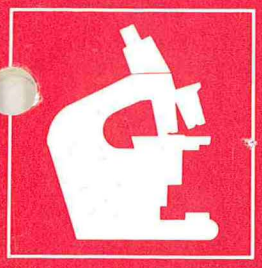
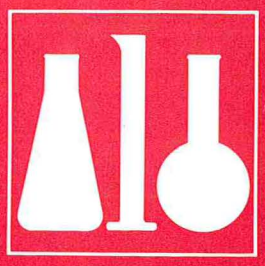
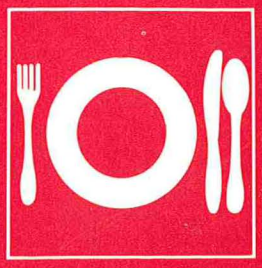
June, 1977
Vol. 40, No. 6
Pages 361-436
CODEN: JFPRDR 40(6):361-436(1977)
ISSN:0362-028X





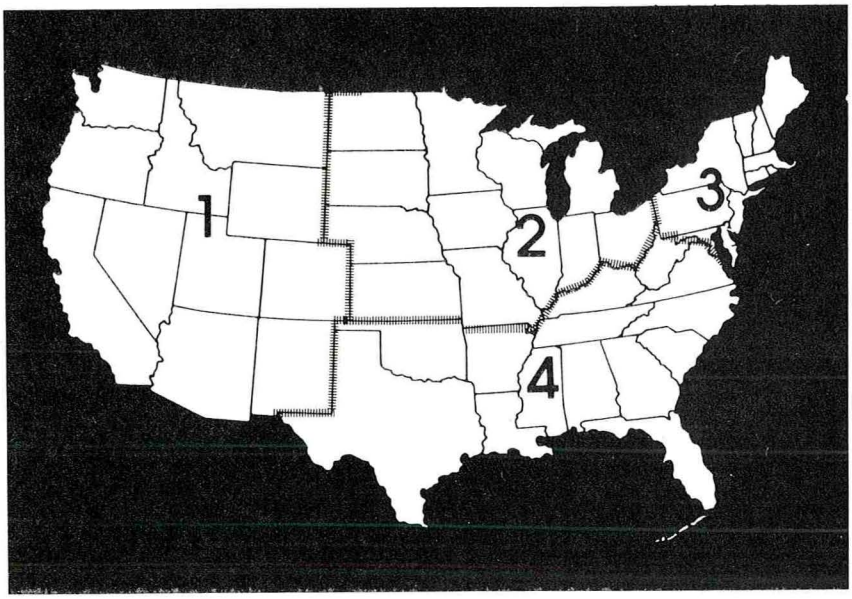


Journal of Food Protection™

An International Journal Concerned With An Adequate Food Supply That Is Safe, Wholesome, Nutritious, and Palatable

64th Annual Meeting
Sioux City Hilton Hotel
Sioux City, Iowa
August 14-18, 1977

Published Monthly by the International Association of Milk, Food, and Environmental Sanitarians, Inc., Ames, Iowa, U.S.A.



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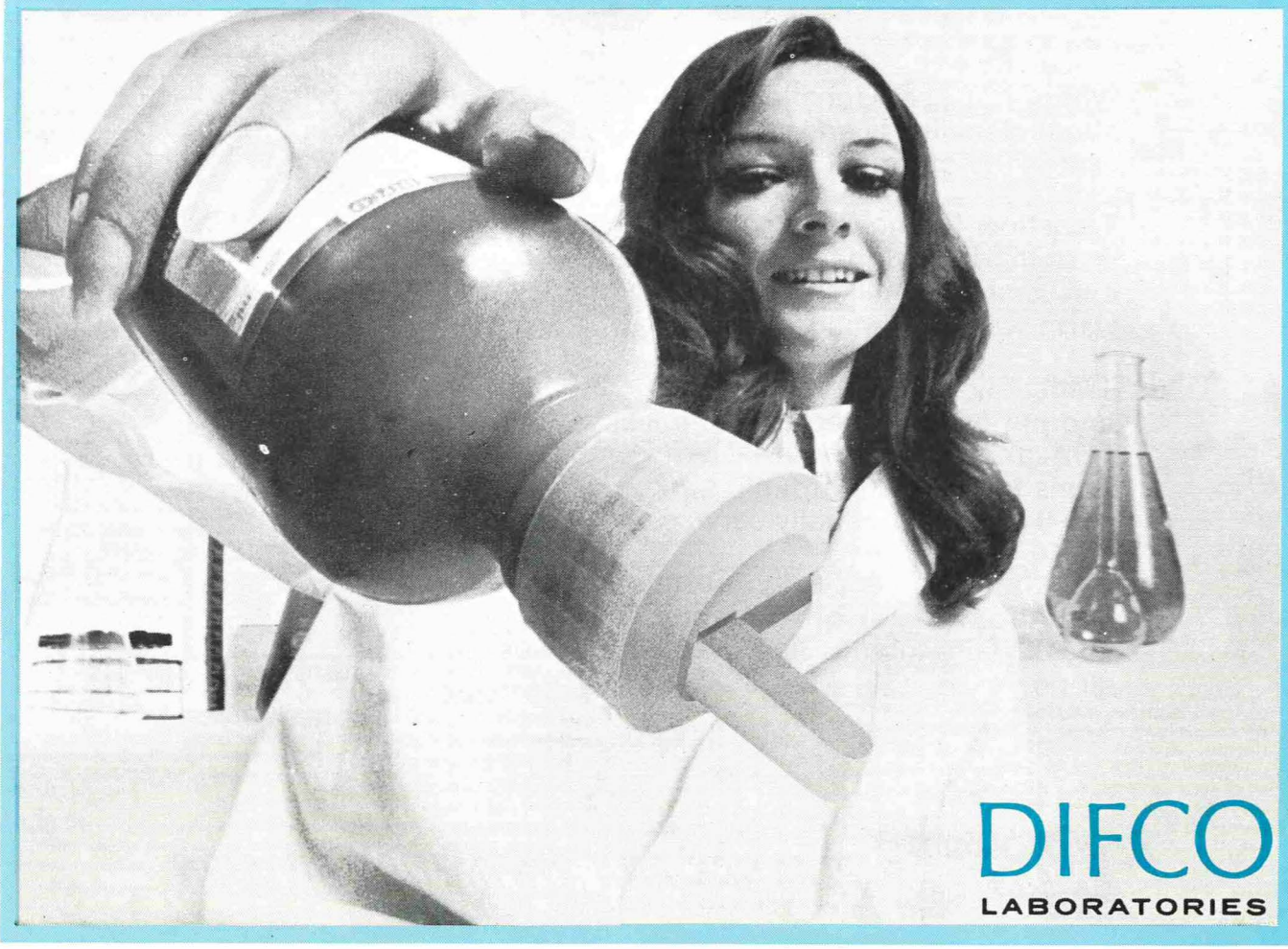

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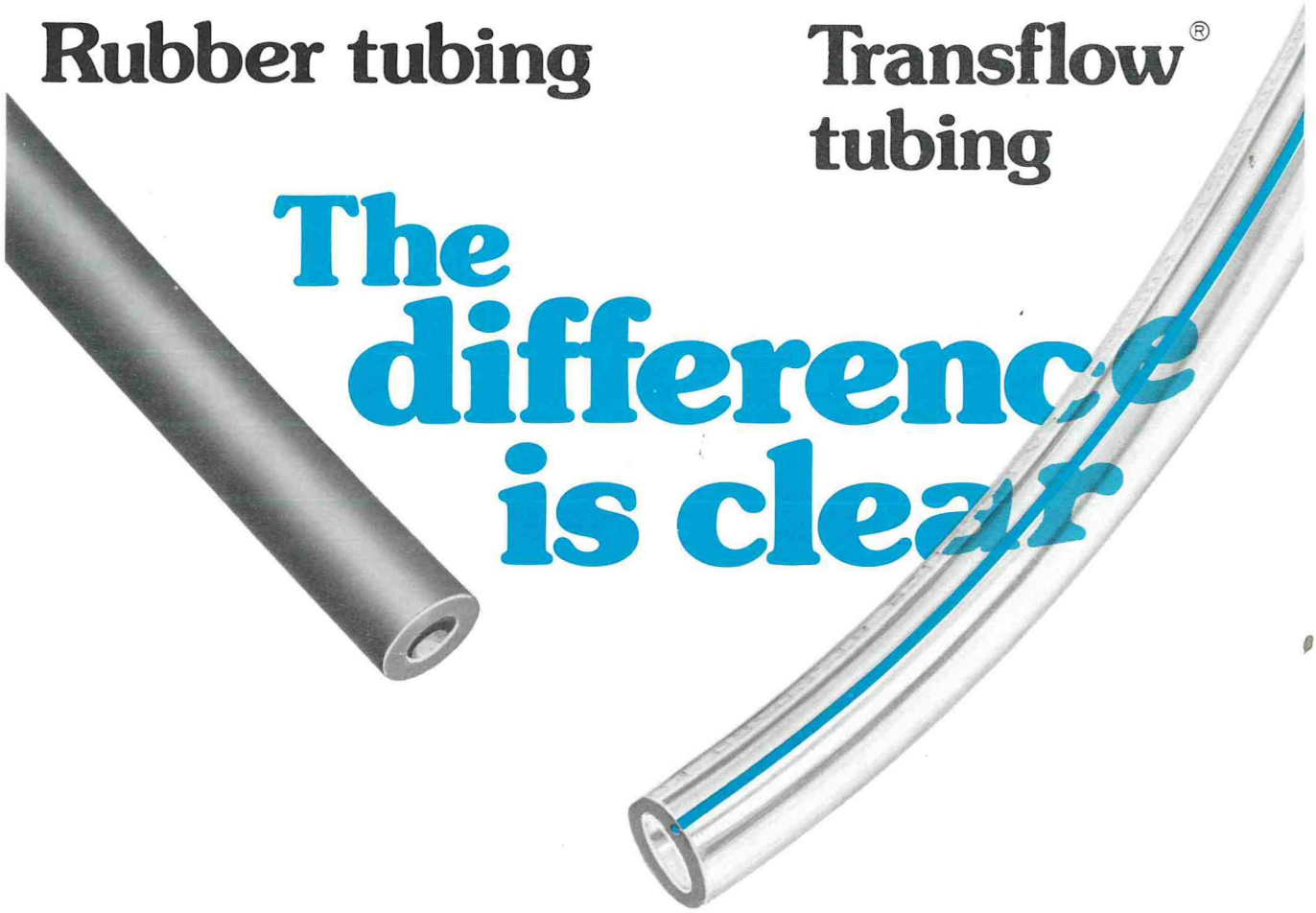

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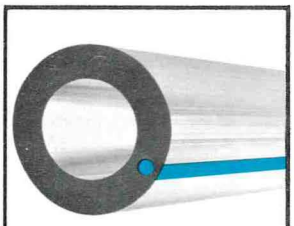

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2nd Class postage paid at Ames, Ia. 50010.

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Journal of Food Protection

(formerly Journal of Milk and Food Technology)

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

Vol. 40 June, 1977 No. 6

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Subscription Rates: $32.00 per volume, one volume per year, January through December. Single copies $3.00 each.

Volumes on Microfilm are available from Xerox University Microfilms, 300 N. Zeeb Rd., Ann Arbor, MI 48106.

Membership Dues: Membership in the Association is available to individuals only. Dues are $16.00 per calendar year and include subscription to the JOURNAL OF FOOD PROTECTION. Student membership is $5.00 per year with certification.

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Journal of Food Protection Vol. 40, No. 6, Pages 364-368 (June, 1977)
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Intermediate-Term Transoceanic Shipments of Fresh Beef

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(Received for publication September 22, 1976)

ABSTRACT

Use of a NaOCl rinse did not consistently affect ($P > .05$) weight losses or visual scores for appearance of beef cuts and quarters shipped from Seattle, Washington to Anchorage, Alaska. Wrapping of beef with PVC film greatly enhanced ($P < .05$) overall appearance of beef but also increased ($P < .05$, six of 24 comparisons) microbial counts and increased ($P < .05$, 12 of 23 comparisons) extent of visual microbial damage. Microbial counts did not increase ($P > .05$) on PVC film-wrapped beef shipped in modified atmosphere (60% CO_2, 25% O_2, 15% N_2) vans. Surface discoloration was minimized and overall appearance was enhanced ($P < .05$) for hindquarters as a result of the combined (additive) effects of NaOCl rinsing, PVC film wrapping, and shipment in a van with modified atmosphere.

Fresh beef shipped within the continental United States is in transit for an average of 4 days, incurs shrinkage during distribution at the approximate rate of 0.5% per day, and may be handled as many as 19 times during the 9-day distribution cycle from slaughter to consumer purchase (*14*). Since a large proportion of fresh beef is still distributed as hanging quarters and primal cuts in refrigerated vans, packaging and transportation systems need to be identified to minimize weight loss, trim loss, microbial damage, and loss of freshness (*12*).

Fresh meat storage time can be prolonged by modifying transit and storage temperatures and/or atmospheres (*3,4,7-10*). Meat stability has been enhanced by use of enviroments which are high in carbon dioxide concentration (*9*); whereas, increased nitrogen concentration in the atmosphere did not significantly improve product condition (*9*). Reduction in microbial growth on meat stored in modified atmospheres may result from a prolonged lag phase and a decreased logarithmic growth rate for the bacteria involved (*1*).

Use of plastic film overwraps for protecting beef quarters from shrinkage and for improving physical appearance has been studied (*10-12*). In one study (*10*) beef protected with polyvinyl chloride film was superior to unwrapped beef in lean color and subcutaneous fat appearance but prolonged storage (more than 9 days) in PVC film resulted in increased microbial growth on the moist surface created beneath the film. In other research (*11*) beef carcasses wrapped in PVC film sustained significantly less shrinkage than unprotected beef and PVC film wrapping could be used for storage of beef quarters for 14 or 21 days.

There is interest in bacterial decontaminating agents for improving appearance and condition of beef. Spraying of beef quarters with a 200 ppm solution of chlorine can reduce bacterial counts by 0.7 to 0.8 (log_{10}) units on the first day following decontamination (*4*). Rinsing of beef quarters with a 200 ppm chlorine solution has been reported (*11*) to significantly reduce shrinkage but did not improve appearance or condition of the product.

This study investigated the effects of two atmospheres, use of a NaOCl rinse, and wrapping with polyvinyl chloride film on the condition, appearance, and shrinkage of fresh beef following an intermediate-term transoceanic shipment. It is of intermediate-term in relation to distance-duration parameters of two previous studies (*7,8*) of the same kind.

EXPERIMENTAL

Two vans containing trimmed hindquarters and primal cuts of fresh beef refrigerated at -1 C were shipped from Bellevue, Washington to Anchorage, Alaska. One van had a normal (ambient air) atmosphere, the other van was charged with a modified atmosphere comprised of 60% CO_2, 25% O_2, and 15% N_2. Data were collected from 40 hindquarters, 40 wholesale rounds, 14 wholesale loins, and 56 wholesale ribs. Half of the quarters and cuts were wrapped in polyvinyl chloride film; the remaining quarters and cuts were unwrapped during transit and storage. In addition, one-half of the hindquarters and one-half of the wholesale ribs in each treatment were rinsed with a 0.02% solution of NaOCl before shipment.

All beef cuts and quarters in each treatment were weighed, swabbed (muscle surfaces on wholesale cuts, subcutaneous fat surfaces on hindquarters) by use of a 12.9-cm^2 template (to obtain bacterial samples), and subjectively evaluated at both origin and destination by two trained evaluators. Rating scales for evaluating quarters and cuts were as follows: an 8-point scale for muscle color (8 =very bright

cherry red; 1 =very dark red); a 7-point scale for surface discoloration (7 = no surface discoloration; 1 = total surface discoloration); a 6-point scale for freshness of subcutaneous fat (6 =very fresh; 1 = extensive deterioration); a 7-point scale for visual microbial damage (7 = dry, no slime formation; 1 =wet, moist, or dry; extensive colony formation or mold growth); an 8-point scale for overall appearance (8 =extremely desirable; 1 =extremely undesirable).

The two test vans were loaded with beef at a warehouse in Bellevue, Washington. The beef had been shipped to the warehouse from packers and was 2-4 days old (postmortem) at the time of loading. Vans were pulled overland to the harbor in Seattle, Washington (8 h after loading), pulled onto an ocean-going vessel (16 h after loading), and shipped to Anchorage, Alaska. Upon arrival at the destination (138 h after loading), the meat vans were pulled overland to four retail outlets where they were unloaded (162 h after loading). The quarters and cuts were monitored in retail stores for 0 to 4 days following unloading. Evaporation losses were obtained by weight differences. At the conclusion of each storage period, each cut was presented to a meatcutter for trimming. The meatcutters were instructed to trim only those cuts which needed it, and to trim very carefully to assure that no salable product was discarded. Trim was weighed for individual cuts.

Temperature during loading, transit and unloading was monitored by Ryan (models D and F) continuous recording thermometers positioned at various locations in each van. Each van also contained 24 thermocouples strategically located to measure air and product temperature by use of a potentiometer. Temperature readings from the thermocouple-potentiometer system were taken at the Seattle Pier approximately 12 h after loading and at the Anchorage Pier approximately 138 h after loading.

Swab samples for bacterial evaluations were stored in sterile 0.1% peptone broth, shipped by air to the Texas A&M University Meat Laboratory, diluted to appropriate concentration in 0.1% peptone broth, plated on standard plate count agar, incubated at 25 C for 3 days, and subsequently counted.

Data were analyzed by analysis of variance (*13*). When significant ($P < .05$) main effects were observed in the analysis of variance, mean separation analysis (*2,5*) was done. In several instances, lack of time precluded collection of complete data from certain cuts and hindquarters. In such instances, partial data are reported (e.g., bacterial counts, Table 6) or incomplete sets of data are not presented in tabular form.

RESULTS AND DISCUSSION

Data from recording thermometers and thermocouples used during loading, transit, and unloading revealed that the internal van temperature during loading was 1.6 C. Average temperature during shipment was -1 C for the van with normal atmosphers; whereas the van with modified atmosphere averaged 0 C.

Muscle color scores for primal cuts and hindquarters following 7 days of transit and 0-4 days of storage are in Table 1. Use of a NaOCl rinse did not significantly ($P > .05$) affect the brightness or stability of muscle color. With one exception, no significant differences in muscle color were observed between shipment atmospheres. Wholesale ribs and loins wrapped with PVC film had higher ($P < .05$) scores for muscle color than unprotected cuts. Hindquarters wrapped with PVC film and shipped in the modified atmosphere van were brighter ($P < .05$) in muscle color than unprotected cuts. Generally, muscle color remained relatively stable during transit-storage when the product was protected with PVC film while unprotected beef sustained greater degradation of muscle color.

Visual scores for surface discoloration of primal cuts and hindquarters following 7-11 days of transit and/or storage are in Table 2. Use of NaOCl rinse had no effect ($P > .05$) on surface discoloration of cuts and quarters in 16 of 19 comparisons. Use of modified atmosphere during shipment increased ($P < .05$) surface discoloration in seven of 11 comparisons of unprotected cuts or quarters, but in only one of 10 comparisons of cuts or quarters wrapped with PVC film. Atmospheres enriched with CO_2 have previously been shown (*6*) to increase surface discoloration of beef. In six of 11 comparisons of beef in normal atmosphere and in 12 of 12 comparisons of beef in modified atmosphere, cuts or quarters wrapped in PVC film had less ($P < .05$) surface discoloration than those which were unprotected, suggesting that cuts protected with PVC film had less discoloration from desiccation of the exposed muscle surfaces. For hindquarters, significant interactions (atmosphere × wrap, 9 days; atmosphere × NaOCl rinse, 11 days; NaOCl rinse × wrap, 11 days) suggested that reductions in surface discoloration could be attributed to additive and combined effects fo NaOCl rinsing, PVC film wrapping, and use of midified atmosphere. For wholesale ribs, PVC film wrapping was more important than use of modified atmosphere in reducing surface discoloration.

Visual scores for appearance of subcutaneous fat on primal cuts and quarters following 7-11 days of transit

TABLE 1. *Mean muscle color scores*[a] *for primal cuts and quarters following 7 days of transit and 0-4 days of storage*

Item	Transit and/or storage interval (days)	Normal atmosphere				Modified atmosphere			
		Unprotected		PVC film wrapped		Unprotected		PVC film wrapped	
		Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed
Wholesale ribs	8	5.9^{cde}	5.8^{de}	6.9^{b}	6.6^{bc}	5.6^{e}	5.8^{de}	6.4^{bcd}	6.7^{b}
Wholesale ribs	10	4.9^{c}	4.8^{c}	6.7^{b}	6.4^{b}	4.8^{c}	4.8^{c}	6.3^{b}	6.6^{b}
Wholesale rounds	7	6.9^{b}	—	6.9^{b}	—	6.0^{b}	—	7.1^{b}	—
Wholesale rounds	9	6.7^{b}	—	6.3^{b}	—	—	—	—	—
Wholesale loins	9	—	—	—	—	5.1^{c}	—	5.7^{b}	—
Trimmed hindquarters	7	5.7^{cd}	5.8^{cd}	5.8^{cd}	5.9^{cd}	5.4^{d}	5.8^{cd}	6.2^{bc}	6.6^{b}
Trimmed hindquarters	9	5.4^{b}	5.4^{b}	5.7^{b}	5.4^{b}	4.0^{c}	4.2^{c}	5.5^{b}	6.0^{b}
Trimmed hindquarters	11	4.0^{cd}	4.6^{c}	—	6.3^{b}	3.1^{d}	3.6^{cd}	5.6^{b}	6.2^{b}

[a]Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red).
[bcde]Means on the same line bearing a common superscript letter are not different ($P > .05$).

TABLE 2. *Mean surface discoloration scores[a] for primal cuts and quarters following 7 days of transit and 0-4 days of storage*

Item	Transit and/or storage interval (days)	Normal atmosphere				Modified atmosphere			
		Unprotected		PVC film wrapped		Unprotected		PVC film wrapped	
		Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed
Wholesale ribs	8	4.4^{d}	3.9^{e}	5.8^{b}	5.4^{bc}	3.4^{e}	3.5^{e}	4.9^{cd}	5.1^{bcd}
Wholesale ribs	10	3.0^{c}	2.4^{c}	4.6^{b}	4.5^{b}	2.0^{c}	2.5^{c}	4.5^{b}	5.0^{b}
Wholesale rounds	7	3.9^{c}	—	4.7^{b}	—	2.5^{d}	—	5.0^{b}	—
Wholesale rounds	9	—	4.8^{b}	—	4.3^{b}	—	—	—	—
Wholesale loins	9	—	—	—	—	1.8^{c}	—	3.3^{b}	—
Trimmed hindquarters	7	3.0^{def}	3.4^{cde}	4.0^{bcd}	3.8^{bcd}	2.4^{ef}	2.3^{f}	4.3^{bc}	4.8^{b}
Trimmed hindquarters	9	2.8^{d}	3.1^{cd}	3.8^{bc}	3.4^{bc}	1.7^{e}	1.6^{e}	3.8^{bc}	4.3^{b}
Trimmed hindquarters	11	1.8^{d}	2.7^{c}	—	4.3^{bc}	1.1^{e}	1.4^{e}	3.5^{c}	4.4^{b}

[a]Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration).
[bcdef]Means on the same line bearing a common superscript letter are not different ($P > .05$).

and/or storage are in Table 3. Use of a NaOCl rinse had no effect (P+ .05) on subcutaneous fat appearance of beef cuts or quarters. In seven of 21 comparisons, cuts or quarters shipped in a normal atmosphere van sustained less ($P < .05$) subcutaneous fat deterioration than corresponding beef shipped in a modified atmosphere van. Appearance of subcutaneous fat was superior ($P < .05$) for PVC film wrapped beef in comparison to unprotected beef in 10 of 23 comparisons in Table 3. Use of PVC film appeared to be more advantageous in maintaining fresh product appearance as storage time increased; nine of 17 comparisons of beef stored 8 days or more favored ($P < .05$) use of PVC film in reducing discoloration of subcutaneous fat.

