corresponding ACI[3] types (See Appendix, Section E), or metal which under conditions of intended use is at least as corrosion resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

C.1.1

Rubber and rubber-like materials may be used for stoppers, bumpers, squeegees, gaskets and parts used in similar applications. These materials shall comply with the applicable provisions of the "E-3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Egg Processing Equipment, Number E-1800."

C.1.2

Plastic materials may be used for bushings, bearings, bearing surfaces, components of cracker and receiving cup assemblies, gaskets, tubing used to deliver product from the machine to pails or pumping stations and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Number 20-00" as amended.

C.1.3

Rubber and rubber-like materials and plastic materials having critical or semi-critical surfaces shall be of such composition as to retain their surface and conformation characteristics when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.1.4

Silver solder that is non-toxic and corrosion resistant may be used where it is not practical to weld.

C.1.5

Cracker knives, portions of the cracker assembly that retain the shell until ejected and parts used in similar applications may be made of non-toxic hardenable, corrosion resistant stainless metal (400 series stainless steel, aluminum bronze alloy or equivalent) or these metals covered with an engineering plating of nickel, chromium or an equally corrosion resistant, non-toxic metal.

C.2

Non-critical surfaces shall be of corrosion resistant material or material that is rendered corrosion resistant. If coated, the coating used shall adhere. Non-critical surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both critical and non-critical surfaces or both semi-critical and non-critical surfaces shall not be painted.

D.

FABRICATION

D.1

Critical and semi-critical surfaces shall be at least as smooth as a No. 4 finish on stainless steel sheets free of imperfections such as pits, folds and crevices. (See Appendix, Section F.).

D.2

Permanent joints in critical surfaces shall be welded, except where it is not practical to weld, joints may be silver soldered. Welded and silver soldered areas on critical surfaces shall be at least as smooth as a No. 4 finish on stainless steel sheets free of imperfections such as pits, folds and crevices.

D.3

The minimum thickness of engineering plating shall be 0.0002-inch for critical and semi-critical surfaces except that when these surfaces are other than stainless steel, the minimum thickness of the engineering plating shall be 0.002-inch.

D.4

Rubber or rubber-like materials and plastic materials having critical or semi-critical surfaces that are a coating or covering shall be bonded in such a manner that the bond is continuous and mechanically sound, and so that when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment, the rubber or rubber-like material or the plastic material does not separate from the base material.

D.5

The machine shall be designed so that the contents of each egg can be organoleptically inspected.

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[1] *QQ-C-320a-Federal Specification for Chromium Plating (Electrodeposited), July 25, 1954. (For sale by the General Services Administration, Seventh and D Streets, NW, Room 1643, Washington, DC). QQ-N-290-Federal Sepcification for Nickel Plating (Electrodeposited), April 5, 1954, and Amendment 1, December 13, 1961. (Available from General Services Administration, Seventh and D Streets, NW, Room 1654, Washington, DC).*

[2] *The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, December 1974, Table 2-1, pp. 18-19. Available from: American Iron & Steel Institute, 1000 16th Street, NW, Washington, DC 20036.*

[3] *Alloy Casting Institute Division, Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116.*

D.6
The machine shall be designed so that inedible egg content may be separated and segregated from product.

D.7
Machines that are to be mechanically cleaned shall be designed so that all critical and semi-critical surfaces of the machine and all non-removable parts thereto can be mechanically cleaned and are accessible for inspection.

D.8
Critical and semi-critical surfaces of machines requiring manual cleaning shall be readily accessible for cleaning and inspection either when in an assembled position or when removed. Removable parts having critical surfaces shall be readily and easily demountable for replacement or cleaning and inspection.

D.9
Critical and semi-critical surfaces shall be self-draining except for normal clingage.

D.10
Unless the product connection is sanitary tubing for a flexible hose to the product take-away system, connections shall conform to the "E-3-A Sanitary Standards for Fittings Used on Egg and Egg Products Equipment and Used on Sanitary Lines Conducting Egg and Egg Products, Number E-0800."

D.11
Internal angles of 135° or less on critical surfaces shall have minimum radii of 1/8 inch, except those in the receiving cup assembly and the shell retaining mechanism which shall not be less than 1/32 inch.

D.12
There shall be no exposed threads on critical surfaces, except where required for functional and safety reasons. Threads shall be mechanically cleaned, or shall be readily accessible for manual cleaning.

D.13
Coil springs having critical surfaces shall have at least 3/32 inch openings between coils, including the ends when the spring is in a free position. Coil springs shall be readily accessible for cleaning and inspection.

D.14
Equipment for supplying processing air, if furnished as a part of the machine, shall comply with the applicable provisions of the "E-3-A Accepted Practices for Supplying Air Under Pressure in Contact with Liquid Egg and Egg Products and Product Contact Surfaces, Number E-60400."

D.15
The exposed portions of legs, including threaded portions, shall be readily accessible for cleaning. Legs made of hollow stock shall be sealed. Legs shall provide a minimum clearance between the lowest part of the machine, with the exception of the legs, and the floor of not less than six inches.

D.16
Any guard(s) required by a safety standard that will not permit accessibility for cleaning and inspection shall be designed so that it (they) can be removed without the use of tools.

D.17
Non-critical surfaces shall be cleanable and shall be smooth, free of pockets and crevices, and be easily accessible for cleaning either when in an assembled position or when removed. Those to be coated shall be effectively prepared for coating.

APPENDIX

E.
STAINLESS STEEL MATERIALS
Stainless steel conforming to the applicable composition ranges established by AISI[2] for wrought products, or by ACI[3] for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved, the carbon content of the stainless steel should not exceed 0.08 percent. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steel of the 300 Series.

Cast grades of stainless steel corresponding the types 303, 304 and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM[4] specifications A296-68 and A351-70.

F.
CRITICAL SURFACE FINISH
Surface finish equivalent to 150 grit or better as obtained with silicon carbide is considered in compliance with the requirements of Section D.1 herein.

G.
CLEANING AND SANITIZING PROCEDURES
A cleaning and sanitizing regimen which is effective should be employed. A description of this regimen should be available at the breaking plant. Because of the possibilities of corrosion, the recommendations of the cleaning compound manufacturer should be followed with respect to the time, temperature, and the concentration of specific detergents and sanitizing agents. To insure proper strength of solution and to avoid corrosion, the detergent or sanitizer should be completely dissolved or dispersed prior to use.

H.
ELECTRICAL SAFETY
Where pressure cleaning is required for sanitary reasons, electrical motors, switches and appurtenances should be waterproof or be apart from the affected cleaning areas.

These standards are effective January 28, 1977.

[4] *Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.*

J. Milk Food Technol. Vol. 39, No. 9, Pages 654-656 (September, 1976)

E-3-A Sanitary Standards for Shell Egg Washers

Number E-1500

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Department of Agriculture
Poultry & Egg Institute of America
Dairy and Food Industries Supply Association

It is the purpose of the IAMFES, USDA, PEIA, and DFISA in connection with the development of the E-3-A Sanitary Standards program, to allow and encourage full freedom for inventive genius or new developments. Specifications for shell egg washers which are developed and which so differ in design, material and construction, or otherwise, as not to conform to the following standards but which, in the fabricator's opinion, are equivalent or better, may be submitted for the joint consideration of the IAMFES, USDA, PEIA, and DFISA at any time.

A.

SCOPE

A.1

These E-3-A Sanitary Standards cover the sanitary aspects for washers of shell eggs. They do not pertain to any device used in moving the product from a flat to the washer rollers or washer infeed system such as hand or vacuum lifters, trays, wire fingers or similar devices nor do they pertain to fixed transfer devices between components nor to an oiling device which is used with a washer in a shell egg cartoning operation.

A.2

In order to conform with these E-3-A Sanitary Standards, shell egg washers shall comply with the following design, material and fabrication criteria.

B.

DEFINITIONS

B.1

Product: Shall mean shell eggs.

B.2

Solution: Shall mean the solutions used to wash, sanitize or rinse the product.

B.3

Surfaces

B.3.1

Product Contact Surfaces: Shall mean surfaces that are in contact with the product. These surfaces may also be in contact with the solutions defined in B.2.

B.3.2

Wash Solution Contact Surfaces: Shall mean surfaces that are in contact with the solution defined in B.2 but not in contact with product. These surfaces include but are not limited to the wash solution contact surfaces of wash solution tanks, pumps, piping, hoses and heat exchange surfaces.

B.3.3

Non-Product, Non-Wash Solution Contact Surfaces: (Referred to as Non-Product Contact Surfaces in these E-3-A Sanitary Standards) shall mean all other exposed surfaces.

B.4

Wash Solution Tank: Shall mean the tank used to contain and/or collect the wash solution used for cleaning the product.

B.5

Sanitizing Systems: Shall mean the equipment used to sanitize the product.

B.6

Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the product contact surfaces to be cleaned, by mechanical means. Note: This definition applies to the washer, not the product.

C.

MATERIALS

C.1

Product contact surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types (See Appendix, Section E.), or metal which under conditions of intended use is at least as corrosion resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

C.1.1

Rubber and rubber-like materials may be used for conveyor spools, and parts used in similar applications. These materials shall comply with the applicable provisions of the "E-3-A Sanitary Standards for Multiple-Use Rubber and Rubber-like Materials Used as

[1]*The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, December 1974, Table 2-1, pp. 18-19. Available from: American Iron and Steel Institute, 1000 16th Street NW, Washington, DC 20036.*
[2]*Alloy Casting Institute Division, Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116.*

Product Contact Surfaces in Egg Processing Equipment, Number E-1800."

C.1.2

Plastic materials may be used for conveyor spools, brush bristles, guide rails and surfaces, conveyor chain, and parts used in similar application. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Number 20-00," as amended.

C.1.3

Rubber and rubber-like materials and plastic materials having product contact surfaces that are a coating or a covering, shall be of such composition as to retain their surface and conformation characteristics when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.2

Wash solution contact surfaces other than those of wash solution tanks shall be one or more of the following: steel, stainless steel, clear heat resistant glass, copper, brass, aluminum, rubber and plastic. These materials shall be non-toxic, relatively non-absorbent and relatively corrosion resistant under the conditions of intended use. The wash solution contact surfaces of wash solution tanks shall be stainless steel or steel that is rendered corrosion resistant by a non-toxic coating, or plastic.

C.3

Sanitizing solution contact surfaces of sanitizing systems, furnished by the washer manufacturer, shall be of material(s) which under conditions of intended use is corrosion resistant and is non-toxic and durable.

C.4

Non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion resistant. If coated, the coating used shall adhere. Non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.