Visual microbial damage scores for primal cuts and quarters following 7-11 days of transit and/or storage are in Table 4. Use of a NaOCl rinse had no effect ($P > .05$) on scores for visual microbial damage in 16 of 19 comparisons. Shipment in modified atmosphere was associated with less ($P < .05$) visual microbial damage in 7 of 21 comparisons of cuts or quarters, otherwise treated alike, in Table 4. Modified atmosphere was most effective in decreasing microbial proliferation and damage on surfaces of unprotected quarters and cuts (five of 11 comparisons, Table 4) suggesting that high CO_2 concentrations were more effective when a protective covering was not used. PVC film wrapping increased ($P < .05$) visual microbial damage in 12 of 23 comparisons

TABLE 3. *Mean subcutaneous fat appearance scores[a] for primal cuts and quarters following 7 days of transit and 0-4 days of storage*

Item	Transit and/or storage interval (days)	Normal atmosphere				Modified atmosphere			
		Unprotected		PVC film wrapped		Unprotected		PVC film wrapped	
		Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed
Wholesale ribs	8	4.0^{b}	3.6^{bc}	4.2^{b}	4.0^{b}	3.2^{c}	3.3^{c}	3.6^{bc}	3.4^{cd}
Wholesale ribs	10	2.8^{cd}	2.5^{d}	3.3^{b}	3.5^{b}	1.4^{e}	1.2^{e}	3.2^{bc}	3.3^{b}
Wholesale rounds	7	4.7^{b}	—	5.0^{b}	—	4.5^{b}	—	5.0^{b}	—
Wholesale rounds	9	4.0^{b}	—	3.4^{b}	—	—	—	—	—
Wholesale loins	9	—	—	—	—	1.5^{c}	—	3.6^{b}	—
Trimmed hindquarters	7	4.7^{bc}	4.0^{bc}	5.0^{bc}	5.0^{bc}	3.9^{c}	4.5^{bc}	5.1^{b}	5.0^{bc}
Trimmed hindquarters	9	3.7^{cde}	3.6^{cdef}	4.0^{bcd}	4.4^{b}	3.0^{f}	3.4^{ef}	4.2^{bc}	3.7^{cde}
Trimmed hindquarters	11	3.8^{bc}	3.1^{cd}	—	4.0^{b}	2.3^{e}	2.9^{de}	4.1^{b}	3.4^{bcd}

[a]Means based on a 6-point scale (6 = very fresh; 1 = extensive deterioration).
[bcdef]Means on the same line bearing a common superscript letter are not different ($P > .05$).

TABLE 4. *Mean visual microbial damage scores[a] for primal cuts and quarters following 7 days of transit and 0-4 days of storage*

Item	Transit and/or storage interval (days)	Normal atmosphere				Modified atmosphere			
		Unprotected		PVC film wrapped		Unprotected		PVC film wrapped	
		Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed
Wholesale ribs	8	6.6^{bc}	4.7^{e}	6.1^{bcd}	5.5^{de}	6.8^{b}	5.7^{cd}	5.9^{bcd}	5.6^{cde}
Wholesale ribs	10	4.6^{c}	3.5^{d}	3.1^{d}	3.4^{d}	7.0^{b}	6.9^{b}	5.9^{bc}	6.0^{bc}
Wholesale rounds	7	6.2^{b}	—	6.1^{b}	—	6.0^{b}	—	5.0^{c}	—
Wholesale rounds	9	5.7^{b}	—	5.1^{b}	—	—	—	—	—
Wholesale loins	9	—	—	—	—	6.9^{b}	—	5.6^{c}	—
Trimmed hindquarters	7	6.8^{b}	6.7^{b}	6.1^{c}	6.0^{c}	6.7^{b}	6.7^{b}	6.0^{c}	6.0^{c}
Trimmed hindquarters	9	6.4^{bc}	6.8^{b}	6.0^{c}	6.0^{c}	6.8^{b}	6.6^{b}	5.8^{c}	6.0^{c}
Trimmed hindquarters	11	4.8^{e}	5.0^{de}	—	5.5^{cde}	7.0^{b}	7.0^{b}	6.0^{c}	5.7^{c}

[a]Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony formation or mold growth).
[bcde]Means on the same line bearing a common superscript letter are not different ($P > .05$).

in Table 4. Quarters and cuts wrapped with PVC film had increased surface moisture which enhanced microbial growth and increased visual microbial damage; unwrapped cuts and quarters were drier and were less able to support microbial growth.

Overall appearance scores for primal cuts and quarters following 7-11 days of transit and/or storage are in Table 5. Overall appearance was not improved ($P > .05$) by use of a NaOCl rinse. Use of modified atmosphere during shipment decreased ($P < .05$) overall appearance scores in 7 of 11 comparisons of unwrapped beef but decreased ($P < .05$) overall appearance of beef wrapped with PVC film in only one of 10 comparisons; results which closely parallel those for surface discoloration (Table 2). Maintenance of high concentrations of CO_2 and/or N_2 around fresh meat causes discoloration of the muscle surface (6,11) either because of anoxia or because CO_2 causes a change in pH (*6*). In 20 of 23 comparisons (Table 5) use of PVC film enhanced ($P < .05$) the overall appearance of primal cuts and quarters. For hindquarters, significant interactions (atmosphere × wrap, 7 days; NaOCl rinse × atmosphere, 9 days) suggested that enhanced overall appearance could be attributed to combined effects of NaOCl rinsing, PVC film wrapping, and use of modified atmosphere. PVC film should be used when the van atmosphere is modified to assure optimal overall appearance of beef.

Microbial counts for primal cuts and quarters following 7-11 days of transit and/or storage are in Table 6. Use of a NaOCl rinse reduced ($P < .05$) bacterial counts in four of 20 comparisons in Table 6. Use of modified atmosphere during shipment reduced ($P < .05$) microbial counts in seven of 21 comparisons of cuts or quarters, otherwise treated alike. Use of PVC film increased ($P < .05$) microbial counts in five of 12 comparisons of beef in normal atmosphere but in only one of 12 comparisons of beef in modified atmosphere. Use of PVC film did not increase ($P > .05$) microbial counts if beef was shipped in a modified atmosphere van. Taxonomic classification of bacteria from representative cuts or quarters in all treatments revealed that *Pseudomonas* was the predominant genus among microorganisms found on beef in the present study. Microbial samples from hindquarters were taken from subcutaneous fat and thus may be lower than if counts had been obtained by swabbing muscle surfaces.

Evaporative and trim losses of beef cuts following 7-10 days of transit and/or storage are in Table 7. Use of a NaOCl rinse did not consistently affect ($P > .05$) weight losses. Use of modified van atmosphere during shipment did not consistently affect ($P > .05$) evaporative or trim losses. Cuts wrapped in PVC film sustained less ($P < .05$) evaporative loss (11 of 12 comparisons) and less ($P < .05$) trim loss (four of 10 comparisons) than those which were unprotected. PVC film can effectively reduce evaporative and trim loss of beef transported and/or stored for 7 to 10 days.

The following conclusions were drawn from these

TABLE 5. *Mean overall appearance scores*[a] *for primal cuts and quarters following 7 days of transit and 0-4 days of storage*

Item	Transit and/or storage interval (days)	Normal atmosphere				Modified atmosphere			
		Unprotected		PVC film wrapped		Unprotected		PVC film wrapped	
		Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed
Wholesale ribs	8	4.9^{c}	4.1^{d}	6.1^{b}	5.4^{bc}	4.0^{d}	4.1^{d}	5.1^{c}	5.4^{bc}
Wholesale ribs	10	3.4^{c}	2.7^{c}	4.6^{b}	4.6^{b}	2.1^{d}	2.3^{d}	4.7^{b}	5.2^{b}
Wholesale rounds	7	4.8^{bc}	—	5.5^{b}	—	3.5^{c}	—	5.9^{b}	—
Wholesale rounds	9	5.0^{b}	—	4.4^{c}	—	—	—	—	—
Wholesale loins	9	—	—	—	—	2.1^{c}	—	4.2^{b}	—
Trimmed hindquarters	7	4.2^{de}	4.0^{e}	4.9^{cd}	4.9^{cd}	4.0^{e}	4.0^{e}	5.4^{bc}	5.7^{b}
Trimmed hindquarters	9	3.8^{cd}	3.7^{d}	4.8^{b}	4.2^{bc}	2.5^{e}	2.7^{e}	4.8^{b}	4.8^{b}
Trimmed hindquarters	11	2.8^{cd}	3.3^{c}	—	5.2^{b}	2.0^{e}	2.3^{de}	4.6^{b}	4.8^{b}

[a]Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[bcde]Means on the same line bearing a common superscript letter are not different ($P > .05$).

TABLE 6. *Mean microbial counts*[a] *for primal cuts and quarters following 7 days of transit and 0-4 days of storage*

Item	Transit and/or storage interval (days)	Normal atmosphere				Modified atmosphere			
		Unprotected		PVC film wrapped		Unprotected		PVC film wrapped	
		Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed
Wholesale ribs	8	8.9^{b}	8.9^{b}	8.2^{bc}	8.2^{bc}	7.4^{cd}	6.7^{d}	7.8^{bcd}	7.6^{cd}
Wholesale ribs	10	7.7^{bcd}	8.7^{b}	7.6^{bcd}	7.9^{bc}	6.0^{d}	6.6^{d}	6.0^{d}	6.8^{cd}
Wholesale rounds	7	8.7^{b}	—	5.5^{bc}	—	4.0^{c}	—	5.6^{bc}	—
Wholesale rounds	9	8.6^{b}	—	6.9^{c}	—	—	—	—	—
Wholesale loins	9	—	—	—	—	7.7^{b}	—	7.3^{b}	—
Trimmed hindquarters	7	5.1^{cd}	6.2^{c}	5.9^{c}	7.1^{b}	5.8^{c}	4.1^{d}	4.8^{cd}	5.9^{c}
Trimmed hindquarters	9	4.6^{c}	3.6^{d}	6.3^{b}	6.5^{b}	4.3^{cd}	4.9^{c}	4.9^{c}	5.2^{bc}
Trimmed hindquarters	11	4.8^{c}	3.9^{d}	7.1^{b}	6.2^{c}	4.8^{c}	4.7^{c}	6.2^{bc}	6.3^{bc}

[a]Microbial counts are reported as the number of microorganisms ($\log_{10}$) per 6.45 cm^2 of surface area.
[bcd]Means on the same line bearing a common superscript letter are not different ($P > .05$).

TABLE 7. *Mean weight loss percentages for primal cuts following 7 days of transit and 0-3 days of storage*

Primal cut	Transit and/or storage interval (days)	Kind of weight loss[a]	Normal atmosphere				Modified atmosphere			
			Unprotected		PVC film wrapped		Unprotected		PVC film wrapped	
			Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed	Not chlorine rinsed	Chlorine rinsed
Wholesale rounds	7	Evaporative	0.53[b]	—	0.16[c]	—	0.63[b]	—	0.27[c]	—
Wholesale rounds	9	Evaporative	0.80[b]	—	0.44[c]	—	—	—	—	—
Wholesale rounds	9	Trim	0.47[b]	—	0.50[b]	—	—	—	—	—
Wholesale loins	9	Evaporative	—	—	—	—	2.13[b]	—	0.36[c]	—
Wholesale loins	9	Trim	—	—	—	—	1.84[b]	—	1.21[c]	—
Wholesale ribs	8	Evaporative	0.68[c]	0.33[d]	0.13[e]	0.36[d]	0.50[d]	1.07[b]	0.14[e]	0.11[e]
Wholesale ribs	8	Trim	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.00[b]
Wholesale ribs	10	Evaporative	1.12[d]	1.14[d]	0.23[e]	0.09[f]	1.31[c]	2.25[b]	0.23[e]	0.24[e]
Wholesale ribs	10	Trim	1.61[d]	1.95[c]	0.62[e]	0.39[f]	3.07[b]	0.62[e]	0.62[e]	0.66[e]

[a]Evaporative weight loss = loss of volatile components during transit and/or storage plus weight of accumulated fluids in the package at the time of PVC film removal; trim weight loss = weight of muscle and fat removed from cut surfaces before retail cutting because of deterioration or spoilage.
[bcdef]Means on the same line bearing a common superscript letter are not different ($P > .05$).

data: (*a*) Use of a NaOCl rinse decreased ($P < .05$) microbial counts in a few instances (four of 20 comparisons) but did not consistently affect ($P < .05$) evaporative losses, trim losses, or visual scores for muscle color, surface discoloration, subcutaneous fat appearance, visual microbial damage, and overall appearance. (*b*) Use of a modified atmosphere (60% CO_2, 25% O_2, 15% N_2) during shipment had no consistent effect ($P < .05$) on muscle color scores, evaporative losses or trim losses; decreased ($P < .05$, seven of 11 comparisons of unprotected beef and one of 10 comparisons of PVC film wrapped beef) scores for surface discoloration and overall appearance and detracted ($P < .05$, seven of 21 comparisons) from subcutaneous fat appearance; but reduced ($P < .05$, seven of 21 comparisons) microbial counts and extent of visual microbial damage. (*c*) Wrapping of beef with PVC film increased ($P < .05$, 16 of 23 comparisons) brightness of muscle color, decreased ($P < .05$, 18 of 23 comparisons) extent of surface discoloration, improved ($P < .05$, 10 or 23 comparisons) appearance of subcutaneous fat, and enhanced ($P < .05$, 20 of 23 comparisons) overall appearance but increased ($P < .05$, six of 24 comparisons) microbial counts and increased ($P < .05$, 12 of 23 comparisons) extent of visual microbial damage. (*d*) Wrapping of beef with PVC film greatly enhanced overall appearance of beef but also increased microbial growth; however, microbial counts did not increase on PVC film-wrapped beef shipped in a van with modified atmosphere. Surface discoloration was minimized and overall appearance was enhanced for hindquarters as a result of the combined (additive) effects of NaOCl rinsing, PVC film wrapping, and shipment in a van with modified atmosphere.

ACKNOWLEDGMENTS

Technical Article 12867 from the Texas Agricultural Experiment Station. This research was supported in part by the Agricultural Marketing Research Institute, Agricultural Research Service, U.S. Department of Agriculture (Contract 12-14-1001-407).

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Journal of Food Protection Vol. 40, No. 6, Pages 369-372 (June, 1977)
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Influence of pH, Temperature, Curing Agents, and Water Activity on Germination of PA 3679 Spores[1]

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(Received for publication August 30, 1976)

ABSTRACT

The influence of pH, temperature, water activity, and curing agents on germination of spores of *Clostridium sporogenes* (PA 3679) was examined. The most influential factor was pH; least germination occurred at pH 5.5, and most at pH 7.0, the highest pH tested. Germination occurred over a temperature range of 4 to 55 C, with maximal germination at 35 and 45 C. NaCl was more inhibitory than $NaNO_2$ and $NaNO_3$ at pH 7.0 at the levels used. At pH 5.5 and 6.0, $NaNO_2$ stimulated germination.

Bacterial endospores are notable because of their resistance to heat and chemicals that may be used for inactivation of bacteria. Bacterial spores are usually present in foods; and, under favorable conditions, these unwanted spores can germinate and multiply to cause spoilage and, in some instances, to create a health hazard. Control of germination and development of growth from bacterial spores in foods have been approached from two viewpoints. One is to induce germination that produces a spore or cell of increased susceptibility to adverse environments. Second is the concept of preventing or inhibiting germination, which eliminates the possiblility of growth of the organism.

The purpose of this work was to determine the influence of environmental conditions including pH, temperature, water activity, and curing agents used for meat products on germination of a spore-forming putrefactive anaerobe. Knowledge of the influence of these factors on germination of sporeforming bacteria may yield insights into the control of spores in food, particularly in those foods depending on nitrates and nitrites for preservation.

MATERIALS AND METHODS

The organism used in this work was *Clostridium sporogenes* ATCC 7955; it is also referred to as Putrefactive Anaerobe 3679 NCA strain or PA 3679. Stock cultures were maintained in Cooked Meat medium (Difco) stored at 5 C. Spores were produced by using the technique of Uehara et al. (*15*) except that the medium used for sporulation consisted of 6.0% trypticase (BBL) and 0.1% glucose. The pH of the medium was adjusted to 7.0. Inoculated flasks were incubated at 35 C for 55 to 60 h at which time 80 to 90% of the cells had sporulated. Cells were collected by centrifugation and washed with sterile, deionized water. After the final wash, the pellet of cells was resuspended in distilled water and stored overnight at 5 C to allow for some lysis of vegetative cells. After an additional wash, further lysis of vegetative cells was induced by treatment with lysozyme as described by Finley and Fields (*5*). After a final wash, the spores were suspended in sterile, deionized water at a concentration of approximately 2.0×10^{10} spores/ml. Approximately 99% of the spores in the suspension were refractile.

Media for determining the degree of germination were prepared from Brain Heart Infusion (BHI) broth to which 0.1% sodium metabisulfite had been added; the metabisulfite inhibited outgrowth without altering germination of spores (*7*). Various combinations of 4.0% sodium chloride, 0.1% sodium nitrate, and 0.02% sodium nitrite in this broth were examined for influence on germination. Other interacting factors included in the study were water activity (a_w) at levels of 0.99, 0.97, and 0.95 and pH levels of 7.0, 6.5, 6.0, and 5.5. The media containing 4.0% sodium chloride and 4.0% sodium chloride plus 0.1% sodium nitrate plus 0.02% sodium nitrite had a_w levels of 0.97 and could not be included in comparisons of combinations having an a_w of 0.99.

The a_w was calculated by first determining the freezing point of the various broths by using the graphical method of Shoemaker and Garland (*12*). This information was then used in the equations given by Daniels et al. (*1*) to calculate a_w. When needed, glycerol was added to each solution to attain the desired a_w.

Five different incubation temperatures (4, 25, 35, 45, and 55 C) were evaluated for their influence on germination of spores in each of the 52 different combinations. The order in which the 260 observations (52 combinations of additives and pH times 5 temperatures) were made was randomized to eliminate possible biases due to aging effect on spores. The total time used for each replication did not exceed 10 days; therefore, spores used in each replication were not more than 10 days old. Three replications were made with different batches of spores. Each tube of medium was brought to the specified temperature before 0.5 ml of aqueous spore suspension was added to 15 ml of medium. The absorbance of this suspension was measured at 600 nm by using a Bausch and Lomb Spectronic-20 colorimeter. Absorbance was measured after 2, 4, 6, and 24 h of incubation.

Data for the percent loss of absorbance were analyzed statistically by analysis of variance to determine the significance of the effect of each single factor and of factor interactions on germination (Table 1). The analysis was made on the arcsin of the square root of percent loss of absorbance. Data for observations made at 4 C and after 24 h were not included in the analysis; the retarding effect of incubation at 4 C made it difficult to determine the influence of other factors on germination. About 5% of the observations made after 24 h showed outgrowth, making it necessary to discuss these observations separately. The analysis in Table 1 and the plotted points in the figures represent data that are percent loss of absorbance averaged for 2, 4, and 6 h.

RESULTS AND DISCUSSION

Germination of spores of *C. sporogenes* PA 3679 occurred in Brain Heart Infusion (BHI) broth at all pH

[1]*Journal Paper No. J-8603 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 2201.*

levels in the range of 5.5 to 7.0; the least amount of germination occurred at pH 5.5, and the greatest at pH 7.0 (Fig. 1). An optimal pH for germination was not established because pH levels above 7.0 were not used. Duncan and Foster (*4*) reported that pH 6.0 was optimal for rapid germination of spores of PA 3679h in a phosphate buffer containing sodium nitrite; on the other hand, an alkaline pH of 8.5 or higher was optimal in systems in which L-alanine was used to induce germination (*6,13,14*).

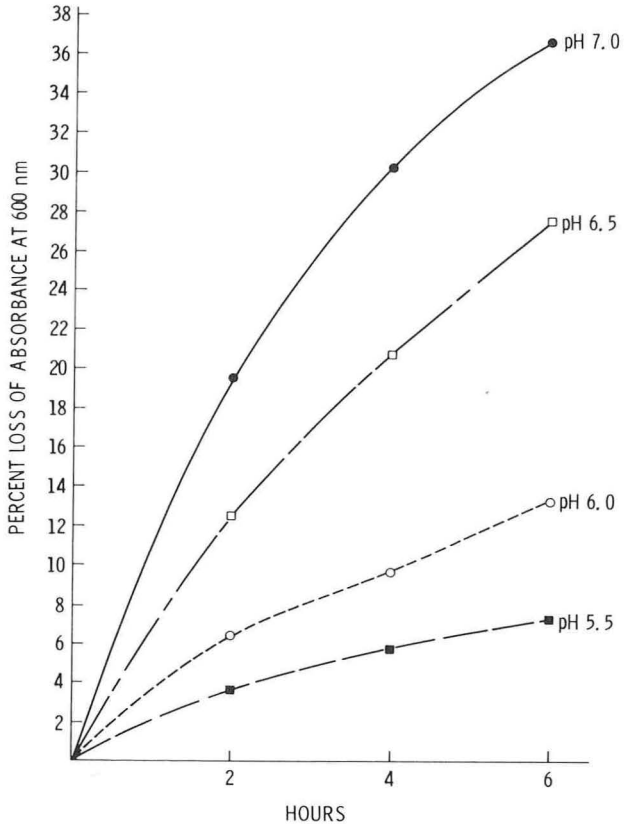

Figure 1. *The effect of pH on germination of PA 3679 NCA spores.*

The pH of the system plays a dominant role in the control of germination of spores of PA 3679 in BHI broth. A comparison of values of the mean squares in Table 1 show that temperature of incubation, curing agents, and water activity also played statistically significant roles, but to a lesser degree than pH. Replications in which different batches of spores were used did not differ significantly from each other.

Temperature and pH interacted in such a manner that, at pH 5.5, the level of germination increased linearly as the temperature was increased from 25 C to 55 C (Fig. 2). At pH 6.0, however, little variation occurred in the amount of germination as the temperature was increased from 35 to 55 C. Germination was least at 25 C for all pH levels. At pH 6.5 and 7.0, the percentage of germinated spores was maximal when the temperature was 35 and 45 C. Germination of PA 3679 spores occurs over a wide range of temperatures, with the optimum rate of germination

TABLE 1. *Least-squares analysis of variance of factors affecting germination of spores of PA 3679*

Source	Degrees of Freedom	Mean Square	F Value[1]
Replications	2	0.0038	0.29
B (Additives)	12	0.1480	11.14**
Curing agents (CA)	4	0.0693	5.21**
Water activity (a_w)	2	0.1291	9.71**
CA × a_w	6	0.0121	0.91
pH	3	8.6828	653.62**
pH_L (linear)	1	25.7182	1933.70**
Temperature (T)	3	3.3112	249.26**
T_L	1	3.4329	258.11**
T_Q (quadratic)	1	6.2143	467.24**
pH × T	9	0.4552	32.27**
$pH_L \times T$	1	0.1600	12.03**
$pH_L \times T_Q$	1	3.4029	255.86**
B × pH	36	0.0881	6.63**
B × T	36	0.0149	1.12
B × pH × T	108	0.0151	1.13
Error (1)	414	0.0133	

[1]Significance at the .01 level denoted by **.

occurring at 35 and 45 C when the pH is favorable. These findings are similar to those of Mehl and Wynne (*9*) who observed germination of PA 3679 spores over a temperature range of 25 to 45 C and reported that maximal germination occurred between 40 and 45 C and that germination was slow and limited at 20 C. This relationship between pH and temperature emphasizes the need for reporting pH when stating maximal germination of spores.