FABRICATION

D.1

Permanent joints in metallic product contact surfaces shall be welded. Welded areas on contact surfaces shall be at least as smooth as the adjoining surface.

D.2

Rubber or rubber-like materials and plastic materials having product contact surfaces that are a coating or covering shall be bonded in such a manner that the bond is continuous and mechanically sound, and so that when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment, the rubber or rubber-like material or the plastic material does not separate from the base material.

D.3

Product contact surfaces not designed to be mechanically cleaned shall be easily accessible for cleaning and inspection either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.4

Internal angles of 135° or less on product surfaces shall have minimum radii of 1/8 inch.

D.5

There shall be no threads on product contact surfaces.

D.6

Wash solution contact surfaces shall be readily accessible for cleaning in place or be removable for cleaning and inspection.

D.7

Wash solution contact surfaces of solution tanks shall be self-draining except for normal clingage. Metallic wash solution contact surfaces of wash solution tanks shall be smooth, readily cleanable and not contain pockets or crevices.

D.8

A thermostatically controlled heating device(s) shall be provided to assure a uniform temperature wash solution.

D.8.1

The thermostatic control shall be capable of functioning on a change in wash solution temperature of not more than 5°F above or below the temperature at which the control is set.

D.9

The wash solution tank or wash solution supply line shall be provided with an indicating thermometer for indicating the temperature of the solution. It shall have a scale range of 80°F to 130°F, extension of range on either side permitted, the scale shall be graduated in divisions of not more than 2 degrees with not more than 30 degrees per inch of scale. The thermometer shall be moisture proof under normal operating conditions.

D.10

If the washer, at times, is to be used only as a dry egg conveyor, provision shall be made to prevent cleaning devices from touching the product.

D.11

Provision shall be made for spraying a sanitizing solution over the entire egg. The quantity of sanitizing solution sprayed shall be sufficient to rinse off all wash solution.

D.12

In washers that are to use an iodine solution for sanitizing, provisions shall be made for a spray(s) of water to remove the washing solution from the egg before sanitizing. (See Appendix F.3)

D.13

Sufficient drainage time or means for removing excessive moisture from the washed and sanitized egg shall be provided for eggs to be broken. Blowers, fans

or other means shall be provided for drying eggs to be cartoned or cased.

D.14

When a proportioning pump and a sanitizing solution tank is provided for sanitizing the product, the outlet of the sanitizing solution tank shall be in the bottom or near the bottom of the tank and the intake of the sanitizing solution pump shall be below the bottom of the tank or means shall be provided to assure the pump is continuously primed.

D.15

Exposed portions of legs, including threaded portions, shall be readily accessible for cleaning. Legs made of hollow stock shall be sealed. Legs shall provide a clearance between the lowest part of the washer, with the exception of the leg itself, and the floor of at least 4 inches when the base outlines an area in which no point is more than 12½ inches from the nearest edge, or a clearance of at least 6 inches when any point is more than 12½ inches from the nearest edge.

D.16

Non-product contact surfaces shall be readily cleanable and shall be smooth, free of pockets and those to be coated shall be effectively prepared for coating.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or by ACI for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved, the carbon content of the stainless steel should not exceed 0.08 percent. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steel of the 300 series. Cast grades of stainless steel corresponding to types 303, 304 and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM[3] specifications A296-68 and A351-70.

F

SUGGESTIONS FOR OPERATING AND CLEANING

F.1

The washer should be drained and cleaned approximately every four hours and at the end of each shift or more often if needed to maintain sanitary conditions. The washer, brushes and conveyor should be thoroughly cleaned at the end of each day. Special care should be given to the brushes.

F.2

Detergent should be maintained at the level recommended by the washer manufacturer and/or by a responsible detergent manufacturer.

F.3

Washers that recirculate the washing solution should have a supply of replacement water of a safe, sanitary quality to maintain a continuous overflow. It is recommended that the amount of replacement water be one gallon per case of shell eggs. Chlorine sanitizing solution may be used for replacement water. Iodine sanitizing solution should not be used for replacement water but should be discharged to waste.

F.4

Eggs should be removed from the washer during rest or scheduled shutdown periods.

F.5

The minimum temperature of the washing solution should be (1) 90°F or (2) 20°F warmer than the temperature of the eggs to be washed, whichever is higher.

F.6

The sanitizing solution should have not less than 100 ppm or more than 200 ppm of available chlorine or if it is an approved iodine compound, not less than 12.5 ppm or more than 25 ppm of available iodine.

F.7

Waste water from the washer should be piped to a drain or to a shell trap.

F.8

Only water of a safe, sanitary quality should be used in washing the sanitizing solutions and for rinsing. The iron content of water used for washing eggs to be cartoned or cased should not exceed 2 ppm.

F.9

Sanitizing systems should be examined at least daily and maintained in operating condition at all times.

G.

SANITIZING SYSTEMS

Sanitizing systems not supplied by the washer manufacturer should comply with the criteria in subsections C.3, D.11 and D.12 of this standard.

These standards are effective January 28, 1977.

[3]*Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.*

News and Events

Calendar of Events

October 29, 1976. FOOD MICROBIOLOGY WORKSHOP. Phillip Morris Research Center, Richmond, Virginia. Fee $15.00. Sponsored by Carolina-Virginia Institute of Food Technology. For more information contact: Dr. Robert M. Ikeda, Phillip Morris Research Center, P.O. Box 26583, Richmond, VA 23261.

November 9-11, 1976. RESEARCH AND DEVELOPMENT ASSOCIATES FOR MILITARY FOOD AND PACKAGING SYSTEMS, INC. U.S. Army Natick Research and Development Command, Natick, Massachusetts. For information contact: Col. Merton Singer, USA (Ret), Executive Secretary, R & D Associates, 90 Church Street, Rm. 1315, New York, NY 10007. (212) 264-7612.

January 12-13, 1977. DAIRY PROCESSORS CONFERENCE. Quality Inn Motel, Madison, Wisconsin. Sponsored by the Food Science Department, University of Wisconsin-Madison.

February 9-10, 1977. DAIRY INDUSTRY CONFERENCE. Center for Tomorrow, Ohio State University, Columbus, Ohio. For information contact: John Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Road, Ohio State University, Columbus, OH 43210.

February 13-16, 1977. INTERNATIONAL EXPOSITION FOR FOOD PROCESSORS. Superdome, New Orleans, Louisiana. Sponsored by the Food Processing Machiner and Supplies Association, 7758 Wisconsin Avenue, Washington, DC 20014.

March 21-25, 1977. MID-WEST WORKSHOP IN MILK AND FOOD SANITATION. Center for Tomorrow, Ohio State University, Columbus. For information contact: John Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Road, Ohio State University, Columbus, OH 43210.

Food Processors Microbiology Short Course

A basic microbiology short course for food processors will be offered at the University of California at Davis during December 13-16, 1976. This short course is designed for food industry personnel who are new to the field of food microbiology or would like a review of basic food microbiology and microbiological techniques. There are no prerequisites to this course. Instructors will assume that students have had no formal training in biology or chemistry.

The objectives of the course are to develop basic skills in and an understanding of food microbiology. Topics to be covered include use of a microscope; basic laboratory skills; isolation, enumeration, and identification techniques; microbes of importance in food processing; and the significance of microbes in processed foods. The short course will consist of lectures, laboratories, and evening discussions. Certificates of course completion will be issued.

Enrollment is limited to 32 students. The registration fee of $100 includes laboratory manual and fees, first evening's social, and last evening's dinner. For registration information and further details contact: Dr. Robert J. Price, Department of Food Science and Technology, Cruess Hall, University of California, Davis, CA 95616. (916) 752-2191.

Government Regulations Symposium Scheduled

Western N.Y. Section of IFT and the Institute of Food Science, Cornell University will cosponsor their 11th Annual Symposium titled, "Working with Government Regulations," November 11, 1976.

There will be a consumer advocate talking about the need for regulations, an industry spokesman describing the conflicts of regulatory agencies, followed by a speaker from each of the following agencies: FDA, USDA, FTC, EPA, and OSHA.

The regulatory spokesmen will discuss the origin of the agency, the major regulations affecting the food industry, how the industry can best keep informed on pertinent activities of the agency, suggestions on how the industry can work best with the agency, including industry recourse on agency's actions, a table of organization and chain of command, what information is available under the Freedom of Information Act, how the conflicts of certain agencies can be overcome, a remark on the cost benefit to regulations, and conclude with a comment on the type of legislation that may be coming.

For more information, contact D. L. Downing, New York State Agricultural Experiment Station, Geneva, NY 14456. (315) 787-2273.

Dairy Processing Workshop Planned

A workshop for milk processing personnel has been scheduled for October 18-29, 1976 at The Pennsylvania State University. It is to be conducted by the staff of the Food Science Department.

Subjects to be covered will include composition and properties and processing of fluid milk products, soft ice cream, buttermilk, yogurt, sour cream and cottage cheese. Quality control procedures will be taught including basic fat tests, cryoscopy and flavor evaluation. Other subjects will include labeling requirements, basic dairy arithmetic, dairy refrigeration, steam and electrical systems, cleaning and sanitizing of dairy equipment. The workshop will be directed to dairy plant processing personnel.

For additional information and a copy of the program bochure contact:

Agricultural Conference Coordinator
410 J. O. Keller Building
University Park, PA 16802

News and Events

Bandler and Bodyfelt—Dairy Technologists in Job Trade

Mr. David K. Bandler, Assistant Professor, Cornell University, Food Science Extension and Mr. Floyd W. Bodyfelt, Associate Professor, Extension Food Science, Oregon State University have recently switched positions for one year. Each of the two extension dairy technologists is shown above symbolically trading job responsibilities, homes, and second cars for a full year. The two food scientists consider their exchange to be primarily a program of professional development and an opportunity to inject new insights into the extension education programs for the dairy processing industries of the two respective states.

In addition to extension responsibilities in Oregon, Bandler will also undertake certain teaching and applied research duties in Oregon. Bandler is a member of the American Dairy Science Assn. (ADSA), the International Assn. of Milk, Food and Environmental Sanitarians (IAMFES), and the Institute of Food Technologists (IFT). The Bandlers and their two boys will reside in the Bodyfelt's home in Corvallis.

At Cornell, Bodyfelt undertakes extension program responsibilities in dairy technology, applied research activities and will serve as acting secretary of the New York State Cheese Manufacturers' Assn. Bodyfelt is a member of the ADSA, IAMFES and the Oregon Section of the IFT. The Bodyfelt family, including four children, reside in the Bandler home.