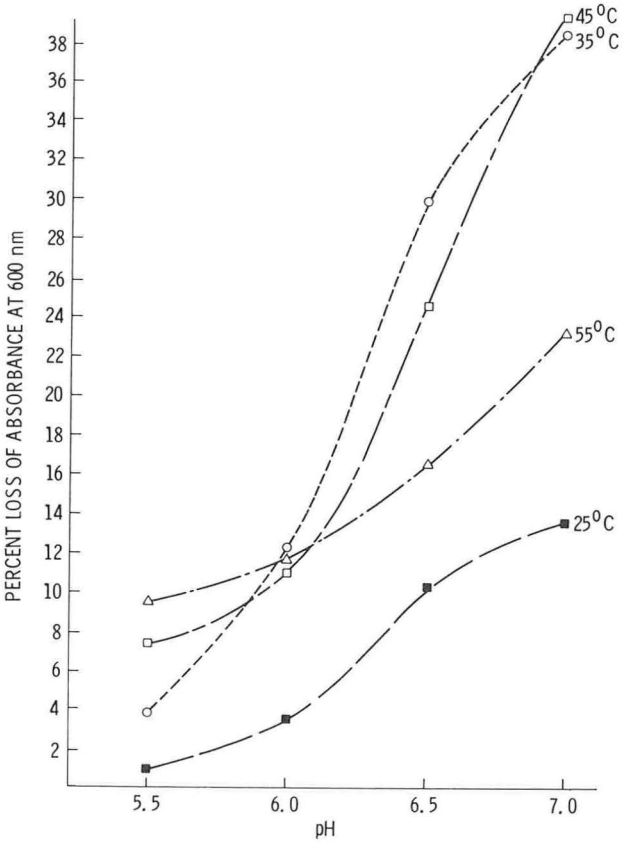

Figure 2. *The effect of pH and temperature on germination of PA 3679 NCA spores.*

Inhibition of germination by curing salts can be attributed to changes in water activity and to the inherent inhibitory activity of curing salts (Fig. 3, 4). At levels of 4.0% NaCl, a_w becomes a dominant factor in preventing germination. When glycerol was added to the system instead of NaCl to adjust the systems to identical a_w levels, the combination of the curing salts produced a reduction in germination exceeding that of any of them alone.

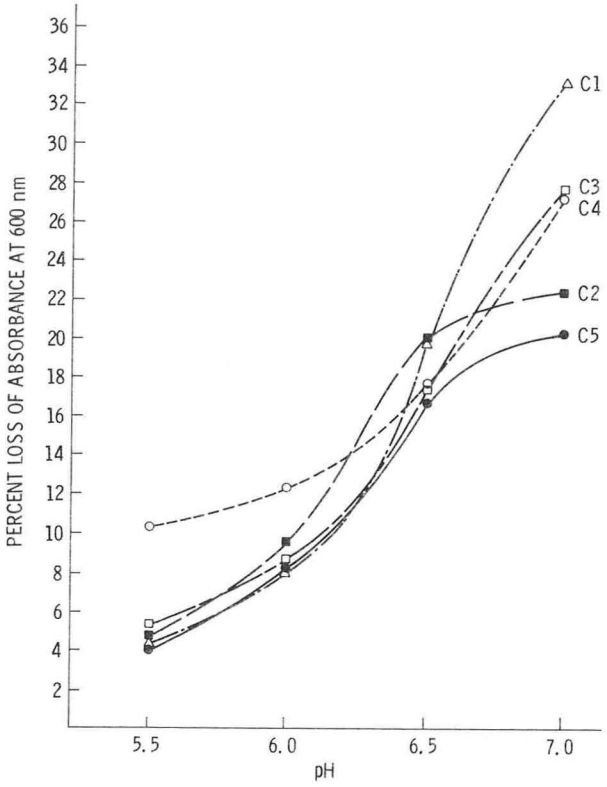

Figure 3. *The effect of pH and curing agents on germination of PA 3679 NCA spores. C1: Basal germinant (BHI + 0.1% sodium metabisulfite), C2: Basal germinant + 4% sodium chloride, C3: Basal germinant + 0.1% sodium nitrate, C4: Basal germinant + 0.02% sodium nitrite, and C5: Basal germinant + 4% sodium chloride + 0.1% sodium nitrate + 0.02% sodium nitrite.*

Addition of 4.0% NaCl to the medium inhibited outgrowth for 24 h under all conditions tested, but inhibited germination only in a few instances in which pH and temperature played dominant roles. At 25 C, addition of 4.0% NaCl produced the most extensive inhibition of all combinations of salts. When the pH was equal to or less than 6.0, little additional inhibition on spore germination was produced by addition of NaCl or by reducing the a_w of the medium. Mundt et al. (*10*) observed germination of 90% of the spores of *C. sporogenes* in the presence of 8.0% NaCl at pH levels down to 5.3 in a temperature range of 4.4 C to 35 C, but outgrowth did not occur under these conditions. Duncan and Foster (*3*) used a microculture technique to demonstrate that germination of spores of PA 3679h occurred in the presence of 3 to 6% NaCl; vegetative cells appeared under these circumstances, but cell division was blocked. Germination of unheated spores of such

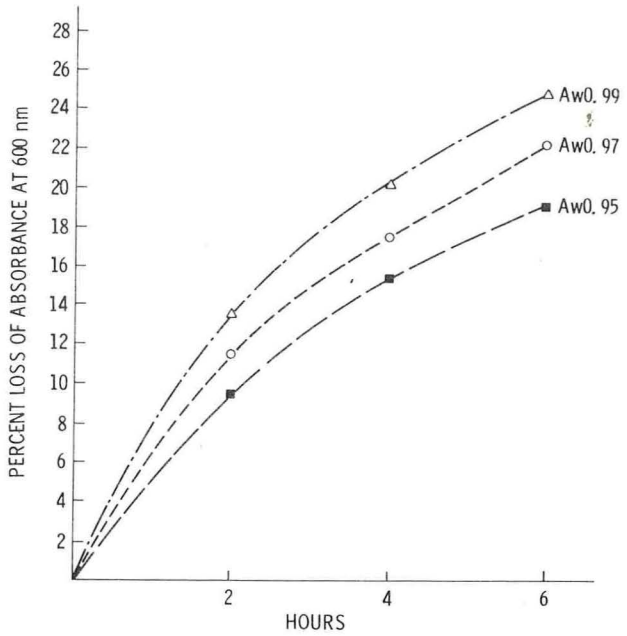

Figure 4. *The effect of water activity on germination of PA 3679 NCA spores.*

anaerobes as *Clostridium roseum* and *Clostridium botulinum* is completely inhibited in tryptone solution containing 10% NaCl (*8*). Relatively high concentrations of NaCl are needed to completely prevent germination of spores of putrefactive anaerobes; the actual amount of salt required depends on the medium, the strain or species, and other experimental conditions.

The pH of the medium influenced the degree to which the various curing salts affected germination (Fig 3). At pH 7.0, nitrate and nitrite suppressed germination to a similar extent, but not to the extent observed in the presence of NaCl; a_w was probably an important factor in the presence of 4.0% NaCl. The combination of the three salts was most effective for inhibiting germination at pH 7.0. At pH 5.5 and 6.0, the amount of germination was similar in all combinations except for $NaNO_2$ alone, which, under these conditions, enhanced germination. Duncan and Foster (2, 3, 4) have reported stimulation of germination in the presence of 0.02% $NaNO_2$ and found that levels of $NaNO_2$ up to 2.0% were incapable of completely inhibiting germination or outgrowth of PA 3679h spores. These workers postulated that, in cured meats, $NaNO_2$ induces germination, thus making the spores susceptible to subsequest heat processing.

In the presence of 0.02% $NaNO_2$, more extensive germination occurred at 45 and 55 C than at the lower temperatures. Duncan and Foster (*4*) also have stated that nitrite-induced germination is accelerated at high incubation temperatures.

Germination occurred at a_w levels of 0.95 to 0.99, but the extent of germination decreased as the a_w was decreased (Fig. 4). The medium containing 0.02% $NaNO_2$ and having an a_w of 0.99 yielded the greatest percentage of germinated spores. Least germination occurred in the medium containing all three curing salts

and having an a_w of 0.95. No outgrowth occurred within 24 h in media with an a_w of 0.95. In media having identical a_w values of 0.97, germination occurred to a greater extent when only glycerol was used to adjust a_w than when NaCl was used.

After 24 h of incubation, approximately 5% of the tubes (38 tubes of 780) showed outgrowth (Table 2). Of

TABLE 2. *Conditions that permitted outgrowth of spores of PA 3679 within 24 h*

Medium	a_w	pH	Temperature (C)	Number of tubes showing growth[a]
I. *After 6 hours of incubation:*				
Basal	0.99	7.0	35	1
Basal + 0.1% $NaNO_3$	0.99	7.0	35	1
Basal + 0.02% $NaNO_2$	0.99	7.0	35	2
II. *After 24 hours of incubation:*				
Basal	0.99	7.0	35	1
	0.99	7.0	25	1
Basal + 0.1% $NaNO_3$	0.99	7.0	35	3
	0.97	7.0	35	1
Basal × 0.02% $NaNO_2$	0.99	7.0	25	2
	0.99	7.0	35	3
	0.99	7.0	45	2
	0.99	6.5	25	1
	0.99	6.5	35	3
	0.99	6.5	45	1
	0.97	7.0	35	2
	0.97	6.5	35	1
Basal + 4% NaCl +	0.97	7.0	35	3
0.1% $NaNO_3$ + 0.02%	0.97	7.0	45	2
$NaNO_2$	0.97	7.0	55	1
	0.97	6.5	35	2
	0.97	6.5	45	1
	0.97	5.5	45	1
	0.95	7.0	35	1
	0.95	7.0	45	2
	0.95	7.0	55	1
	0.95	6.5	35	1
	0.95	6.5	45	2

[a]Number of tubes from three replications showing growth.

all treatments, growth occurred most frequently in the presence of 0.02% $NaNO_2$; indeed, 15 instances of growth were observed in the presence of nitrite as compared with two instances in the control group. Sodium nitrite not only stimulated germination, but also reduced the lag time for initiation of growth. Temperatures optimal for germination also favored outgrowth. A satisfactory explanation for these occurrences is not obvious, but perhaps the greater number of germinated spores in the presence of $NaNO_2$ increased the chance of growth occurring. Riemann (*11*) has emphasized that curing agents and heat are much more effective in controlling growth when the numbers of organisms are small. However, this explanation is lacking when it noted that least germination of spores occurred in the presence of NaCl plus $NaNO_3$ plus $NaNO_2$ and that 17 out of 120 of the tubes containing this combination showed outgrowth. Additional information is needed on the interactions of numbers of cells, pH, curing agents, time, and growth before a satisfactory explanation can be made.

At 4 C, germination was quite slow or absent, depending upon the pH. After 24 h, germination occurred to a limited extent in all media with a pH of 7.0. At pH 5.5, no or very slight germination was evident. No outgrowth was observed at 4 C within 24 h.

In summary, of the various parameters studied, pH of the medium was the most influential on germination of spores of PA 3679, followed in order of importance by incubation temperature, water activity, and curing salts. Maximal germination was observed at pH 7.0, the highest pH used. Germination occurred over a wide temperature range of 4 to 55 C, with 35 to 45 C being optimal. Addition of 4.0% NaCl caused greater reduction in germination than did 0.1% $NaNO_3$, or 0.02% $NaNO_2$ at pH 7.0; addition of 0.02% $NaNO_2$ stimulated germination at pH 5.5 and 6.0. The combination of the three salts produced the greatest inhibitory effect on germination at pH 6.5 and 7.0. No outgrowth occurred within 24 h in media containing 4.0% NaCl. Outgrowth occurred most frequently at temperatures of 35 C and 45 C when the pH was 6.5 and 7.0.

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Journal of Food Protection Vol. 40, No. 6, Pages 373-374 (June, 1977)
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Acid Phosphatase Activities of the Skin, Flesh, and Seed in *Sechium edule*, Sw, the Chayote

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ABSTRACT

The skin, flesh, and seed of the green variety of chayote (*Sechium edule*) were analyzed for relative phosphatase activities on phenyl phosphate, sodium phytate, fructose-1, 6-diphosphate, β-glycerol phosphate, glucose-1-phosphate, glucose-6-phosphate, and ATP. Relative rates of phosphatase activities were highest with ATP and lowest with glucose-1-phosphate. Activities of all phosphatases were highest in the skin and lowest in the seed.

Sechium edule (vegetable pear), also called mitliton by the French and chayote by the Spanish (12), is a squash-like vegetable grown primarily in tropical countries. The plant is a native of the West Indies (*13*); however, interest in this vegetable is increasing in the southern United States (Louisiana, Mississippi, Florida, and California) (*5*). The perennial creeping vine resembles the climbing cucumber (*8*). The fruit is pear-shaped, wrinkled, approximately 10 to 14 cm in length, sparsely covered with spines (*13*), and contains a single flat oval-shaped seed which protrudes through one end. Recently a seedless fruit has been produced (*1*). Of the two varieties available, green and creamy white, the green is most often cultivated. Recent data have shown that chayotes are a good supplementary vegetable (*5*) for a given meal. The flesh is a good source of dietary fiber and contains neglible lipid (*5*).

Acid phosphatases occur widely in the plant kingdom (*3*) and have an important effect on carbohydrate metabolism in plant tissue. (*6*) One function is to catalyze hydrolysis of phosphate esters (*7*) which may be bound to compounds such as lipids, sugars, or nucleic acids (*5*). Another role could be related to onset and development of senescence (*3*). The enzyme activity has been reported to vary in different anatomical parts of banana fruit, in part due to varying concentrations of enzyme inhibitory substances (*2*). The purpose of this preliminary investigation was to determine the relative rates of phosphatase activity on each of seven substrates. Studies (skin, flesh, and seed) of the chayote by comparing acid phosphatase acitvity on each of seven substrates. Studies to determine the significance and purpose of acid phosphatases have been initiated.

MATERIALS AND METHODS

All chayotes were grown by Reynoso Brothers in Tijuana, B.C. Mexico, harvested at the same maturity, and shipped to Blacksburg, Virginia. Acid phosphatase activity was measured according to procedures outlined in the Worthington Enzyme Manual (*14*) at pH 5 (*11*). The flesh, seed, and skin tissue was quickly cut into 1-cm³ pieces and immediately homogenized in cold deionized water for 3 min in a food blender at high speed. The homogenate was filtered with Whatman No. 42 filter paper and an aliquot of filtrate was pipetted (0.5 to 1.0 ml) into a test tube containing 0.1 M substrate (phenyl phosphate disodium salt, sodium phytate, fructose-1,6-diphosphate, β-glycerol phosphate, glucose-1-phosphate, glucose-6-phosphate, or ATP), 0.05 ml of 0.01 M $MnCl_2$, 0.1 ml of buffer (0.15 M sodium acetate, pH 5.0) plus deionized water to give a total volume of 1.2 ml. All reagents were purchased from Sigma Chemical Company.

The enzyme reactions were allowed to proceed for 90 min and were stopped by the addition of 0.1 ml of 8% trichloroacetic acid (TCA). A modification of the Fiske-Subbarow test (*4*) was used to determine inorganic phosphorus released during the reaction. The entire contents of the reaction mixture (1.2 ml plus 0.1 ml of TCA) were added to a test tube containing 1.0 ml of molybdate reagent (2.5% ammonium molybdate in 5 N H_2SO_4), 1.0 ml of reducing reagent (1.5 g of $NaHSO_3$ plus 5 g of p-methylamino-phenol (Elon) in 500 ml of H_2O) and 6.7 ml of H_2O. The volume of each tube was adjusted to 10 ml by addition of deionized water.

All enzyme assays were done at a pH of 5 as described by Sacher (*11*). This pH, however, may not provide maximum enzyme activity for all seven chayote acid phosphatases studied.

Following a 20-min color development, samples were centrifuged at $3458 \times g$ and the optical density of the supernatant fluid was determined spectrophotometrically at 710 nm using a Perkin-Elmer Coleman 154 Spectrophotometer at room temperature (*25-26 C*). These results were compared to a standard curve obtained in the same manner using serial dilutions of 0.001 M K_2PO_4 (dry). Nitrogen content of the original flesh, skin, and seed filtrate was determined using the macro-Kjeldahl method.

RESULTS AND DISCUSSION

Figure 1 shows the size and shape of a chayote and its seed. Relative activities of the acid phosphatases in the skin, seed, and flesh of the chayote are contained in Fig. 2

In all instances, the skin exhibited the greatest phosphatase activity and the seed the least. Different amounts of phosphatase activity in the skin and the seed were observed when different substrates were used. The flesh exhibited a relatively constant acitvity with all substrates except ATP. The skin, seed, and flesh all

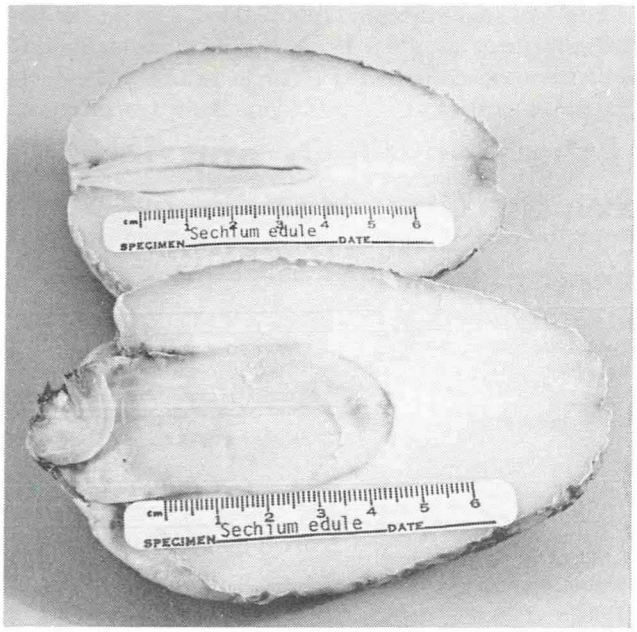

Figure 1. *Chayote: A longitudinal cut first perpendicular and then parallel to the wide axis of the seed to indicate thickness and width of the seed in the fruit.*

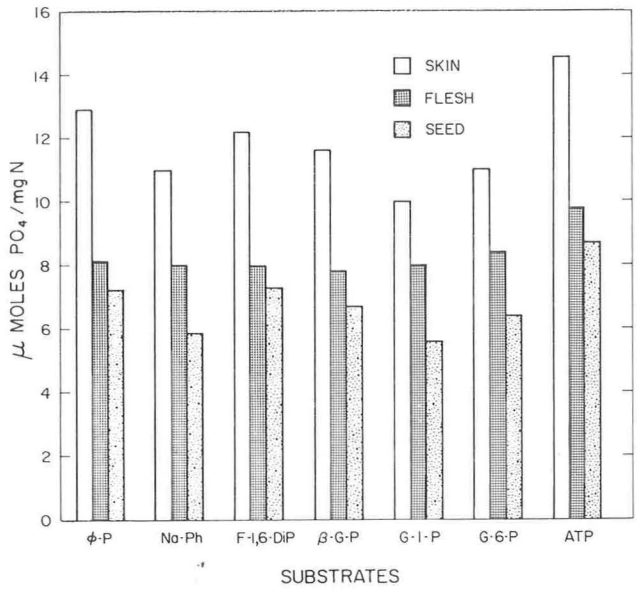

Figure 2. *Activities of acid phosphatases in skin, flesh, and seed of chayotes. Substrates: Ø-P (phenylphosphate, disodium salt), Na-Ph (sodium phytate), F-1,6-DiP (fructose-1,6-diphosphate), β-G-P (β-glycerol phosphate), G-1-P (glucose-1-phosphate), G-6-P (glucose-6-phosphate) and ATP.*

showed increased ATPase acitvity in comparison to the other phosphatases. This high ATPase activity could be indicative of greater phosphate-dependent reactions present than those involving hexose mono- and diester phosphates (*6*). Phenyl phosphate is not considered as a natural substrate in plants, therefore it has been suggested that some of the phenyl phosphatase acitivity may be due to non-specific esterases as was reported in peanuts (*9*). If this were true, then phenyl phosphatase activity should have been more than ATPase activity. The relative activity of acid phosphatase in the seed and skin was as follows: ATP > phenyl phosphate, fructose-1, 6-diphosphate, and β-glycerol phosphate > sodium phytate and glucose-phosphate > glucose-1-phosphate. Relative activities in the flesh were similar to those found in the seed and skin except β-glycerol phosphate exhibited the lowest activity. These results agree with Sacher (*11*) who reported that phosphatases are not substrate-specific in different anatomical parts of an avocado. it has been reported that the same enzyme may release inorganic phosphate from different substrates.

Work recently completed on acid phosphatase activity in purple, green, and white variations of eggplant (*Solanum melongena*) (*6*) indicated the order of affinity for different phosphate substrates followed the same order as that for chayotes.

ACKNOWLEDGMENT

The authors thank J. J. Bergquist for the photography and V. C. Scott for drawing Fig. 2.

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Journal of Food Protection Vol. 40, No. 6, Pages 375-377 (June, 1977)
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Purification and Properties of Ribonuclease From Buffalo Milk Whey

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ABSTRACT

A procedure was developed for isolation and identification of ribonuclease from buffalo milk whey. Ribonuclease was precipitated with $(NH_4)_2SO_4$ between 65 and 90% saturation. The precipitate was dissolved, dialyzed, and fractionated on DEAE-cellulose. Two ribonuclease-rich fractions were collected, i.e. ribonuclease A and B. Ribonuclease A had an optimum pH of 7.0, and ribonuclease B had an optimum pH of 8.6. Both had an optimum temperature at 38 C. The ribonucleases in the purified state were unstable to heat and their activity decreased as the time of exposure increased. Both enzyme fractions were sensitive to inhibitors. NaCl and NaN_3 were stimulatory for ribonuclease A, while ribonuclease B was stimulated only by NaCl.

Buffalo milk has been reported to be a fairly rich source of ribonuclease (*5,7*). However, there is no work about isolation of ribonuclease from buffalo milk. Bovine milk was investigated for ribonuclease activity and two separate enzymes, A and B, were isolated in a pure form (*1,2*).

Therefore, the aim of this study was to isolate ribonucleases from buffalo milk whey and to study their properties.

MATERIALS AND METHODS

Protein determination

The protein of the pure enzyme was estimated from absorbance at 280 nm using a Carl Zeiss spectrophotometer, with 1-cm quartz cells. Protein was also determined by the procedure of Lowry et al. (*6*).

Enzyme activity

Ribonuclease activity was estimated according to Bingham and Zittle (*1*). Yeast ribonucleic acid (B.D.H.) was used as a substrate. Also veronal acetate buffer pH 7.5 was used and incubated at 38 C for 5 min. The optical density was read at 260 mn in a Unicam Spectrophotometer using a 1-cm light path.

Purification procedure

Ammonium sulfate precipitation. The rennent whey (10 liters) was adjusted to pH 6.0 using 1.6 N NH_4OH. Solid $(NH_4)_2SO_4$ was added until the solution became 20% saturated. The solution was centrifuged at 10,000 r.p.m. at 0 C for 30 min. The precipitate was dialyzed against distilled water. The precipitation was repeated with the supernatant fluid for 30, 40, 60, 70, and 80% saturation with $(NH_4)_2SO_4$ as shown in Table (1). The highest enzyme acitvity was found when saturation was 65 to 90% with $(NH_4)_2SO_4$.