This exchange of jobs was facilitated by the fact that the professional areas of interest and expertise for Bandler and Bodyfelt closely parallel each other. Both food scientists have developed extensive educational programs for dairy processing, marketing, and regulatory agency personnel. The programs have primarily related to better processing methods, improved quality assurance efforts, consumer acceptance of dairy products, extended shelf-life, more uniform and relevant dairy industry standards and regulations, and consumer education for better milk product utilization.

Brucellosis Efforts

A commission of agricultural economists, veterinary scientists, microbiologists and public health experts has been formed to evaluate the effectiveness of the U.S. Department of Agriculture (USDA) brucellosis eradication program.

The group, the National Brucellosis Technical Commission, will solicit views on the USDA program from individuals and groups. A hearing has been set for the Americana Hotel, Miami Beach, Florida, on November 7-12, with other hearings to follow on the West Coast, in the South Central states and in the East-North Central area.

Chairman of the commission is Dr. David T. Berman, University of Wisconsin-Madison veterinary scientist. Others on the commission are R. K. Anderson, D.V.M., of the University of Minnesota School of Public Health; W. T. Berry, of the Victorio Land and Cattle Co., Phoenix, Arizona; John A. Hopkin, agricultural economist at Texas A & M University; and Robert Wise, M.D., of the Veterans Administration Center, Togus, Maine.

The commission was formed last fall when livestock producer groups asked the U.S. Animal Health Assn. to study the eradication program, investigate deficiencies and recommend changes, if necessary. The association nominated the commission members, who were approved by the USDA. A contract with UW-Madison and Texas A & M to provide staff followed.

The commission expects to issue interim reports periodically and the final report in June, 1978, according to Berman, who will be on part-time leave to work on the commission.

Berman stresses that the review of government programs will be free of any agency or individual influence and invites those with views on the eradication program to send comments or position papers to him. His address is: Department of Veterinary Science, UW-Madison, 1655 Linden Dr., Madison, WI 53706.

News and Events

Group Award of Merit to FDA's Laboratory Certification Section

Dr. A. Richard Brazis (right), Chairman of the Applied Laboratory Methods Committee of the IAMFES recently received the FDA Group Performance Award on behalf of the Laboratory Certification Section of the Division of Microbiology, Bureau of Foods. The FDA Group "Award of Merit" was presented at the Sixteenth Annual Honor Awards Ceremony of the Food and Drug Administration in Rockville, Maryland. The award was presented to this eight-member group in recognition of superior performance in conducting national and international laboratory quality assurance programs. Other members of IAMFES who work in this group include Past President Raymond Belknap and James W. Messer, Ph.D.

Expo '76 Forum to Focus on Energy for Food Production

The impact of energy on the world's food production will be viewed from different perspectives at the 1976 Food Forum, to be held October 12, 1976, during Food and Dairy Expo '76 at Atlantic City Convention Hall.

Dependence of the food industry on energy availability, relationship of energy to food production requirements worldwide and an assessment of the deteriorating energy situation as fuel consumption increases and domestic supplies decline will be discussed.

Speakers will be Dr. Kenneth D. Frederick, senior research associate, energy and materials division, Resources for the Future; Dr. David Pimentel, professor of insect ecology and agricultural science, department of entomology, Cornell University, and Raymond R. Wright, refining director, American Petroleum Institute. A fourth presentation will be made by the Energy Research and Development Agency, Federal Energy Administration.

Dr. Walter M. Urbain, retired director of engineering research at Swift and Co., will deliver the 1976 Food Engineering Award address and accept the award for outstanding original research on food irradiation and development of meat processing techniques.

The Forum is conducted biennially for food processors attending Food and Dairy Expo. It will start at 10 a.m. October 12 and continue in the afternoon. It is sponsored jointly by American Society of Agricultural Engineers and Dairy and Food Industries Supply Association.

Dutch Cows are Guided, Fed by Computer

The D.A.C.A. Electronic Engineering & Contracting Co. of Lelystad, the Netherlands, recently introduced an electronic system which records the amounts of feed concentrate given to a maximum of 254 cows and controls a mechanical feeding actuator. The system is designed to scientifically supervise the food intake of each specific animal according to its own needs as well as to increase milk yields, avoid wastage of concentrate and save human labor. After a year-long trial at a Dutch model farm, all of these objectives have been achieved.

A small transceiver worn by each animal on its collar is the key to the entire system. In these transceivers, use is made of electromagnetic transfer of energy, thus eliminating batteries.

As soon as a cow approaches a manger, its number is received by the manger electronics unit via the signal from the transceiver. This number is relayed to the central processing unit where all of the specific data relating to that cow is stored in a memory.

From this data, the central control panel then ascertains whether the animal should receive concentrate at that time. If so, a vibrating pipe above the particular manger is opened automatically by the central processing panel and a predetermined quantity of concentrate is given to the cow. Simultaneously, the cow's number and the concentrate dose are indicated digitally on a keyboard and recorded in the memory of central processing. The memory is protected against breakdowns in the power supply so that none of the programmed data can ever be lost. Thus, it is possible to maintain constant supervision of each cow's individual daily regimen, and to increase or reduce its concentrate intake according to its needs.

For further information, contact the Netherlands, Consulate General, Commercial Division 529, One Rockefeller Plaza, New York, NY 10020.

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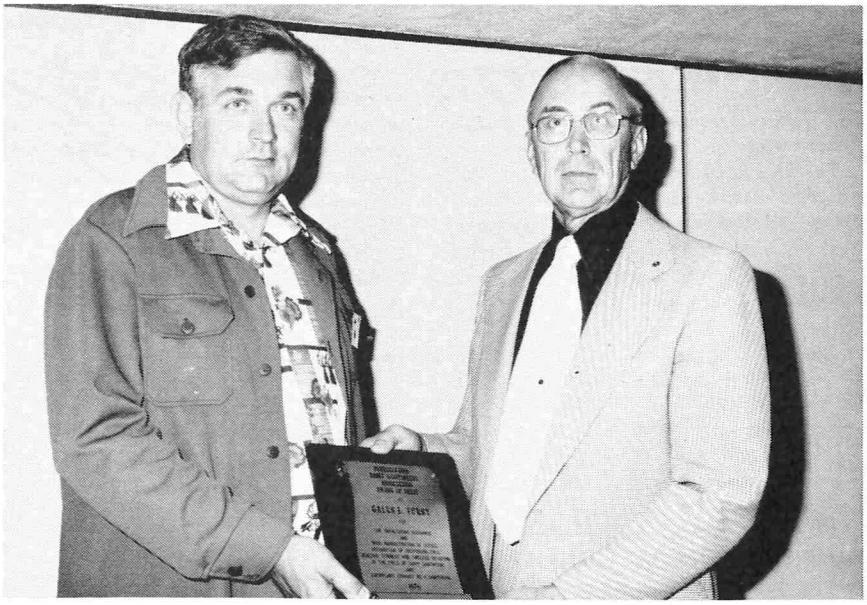
Pennsylvania Dairy Sanitarians Annual Meeting

Officers for the coming year are: Front row, l-r: George Mansell, past president and Ray Ackerman, president-elect; Second row l-r: Alfred Gottfried, Secretary-treasurer, Don Breiner, president, and John Blyholder, vice-president.



Mr. Galen Furry (right) received the 1976 Dairy Sanitarian Award at the Dairy Fieldmen's Conference banquet. A dairy farmer from Curryville, Pensylvania, he has been a full time sanitarian for Abbotts and Interstate Milk Producers Cooperative for more than 20 years. Gerald Schick, chairman of the awards committee is shown presenting the award.

More than 250 persons participated in the recent two-day program of the Pennsylvania Dairy Fieldmen's Conference. A total of 13 papers were presented and three discussions of new programs were held.

The Pennsylvania Dairy Sanitarians Association has eight active committees and holds three executive sessions each year. Four newsletters are prepared and distributed to members.

The nearly 300 members of the Pennsylvania Dairy Sanitarians Association support scholarships for undergraduate students in Food

Association Affairs

Science at the Pennsylvania State University. For the past year, the $800 was divided among David Fissel, Julia Konopa and Bruce Miller. The awards are based on academic record and financial need.

Interim Report of the Applied Laboratory Methods Committee-1975

The Applied Laboratory Methods (ALM) Committee of the International Association of Milk, Food and Environmental Sanitarians (IAMFES) has continued to be active in those affairs of this Association which require laboratory resource information. Subcommittee members have developed and conducted collaborative and/or comparative studies on laboratory methods, most of which have been published in the *Journal of Milk and Food Technology* (JMFT). Subcommittee members and the Chairman are also assisting in the preparation of the 14th edition of *Standard Methods for the Examination of Dairy Products* (SMEDP). The Chairman is also assisting in preparation of two additional publications: *Compendium of Methods for the Microbiological Examination of Foods* and *Quality Assurance Practices for Health Laboratories.*

The 1973 interim report of this Committee projected reactivation of the ALM Subcommittee on Laboratory Methods for the Examination of Food before the 1975 meeting. It is essential that an effective laboratory subcommittee has a chairman who is able to work closely with the members of his subcommittee. Mr. Charles N. Huhtanen, U.S. Department of Agriculture, has indicated that he is willing to accept this new responsibility as Chairman of the Food Subcommittee. Due to this reassignment, a new chairman for the Subcommittee on Laboratory Methods for the Examination of Milk and Milk Products must be selected. It is hoped that one of the members of this subcommittee will volunteer to accept this responsibility.

A. RICHARD BRAZIS
Chairman
Applied Laboratory Methods Committee

The Chairman of the ALM Subcommittee on Laboratory Methods for the Examination of Water and Other Environmental Samples reported that two comparative studies have been completed and a third study is nearing completion. The intent of this subcommittee is that all three studies will be written as separate research papers and submitted to JMFT for publication.

Unfortunately, three members of this subcommittee have had changes of job responsibility and can no longer participate in the actions of this subcommittee. Members of the IAMFES and others who may wish to contribute to the objectives of the ALM Committee by participating in this subcommittee are requested to contact the Subcommittee Chairman.

ARNOLD SALINGER
KENNETH WHALEY
GENE RONALD, *Chairman*
Subcommittee on Laboratory Methods for the Examination of Water and Other Environmental Samples

The Subcommittee on Laboratory Methods for the Examination of Milk and Milk Products has been the most active laboratory subcommittee of IAMFES since 1964. Briefly, two studies were completed by subcommittee members which concerned methods described in the 13th edition of SMEDP. Data from these studies indicated that: (*a*) additional emphasis be placed on the importance of water bath equilibration times on tempering of agar before plating, and (*b*) phosphate buffer, in the concentration used, does not appear to exert a strong buffering capacity in dilution blanks when raw milk samples are examined. Both of these studies have been published in the JMFT.