Column chromatography. Anion exchange Whatman DEll (DEAE-cellulose) was stirred into buffer pH 7.0 (15 ml/g). The suspension was titrated with buffer acid component until the correct pH was reached. Then it was decanted and washed again with the buffer and the pH was checked. The dialyzed preparation was applied to DEAE-cellulose column equilibrated with pH 6.0, 0.2 M veronal buffer. The preparation was eluted by a linear NaCl gradient of 0 to 1 M, and the eluate was collected in-5 ml fractions at a rate of 3.5 ml per 10 min. The ribonuclease activity and protein were measured in each of the 5-ml fractions. The protein content of the fraction was spectrophotometrically determined at 280-nm wavelength. Ribonuclease in the dialyzed preparations was eluted in two main peaks. Contents of tubes which contained the fractionated ribonucleases were collected in two brown bottles. The protein in both enzyme fractions was also determined.

Enzyme properties

Optimum pH. Curves of pH activity were determined for ribonuclease A and B using the barbital buffer 0.2 M with the following pH values: 6.8, 7.0, 7.6, 8.0, and 9.0.

Optimum temperature. Velocity was determined by heating the reaction mixture containing ribonuclease A or B at 25, 30, 35, 38, 40 and 45 C for 5 min.

Heat stability. Activities of ribonuclease A and B were measured after exposure to 90 C for 1, 2, 3, 4, and 5 min. The assay was carried out on each treatment.

Inhibitors and activators. The compounds silver nitrate ($AgNO_3$), calcium chloride ($CaCl_2 \cdot 6H_2O$), zinc sulfate ($ZnSo_4 \cdot 7H_2$?), cupric sulfate ($CuSO_4$ anhydrous), sodium chloride (NaCl), sodium azide (NaN_3), manganese sulfate ($MnSO_4 \cdot 4H_2O$), and mercuric chloride ($HgCl_2$) were each used at a concentration of 0.001 M in the reaction mixture containing ribonuclease A and B.

Determination of Michaelis constant. The initial activites of ribonuclease A and B were measured at yeast nucleic acid concentrations of 0.05, 0.075, 0.1, 0.2, and 0.3 ml of the reaction mixture. The reaction velocity of each ribunuclease was calculated from the activity curve slope. The reciprocal of the velocity was plotted against the reciprocal of the substrate concentration using the Lineweaver and Burk straight line equation. The Michaelis constant (Km) and maximum velocity (v_{max}(were calculated for each substrate.

RESULTS AND DISCUSSION

Ribonuclease precipitation by ammonium sulphate

As shown in Table 1, the rich enzyme fraction was precipitated in the range of 65-90% ammonium sulfate saturation. This result is in line with those of Bingham

and Zittle (2) working on bovine milk ribonuclease, and Uchida and Egami (8) working on Taka-Diastase-RNase.

TABLE 1. *Ribonuclease activity determination in the ammonium sulfate fractions*

Percent saturation	Activity units)[a]	Protein (mg/ml)	Specific activity (unit/mg protein)
0-20	28	0.038	736
20-30	22	0.028	785
30-40	6	0.019	0
40-50	5	0.011	454
50-60	6	0.011	545
60-70	34	0.024	1416
70-80	76	0.0093	8172

[a]1 unit of enzyme is the change in O.D. at 260 nm per min at 38 C.

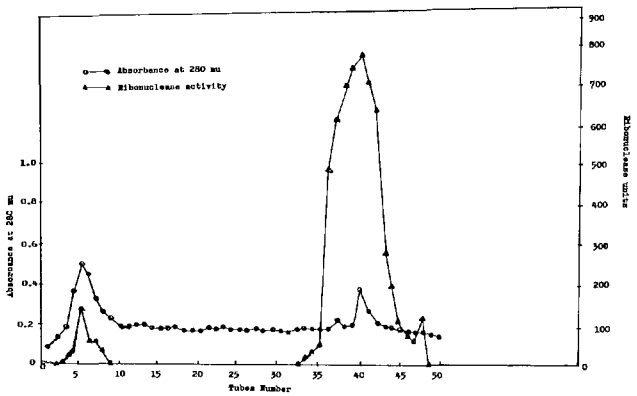

Figure 1. *Chromatography of whey ribonuclease in DEAE-cellulose.*

DEAE-cellulose chromotaography

Results in Table 2 and Fig. 1, show that two ribonucleases from the DEAE-cellulose column were obtained by the concentration of NaCl in the gradient. At a low NaCl gradient, one ribonuclease was obtained in fractions 3 to 9. The ribonuclease activities in such fractions ranged from 140 to 20 units. The second ribonuclease was obtained for the higher concentrations in fractions 32 to 48 and had activity ranging from 780 to 20 units.

TABLE 2. *Ribonuclease purification of buffalo milk whey*

Fraction	Value (ml)	Total ribo-nuclease activity	Total protein (mg)	Specific activity
Whey	10,000	40,000	13,000	30
Ammonium sulfate ppt. 65-90%	11,50	30,3600	10,7	28,37
Absorption to DEAE-cellulose				
Ribonuclease A	50	12,000	40.0	39,3
Ribonuclease B	85	63,240	10.6	59,66

Data dealing with the bufflao milk are scanty. However, Bingham and Zittle (1) reported that bovine milk contained 25.0 μg of ribonuclease per ml, and whey contained 26.7 μg of ribonuclease per ml. They also isolated two different ribonucleases, A and B; ribonuclease A was chromatographically and electrophoreticaly identical to pancreatic ribonuclease A. Bingham and Kalan (3) found that ribonuclease B has an amino acid composition which is identical to pancreatic ribonuclease A, milk ribonuclease A, and pancreatic ribonuclease B. Rifaat et al. (7) reported that bufflao milk contained 158.3 μg of ribonuclease per ml, and Egyptian cow milk contained 225.6 μg of ribonuclease per ml. Such results were higher than bovine milk by 6.32, and 9.0 times for each respectively.

Optimum pH

Ribonuclease A had a pH optimum of 7.0 while ribonuclease B had an optimum at 8.6 as shown in Table 3. The present results differ from those for the unpurified enzyme in buffalo whey, as reported by Ismail et al. (5) who mentioned that the maximum enzyme activity was at pH 6.8. However, Bingham and Zittle (1) reported optimum pH at 7.5 for cow milk ribonuclease.

TABLE 3. *Ribonuclease pH activity of buffalo milk whey*

	Unit		Protein (mg/ml)		Specific activity	
pH	A	B	A	B	A	B
6.8	48	144	8	4	6.0	36.0
7.0	228	168	8	4	28.5	42.0
7.6	36	96	8	4	14.5	24.0
8.0	96	312	8	4	12.0	78.0
8.6	24	372	8	4	3.0	93.0
9.0	120	84	8	4	15.0	21.0

Optimum temperature

The optimum temperature was 38 C for both ribonuclease A and B as shown in Table 4.

TABLE 4. *Ribonuclease activity of buffalo milk whey at various temperatures*

Temp-erature	Unit		Protein concent.		Specific activity	
(C)	A	B	A	B	A	B
25	252	196	8	4	31.5	49
30	192	120	8	4	24	30
35	792	523	9	4	99	134
38	1132	792	8	4	143	198
40	660	684	8	4	85	171
45	464	552	8	4	58	138

Heat stability

The results in Table 5 show that the ribonuclease in the purified state was unstable to heat and the activity was completely inhibited. The unpurified enzyme was found to be resistant to heating and the activity decreased as the exposure time increased (5). Also, Uchida and Egami (8) reported that RNase T_1 (Ribonuclease T_1 from Taka-Diastase) was a stable enzyme around pH 6.0 and at acidic pH values was fairly stable. However, to a certain extent it was unstable at alkaline pH values, and was rapidly and irreversibly inactivated above pH 9.0. RNase T_1 possessed full activity after heating at 100 C for 10 min. They also, found that RNase T_2 was somewhat less stable than RNase T_1 but was fairly stable as compared with most enzymes. The enzyme was most stable near neutral pH at room temperature and also to heating at 90 C. It may be heated at 20 C for 5 min. at pH 6.0 without loss of activity, but it loses activity fairly rapidly above 90 C. At weak alkaline pH values it suffers little inactivation at room temperature; in contrast to the stability of RNase T_1 at alkaline pH, however, in the acidic pH region, RNase T_2 is somewhat less stable than is RNase T_1.

TABLE 5. *Temperature effect on the ribonuclease stability of buffalo milk whey*

Exposure time at 90 C min.	Unit		Inhibition (%)	
	A	B	A	B
1	—	48	100	54
2	—	—	100	100
3	—	—	100	100
4	—	—	100	100
5	—	—	100	100

Activators and inhibitors

Ribonuclease A activity was completely inhibited by addition of $AgNO_3$, $CaCl_2$, and $HgCl_2$, whereas it was activated by NaCl and NaN_3, as shown in Table 6.

Ribonuclease B activity was completely inhibited by addition of $AgNO_3$, $CaCl_2$, and $HgCl_2$, while it was activated only by NaCl as shown in Table 6. On the other hand,RNase A was partially inhibited by $CuSO_4$ and RNase B was partially inhibited by $ZnSO_4$, $CuSO_4$, NaN_3, and $MnSO_4$. These results differ from those reported by Ismail et al. (5) on the unpurified enzyme, which was activated by NaCl, NaN_3 and $MnSO_4$. However, Uchida and Egami (8) reported that RNase T_1 is inhibited by Zn^{++} and heavy metal ions, and slightly by Mg^{++} and Ca^{++}. Such results agree with the previously mentioned results. They found that RNase T_2 was inhibited by heavy metal ions, especially Cu^{++}, but only slightly by Mg^{++} and Ca^{++}. Most purified RNase T_2 preparations were not activated by EDTA as consistently as the partially purified preparations. EDTA was apparently stimulatory to RNase T_1 because it eliminated the effect of metallic ions. It was concluded from these results that NaCl acted as activator for both types while azide was an activator only for RNase A.

TABLE 7. *Michaelis constants and maximum velocities for ribonuclease A and B of buffalo milk whey*

Ribonuclease	Km substrate (%)	V_{max} (units/mg protein)
A	0.147	28.2
B	0.263	37.0

Determination of Michaelis constant

The results in Fig. 2 and Table 7 show the Michaelis constants (Km) and the maximum velocities (V_{max}) for ribonuclease A and B.

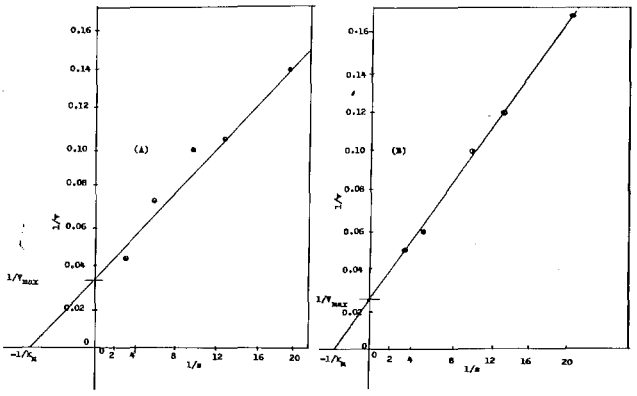

Figure 2. *Michaelis constant and maximum velocity in ribonuclease A and B.*

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TABLE 6. *Effect of some inhibitors and acitivators on nuclease activity of buffalo milk whey*

Compound 1 X 10^{-3}M of reaction mix.	Unit		Specific activity		Inhibition (%)		Activity increase (%)	
	A	B	A	B	A	B	A	B
$AgNO_3$	—	—	—	—	100	100	—	—
$CaCl_2 \cdot 6H_2O$	—	—	—	—	100	100	—	—
$ZnSO_4 \cdot 7H_2O$	—	144	—	38	100	59	—	—
$CuSO_4$, anhydrous	108	48	13.5	12	53	87	—	—
NaCl anhydrous	912	960	114.0	240	—	—	307	180
NaN_3 anhydrous	300	144	37.0	38	—	59	32	—
$MnSO_4 \cdot 4H_2O$	—	31.2	—	7.8	100	91	—	—
$HgCl_2$ anhydrous	—	—	—	—	100	100	—	—

Journal of Food Protection Vol. 40, No. 6, Pages 378-381 (June, 1977)
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Bacteriological Evaluation of Retail Ground Beef, Frozen Beef Patties, and Cooked Hamburger

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(Received for publication September 29, 1976)

ABSTRACT

A total of 108 samples of fresh refrigerated ground beef, 99 samples of frozen hamburger patties, and 107 fried hamburgers, purchased from retail stores and fast-food outlets in Ontario, were analyzed for their bacteriological quality. About 44% of non-frozen ground beef samples had aerobic plate counts exceeding 50 million/g; 50 of 108 samples (46.3%) contained *Staphylococcus aureus* and 46 of these 50 samples (88%) exceeded 1000 organisms/g; 43 of 108 samples were positive for *Escherichia coli* with 38 samples (88.,4%) exceeding 500 organisms/g. About 19% of frozen hamburger patties had aerobic plate counts in excess of 10 million/g; 93 of 99 samples (93.9%) contained *S. aureus* with 83 of these samples (89.3%) exceeding 1000 organisms/g; 28 of 99 samples were positive for *E. coli* with 7 of these samples (25%) exceeding 500 organisms/g. About 96.3% of fried hamburger samples had aerobic plate counts of less than 10,000/g.

The bacteriological quality of raw ground beef has been of interest to many people and has resulted in publication of bacteriological data in several reports (*1,3-8, 11-16*). These reports indicate that ground beef may contain large numbers of bacteria at points of sale. Examination of ground beef in this laboratory (*3*) showed the bacterial quality of many samples of ground meat to be poor and supported the belief that bacterial standards for ground beef are necessary to ensure quality and safety. Recent reports (*2,4,17*) discuss experiences with respect to desirability and practicability of establishing bacterial standards for meats. Because of the results of our 1973 study, a follow-up study was carried out in 1975 to gather additional data on bacterial quality of fresh and frozen ground beef and fried hamburgers as sold in fast-food outlets.

MATERIALS AND METHODS

Collection of samples

Two hundred and seven retail packages of ground beef, each weighing about 500 g, were collected from retail display cabinets in six major food chain stores over a period of 18 weeks during the summer months of 1975. They included 69 packages of hamburger, 14 soyaburger (composed of 30% soya flour and 70% meat), 18 ground chuck, 7 ground steak, all fresh, and 99 samples of frozen ground beef patties. The internal temperature of each package of unfrozen meat was recorded and all samples were transported in iced containers to the laboratory. Also, 107 fried hamburgers on the bun, but without added condiments, were purchased from five different major fast-food outlets. Aseptic procedures were observed in removing the hamburgers from the buns and placing them in sterile plastic bags for immediate refrigerated transport to the laboratory. All samples were held at 0-2 C and analyzed within 24 h.

Analytical procedure

Each package of fresh ground beef was divided into six equal sections and 5-g portions removed from each section or a total of 30 g of raw beef; other samples consisted of 60 g of frozen beef patty which had been thawed 6 to 8 h at 5-7 C and whole fried hamburger patties. All samples were homogenized in Waring blendors with 0.1% peptone water to give a 0.1 dilution. The following tests were conducted on the homogenate: (*a*) Total aerobic plate count (APC); using standard methods agar, plates were incubated for 72 h at 21 C. (*b*) Coliforms and *Escherichia coli*; presumptive coliforms were enumerated on violet red bile agar for 18 to 24 h at 35 C. Colonies (square root of count) were picked by random design into brilliant geen bile lactose broth. Growth from positive tubes was streaked on eosin methylene blue plates and an isolated typical colony picked and streaked on a plate count agar slant. The isolates were subjected to indole, methyl red, Voges-Proskauer, citrate (IMVIC) tests and inoculated into EC broth for incubation at 45.5 C and confirmation for *E. coli.* (c) Enterococci; they were enumerated on KF-Streptococcus agar that was incubated for 48 h at 37 C. (d) Staphylococci; suspected staphylococci were enumerated by pour-plating on Baird-Parker's egg yolk tellurite agar and incubating for 48 h at 37 C. Colonies (square root of count) from the countable plate, representing each type, were picked and streaked on plate count agar. The isolates were tested for coagulase production by the tube method using rabbit plasma containing EDTA. (e) Salmonellae. The procedure included using lactose broth pre-enrichment (24 h at 37 C), enrichment in selenite-cystine and tetrathionate-novobiocin broths (24 h at 37 C) followed by streaking on brilliant-green-sulfa, Salmonella-Shigella, and xylose-lactose-desoxycholate agars (24 h at 37 C). Because no suspicious colonies appeared, no further tests for confirmation were conducted. (f) *Clostridium perfringens*; enumeration and confirmation of *C. perfringens* was carried out using the method of Shahidi and Ferguson (*10*).

RESULTS AND DISCUSSION

Fresh ground beef

A summary of the APC, *S. aureus* and *E. coli* levels in the different types of fresh ground beef is presented in Table 1. Aerobic plate counts ranged from 1 million to more than 50 million/g. About 43% of the round steak samples had counts of less than 5 million/g, while only about 10% of the other samples were in this range. This difference was probably related to the bacteriological quality of the meat before grinding and/or to the length

TABLE 1. *Summary of the bacteriological analysis of 108 samples of non-frozen ground*

Type of ground beef in ranges	No. of samples (total)	Aerobic plate count/g Ranges	% of samples in ranges	*S. aureus*/g No. of samples positive	Ranges	% of samples in ranges	*E. coli*/g No. of samples inranges	Ranges	% of samples in ranges
Hamburger	69	1,000,000-5,000,000	5.8	32	101-1,000	12.5	26	101-500	19.2
		5,000,001-50,000,000	50.7		1,001-10,000	75.0		501-1,000	0
		>50,000,000	43.5		>10,000	12.5		>1,000	80.8
Chuck	18	1,000,000-5,000,000	16.7	9	101-1,000	0	6	101-500	0
		5,000,000-50,000,000	38.9		1,001-10,000	33.4		501-1,000	16.7
		>50,000,000	44.4		>10,000	66.6		>1,000	83.3
Round steak	7	1,000,000-5,000,000	42.8	3	101-1,000	33.3	3	101-500	0
		5,000,000-10,000,000	42.8		1,001-10,000	66.7		501-1,000	12.5
		>50,000,000	14.3		>10,000	0		>10,000	87.5
Soya extended	14	1,000,000-5,000,000	0	6	101-1,000	16.7	8	101-500	0
		5,000,001-50,000,000	35.7		1,001-10,000	66.6		501-1,000	12.5
		>50,000,000	64.3		>10,000	16.7		>10,000	87.5
Total	108			50 (46.3%)			43 (39.8%)		

of time and conditions under which the ground meat was held before purchase. Data in Table 2 show that the aerobic plate counts were rather evenly distributed

TABLE 2. *Distribution of aerobic plate count (APC),* S. aureus, *and* E. coli *in non-frozen ground beef*

	Arbitrary grouping of No. per g	No. of samples	% of samples
APC	1,000,000-5,000,000	10	9.3
	5,000,001-50,000,000	50	46.3
	>50,000,000	48	44.4
		108	
S. aureus	100-1,000	6	12
	1,001-10,000	33	66
	>10,000	11	22
		50[a]	
E. coli	100-500	5	11.6
	501-1,000	2	4.7
	>1,000	36	83.7
		43[a]	

[a]Of a total of 108 samples examined, 50 and 43 were positive for *S. aureus* and *E. coli* respectively.

between 5 million and 50 million/g (46.3%) and greater than 50 million/g (44.4%). Not all samples contained *E. coli*, but those samples that were positive (39.8%) exceeded the level of 100/g. Most samples (83.7%) had counts of more than 1000/g. *S. aureus* was isolated from 43.6% of the samples with most of those samples (66%) having counts in the range of 1000 to 10,000/g.

It should be noted that 90.7% of the samples exceeded the standard for APC set by the State of Oregon (5 million/g), while 100% exceeded the guidelines (1 million/g) employed by the States of New York, Rhode Island, and Wyoming (*16*). Of more concern is the fact that 44.4% of the samples exceeded the recently proposed Canadian standard of 50 million/g. Also, 88.4% of the samples positive for *E. coli* and 88% of the samples positive for *S. aureus* would not be in compliance with the new Canadian standard for these organisms (*9*).

Temperature of the ground beef at time of purchase ranged from 8 to 13 C. Such elevated holding temperature in the store display cases was probably one of the factors contributing to high counts.

Frozen ground beef

Ninety-nine samples of frozen ground beef patties were examined and the APC, *S. aureus* and *E. coli* counts are presented in Table 3. In general, the microbiological quality was better than that of the fresh ground beef. This was not surprising as counts in frozen meat are known to stabilize or even decrease during storage, while counts in fresh ground beef may increase depending on temperature abuse during retail handling. Data in Table 4 and Fig. 1 indicate condiderable variation in bacterial counts among meats of the nine different suppliers. Even though the meat was frozen when purchased, data in Fig. 1 show that only the product of

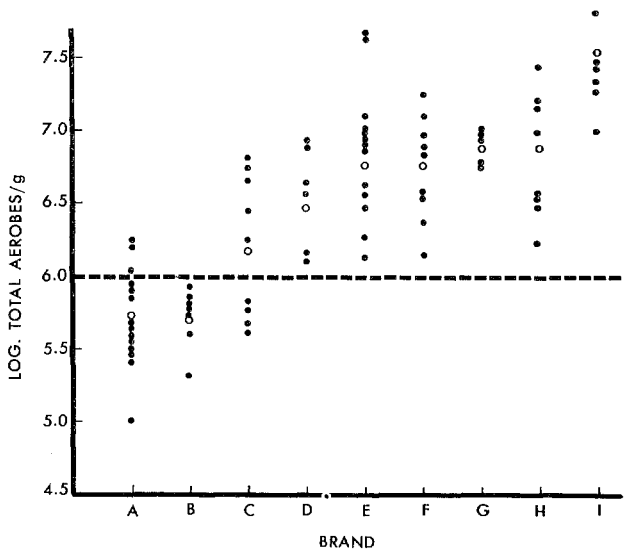

FIGURE 1. *Total Aerobic Plate Count of frozen beef patties from nine different suppliers. O geometric mean; .... arbitrary standard of 1 million/g.*

TABLE 3. *Summary of the bacteriological analysis of 99 samples of frozen ground beef patties from 9 different manufacturers*

Manufacturer	No. of samples	Aerobic plate count/g		*S. aureus*/g			*E. coli*/g		
		Ranges	% of samples	% of samples positive	Ranges	% of samples	% of samples positive	Ranges	samples
A	19	100,000-1,000,000	84.2	19	100-1,000	21.0	8	<10	12.5
		1,000,001-5,000,000	15.8		1,001-10,000	31.6		11-50	50.0
		>5,000,000	—		>10,000	47.4		>50	37.5
B	10	100,000-1,000,000	100	10	100-1,000	—	0		
		1,000,001-5,000,000	—		1,001-10,000	100			
		>5,000,000	—		>10,000	—			
C	10	100,000-1,000,000	40	8	100-1,000	12.5	2	<10	—
		1,000,001-5,000,000	60		1,000-10,000	75.0		11-50	50
		>5,000,000	—		>10,000	12.5		>50	50
D	8	100,000-1,000,000	—	8	100-1,000	37.5	0		
		1,000,001-5,000,000	100		1,000-10,000	50.0			
		>5,000,000	—		>10,000	12.5			
E	18	100,000-1,000,000	—	17	100-1,000	5.9	7	<10	—
		1,000,001-5,000,000	44.4		1,000-10,000	58.8		11-50	14.3
		>5,000,000	55.6		>10,000	35.3		>50	85.7
F	10	100,000-1,000,000	—	9	100-1,000	11.1	0		
		1,000,001-5,000,000	40		1,000-10,000	88.9			
		>5,000,000	60		>10,000	—			
G	5	100,000-1,000,000	—	5	100-1,000	—	2	<10	—
		1,000,001-5,000,000	—		1,000-10,000	—		11-50	50
		>5,000,000	100		>10,000	100		>50	50
H	10	100,000-1,000,000	—	9	100-1,000	—	9	<10	—
		1,000,001-5,000,000	40		1,000-10,000	77.8		11-50	—
		>5,000,000	60		>10,000	22.2		>50	100
I	9	100,000-1,000,000	—	8	100-1,000	—	0		
		1,000,001-5,000,000	—		1,000-10,000	12.5			
		>5,000,000	100		>10,000	87.5			
Total	99			93 (93.9%)			28 (28.3%)		

suppliers A and B could meet a APC standard of one million/g. This indicates that the other meats were perhaps excessively contaminted or were held too long at elevated temperature before freezing. The results for suppliers A and B indicate that it is possible to process and market frozen beef patties of acceptable quality.