An additional study has been completed on the comparison of 48- and 72-h incubation times as well as 30 and 32 C incubation temperatures. The results of this study indicate that a higher Standard Plate Count (SPC) on samples occurs when the plates are incubated for 72 h. The data indicate also that some counts may be higher at 30 C, but statistical tests of counts of 30 and 32 C did not show the differences to be significant. The report of this study has been published in JMFT.

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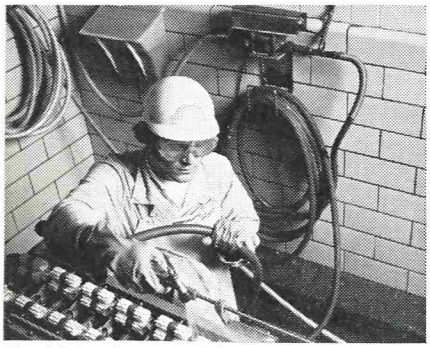

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For full details, see your Klenzade Representative, or call Glenn Weavers, Director of Sales.

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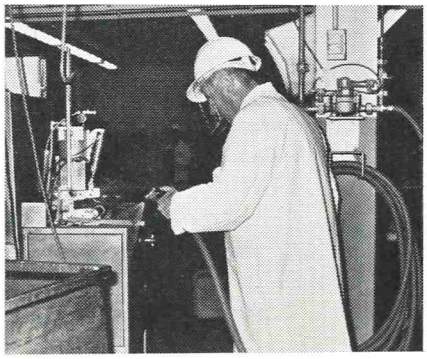

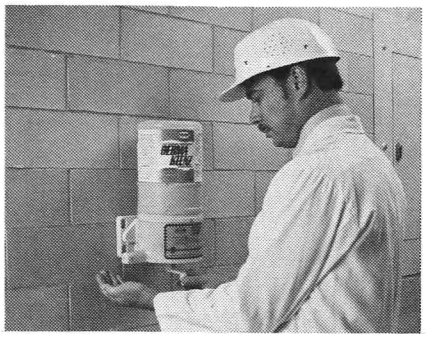

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CALIFORNIA—October 18-20, 1976. Airport marina Hotel, Burlingame (San Francisco Bay Area).

CONNECTICUT—January 1977. Hartford Area.

IOWA—October 27, 1976. Scheman Continuing Education Bldg., Ames.

ILLINOIS—December 1976. Elgin

KANSAS—October 13-15, 1976. Holiday Inn, Emporia.

KENTUCKY—February 22-23, 1977. Stouffer's Inn, Louisville.

MISSOURI—April 1977. Ramada Inn, Columbia.

ONTARIO—March 1977. Holiday Inn, Highway #427, Etobicoke.

EASTERN ONTARIO—November 1976. Kemptville.

PENNSYLVANIA—June 13-15, 1977. State College.

VIRGINIA—March 8-9, 1977. Donaldson Brown Center, VPI & SU, Blacksburg.

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and for ALL OTHER SANITARY MACHINE PARTS which are cleaned daily.

The Modern HAYNES-SPRAY Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service

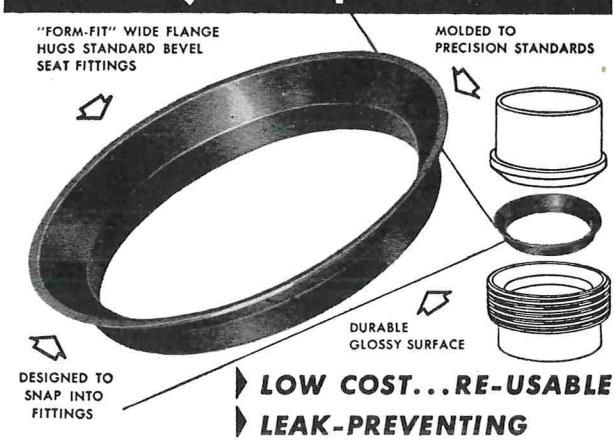
The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON
SHIPPING WEIGHT—7 LBS.

THE HAYNES MANUFACTURING CO.
4180 Lorain Ave. • Cleveland, Ohio 44113

HAYNES-SPRAY INGREDIENTS ARE APPROVED ADDITIVES AND CAN BE SAFELY USED AS A LUBRICANT FOR FOOD PROCESSING EQUIPMENT WHEN USED IN COMPLIANCE WITH EXISTING FOOD ADDITIVES REGULATIONS.

HAYNES SNAP-TITE GASKETS



LOW COST...RE-USABLE
LEAK-PREVENTING
NEOPRENE GASKET for Sanitary Fittings

Check these SNAP-TITE *Advantages*

- Tight joints, no leaks, no shrinkage
- Sanitary, unaffected by heat or fats
- Non-porous, no seams or crevices
- Odorless, polished surfaces, easily cleaned
- Withstand sterilization
- Time-saving, easy to assemble
- Self-centering
- No sticking to fittings
- Eliminate line blocks
- Help overcome line vibrations
- Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

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HAYNES
SELF-CENTERING
SNAP-TITE®
Gaskets

SIZES 1" - 1½"
2" - 2½" - 3" - 4"

*MADE FROM
TEFLON®

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . .
slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solutions, steam and solvents. They will not embrittle at temperatures as low as minus 200° F. and are impervious to heat up to 500° F.

FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

Specify . . . HAYNES SNAP-TITES of TEFLON

- *TEFLON ACCEPTED SAFE FOR USE ON FOOD & PROCESSING EQUIPMENT BY U. S. FOOD AND DRUG ADMINISTRATION*

* Gaskets made of DuPont TEFLON ® TFE-FLUOROCARBON RESINS

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A HEAVY DUTY SANITARY LUBRICANT

Available in both
SPRAY AND TUBE

All Lubri-Film ingredients are approved additives and can be safely utilized as a lubricant for food processing equipment when used in compliance with existing food additive regulations.

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

For Use in Dairies — Ice Cream Plants — Breweries — Beverage Plants — Bakeries — Canneries — Packing Plants

SANITARY • NON TOXIC • ODORLESS • TASTELESS

SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

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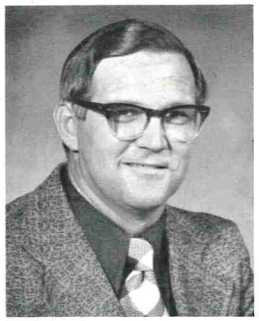
Dairy authorities speak out on better cow milking



Dr. Robert D. Appleman
Professor of Animal Science
University of Minnesota

Automatic take-off milking units: They can save and protect.

There are two primary reasons why an investment in more mechanized milking is being considered by many dairymen. One is to reduce labor. The other is to improve udder health and maintain production of high quality milk.

LABOR TAKES A BIG BITE

Labor accounts for 15 to 30 percent of all costs in a dairy operation. About 55 percent of this labor is expended in the milking operation. In general, the total labor cost to produce 100 pounds of milk in a herd averaging 12,000 pounds per cow annually when labor* is valued at $3.00 per hour approaches $2.50 per cwt. in 30-cow herds; $2.10 in 50-cow herds; $1.68 in 100-cow herds; $1.13 in 250-cow herds; and $.91 in 500-cow herds**.

With an investment to modernize milking parlors, including unit take-off, it is not unusual to substantially lower the labor costs of producing milk.

Many of the milking chores are repetitious and result in drudgery. According to our studies, 5 to 10 percent of the milker's chore time is spent removing the milking unit. On top of that, the typical milker spends from 12 to 30 percent of his time machine-stripping cows.

THE OPERATOR IS A BUSY MAN

Proper stimulation of cows in a milking parlor is important to good milk letdown. Recent New Zealand work shows there is a loss of up to 1,000 pounds of milk per cow yearly when cows are not properly stimulated. In many barns the milker cannot effectively handle as many milking units as today's economy demands. Frequently, washing and stimulation time is limited to less than 15 seconds per cow because the milker is too "busy" with machine stripping or handling other units. The result is slow milking combined with considerable overmilking. Automatic unit take-off should improve this situation. Addition of automated prep stalls will help even more, provided they function properly.

SOME RESEARCH RESULTS

Research studies comparing automatic take-off and conventional milking units involving 550 cows in a Louisiana herd resulted in these conclusions:

1. Automatic unit take-off significantly reduced the number of quarters infected with mastitis.
2. Automatic unit take-off reduced udder irritation as evidenced by lower CMT scores.
3. The men operating the automatic take-off units reduced their walking distance in the parlor by more than 25%.

Dr. Nelson Philpot, leader of this study, says one should not expect miracles. Automatic take-off units do not make a poor operator better. They do, however, allow a conscientious operator to do an even better job on more cows.

According to dairymen using this equipment, proper maintenance and proper operation of equipment is even more important with non-automated systems. The ability, cooperative attitude and location of your local serviceman should become a primary factor in deciding whether to install this more sophisticated and expensive equipment.

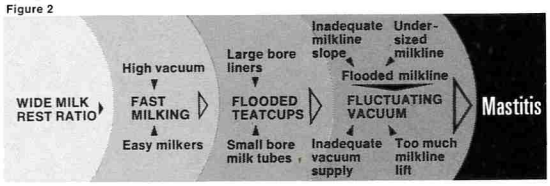
MASTITIS PREVENTION NOT SIMPLE

Dairymen should not necessarily expect a reduction in the number of cows requiring treatment for clinical mastitis (gargot). In a marginal system, more cases may result because the significance of a single variable is not the same in every milking system or in every situation.

Frank Smith, California milking system specialist, illustrates this point well. He indicates that too many researchers and educators have attempted to over-simplify the cause of mastitis. In turn, they have over-simplified its prevention. *The concept of a direct, independent relationship shown in Figure 1 is incorrect.* Figure 2 arranges these same variables in a manner which is sequential, additive, and interdependent.

Figure 1

Figure 2



As mentioned earlier, installing automatic unit take-off may allow one to milk cows faster and reduce overmilking. However, if such a change resulted in flooded milk lines and fluctuating vacuum, the incidence of mastitis might increase rather than decrease. Providing all other deficiencies in the system were corrected, automatic take-off would prove highly beneficial.

AUTOMATIC TAKE-OFFS A COMMON SIGHT?

Where cost of this mechanization is not excessive and such installations prove to be reasonably trouble-free over time, I'm sure that automatic take-off units will become an increasingly more common sight on dairy farms.

*For our purposes, the labor figures include all dairy chore labor, feeding labor, and the raising of offspring. Field labor isn't included.

**In 250-cow and 500-cow herds, we assume the existence of a parlor and a free-stall barn with mechanized feeding and waste handling.