Fried hamburger

Total aerobic plate counts ranged from less than 100 to 1,700,000/g. Most samples (75.7%) had counts of less than 1000 organisms/g (Table 5). Enterococci, coliforms, staphylococci, salmonellae, and *C. perfringens* were not isolated. The contributions of buns to the total counts was considered negligible; an assortment of 50 buns examined had counts of less than 100/g. Bacterial growth in hamburger subsequent to frying is possible since hamburgers may be held at ambient temperature by the consumer before consumption. Although no organisms known to cause foodborne illness were isolated, the high surviving population in seven samples makes questionable the quality of the meat and the time-temperature relationship of the cooking procedure.

Results of this study show some improvement in the bacterial quality of raw ground beef as compared to results of the 1973 study (*3*). Incidence of high count ground beef is still excessive and the fact that over 44% of the samples exceeded the recently proposed Canadian standard (APC 50 million/g) indicates the magnitude of the task facing those responsible for the bacterial quality

TABLE 4. *Distribution of aerobic plate count (APC),* S. aureus, *and* E. coli *in frozen hamburger patties*

	Arbitrary grouping of No. per g	No. of samples	% of samples
APC	100,000-1,000,000	30	30.3
	1,000,001-5,000,000	29	29.3
	5,000,000-10,000,000	21	21.2
	>10,000,000	19	19.2
		99	
S. aureus	100-1,000	10	10.7
	1,001-10,000	52	55.9
	>10,000	31	33.4
		93[a]	
E. coli	<100	15	53.6
	100-500	6	21.4
	>500	7	25.0
		28[a]	

[a]Of a total of 99 samples, 93 and 28 were positive for *S. auresu* and *E. coli* respectively.

TABLE 5. *Range of aerobic plate count [APC, 21 C, 72 h] in fried hamburger*

Arbitrary grouping of No. per g	No. of samples	Percent
<100	50	46.7
101-1,000	31	29.0
1,001-5,000	19	17.8
5,000-10,000	3	2.8
>10,000	4	3.7
	107	

ACKNOWLEDGMENTS

This investigation was supported in part by the Canadian Broadcasting Corporation (Toronto) and by the Ontario Ministry of Agriculture and Food. The technical assistance of Ms. Anne Patience and Mr. John Van Esch is greatly appreciated.

of the meat. About 19% of the frozen ground beef samples would have been unacceptable based on APC (Canadian standard 10 million/g) but the majority (89.3%) would be unacceptable based on excessive numbers of *S. aureus* (Canadian standard 1000/g). Most fried hamburger samples were of acceptable bacterial quality. Unacceptable counts after frying can reflect both high intial counts or insufficient heat treatment and are of concern if the handling of the hamburger after cooking is inadequate.

Aerobic plate counts in the present study were obtained at 21 C, an incubation temperature at which more bacteria are expected to be recovered as compared to 35 C (*9,16*). The proposed Canadian standard (APC) for ground beef is based on incubation at 35 C. These standards are rather lenient compared to some standards in the United States, but may reflect the bacterial quality of ground meat in Canada. To achieve a ground meat of improved bacterial quality, Canadian regulatory officials should consider a progressive tightening of standards. APC maxima proposed for ground beef as a standard or guideline in two states of the U.S.A. are 10 million/g (*16*). Most other states have proposed standards or guidelines ranging from 0.5 to 5 million/g.

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Journal of Food Protection Vol. 40, No. 6, Pages 382-384 (June, 1977)
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Bacteriological Evaluation of Some Luncheon Meats in the Canadian Retail Market

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(Received for publication October 8, 1976)

ABSTRACT

Four types of luncheon meats, bologna, chicken loaf, ham, and macaroni cheese, each manufactured by four different companies, were purchased from four major retail outlets in Ontario over a period of 16 weeks during the summer of 1975. Bacterial evaluation included determination of total aerobic plate count, coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, salmonellae, and enterococci. Bacteria of public health significance were not a problem except for a high incidence of enterococci in all samples. *S. aureus* counts exceeded 1000/g in 20% of 30 positive samples out of a total of 159 samples. Total aerobic plate counts exceeded 5,000,000/g in 46.5% of the samples. Wide variation in bacteriological quality of the products between manufacturers was found.

A survey was undertaken to determine bacterial levels in four popular types of vacuum-packaged sliced luncheon meats (bologna, ham, chicken loaf, macaroni cheese) sold in supermarket grocery stores in Ontario. These types of meat products are cured and have been subjected to a mild heat process sufficient to yield pasteurized, cooked products. They are generally not heated further by the consumer which would destroy most of the contaminating microflora before consumption. Thus, the bacteriological quality of luncheon meats depends on the quality of raw materials, sanitation during production, and maintenance of the refrigeration chain from processor to consumer. It is evident, therefore, that responsibility for effective control of quality must be shared equally by producer and retailer. There are relatively few published data concerning the bacterial content of ready-to-eat luncheon meats (*2,5,6,7*) as purchased from the retail display cabinets. Allen and Foster (*1*) and Kempton and Bobier (*4*) reported that only lactic acid bacteria multiply during refrigerated storage in the types of meats they examined.

Canada has no bacterial standards or guidelines for this type of food. Other countries have such regulations (*2*). Most Canadian companies manufacturing these products probably use their own standards. A desire for information on the bacterial quality of some luncheon meats as they appear on the retail market in Canada prompted this study.

MATERIALS AND METHODS

Collection of samples

A total of 159 samples of four types of luncheon meats representing four national manufacturers were collected at random from four major retail outlets in Ontario over a period of 16 weeks during the summer of 1975. All samples were retail packs weighing from 200 to 500 g and were taken directly from the market display case. Two of each type representing each manufacturer were purchased and used for determination of the temperature of the meat at time of purchase. Only samples which did not exceed the code date by 5 days were obtained. Samples were transported to the laboratory in an iced container and analyzed immediately.

Analytical procedure

For bacterial analyses a 30-g sample was obtained by aseptically removing a wedge from the center to periphery, thus including an equal amount of each slice of meat in the package. Samples were blended for 3 min in Waring blendors with 0.1% peptone water to give a 0.1 dilution. Aerobic plate counts (APC), coliforms, *Escherichia coli*, *Staphylococcus aureus*, enterococci, salmonellae, and *Clostridium perfringens* were determined by methods previously described (*3*).

RESULTS AND DISCUSSION

Neither salmonellae nor *C. perfringens* were isolated. Four samples contained *E. coli* with 15,14,210 and 50/g, respectively. Table 1 gives a summary of data on the range of total aerobic plate counts, enterococci, and *S. aureus* and the fraction of total samples that contained organisms for each type of sample. Enterococci were recovered from all samples, while *S. aureus* was isolated from 30 (18.8%) of 159 samples. About 46% of all samples had aerobic plate counts exceeding 5×10^6 organisms/g and 23.9% of all samples were in the range of 1×10^8 - 5×10^8 organisms/g.

A certain pattern in the bacterial quality of the luncheon meats of the different manufacturers was noticeable. Comparing the four manufacturers, manufacturer A contributed 34 (50.7%) of 67 grossly contaminated samples (APC $> 1 \times 10^7$/g) in contrast to manufacturer C who had only five samples (7.4%) in this category. About 62% of the samples of manufacturer C had APC values below 10^5/g as compared to 2.5, 20, and 26.5% for manufacturers A, B, and D, respectively. All samples contained enterococci and the same trend in

TABLE 1. *Number of samples and arbitrary count ranges (total aerobic plate count, enterococci, and* S. aureus*) for 159 luncheon meat samples from four different manufacturers*

Manufacturer	Type of meat[a]	No. of samples in APC range/g							No. of samples in enterococci range/g								No. of samples in *S. aureus* range/g					
		No. of samples	$<10^5$	10^5-10^6	10^6-5×10^6	5×10^6-10^7	10^7-10×10^7	10×10^7-50×10^7	No. of samples	$<10^3$	10^3-10^4	10^4-10^5	10^5-10^6	10^6-5×10^6	$>5\times10^6$	No. of samples positive	$<10^1$	10^1-5×10^1	5×10^1-10^2	10^2-10^3	$>10^3$
A	Bologna	10			1		4	5	10	2	1	2	2	3		3		2		1	
	Chick. loaf	10		1	1		2	6	10	1		4	3	1	1	2					2
	Ham	10		1	1		2	6	10		1	3	6			3				2	1
	Mac. cheese	10	1				2	7	10	1	2	2	1	2	2	1				1	
	Subtotal	40	1	2	3		10	24	40	4	4	11	12	6	3	9		2		4	3
	%		2.5	5.0	7.5		25	60		10	10	27.5	30	15	7.5			22.2		44.4	33.3
B	Bologna	10	1	2	1	1	3	2	10	5	2	3				2		2			
	Chick. loaf	10	4	1	3	1	1		10	4	5	1				2			2		
	Ham	10	2		2		5	1	10	4	2	2		2		2				1	1
	Mac. cheese	10	1	1	2	1	2	3	10	4	1		2	3		2			1		1
	Subtotal	40	8	4	8	3	11	6	40	17	10	6	2	5		8		2	3	1	2
	%		20	10	20	7.5	27.5	15		42.5	25	15	5	12.5				25	37.5	12.5	25
C	Bologna	10	8	2					10	7	2	1				0					
	Chick. loaf	10	8				2		10	10						3	1	2			
	Ham	10	4	2	2		1	1	10	8		1	1			1	1				
	Mac. cheese	10	5	4			1		10	6	2	1	1			0					
	Subtotal	40	25	8	2		4	1	40	31	4	3	2			4	2	2			
	%		62.5	20	5		10	2.5		77.5	10	7.5	5				50	50			
D	Bologna	10	3	1	1		3	2	10	7	1	1		1		2		1		1	
	Chick. loaf	10	2	3	2	2	1		10	5	1	3	1			2				2	
	Ham	9	3	1	1			4	9	6	1		1	1		1					1
	Mac. cheese	10	2	4	1	2		1	10	3	2	3	1	1		4	1		2	1	
	Subtotal	39	10	9	5	4	4	7	39	21	5	7	3	3		9	1	1	2	4	1
	%		25.6	23	12.8	10.3	10.3	18		53.9	12.8	17.9	7.7	7.7			11.1	11.1	22.2	33.3	11.1
	Total	159	44	23	18	7	29	38	159	73	23	27	19	14	3	30	3	7	5	9	6
	%	100	27.7	14.5	11.3	4.4	18.2	23.9	100	45.9	14.5	17	11.9	8.8	1.9	18.9	10	23.3	16.7	30	20

[a]The 4 types of luncheon meats were: bologna, chicken loaf, ham and macaroni cheese.

incidence of these organisms was observed as for the total aerobic plate count. The incidence was highest in samples from manufacturer A and lowest in those from manufacturer C. Approximately 19% of samples contained *S. aureus* and 20% of the samples exceeded 1000 staphylococci/g. Again, manufacturer A had the highest incidence, while the incidence of these organisms was lowest in samples of brand C.

Earlier work (*1,4,6*) showed that lactic acid bacteria rapidly became the predominant microflora in processed meats during storage. In the present work, no attempt was made to determine the lactic acid bacteria. There was no indication that large numbers were present. Regardless of meat type (0.1% homogenate in distilled water) the pH range was 5.7-6.5. The pH would have approached 5.0 if lactics were the main contaminants.

About 80% of the colonies from a countable plate were catalase-positive. Since none of the samples exceeded the code date by more than 5 days, it is possible that a shift from a catalase-positive to a catalase-negative flora would have occurred upon longer storage.

Results of this survey indicate that the bacterial quality of luncheon meats in the retail market is quite variable and that wide variations between manufacturers can exist. Of the four manufacturers examined, only products from manufacturer C, except for a few samples, could generally be considered satisfactory, while those from manufacturer A were of the lowest bacterial quality.

Temperature abuse of the products at the retail level was probably one of the principal contributing factors to high counts, particularly where the initial bacterial content was already high. The internal temperature of the meat samples varied between 5 and 14 C (85% of the samples between 10 and 14 C) at time of purchase.

This study, although restricted to gathering data on the bacterial content of the luncheon meats as purchased from the retail cabinet by the consumer, nevertheless suggests that the high APC values could not be solely ascribed to mishandling during retail marketing. Based on the temperature recordings, the storage conditions and handling appeared to be similar for each product in each store visited.

Reports on the sanitary conditions in each manufacturing plant were not available; such information would be valuable in interpreting the results, especially since there existed such wide variations between manufacturers. Considering that the cooking procedures used for every product should be sufficient to substantially reduce the microbial population normal to the raw product materials and eliminate the lactic acid bacterial flora in raw meat, there is no alternative but to imply a great need for improvements in plant sanitation for manufacturers A, B, and D, particularly A. The need for better temperature control in the merchandising channels is also evident from this work.

Salmonellae, *C. perfringens, E. coli,* and *S. aureus* were not found to be a problem in this type of product, but the relatively high incidence of enterococci is indicative of a need for better sanitation practices.

ACKNOWLEDGMENTS

This investigation was supported in part by the Canadian Broadcasting Corporation and by the Ontario Ministry of Agriculture and Food. The technical assistance of Ms. Anne Patience is greatly appreciated.

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Journal of Food Protection Vol. 40, No. 6, Pages 385-388 (June, 1977)
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Sources of Variation at the Retail Level in Bacteriological Condition of Ground Beef[1]

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(Received for publication October 12, 1976)

ABSTRACT

Ground beef from two locker plants using beef trimmings, and two retail stores, using 4.5-kg chub packs of coarse-ground centrally packaged beef as a source of meat, was evaluated microbiologically on the first Monday and Wednesday of every other month for 14 months. Only slight variations in coliform, fecal coliform, coagulase-positive staphylococci, and aerobic plate counts by plant or retail store were noted. Both retail stores using chub packs showed average total aerobic plate counts of 3.5×10^6 /g while one locker plant averaged 1.7×10^6 /g and the other 7.7×10^6 /g. Source of meat for ground beef and holding time of the meat contributed most to differences in microbiological quality. Overall, ground meat packaged on Monday had higher ($P<.05$) average total aerobic plate counts (5.7×10^6/g) than that packaged on Wednesday (2.5×10^6 /g) when more recent beef shipments were available. Coliform, fecal coliform, and coagulase-positive staphylococcus counts followed the same patterns as noted for total aerobic plate counts. Total aerobic plate counts in beef increased slightly in the locker plants where game was processed in the fall, in addition to beef. However, game meat did not cause *Salmonella* contamination of ground beef in plants where both game and beef were processed. Three *Salmonella* positive samples out of the 112 total ground beef samples and the 112 swabs from used grinders were isolated and serotyped.

Bacteriological condition of ground beef and other meat is of concern to all segments of the industry. Reduced shelf life, discoloration of the product, and economic losses as a result of bacteria are often encountered. Current interest is centered around microbiological standards for ground beef that have been established by some states. Researchers have enumerated aerobic plate counts, coliforms, and *Escherichia coli* in ground beef at the retail level (*3,5,10,15*). Efforts to reduce bacterial content at the retail level have concentrated on proper handling of live animals and on the carcass at the packing plant. Several researchers (*4,6,7,12*) have shown a progressive increase in the incidence of *Salmonella* as animals move from the farm to the stockyard and from the holding pen to the abattoir. Mann (*13*) found bacteria in the mesenteric lymph nodes of pigs, sheep, and cattle at the packing house. A study of the sources of microbial contamination of meat at the packing plant indicated that some contamination from equipment occurred and that washing the carcass carried the contaminants lower on the carcass rather than removing them (*2*). More recently it has been shown that a Clor-Chil spray on carcasses reduces bacterial counts by 94.5 to 99.9% (*8*).

Although bacterial numbers in ground beef at the retail level have been surveyed and several studies aimed at reducing carcass contamination at the packer level have been completed, there is relatively little information showing how variations in fabrication of the carcass affect microbial load. The purpose of this study was to show how centralized or traditional preparation of ground meat affects coagulase-positive staphylococci, *Salmonella* spp., fecal coliforms and coliforms, as well as total aerobic plate counts (APC). Differences in bacterial numbers in ground beef as they relate to day of week and processing of big game carcasses in the same plant as beef were also studied.

MATERIALS AND METHODS

Ground beef from two retail stores (plants 1 and 4) and two lockers (plants 2 and 3) was evaluated on the first Monday and Wednesday of every other month for 14 months. Plant 1 received fresh coarse ground beef in chub packs from a centralized warehouse which used boneless cow in addition to flanks, plates, and trimmings from choice grade cattle as its source of meat. The meat was purchased from other packers and shipped to the centralized warehouse as carcasses or carcass parts. Plant 2 was a locker plant which purchased frozen bull and cow meat, and frozen diaphragm muscles from USDA-inspected plants in addition to using their own beef trimmings. Plant 3 slaughtered and processed its own beef. Trimmings from carcasses were obtained on the same day the carcasses were cut. Plants 2 and 3 processed some deer, elk, and antelope meat during November of 1974 and during January and November of 1975 when ground beef samples were obtained. The big game carcasses were processed at the end of the day after beef processing for that day had been completed. Plant 4 purchased fresh coarse ground beef in chub packs from a packer that had the capability of making 147,000 kg of ground beef per day from cattle (mainly cows), slaughtered, cut, and packaged in the same plant. Therefore, ground beef in chub packs from two different centralized

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processing plants (plants 1 and 4) and from two different locker plants (plants 2 and 3) preparing ground beef under older more traditional methods was evaluated. Each of the plants allowed meat to be taken directly from the freshly opened 4.5-kg chub packs or from the mixed beef trimmings before the beef was placed on the grinder table. The beef was then ground and packaged for sale. No other meat, fat, or other additives were incorporated into the meat. As a result, any differences in bacterial numbers before or after grinding were due to contamination from the grinder, contamination from air, or contamination from hands of plant personnel. The 2-4 C temperature rise during grinding could also influence bacterial numbers. Two sterile, moist cotton swabs from the used grinder tables were taken the same afternoon and at the same time meat samples were obtained. One swab of a surface area of 25 cm^2 was obtained with the aid of sterile aluminum cutouts and placed into sterile lactose enrichment broth for aerobic plate counts. The second swab covered an area approximately 615 cm^2 and was placed into a second lactose pre-enrichment broth tube for detection of *Salmonella* spp. At the beginning of the study, and on several occasions through the study, swabs of clean grinders before the start of the day's work were also obtained to monitor the cleaning job of the plant personnel. All meat samples and swabs were returned to the meat laboratory within 1 h after they were obtained and chilled to 3 C. Samples were then held overnight at 3 C. The coarse ground chub packs and the beef trimmings were then finely ground in a sanitized laboratory grinder to obtain a more homogenous sample for analysis.

Emuneration

Fifty grams of each meat sample were weighed into a sterile, 1-liter, Waring blendor container. Four hundred fifty ml of sterile buffered distilled water (1.25 ml of Butterfield phosphate diluent/liter) were added and the contents blended at high speed for 2 min, to obtain a 10^{-1} dilution of the sample. Dilutions of 10^{-2} through 10^{-6} then were prepared for subsequent bacterial enumerations (*19*). The total number of aerobic bacteria in each sample and in the tubes of lactose broth containing the swabs (6.25 cm^2), of the grinder surface were determined using plate count (PC) agar with a 48-h incubation at 35 C. Single lauryl sulfate tryptose (LST) broth fermentation tubes were inoculated with 1.0 or 0.1 ml from the appropriate dilution blanks and incubated at 35 C for 24 h. The highest dilution showing gas production was reported as the number of coliform organisms/g of meat sample. One loopful of the contents of each LST tube showing gas was transferred to an EC broth tube. The EC broths were incubated at 45 C for 24 h and the numbers of fecal coliforms were reported according to the highest dilution showing gas formation in the EC broth. To determine the presence of coagulase-positive staphylococci single tubes of trypticase soy (TS) broth containing 10% NaCl were inoculated with 10^{-1} through 10^{-6} dilutions of the blended meat samples. Following incubation at 35 C for 48 h a loopful of broth from each TS tube showing growth and from the next higher dilution tube was streaked on previously prepared plates of Vogel Johnson (VJ) agar. Following incubation of these plates for 48 h at 35 C representative shiny black colonies showing tellurite reduction were streaked on PC agar slants which were then incubated at 35 C for 24 h. A small amount of growth from the PC agar slants was transferred into duplicate 13 × 100 mm tubes containg 0.2 ml of brain heart infusion (BHI) broth which was held at 35 C for 24 h. Following incubation, 0.5 ml of reconstituted coagulase plasma with EDTA was added to the BHI tubes and mixed thoroughly. The tubes were placed in a 37 C waterbath for 4 h for clot formation. All tubes showing an organized clot in 4 h were recorded as coagulase-positive staphylococci calculated as the reciprocal of the highest positive dilution.

Isolation of Salmonella

In an effort to isolate *Salmonella* spp., 225 g of the blended 10^{-1} dilution of each meat sample were transfered to a sterile one-pint Mason jar along with 25 ml of 10-fold concentration of sterile lactose broth and 1.5 ml of Tergitol 7 and the pH was adjusted to 6.8 - 7.0 with 1 N NaOH. After incubating the jars for 24 h at 35 C, 0.5 ml of the contents was transferred to 10 ml of tetrathionate broth (TT). TT broth tubes were also inoculated with material from 625 cm^2 area of the grinders after the swabs were held in lactose broth tubes 24 h at 35 C. The TT broth tubes were incubated at 35 C for 24 h for enrichment purposes after which the contents were streaked on separated plates of SS agar, XLD agar, and briliant green agar plus sulfadiazine (BGS). The streaked plates were incubated at 35 C for 24 h. After incubation, selected colonies were inoculated into both triple sugar iron (TSI) agar and lysine iron (LI) agar slants as well as lactose broth tubes containing phenol red dye. After preliminary screening those organisms showing typical *Salmonella* reactions in all three media were serotyped by the Veterinary Services Diagnostic Laboratories of the Animal and Plant Health Inspection Service at Ames, Iowa.

RESULTS AND DISCUSSION

Coliforms, fecal coliforms and APC/g of ground beef varied by plant (Table 1). The retail store (plant 4), which purchased chub packs from a packing plant capable of producing 147,000 kg of ground beef per day, had ground beef with the lowest coliform and fecal coliform counts and an APC similar to the other retail store (plant 1) with centralized processing. The lowest APC occurred in ground beef from plant 3 where beef trimmings were sampled on the same day they were cut from the carcass. Rogers and McCleskey (*14*) found that among markets categorized as small, medium, and large there was a trend toward less contamination as size increased. Shoup and Oblinger (*15*) concluded that ground beef prepared in centralized operations was of better microbiological quality than traditionally prepared ground beef. Microbiological quality of ground beef depends not only on size but also on length of storage time between

TABLE 1. *Least-squares means and standard errors for bacteria/g of ground beef and for bacteria on the grinder surface*

Variable	Ground beef (N = 112)				Used grinder surface
	Coliforms	Fecal coliforms	APC	Staph	APC/6.25 cm^2
Plant number					
1 chub packs	5.0×10^3	4.4×10^3	3.5×10^6	167	9.8×10^3
2 beef trimmings	8.9×10^3	8.1×10^3	7.7×10^6	51	2.9×10^4
3 beef trimmings	9.3×10^2	5.5×10^2	1.7×10^6	87	2.4×10^4
4 chub packs	5.8×10^2	5.2×10^2	3.5×10^6	26	1.8×10^4
Standard error	3.0×10^3	3.0×10^3	1.8×10^6	45	3.3×10^3
Sampling period					
Before grinding	3.2×10^3	2.8×10^3	3.8×10^6	77	—
After grinding	4.5×10^3	3.9×10^3	4.4×10^6	89	6.2×10^4
Standard error	2.1×10^3	2.2×10^3	1.3×10^6	32	1.7×10^4
Day of week					
Monday	5.0×10^3	4.2×10^3	5.7×10^6	109	1.0×10^5
Wednesday	2.7×10^3	2.5×10^3	2.5×10^6	56	2.3×10^4
Standard error	2.2×10^3	2.2×10^3	1.3×10^6	32	2.4×10^4

grinding and use. The large centralized operations making coarse ground chub packs may have packaged beef with lower initial counts than the locker plants but the elapsed time after the coarse ground chub packs were made and before they were shipped to the stores and reground contributed to growth of bacteria. Slightly higher coliform, fecal coliform, and APC values were found in ground beef from plant 2 when compared to those of the other plants even though much of the beef purchased by plant 2 was frozen. It has long been known that diaphragm meat such as that used by plant 2 has higher bacterial levels due to more optimum conditions for bacterial growth in this area of the carcass. These data support the findings of Surkiewicz et al. (*17*) who observed that bacterial quality of ground meat is more dependent on quality of the meat than on conditions during fabrication. No close relationship between APC in ground beef and APC/6.25 cm^2 on the grinder surface was evident (Table 1). APC values/6.25 cm^2 on the clean grinder table before meat was ground ranged from 40 to 210/6.25 cm^2 with the great majority of the swabs showing counts of less than 100. Because of the low counts variation in cleaning of the grinders in the plants involved had little influence on numbers of bacteria in ground beef.

No *Salmonella* were isolated from the clean grinder surfaces. However, *Salmonella anatum* was isolated on Monday from the used grinder surface of plant 2 during July. Other *Salmonella* isolations were *Salmonella sundsvall* from fine ground beef as it was packaged for sale in plant 1 on a Monday in November and an untypeable *Salmonella* spp. isolated from fine ground beef as it was packaged for sale in plant 1 on a Monday in February. Although plant 1 had *Salmonella*-positive samples in ground beef packaged for sale, no *Salmonella* were isolated from the grinder surface or from the coarse ground chub packs in plant 1. It is possible that the *Salmonella* came from the butchers' hands. Since small numbers of *Salmonella* are not homogeneously distributed in ground beef or on grinder surfaces, either of these also could have been the source of *Salmonella* in the ground beef packaged for retail sale. Neither of the locker plants (plants 2 and 3) processing game during November and January had ground beef samples in which *Salmonella* were found. Smith et al. (*16*) were unable to detect *Salmonella* in any of the 121 ground game samples which they collected. The present research confirms that game meat is not likely to cause *Salmonella* contamination of domestic meat in plants where both are processed.

Recoveries of coagulase-positive staphylococci shown in Table 1 were low. Coagulase-positive staphylococci lack the ability to compete well with other bacteria in ground beef especially at temperatures under which ground meat is stored.

The slight increases in coliform, fecal coliform, and APC numbers after grinding are probably due to sampling error (Table 1). Nevertheless, increased numbers may be a result of the holding period of 1 to 4 h at 2-6 C after grinding and before sampling. The total APC in ground beef after packaging in this study is in the range of those reported previously (*1,5,9,11,17,18*).

The most consistent difference in microflora of ground beef in this study was the difference between samples taken on Mondays and those taken on Wednesday (Table 1). Since no beef deliveries were made to the plants 1 and 4 on weekends, meat packaged for sale on Monday was from beef which had been delivered the previous week. Frozen meat used by plant 2 on Monday had often thawed more completely over the weekend than meat that was ground on Wednesday. Even plant 3, where trimmings were ground from carcasses the day the carcasses were cut, often had higher bacterial counts evident on Monday than on Wednesday. It is evident that the longer holding periods over the weekend resulted in more microbial growth than was present in the middle of the week when holding periods were reduced. The higher APC on the used grinder surfaces on Monday than on Wednesday also reflects the fact that meat with higher microflora was being processed.

Bacteriological condition of ground beef by time of year in individual plants is listed in Table 2 so that differences due to game processing in November and January in plants 2 and 3 could be detected. In November and January coliform and fecal coliform counts were higher in ground beef from plant 1 where no

TABLE 2. *Relationships between time of year and numbers of bacteria/g of ground beef*

Time of year	Plant no.	No. of samples	Coliforms	Fecel coliforms	APC
Nov. and Jan.	1	12	1.0×10^4	9.3×10^3	1.5×10^6
	2[a]	12	3.5×10^3	1.8×10^3	1.0×10^7
	3[a]	12	1.1×10^3	1.7×10^2	3.6×10^6
	4	12	2.8×10^2	1.9×10^2	1.5×10^6
Feb. and May	1	8	1.9×10^3	7.7×10^2	3.3×10^6
	2	8	1.4×10^2	1.4×10^2	2.8×10^6
	3	8	1.3×10^3	1.2×10^3	2.6×10^5
	4	8	1.3×10^3	1.3×10^3	9.8×10^5
July and Sept.	1	8	6.6×10^2	4.2×10^2	6.7×10^6
	2	8	2.5×10^4	2.5×10^4	8.5×10^6
	3	8	1.5×10^2	1.5×10^2	2.5×10^5
	4	8	5.5×10^1	2.1×10^1	9.1×10^6

[a]Processed game in Nov. and Jan.

game was processed than they were in plants 2 and 3 processing game. According to Goepfert (5) the primary source of fecal coliforms on beef carcasses is the hide of the animal. It was thought that transporting field dressed deer, elk, and antelope with the hide intact into lockers containing beef might raise the coliform and fecal coliform counts in the ground beef being produced in those plants. Evidently this was not true in these locker plants where the efficiency of sanitation in the coolers was average or below. The highest average values for APC were found in plant 2 during November and January when game was processed. It has been observed (5) that beef samples with high APC had a greater likelihood to have fewer than 50 fecal coliforms/g. Samples of meat having an APC of less than 10,000,000/g were almost twice as likely to exceed 50 fecal coliforms/g as those with APC values exceeding 10,000,000/g. They suggested that the major meat flora influenced either viability or detectability of fecal coliforms in meat. Since APC in plants 1 and 4 not processing game in November and January were lower than APC in plants 2 and 3 where game was processed in November and January; it would appear that processing game, even though it was done at the end of the day after beef processing had stopped, did have an influence on the total number of bacteria in ground beef. In addition, plants 2 and 3 processing game in November and January had a higher APC/g of ground beef than at any other time of the year. The higher counts may have been related to an overload of work during game season and not to game as such. If this were true, the overload of work was not reflected in elevated APC values on the cleaned grinder surface. In Tables 1 and 2 a close correlation between coliforms and fecal coliforms/g of ground beef was noted. This would be expected since fecal coliforms constitute 70 to 77% of the coliforms in ground beef (*18*).

Others (*14,15*) have concluded that large centralized meat processing operations tend to produce ground beef of better microbiological quality than traditionally prepared ground beef. Nevertheless, the data in this research do not support this view. Ground beef packaged in centralized operations may have lower microbial loads immediately after packaging, nevertheless, the time which elapses between packaging and retail sale could have a much greater influence on total microbial population than the original microbial load. Centralized processing of meat under USDA inspection is growing rapidly. There is every reason to believe that total microbial counts in ground beef when it is packaged will be kept low under this system. In addition, *Salmonella*, (*18*) and *Staphylococcus aureus* (*15*) do not grow in ground beef held at 5 C. Therefore, standards for centrally packaged ground beef which would regulate the time between packaging and retail sale and the temperature at which the meat should be held seem worthy of consideration. These standards could replace some state regulations on bacterial numbers in ground beef which are difficult to administer. This would not limit the controversial debate on regulations for small plants where ground beef is produced within the plant. Nevertheless, small plants are decreasing in number and as a result microbial standards in small plants should take less time to administer.

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Journal of Food Protection Vol. 40, No. 6, Pages 389-392 (June, 1977)
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Survival of Foot-and-Mouth Disease Virus in Casein and Sodium Caseinate Produced from the Milk of Infected Cows[1]

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(Received for publication June 28, 1976)

ABSTRACT

Milk-borne transmission of foot-and-mouth disease virus (FMDV) was implied during the 1967-1968 epizootic in England. Consequently, experiments were designed to study survival of FMDV in milk and dairy products. As part of these studies, eight batches of casein were prepared from milk collected at various times after infection of cows with FMDV. Raw skimmilk was used in two batches and pasteurized (72 C, 15 sec), skimmilk was used in six. Casein was obtained by acidulation of skimmilk to pH 4.6 with HCl. Each batch was tested for FMDV infectivity either as casein or sodium caseinate in cell cultures and by inoculation of cattle. Samples assayed in cell cultures did not show evidence of infectious FMDV. However, cattle inoculated with these samples became infected with FMDV in one of two trials in which the casein was prepared from raw skimmilk and in three of six trials with skimmilk which was pasteurized. Samples from one of two dried casein batches infected test cattle. Samples from four of six batches of casein prepared from uninfected cows milk to which FMDV was added before pasteurization also infected cattle.

Milk-borne transmission of foot-and-mouth disease virus (FMDV) was implied during the 1967-1968 epizootic in England (*7,10,13*). Investigations during that epizootic indicated that milk from infected cows may have been collected from dairy farms before clinical signs of FMDV were observed in the cows. These studies showed that FMDV could be secreted in cow's milk as long as 33 h before the onset of clinical FMD. Thus, movement of milk from dairy farms during the early phase of an FMD epizootic could be a factor complicating the eventual control of the disease.

Recent studies (*1,2,14,16*) indicated that some FMDV could survive the conditions used during the manufacture of products such as casein and cheese. These observations are of concern because the possible salvage of FMDV-contaminated milk by conversion to edible by-products is currently being considered. Recent marketing trends in world trade involving products such as casein provide potential hazards in transmission of virus diseases infecting animals and man.

The purpose of experiments reported here was to determine whether FMDV would survive the processes used in the manufacture of edible grade casein.

MATERIALS AND METHODS

Cattle

The three dairy cows (*14*) used in this study were obtained from commercial sources and were 3 to 7 years old. Steers used to assay infectivity of casein samples were grade Herefords, approximately 18 months old. Housing and handling of these animals have been described (*9, 12, 14*).

Virus

Type A FMDV, subtype 3, (A_3) was used in these studies. This stock virus was prepared as a 20% suspension of fresh vesicular lesion material from tongues of infected steers. The suspension was clarified by low speed centrifugation.

Casein production

Two batches of casein were made from raw skimmilk and six batches from the pasteurized skimmilk of FMDV infected cows. An additional six batches were made using skimmilk from noninfected cows to which FMDV was added. Production procedures were as follows:

(*a*) Skimmilk from FMDV-infected cows was placed in a 1-liter beaker containing a magnetic stirring bar and equilibrated to 25 C in a water bath. Reagent grade 1N HCl was slowly added while the mixture was stirred until the casein precipitated (pH 4.6). Precipitated curds were kept in suspension by stirring for 1 h at 25 C and then allowed to settle for 10 min. Whey was decanted and curds were washed by resuspension in two to three original volumes of distilled water adjusted to pH 4.7 with HCl. The washing procedure was repeated three times for 10 min each, after which the casein was collected on filter paper and pressed to expel excess water.

(*b*) Ten-ml aliquots of skim milk from FMDV-infected cows were heated at 72 C for 15 sec as previously described (*13*). After pasteurization, the different aliquots were mixed in a 1-liter beaker and equilibrated to 32 C in a water bath with stirring. The milk was adjusted to pH 4.5 with 1N HCl. Precipitated casein was kept in suspension by stirring for 1 h at 32 C and then allowed to settle for 10 min. Whey was decanted, and the curds were washed three times as described above, except that curds were collected on filter paper in a Buchner funnel with vacuum or sedimented by centrifugation after each wash.

(*c*) Commercial cow's milk was used to produce skimmilk to which FMDV was added. Pasteurization and casein production were as described in the second procedure.

[1]*Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.*

In each procedure, 300 to 600 ml of skimmilk was used to prepare the casein. The complete preparation procedure took 2 to 3 h during which the casein was maintained at pH 4.5-4.7. To make sodium caseinate, we suspended wet or dried casein in Eagles' Minimum Essential Medium (MEM) with Earles' salts and then added 1N NaOH. The slurry was vigorously mixed until pH was 7.6. All casein and sodium caseinate samples with the exception of two casein batches made from the milk of infected cows were stored at -20 C for later testing.

These latter two batches of wet casein were dried, as described below, to 8 to 10% moisture with equipment fabricated at the Plum Island Animal Disease Center. Wet curd was first pressed through a #10 mesh screen, then exposed to a stream of air while being mechanically vibrated. The temperature did not exceed 32 C for the first 40 min. Heat was then applied, and the temperature was brought to 65 C and maintained for 10 min. Dried casein was then stored at 4 C until conversion to sodium caseinate as described above.

Infectivity studies

Samples of wet casein for infectivity studies were prepared as 10% suspensions in MEM in a high-speed mixer. After centrifugation, the supernatant fluid (pH 5.5) was used to inoculate cell cultures and cattle.

Samples of sodium caseinate were tested as is or were further treated by extraction with an equal volume of trichlorotrifluoroethane (TTE) in a high-speed mixer. Treatment with TTE was used because TTE dissociates virus-protein complexes such as the FMDV-specific antibody complex (*6*). The aqueous phase of TTE-treated samples was collected after centrifugation and tested for infectivity in cell cultures and cattle. Samples of sodium caseinate prepared from dried casein were tested for infectivity by inoculation into cattle (Batches 5 and 6, Table 2).

Plaque assays were made on primary bovine kidney (BK) cell cultures in 4-oz. prescription bottles (3); 0.1-ml inoculum was used in each of three cultures per virus dilution. Cultures were rinsed with MEM before use. Virus was allowed to absorb for 30 min at 37 C, after which each culture was overlayered with gum tragacanth. Incubation was for 24 h at 37 C, after which the cultures were stained and the plaques counted.

For determination of infectivity, 0.5 ml of sample was inoculated on each of 10 BK cultures. Maintenance medium was MEM containing 2% normal bovine serum and antibiotics. Cultures were incubated at 37 C for 7 days and examined daily for signs of cytopathic effect (CPE).

Each casein and sodium caseinate sample was inoculated into steers when infectivity tests in BK cultures were negative. The steers were first given 10 mg of xylazine ("Rompun," Chemagro Div. Daychem Corp., Animal Health Dept., Kansas City, MO 64120) tranquilizer intravenously, after which 2 ml of each sample was inoculated in approximately 20 sites in the tongue epithelium. The rest of the sample (20 to 40 ml) was injected into the gluteal muscles.

After inoculation, the steers were observed 14 days for signs of FMD. Steers that did not develop disease were challenged by inoculations in the tongue with 10^6 pfu of FMDV A_3. Immediately before this challenge, serum samples were taken for virus neutralization tests. Vesicular fluids and epithelium from steers that developed FMD after inoculation were serotyped by the CF test (*17*).

RESULTS

Most of the milk used from infected cows was obtained 1 to 3 days after inoculation before clinical signs of FMD were apparent. CPE was not observed in cell cultures inoculated with samples of casein or sodium caseinate, although cattle inoculations subsequently demonstrated infectious FMDV in many of the samples.

One of the two batches of casein made from raw milk of infected cows produced FMD within 2 days in each of two inoculated steers (Table 1).

TABLE 1. *Survival of foot-and-mouth disease (FMD) virus in acid casein made from raw skim milk of FMD-infected cows*[a]

Bath No.	Days after infection	Skimmilk	FMDV survival in casein	
			Cell culture	Cattle inoculation
1	1	3.0[b]	<0.7[c]	0/2[d]
2	2	4.0	<0.7	2/2

[a]Casein precipitated at pH 4.6 by addition of HCl. Reaction carried out at 25 C.
[b]$\log_{10}$ pfu/ml.
[c]$TCID_{50}$/ml.
[d]Number positive/number inoculated.

Three of six batches of casein made from pasteurized milk of infected cows were positive when tested in cattle (Table 2). Sodium caseinate was prepared from each of six casein batches and two of the six were positive in cattle, although one produced FMD in only one of two steers. One of the two sodium caseinate samples prepared from dried casein also infected cattle (Batch 5, Table 2).

Results of tests on six batches of casein made from milk that was artificially contaminated with FMDV and

TABLE 2. *Survival of foot-and-mouth disease (FMD) virus in acid casein and sodium caseinate made from pasteurized skim milk of FMD-infected cows*[a]

Batch No.	Days after infection	Skimmilk		Casein		Na caseinate
		Before pasteurization	After pasteurization[b]	Cell culture	Cattle inoculation[f]	Cattle inoculation
1	2	6.4[c]	<0.7[d]	NT[e]	NT	0/2
2	2	5.3	<0.7	NT	NT	0/2
3	1,2	6.2	<0.7	NT	NT	0/2, 1/2[g]
4	1,1	6.0	<0.7[d]	2/2	2/2	2/2, 2/2[g]
5	1	5.2	<0.7	<0.7	2/2	2/2[h]
6	1	5.2	<0.7	<0.7	2/2	0/2[h]

[a]Casein precipitated at pH 4.6 by addition of HCl. Reaction carried out at 32 C.
[b]72 C for 15 sec.
[c]$\log_{10}$ pfu/ml.
[d]$\log_{10}$ $TCID_{50}$.
[e]Not tested.
[f]Number positive/number inoculated.
[g]Before and after treatment with trichlorotrifluoroethane.
[h]Na caseinate solubilized from dried casein.

then pasteurized are presented in Table 3. Four of the six batches were positive when tested in cattle although one batch infected only one or two inoculated steers.

Generalized FMD occurred in the test cattle within 24 h after tongue lesions were detected. Virus from such lesions was identified as FMDV, A-3, by CF tests.

Although the mechanism that protects FMDV in milk has not been determined, it probably involves entrapment within casein micelles that undergo co-aggregation and ultimate precipitation (*5*). Thus, some FMDV might become encased in a mass of protective protein.

Before recent reports (*14,15*), the heat applied during pasteurization and the acid treatments used to manufacture milk products such as casein were often considered sufficient to destroy FMDV (*11,15*). Findings reported here show that infectious FMDV can survive the acid conditions of casein production even after the milk has been pasteurized at 72 C for 15 sec. The fact that dairy cattle may secrete FMDV in their milk 1 to 7 days before FMD is apparent (*7,8,14*) provides sufficient time for wide distribution of infectious milk into commercial and retail markets before the disease is suspected.

Although FMDV-contaminated milk products may be acceptable for human consumption, the ability of such products to initiate FMD if included in animal feeds, continues to be of concern to animal health authorities.

TABLE 3. *Survival of foot-and-mouth disease (FMD) virus in acid casein and sodium caseinate made from skim milk contaminated with FMD virus and then pasteurized*[a]

Batch No.	Skimmilk		Casein		Na caseinate	
	Before pasteurization	After pasteurization[b]	Cell culture	cattle	Cell culture	cattle
1	6.1[c]	<0.7	NT[d]	NT	<0.7	1/2[e]
2	5.2	<0.7	NT	NT	<0.7	2/2
3	5.2	<0.7	NT	NT	<0.7	2/2
4	5.5	<0.5	0.7	2/2[e]	<0.7	2/2
5	6.1	<0.5	NT	NT	<0.7	0/2
6	6.6	<0.5	NT	NT	<0.7	0/2

[a]Casein precipitated at pH 4.6 by addition of HCl. Reaction carried out at 32 C.
[b]72 C for 15 sec.
[c]$\log_{10}$ pfu/ml.
[d]Not tested.
[e]Number positive/number inoculated.

DISCUSSION

The production methods used in this study were selected as representative of processing principles in a large number of slightly differing commercial procedures. There was no correlation between the FMDV infectivity of casein samples and the virus concentration of milk from which they were prepared: low infectivity titers in milk did not necessarily predict difficulty in demonstrating infectious virus in casein produced from such milk. However, regardless of original milk titers, only very low concentrations of infectious FMDV from casein were detected in cattle as evidenced by positive reactions at only one to four of the 20 inoculations sites on each tongue.

It has been observed that bovine tongue epithelium (in vivo) has greater sensitivity for FMDV than FMDV-sensitive cultured cells (primary BK and primary bovine thyroid cells). Both cell cultures remained negative to FMDV replication after adsorption with inocula of 10-,20-,30-,50-,or 100-ml samples of milk. However, FMD was induced in susceptible cattle after tongue inoculations with 2-ml samples of the same milk (personal communication, J. H. Blackwell, 1975). Similarly, others have observed that the bovine tongue proved superior for detecting FMDV in heated milk samples when compared to several cell cultures (personal communication, Dr. J. G. van Bekkum, 1975).

Thus, it is our hypothesis that infectious FMDV in the milk of infected cows may be complexed with host constituents which prevent adsorption to receptor sites on susceptible cultured cells. However, in the tongue epithelium of the living bovine, humoral factors, such as enzymes, dissociate the FMDV-host constituent complex releasing FMDV to react with receptor sites of susceptible cells.

ACKNOWLEDGMENTS

The authors are indebted to Drs. R. J. Yedloutschnig and J. W. McVicar and to Mr. R. Trower for their valuable assistance in this work.

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Journal of Food Protection Vol. 40, No. 6, Pages 393-397 (June, 1977)
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Temperatures in Home Refrigerators and Mold Growth at Refrigeration Temperatures

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(Received for publication October 27, 1976)

ABSTRACT

Air temperature in two home refrigerators ranged from 1.7 to 20.2 C during 4-day periods. Mean air temperatures at thermocouple locations varied from 3.9 to 11.9 C, and temperature changes resulting from opening refrigerators were usually 3 C or less, although increases as great as 18.5 C were recorded. Temperatures in the range at which refrigerators operated were evaluated for their effect on mold growth. Mycelia from a strain of aflatoxin-producing *Aspergillus* did not grow at 8 C during 504 h of incubation. Isolates of *Pencillium* obtained from refrigerated food had optimum growth rates of mycelia at 15 C or above but grew at 5 C. However, incubation at 5 C prevented germination of spores of all but one of the isolates that were tested.

During an investigation in this laboratory of molds in home-stored foods, some molds isolated from certain refrigerated foods produced toxic substances (*6*). A survey by Van Walbeek et al. (*7*) of temperatures in domestic refrigerators indicated that the minimum operating temperatures ranged from -0.5 to 10 C; however, no atempt was made to measure fluctuations in temperature that might occur during home use. Fluctuations in storage may enhance mold spoilage of certain foods (*3*) and would influence preservation of all refrigerated foods. A detailed study of activity rates for several species of bacteria indicated that more growth resulted when temperatures fluctuated to extremes equidistant from a given temperature than when incubation was constant at the temperature (*4*).

The present study was prompted by a concern for the presence, growth characteristics, and temperature response of toxigenic molds in refrigerated foods, and by a need to gain information about changes that occur in home refrigerators during use.

MATERIALS AND METHODS

Determining air temperatures in home refrigerators

Two families, each having two children between the ages of 5 and 14 years, were used for this study. The home refrigerator-freezers monitored were termed by the manufacturers to be frostless of frost-free. Refrigerator A had a capacity of 12.7 ft^3, whereas that of refrigerator B was 14.0 $ft.^3$

A Honeywell (Fort Washington, Pennsylvania) Type 15 Electronik Recorder, a continous balance potentiometer with strip chart recorder equipped with copper-constantan thermocouples, was used to monitor temperatures in refrigerators during 4-day periods. Thermocouples were taped in place to prevent their movement. Air temperatures recorded at each thermocouple, resulting from opening and closing the door, were evaluated to determine the initial temperature before the door opened, maximum temperature attained, and the time required for temperature to return to the initial temperature (recovery time).

Mold strains

Isolates of *Aspergillus, Pencillium, Mucor,* and *Rhizopus* obtained from moldy food (*6*) were inoculated onto yeast extract-sucrose-gelatin medium, described below, and were evaluated for growth at 15 and 5 C. Of these, two isolates identified as belonging to the genus *Pencillium* and designated *Penicillium* sp. 0543 and *Penicillium* sp. 0546, were judged by dry weight and linear growth measurements of colonies to grow better than the other isolates at these temperatures, and were used in the growth experiments along with the aflatoxigenic *Aspergillus parasiticus* NRRL 2999 and nonaflatoxigenic *Aspergillus flavus* WB 1957. Spores used as inocula for growth experiments were obtained by flooding 10-day old cultures grown at 25 C on Mycological Agar (Difco) with sterile water and then gently rubbing the agar surface with an inoculating loop. The resultant spore suspension was filtered through a double layer of sterile cheesecloth into a sterile container, washed twice with distilled water to remove nutrients from the suspending medium, and held in distilled water at 5 C for 2 weeks before use. When used, the inoculum was again filtered through cheesecloth to remove chains of spores and provide a suspension of mostly single spores as judged by microscopic examination.

Medium

The medium for growth analyses contained 20 g of yeast extract, 200 g of sucrose, and 150 g of gelatin per liter of distilled water.

Inoculation and incubation of cultures

Spores were suspended in 0.5% molten agar at 45 to 50 C and inoculated at the center of medium in petri plates. One group of inoculated plates was immediately distributed at incubation temperatures of 25, 15, 8, and 5 C. During incubation, spores were examined with the aid of a microscope to detect outgrowth. A second group of inoculated plates was incubated at 21 C and spores were observed for germination and outgrowth. Mycelia appeared in all cultures within 30 to 54 h upon incubation at 21 C. After outgrowth of spores was observed by examination of the medium surface with a microscope, plates were distributed to incubators maintained at 15, 8, or 5 C.

Petri plates were placed in polyethylene bags containing moistened pieces of cheesecloth, and wetted strips of cheesecloth were hung inside incubators to maintain a moist atmosphere.

Determination of dry weight

Three plates containing a given mold culture were used to determine dry weight at each sampling period and were placed on a steam bath to liquify the gelatin. Mold growth floating on the surface of the medium was removed with a spatula and floated on water at 50 C to remove medium adhering to the colony. The washed colony was then dried at 60 C for 24 h and weighed.

RESULTS AND DISCUSSION

Monitoring refrigerator temperatures

Data obtained from Refrigerator A are in Table 1. Operating temperature, considered to be the initial air temperature before the refrigerator door was opened, varied from 1.7 to 14.0 C, and mean values for initial temperature at all locations ranged from 3.9 to 6.7 C. The maximal air temperature attained at each thermocouple location during door opening was from 15.4 to 20 C. Mean temperature attained on opening the door ranged from 7.8 to 19 C. Comparison of means for initial and maximal temperatures increases during door indicates that mean temperature increases during door openings ranged from 1.1 to 9.0 C. Also, from comparison of mean air temperature values, the least temperature change on door opening occurred at the upper rear of the refrigerator cabinet, and the greatest change occurred at the bottom of the cabinet. Temperature increases at the top and bottom of the door were intermediate to extremes detected in the cabinet. Mean recovery time for temperatures at all locations ranged from 9.5 to 13.0 min.

TABLE 1. *Air temperature changes in refrigerator A during home refrigeration*

Thermocouple location	Temperature, C		Recovery time[d], min
	Initial[a]	Maximal[c]	
Box			
Upper back by light panel			
Left side	6.7[b]	7.8	13.0
	6.7	8.1	12.0
	2.5-11.8	2.8-19.6	2.0-24.0
Right side	6.7	7.8	13.0
	6.7	8.1	12.0
	2.5-11.8	2.8-19.6	2.0-24.0
Near door latch	5.0	9.8	10.0
	4.8	10.1	8.0
	3.1-14.0	5.3-15.4	2.0-30.0
Bottom front (under vegetable crisper)	3.9	12.9	11.5
	3.5	12.3	9.0
	1.7-9.5	4.5-20.2	2.0-30.0
Door			
Top, butter keeper	6.7	11.2	9.5
	7.0	11.2	8.0
	4.8-11.8	7.3-15.7	2.0-30.0
Bottom	4.2	9.5	9.5
	3.9	10.1	8.0
	2.0-10.1	3.4-16.2	2.0-30.0
Room temperature (June)	23.9		
	23.8		
	21.0-26.9		

[a]Temperature immediately preceding door opening; also operating temperature for each location.
[b]Each set of numbers represents, reading down, the mean, median, and range.
[c]Highest temperature reached during door opening.
[d]Time required to return to initial temperature after door opening.

A summary of air temperature changes detected in Refrigerator B is in Table 2. Initial or operating temperatures varied from 4.8 to 12.9 C, and mean values at all locations ranged from 5.8 to 11.9 C. The maximal temperature during door openings at each location was from 7.8 to 18.8 C, and mean temperatures attained on opening the door ranged from 6.4 to 12.5 C. Mean values for initial and maximal temperatures at the same location indicate that mean temperature increases during door openings ranged from 0.4 to 3.5 C. In contrast to temperatures observed in Refrigerator A, the greatest temperature change in response to door opening at any location in Refrigerator B occurred at the upper rear of the refrigerator cabinet. Mean recovery time for all locations was from 5.4 to 13.9 min.

TABLE 2. *Air temperature changes in refrigerator B during home refrigeration*

Thermocouple location	Temperature, C		Recovery time[d], min
	Initial[a]	Maximal[c]	
Box			
Upper back near light panel	7.1[b]	10.6	13.9
	6.4	9.0	10
	5.9-9.2	8.1-18.8	3-71
Near door latch	6.6	8.6	10.8
	6.7	7.8	6
	5.6-7.3	7.3-12.0	2-42
Bottom (under vegetable crisper)			
Rear	6.0	6.4	5.4
	6.1	6.4	0
	4.8-7.0	5.6-7.8	0-26
Left front	6.9	7.6	7.6
	7.0	7.8	3
	6.2-7.6	6.7-9.2	0-26
Right front	5.8	6.5	5.3
	6.4	7.0	2
	4.8-7.0	5.3-9.0	0-23
Door			
Top, butter keeper	11.9	12.5	9.6
	11.8	12.2	0
	10.6-12.9	11.2-13.7	0-42
Room Temperature (December)	23.2		
	23.5		
	20.4-25.2		

a, b, c, and d See Table 1 for explanation of these footnotes.

No effort was made to standardize conditions in the two refrigerators, therefore detailed comparisons of data on air temperature have not been attempted. Instead, emphasis was on monitoring fluctuations in temperature that occurred during normal use.

Several factors that were not controlled probably influenced air temperature profiles. The temperature and surface area of cold refrigerated food, acting as a driving force to cool ambient air introduced during door openings, was not controlled. Location of foodstuffs near thermocouples also was not controlled, and it is possible that in some instances food almost touching thermocouples influenced the time required to return the air temperature to its initial value. Other uncontrolled factors considered to have influenced temperature rise and recovery time during door openings were length of time the door was open, disturbance of air flow patterns within the refrigerator caused by rearrangement or removal of food, and the manner in which the refrigerator door was opened and closed - fast, slow, or jerking motion.

Some comparisons of data in Table 1 and 2 are of interest. Door openings for Refrigerator A numbered 73 during the 4-day monitoring period in June, while

Refrigerator B was opened 39 times during a December period of identical length. Ranges for mean initial temperatures and mean recovery times at all thermocouples in both refrigerators for the 4-day test period (3.9 to 11.9 C, 5.4 to 13.9 min) indicate that both were operated at temperatures recommended for home refrigeration (5) and were efficient in cooling air that entered during opening. However, butter and cheese storage areas in both refrigerators maintained temperatures (mean values of 6.7 and 11.9 C) in excess of the 2.2 to 4.4-C range preferred for storage of these dairy products (2). Dairy products including cheese were frequently found among moldy samples of refrigerated foods in a survey of food stored in the home (6).

Growth studies

Initial plans were to study how air temperature in refrigerators fluctuated and then apply results of that study to an analysis of the effect of temperature fluctuations on growth of molds isolated from home-stored foods. However, air temperature profiles of the two refrigerators examined (Table 1 and 2) indicated that, although occasional increases in air temperature of 18.5 C occurred and recovery times as long as 71 min were detected, most temperature fluctuations were less than 3 C and lasted 12 min or less. We, therefore, limited our experiments to temperature influence on mold growth at constant temperatures, and determined how growth from mycelia and from spores of selected cultures responded to those temperatures. Comparison of rates of growth by different isolates seemed to be done best by use of dry weight measurements, and this required a solid substrate that could be removed easily before mycelial growth was weighed. A medium made solid with gelatin suited this purpose since all incubations were below the temperature at which gelatin is a liquid. The average of triplicate dry weight values were plotted and lines were visually fitted to the points. Growth rates for mycelium at 5 and 8 C appeared to decrease before dry weight values reached 100 mg, and linear regions were difficult to assign with certainty to most of the curves.

Mycelial growth of aflatoxin-producing *A. parasiticus* NRRL 2999 was appreciably reduced at 15 C compared with growth at 25 C (Fig. 1). Mycelium developing at 15 C remained a creamy white, and sporulation was not detected. No lag was detected for mycelial growth at 25 or 15 C. No growth of mycelium occurred at 5 and 8 C. Spores germinated at 15 C after a lag period that exceeded 100 h. Spores incubated at 5 and 8 C did not germinate during the 504-h (21 days) incubation.

Spores and mycelia of *A. flavus* WB 1957 incubated at 5 and 8 C failed to germinate or grow. Growth of spores or mycelia at other temperatures was not attempted with this strain.

Van Walbeek et al. (7) examined six aflatoxigenic strains of *A. flavus* for growth and toxin production in broth and agar cultures at 7.5 and 10.0 C with or without preincubation at room temperature. Spores germinated and aflatoxin was detected in one instance after incubation for 1 week. Two cultures grew at 7.5 and 10 C with no preincubation at room temperature, but failed to produce aflatoxin during incubation for 4 weeks. The authors suggested that absence of spores from cultures growing at 7.5 and 10.0 C presented an additional health hazard since the green color associated with *A. flavus* growth was not available to aid in detection. Lack of growth at 5 and 8 C by strains of *A. flavus* and *A. parasiticus* reported in our study contrasts with results of van Walbeek et al. (7), who used strains of the same species. Differences in the media employed and/or variation in temperature response of the strains may be responsible for this lack of growth.

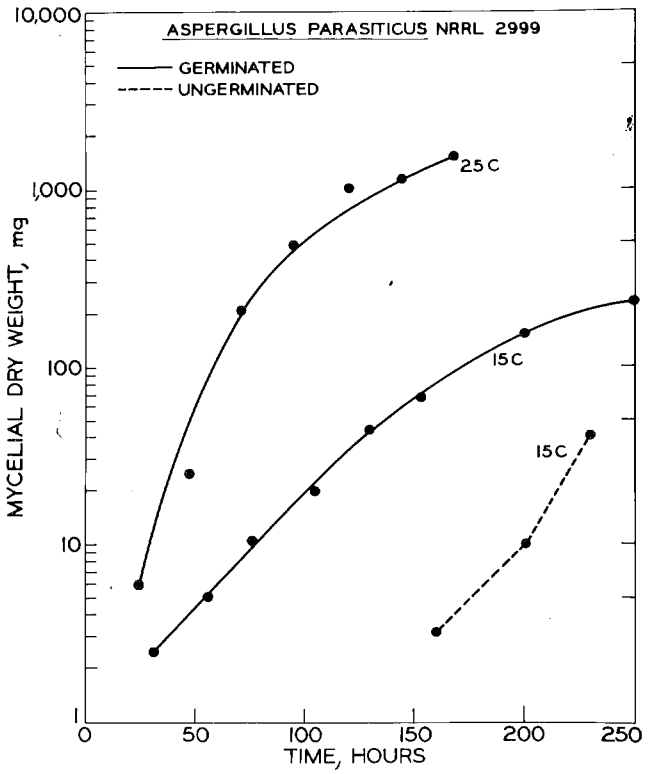

Figure 1. *Growth of mycelium and growth from ungerminated spores of* Aspergillus parasiticus *NRRL 2999 on yeast extract-sucrose-gelatin medium at 5, 8, 15, and 25 C. At 25 C, spores germinated rapidly and no distinction was made between germination and outgrowth of spores, and growth of mycelium. Spores did not germinate and mycelium did not grow at 8 and 5 C during 504 h of incubation.*

Growth curves representing the temperature response of two isolates of *Penicillium* appear in Fig. 2 and 3. The isolates grew at refrigeration temperatures in preliminary studies described in Materials and Methods.

Visual examination of slopes of growth curves for *Penicillium* sp. 0546 (Fig. 2) indicates that the greatest growth rate occurred when mycelia from germinated spores were incubated at 25 C. Comparison of mycelial growth curves fro 5, 8 and 15 C in the region from about 10 to 100 mg of dry weight indicates that growth rates for these regions are similar to each other. The difference between curves appears to reflect a lag time after

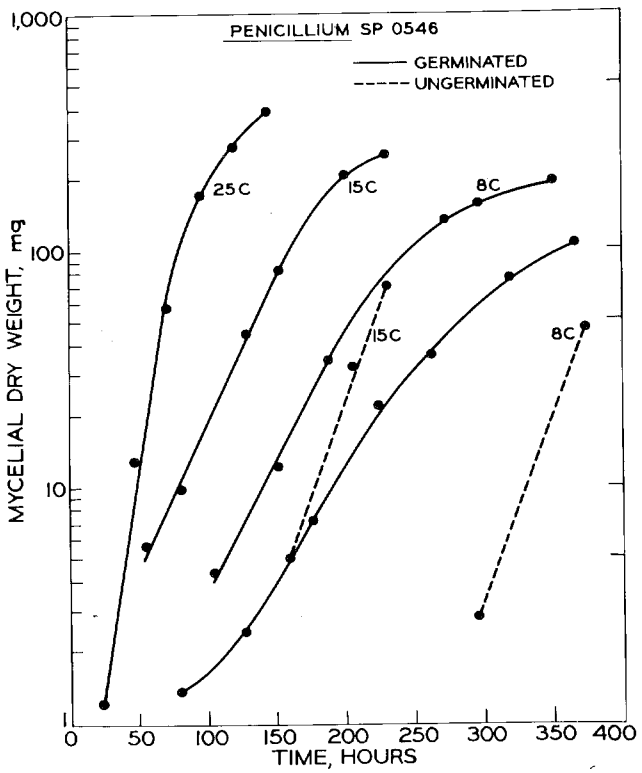

Figure 2. *Growth of mycelium and growth from ungerminated spores of* Penicillium *sp. 0546 on yeast extract-sucrose-gelatin medium at 5 (unlabeled curve), 8, 15, and 25 C. Spores incubated at 8 C had not germinated when examined at 255 h. Spores incubated at 5 C did not germinate during 504 h of incubation.*

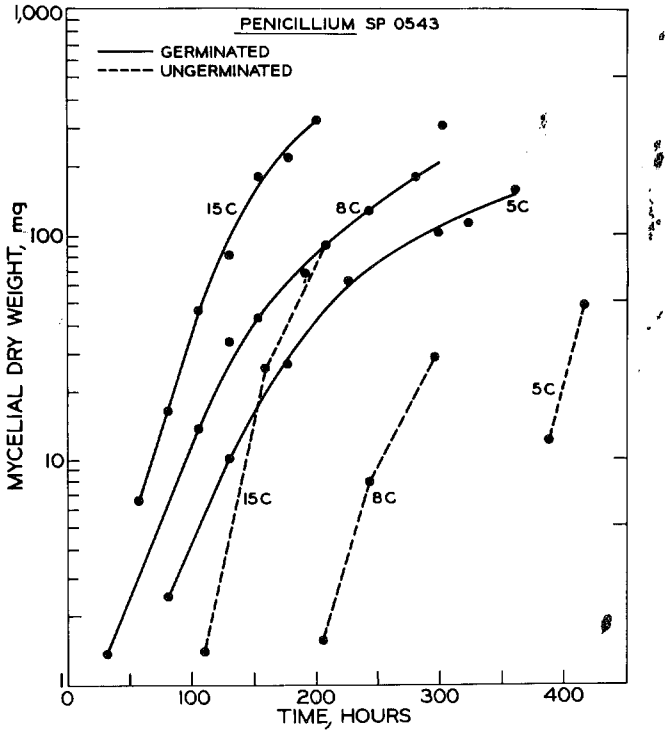

Figure 3. *Growth of mycelium and growth from ungerminated spores of* Penicillium *sp. 0543 on yeast extract-sucrose-gelatin medium at 5, 8, and 15 C. Spores incubated at 5 C had not germinated when examined at 309 h.*

incubation of mycelia was begun. The lag increased with decreasing temperature of incubation.

Spores of isolate 0546 held at 15 C had germinated by 150 h, but almost 300 h were required before spores incubated at 8 C germinated. Spores at 5 C did not germinate within 504 h (21 days).

Growth of strain 0543 at 5, 8, and 15 C is described in Fig. 3. The lag preceding the maximum growth rate is much less evident at 5 and 8 C for strain 0543 than that described for 0546 in Fig. 2. Time required for spore germination at 8 C was about twice that needed for germination at 15 C. Spores of strain 0543 did germinate at 5 C.

Since an increased lag period characterizes growth as the temperature is decreased, it is necessary to consider this lag in growth as well as the slope of the growth curves when temperature sensitivity of different strains is being compared.

Growth curves of 0543, 0546, and NRRL 2999 in Fig. 4 take into account lag periods that precede apparent linear portions of the mycelial growth curves (Fig. 1,2, and 3). Values plotted are the reciprocal times required to attain an arbitrarily selected dry weight. Parallel lines representing different weights of the same strain are an indication that growth curves maintain a constant relationship with change in dry weight. The nonlinear character of plotted values (Fig. 4) for times required to attain 10 mg may be the result of weighing errors.

A. parasiticus NRRL 2999 had a growth rate greater than that of 0546 at 25 C, as indicated by its larger value for reciprocal time. At 5, 8, and 15 C strain 0543 had the greatest rates of growth.

Strain NRRL 2999 is the most temperature-sensitive over the range investigated since no growth was detected at 8 C, and the slope determined by values for growth at 15 and 25 C is the greatest.

Temperature sensitivity of isolate 0546 is slightly more than that of isolate 0543 as determined from the greater slope (-0.29) for isolate 0546 compared to a slope of -0.32 for isolate 0543 (calculated for 100 mg dry weight values).

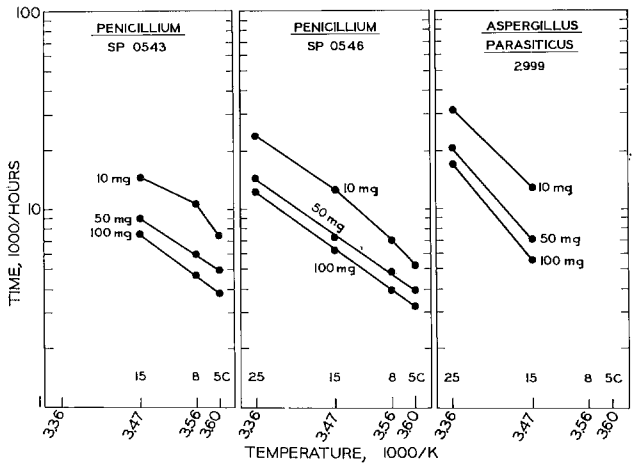

Figure 4. *Growth rates of mold mycelia computed from time required to attain 10, 50, and 100 mg dry weight when grown at constant temperatures from 5 to 25 C.*

Deverall (*1*) has suggested that psychrophilic molds should have a growth optimum near or below 10 C. Growth rates for the isolates of *Penicillium* used in this study continued to increase with increasing temperature (Fig. 4), over the range of 5 to 15 C for isolate 0543 and of 5 to 25 C for isolate 0546. Although they were isolated from refrigerated food and can grow at 5 C, they are not considered psychrophiles according to Deverall's definition.

Profiles for air temperature in home refrigerators and growth studies of molds presented in this report indicate that even when refrigerators are operated according to manufacturer's recommendations, growth of molds may occur. Although isolates of *Aspergillus* that we studied did not grow at 8 C, results of studies by Van Walbeek et al. (*7*) indicate that some toxic strains of *A. flavus* can grow at that temperature.

The recommended temperature for refrigerated storage of some foods is a compromise between maintenance of quality and safety. In light of the isolation of toxigenic molds from refrigerated foods (*6*) and the general concern about molds in foods, refrigerator manufactureres may need to reexamine the design of cabinets so that temperature can be monitored, and to assure that critical items be stored at the recommended temperature. In the final analysis, consumer knowledge of safe food handling and storage practices, and awareness of potential health hazards presented by molds in food may be the most important defense against these hazards.

ACKNOWLEDGMENTS

This research was supported by the College of Agricultural and Life Sciences, by Public Health Service Grants FD000143 and FD00009 from the Food and Drug Administration, and by the Food Research Institute, University of Wisconsin-Madison. One of us (G.S.T.) received an educational allowance from the U.S. Veterans Administration.

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Journal of Food Protection Vol. 40, No. 6, Pages 398-401 (June, 1977)
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Cured Pigment and Color Development in Fermented Sausage Containing Glucono-Delta-Lactone[1]

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(Received for publication October 6, 1976)

ABSTRACT

Addition of 0.41% glucono-delta-lactone (GDL) to meat mixtures for fermented sausages produced an immediate acidulation response, lowering the initial pH from 6.0 to 5.4. After fermentation, control sausages had a pH of 5.1 which decreased to 5.0 at 16 days of drying. Sausages containing GDL had a pH of 4.8 at the end of the fermentation phase and the pH remained constant through heat processing and drying. Additional acidity from GDL usage promoted greater ($P < 0.05$) conversion of the total heme pigments to the nitric oxide heme pigment at each stage of processing examined. Although more pigment production occurred, sausages with GDL lost cured pigment upon dehydration at the same rate as control sausages. Color values showed a more rapid increase in 'a_L' values for sausage containing GDL. Sample L and 'b_L' values were similar for both sausage groups at each process phase.

The primary conditions in meat mixtures favorable to development of a cured color are high hydrogen ion concentration (*6,7*) and input of heat during the last stage of processing (*7,13*). Favorable acidic conditions for the reaction of nitric oxide with myoglobin are developed during the fermentation phase of dry sausage preparation when lactic acid is produced from added carbohydrate. The pH generally found in initial sausage preparations is near 6.0 and decreases to 5.4 to 4.8 during fermentation (*3,10*). In fermentation, usually conducted at 30 to 37 C, there is a slow but substantial heat input. With heat processing, as temperature increases in the range from 49 to 60 C, rate of color development increases (*7*).

Glucono-delta-lactone (GDL) was introduced during the 1960s to give a more rapid and improved color development to cooked comminuted products such as frankfurters, bologna, and luncheon meats (*16*). Although introduced for production involving rapid processing operations of several hours, GDL has been successfully used in the manufacture of dry sausages (*9,16*) where final products are not obtained for 10 to 120 days. On hydrolysis and with the application of heat, GDL yields gluconic acid, effectively reducing the pH of the sausage to "accelerate" color development (*16*). Fox et al. (*7*) and Monagle et al. (*13*) found that maximum color development occurred faster when GDL was included in frankfurter preparations. However, both research groups (*7,13*) reported no color differences between final product samples with or without GDL when these were processed to the same final internal temperature (68 to 70 C).

In fresh (uncured) meats, Landrock and Wallace (*11*) stated that dehydration increases the concentration of meat pigments at the meat surface. Dehydration of fermented, dry sausages results in a loss of the nitric oxide heme pigment (*1*). Townsend (*19*) reported significantly less conversion of the total heme pigements to the nitric oxide heme pigment form in sausages containing 25 to 30% moisture when compared to those containing 45 to 60% moisture.

This study was conducted to determine the effect of GDL on development of fermented sausage color and to examine color stability on dehydration. Sausage preparations were analyzed at several processing phases for percent of total pigments converted to the cured pigment form. Color was determined by color difference measurements.

MATERIALS AND METHODS

Sausage preparation and processing

Two sausage mixes were prepared using a blend of fresh boneless beef and beef fat adjusted to approximately 24% fat. Additional ingredients and their quantities per kg of meat were as follows: 0.078 g of $NaNO_2$, 0.156 g of $NaNO_3$, 0.47 g of sodium erythorbate, 30.0 g of NaCl, 10.61 g of seasoning mix, 5.05 ml of *Pediococcus acidilactici* suspension (LACTACEL), 7.51 g of dextrose, and 25.0 ml of water. The starter culture yielded an approximate initial level of 1.7×10^7 cells/g mix. The effect of GDL was determined with batches containing 4.45 g of GDL per kg of meat.

The curing agents, seasonings, and dextrose were mixed into the meat for 3 min in a Hobart H-600 mixer. The water and starter culture were then added and the mixture blended for an additional 2 min. When used, GDL was added after the water but before adding the starter culture. The initial mix temperature was approximately 2 C and increased to 8 C during the 5 min of blending.

Sausages were stuffed into 52-mm diameter dry sausage fibrous casings (Union Carbide) to approximately 460 g. The chubs were

[1]*Technical Contribution No. 1403 of the South Carolina Agricultural Experiment Station, Clemson University, Clemson, South Carolina 29631.*

fermented at 38 C and 95% relative humidity for 21 h. After fermentation, all sausages were initially heated at 71 C for 45 min, increased to 77 C for 45 min, and finally heated at 82 C until an internal temperature of 60 C was attained (2.0 to 2.5 h total). The sausage chubs were cooled to 24 C by a cold water spray and placed in a 7.5 ± 2 C drying room having 20 to 25 air changes/h. Drying room humidity ranged from 80 to 85%. Sausages were removed for color analyses at 8 and 16 days of drying.

Sampling and compositional analyses

Two sausage chubs of the control and GDL groups were collected for compositional and color analyses at each of the following process phases: (*a*) on completion of mixing and stuffing, (*b*) after fermentation for 21 h (*c*) after heat processing to 60 C internal, (*d*) after 8 days of drying, and (*e*) after 16 days of drying.

All samples were analyzed in duplicate or triplicate for moisture, fat, ash, and protein (Kjeldahl N × 6.25) following AOAC (*4*) procedures. The sausage pH and percent weight loss on drying were determined as described by Acton and Keller (*3*). Two sausage chubs were composited for duplicate heme pigment analyses and color measurements.

Data were analyzed by analysis of variance and the differences between means tested by the least significant difference method (*14*).

Heme pigment analyses

The methods for nitric oxide heme pigments and total pigment were those described by Hornsey (*8*) with the extraction procedure modifications outlined by Acton and Dick (*1*). The results, reported as percent conversion, are the percent of total heme pigment converted to the nitric oxide heme pigment (wet sample basis).

Color determinations

Color measurements were conducted with a Gardner Color Difference Meter, Model C4 (Gardner Laboratory, Bethesda, Maryland). The instrument was standardized with a pink standard plate (No. CG-6632; L = 52.9, a_L = 31.9, b_L = 11.4), Results were obtained in terms of L,a_L, and b_L values.

RESULTS AND DISCUSSION

The pH differences attained during the course of sausage processing are shown in Fig. 1. Within 15 min of GDL addition, the initial mix pH had decreased ($P < 0.05$) from pH 6.0 to pH 5.4. The initial drop in pH was maintained through the drying phase and was not affected by the starter culture. This is in agreement with the findings of Skjelkvale et al. (*17*). The control sausages had an ultimate pH of 5.0 in the drying phase as compared to a pH of 4.8 for the sausages containing GDL. Fermented sausages range in pH from 5.4 to 4.4, with most sausage types having a pH near 5.0 (*2*).

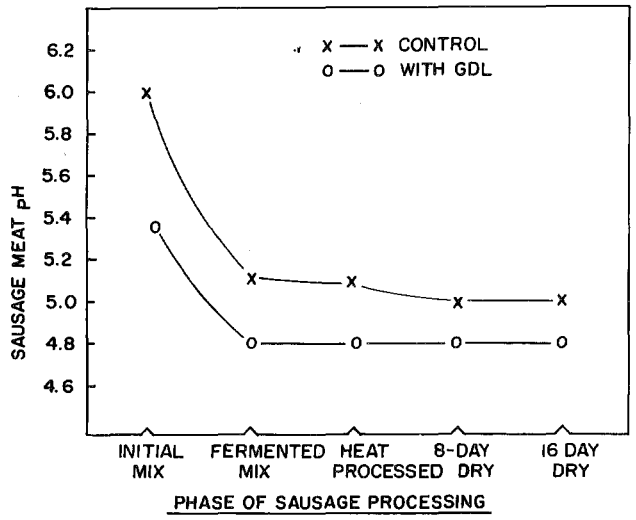

Figure 1. *Sausage pH at various phases of processing.*

The composition (Table 1) and weight losses (Table 2) of both sausage groups at each stage of processing were similar. In curing hams, Pate et al. (*15*) reported that moisture content significantly decreases as levels of GDL in the pumping medium increased. A similar decrease in moisture content of dry sausage containing 0.6% GDL was reported by Kotter et al. (*9*). In our study, GDL at a level of 0.41% did not affect the moisture content or weight loss of the sausages. Based on compositional data and percent weight losses, both sausage groups would be classified as "semidry" at 8 days and "fully dry" at 16 days of drying (*2*).

TABLE 1. *Composition*[a] *of sausage at various phases of processing*

Process stage	Sample	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
Initial mix	control	55.8^{ab}	23.5^{b}	14.8^{a}	3.7^{cd}
	with GDL	56.2^{a}	22.5^{ab}	14.8^{a}	3.5^{bc}
Fermented mix	control	56.9^{a}	22.0^{a}	15.8^{bc}	3.2^{ab}
	with GDL	56.9^{a}	21.5^{a}	15.4^{ab}	3.2^{ab}
Heat processed	control	56.3^{a}	22.3^{ab}	16.7^{d}	3.1^{a}
	with GDL	55.0^{b}	23.5^{b}	16.1^{cd}	3.1^{a}
Dried, 8 days	control	46.4^{c}	28.2^{c}	19.4^{e}	3.9^{d}
	with GDL	45.7^{c}	28.3^{c}	19.2^{e}	4.0^{d}
Dried, 16 days	control	34.6^{d}	35.1^{d}	23.4^{f}	5.0^{e}
	with GDL	34.2^{d}	35.6^{d}	23.0^{f}	4.7^{e}

[a]Means in columns having the same superscript letter are not different ($P < 0.05$).

TABLE 2. *Percent weight loss of sausage during drying*[a]

Sausage sample	Days of drying				
	2	6	8	12	16
Control	5.50	14.95	19.30	26.67	32.24
With GDL	5.84	14.61	19.35	27.25	33.09

[a]Column means are not different ($P < 0.05$). Each horizontal mean is different ($P < 0.05$).

The percent of the total heme pigment converted to the nitric oxide heme pigment form is shown in Fig. 2. The control sausage showed a substantial increase ($P < 0.05$) from an initial 13.5% conversion to 60.1% conversion at the end of fermentation. A further increase ($P < 0.05$) to 81.5% was found after heat processing. Cured pigment production was favored by two occurrences: (*a*) the decrease in pH during fermentation, and (*b*) the heat input during fermentation at 38 C and heat processing to 60 C internal. Fox and Thompson (*6*) reported that the overall reaction rate in the production of the nitric oxide pigment increases sharply with decreasing pH, particularly in the pH range of 5.5 to 4.5. The percent conversion during heat processing of frankfurters progressively increased as product temperature increased from 49 to 60 C (*7*). Thus temperature and hydrogen ion concentration appear interrelated under the experimental conditions used in this study.

At each phase of processing, increased ($P < 0.05$)

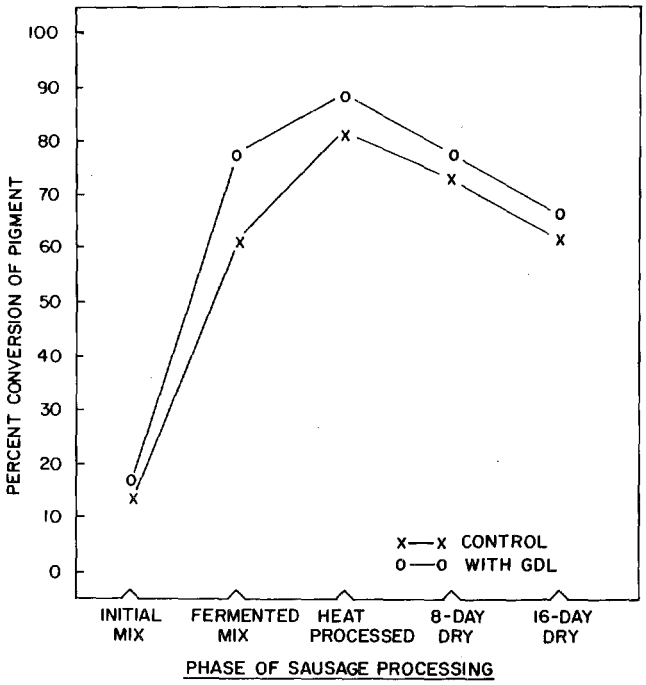

Figure 2. *Percent conversion of total heme pigment to the nitric oxide heme pigment at various phases of sausages processing.*

cured pigment production occurred in sausages containing GDL compared to control sausages (Figure 2). Higher levels of nitric oxide pigment were maintained after heat processing. The additional acidity contributed by GDL addition in early preparation (initial mixing) may have caused a more rapid formation of the nitric oxide heme pigment, which is in agreement with the report of Sair and Henry (*16*).

A loss of cured pigment on sausage dehydration was reported by Acton and Dick (*1*). The loss may involve dissociation of nitric oxide from the nitric oxide heme pigment (*5,18*). Townsend (*19*) found that lower conversion percentages occur in sausage products containing 25 to 30% moisture than in those containing 45 to 60% moisture. While GDL contributed to pigment formation, it did not (Fig. 2) contribute to "stability" of the cured pigment (once formed) as suggested by Sair and Henry (*16*). The loss of converted pigment in the sausages containing GDL occurred at approximately the same rate as for the control sausages.

Gardner color values, Saturation Index, and hue angles of the sausages are given in Table 3. In the processing sequence, the sample 'L' values decreased slightly, showing a darkening of the sausages. The 'a_L' values increased, denoting positive movement toward redness while the 'b_L' values decreased, indicating a decrease in yellowness. Through heat processing the 'a_L' and Saturation Index values for sausages containing GDL increased more rapidly as compared to the respective values of the control sausages. Hue angles also indicated a more rapid "redness" development in GDL sausages than in the control sausages. These results are in agreement with the increased levels of nitric oxide heme pigment production, due to GDL use, as previously discussed.

At 16 days of drying, the Saturation Index for both sausage groups indicate approximately equivalent color intensity. However, the lower hue angles for the GDL sausages showed a greater redness development which may be related to the pH difference between the sausage groups. As a result of moisture loss on dehydration, there is a concentrating of the remaining undissociated cured pigment (*1*) and a visual "browning" of the sausage color. The development of a brownish-red color on sausage dehydration has also been described by Lu and Townsend (*12*) which may be due to oxidation of the remaining free pigment (*20*).

ACKNOWLEDGMENT

The authors express their appreciation to Griffith Laboratories, Lithonia, Georgia and Chicago, Illinois for spices, seasonings, and glucono-delta-lactone samples, and to the Merck Chemical Division of Merck and Company, Inc., Rahway, New Jersey for providing samples of LACTACEL.

Reference to a firm or trade name does not imply endorsement over firms or products not mentioned.

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TABLE 3. *Gardner color values, Saturation Index and hue angles for sausage at various phases of processing.*[a]

Process stage	Gardner L value		Gardner a_L value		Gardner b_L value		Saturation index[b]		Hue angle[c]	
	Control	With GDL	Control	With GDL	Control	With GDL	Control	With GDL	Control	With GDL
Initial mix	45.1[a]	44.6[a]	5.0[a]	4.7[a]	10.5[a]	10.0[a]	11.6	11.0	64°32'	64°56'
Fermented mix	42.6[b]	42.4[b]	9.5[b]	12.6[b]	8.1[b]	7.7[b]	12.5	14.8	40°27'	31°26'
Heat processed	44.7[a]	45.0[a]	12.5[c]	13.1[b]	7.6[bc]	7.5[b]	14.6	15.1	31°18'	29°48'
Dried,										
8 days	42.7[b]	44.2[a]	11.8[bc]	12.6[b]	7.1[cd]	7.0[b]	13.8	14.4	31°02'	29°03'
16 days	40.9[c]	40.8[b]	12.7[c]	13.5[b]	6.7[d]	6.0[c]	14.4	14.8	27°49'	23°58'

[a]Means in columns having the same superscript letter are not different ($P < 0.05$).
[b]Using mean Gardner a_L and b_L values, Saturation Index = $(a^2 + b^2)^{1/2}$.
[c]Using mean Gardner a_L and b_L values, hue angle is the angle whose $\tan^{-1} = b/a$.

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Journal of Food Protection Vol. 40, No. 6, Pages 402-405 (June, 1977)
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Viable Counts Versus the Incidence of Machinery Mold (*Geotrichum*) On Processed Fruits and Vegetables

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(Received for publication October 28, 1976)

ABSTRACT

Aerobic plate counts on peas, green beans, corn, and beets collected at different processing stages were commonly 10^6-10^7 per g while counts on tart cherries were 10^3-10^4 per g. Bacteria made up 99% of the mesophilic microflora on the vegetables while yeasts predominated on the fruit. Many of the organisms appeared to be introduced with the raw product. *Geotrichum* was a common contaminant only of green beans; 49% of the samples from five canneries were positive with the average count being 130 fragments per 500 g. *Geotrichum*-positive samples did not yield higher viable counts than did those that were negative for this mold.

Viable counts have been a common method for evaluating the sanitary quality of foods. High microbial populations on frozen fruits and vegetables, for example, generally reflect insanitary processing equipment (*4,6*). Growth occurs in areas where food solubles collect and microorganisms are picked up by the food when it comes in contact with these surfaces.

Another procedure for assessing sanitary quality is to examine food for the presence of filaments of the mold *Geotrichum. Geotrichum,* a cause of slime, has long been recognized as a common contaminant of processing equipment and often is referred to as "machinery mold" (*3,7*). With the development of improved quantitative methods (*2*), machinery mold counts have been used with increased frequency as an aid to evaluating sanitation. The advantages to the machinery mold analysis are that results can be obtained shortly after the sample has been collected, and the procedure is also applicable to foods such as canned goods that have been subjected to a lethal process.

During this past summer, fruit and vegetable samples, collected from area processing factories, were cultured for both "total" aerobes and fungi, and were examined under the microscope for *Geotrichum* fragments. The objective was to determine the relationship between these different indices of sanitation.

MATERIALS AND METHODS

Samples

Surveys were conducted in which line samples were obtained from different processing stages. Most were from unit operations before the blanch, thus before the food had received a treatment that would be lethal for vegetative microorganisms.

Samples were sealed in polyethylene bags and transported over ice to the laboratory. When time permitted, foods were cultured for viable organisms and processed for machinery mold the same day they were collected. When this was not feasible, they were stored frozen in a -23 C room until the analyses could be done, usually within 1 to 2 days.

Viable counts

Samples of 22 g were homogenized with 198 ml of water in a Waring blender. Appropriate decimal dilutions were plated for aerobic bacteria and yeasts and molds. Bacteria were enumerated on Plate Count Agar (Difco); plates were incubated 2 days at 32 C. Potato dextrose agar, adjusted to pH 3.5 with tartaric acid, was used for the yeasts and molds. Plates were counted after an incubation of 5 days at 20 C.

Machinery mold enumeration

The methods of Cichowicz and Eisenberg (*2*) along with recommendations of the Berkeley laboratory of the National Canners Association served as the basis for our procedure.

The fruit or vegetable was weighed into a tared, 24 × 33 cm, enamel pan. The sample size varied with the product: peas, corn, and green beans, 250 g; cherries, 400 g; small beets, 500 g; large whole beets and slices, 600 to 800 g. When frozen, the product was first thawed by placing the pan in a steamer for 5 to 10 min. The food then was transferred to a No. 16, 8-inch sieve which was suspended over a second pan. The pan used for thawing was rinsed with about 100 ml of water which was poured over the product and collected in the second pan. The food then was rinsed with about 300 ml of water from a wash bottle. During sing, the material was stirred frequently to assure that all surfaces were washed. After draining, the food was removed and the sieve was given a final rinse.

Washings were transferred to a No. 230, 5-inch sieve. The pan was then rinsed with distilled water, and the rinsings were added to the sieve. The residue was flushed to one edge using a wash bottle and was then transferred quantitatively to a 50-ml, graduated conical centrifuge tube. After transfer, the volume usually was 10 to20 ml. The suspension was stained by adding 1 to 3 drops of 10% crystal violet in ethanol solution, after which an equal volume of stabilizer solution was added. The stabilizer solution contained per 500 ml of water, 2.5 g sodium carboxymethyl-cellulose and 10 ml of formaldehyde.

After thorough mixing, 0.5 ml of the suspension was placed on a rot fragment slide (*1*) for counting under a steroscopoc microscope at 30 × magnification. Generally, examination was limited to one slide when no mycelial fragments resembling *Geotrichum* were found, while two to four slides were counted when the mold was detected. To be recorded as *Geotrichum,* the hyphae had to possess a characteristic morphology which included branches that extended at approximantely 45° angles from the main filament. Fragments containing less than three branches were not counted. Questionable filaments were examined under higher magnification using a compound microscope.

The concentration of machinery mold was calculated according to the formula:

[1]*Buffalo District, Food and Drug Administration, Buffalo, N.Y.*

$$Gf = S \times \frac{V}{0.5} \times \frac{500}{W}$$

Where Gf = the *Geotrichum* count per 500 g of food, S = the average number of fragments per slide, V = the volume of suspension in the centrifuge tube, and W = the weight of the food sample.

It can be seen from the above formula that the sample size, the volume of suspension, and the number of slides counted, all influenced the sensitivity of the determination. In most of our analyses for machinery mold, 40 fragments per 500 g was the minimal concentration that could be detected.

RESULTS AND DISCUSSION

During the course of this study, 29 surveys were made at nine different factories that processed tart cherries, peas, green beans, corn or beets. Seven of the plants were canneries while two were freezing operations. A total of 230 samples from these surveys were examined for machinery mold and cultured for viable microorganisms.

The viable populations found in four of the products are summarized in frequency distribution histograms (Fig. 1 aand 2). It can be seen that most of the pea and

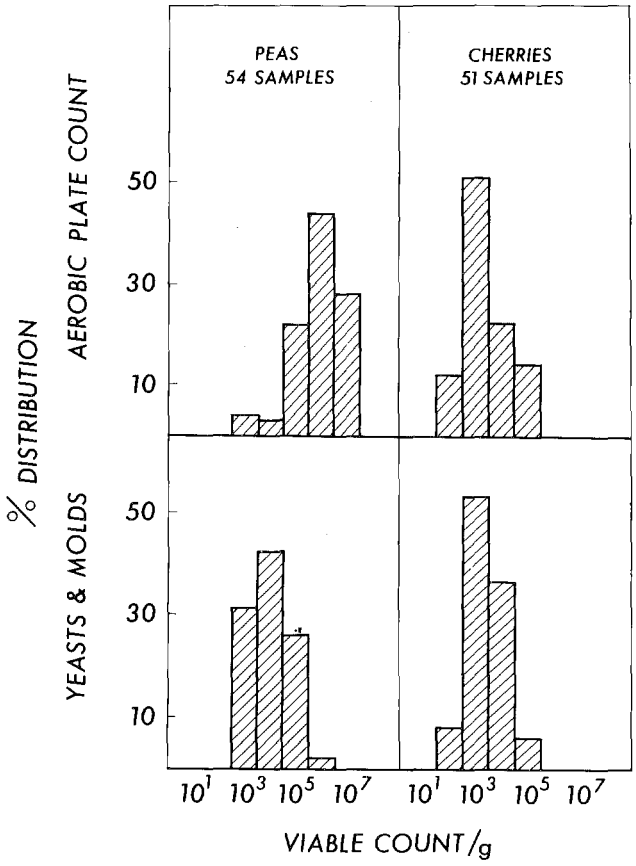

Figure 1 *Viable counts on peas and tart cherries. Frequency distribution histograms.*

green bean samples gave aerobic plate counts of 10^6 - 10^7 per g as did over 80% of the corn samples (not shown). The bacterial populations on beets were somewhat lower; over 30% of the samples gave counts of 10^5 per g or less. Beets were steam blanched and hot lye peeled as early processing steps which explains their lower counts. The

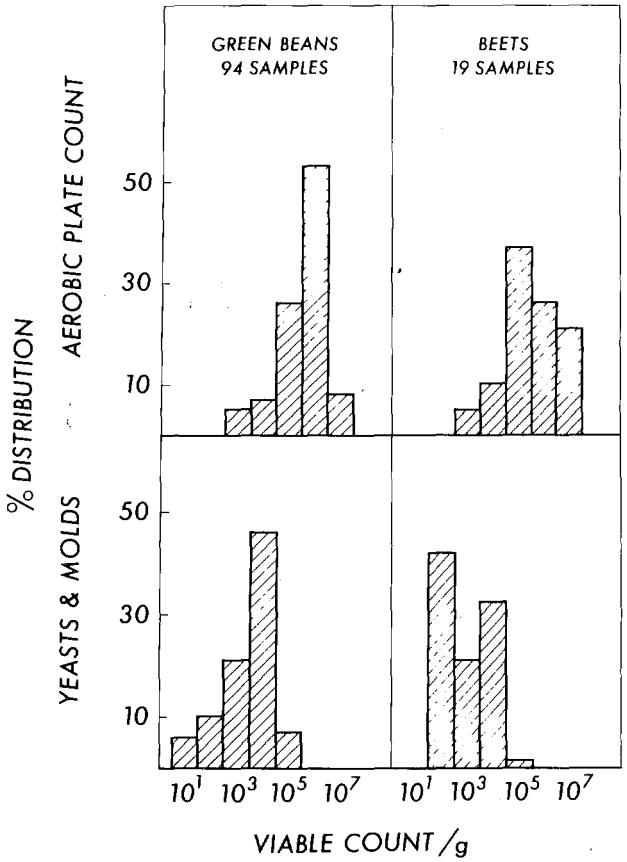

Figure 2. *Viable counts on green beans and beets. Frequency distribution histograms.*

yeast and mold populations on the four vegetables were usually about two logs lower than the aerobic plate count; thus fungi made up only about 1% of the aerobic, mesophilic microflora.

Most of the microorganisms contaminating cherries were yeasts and most samples gave counts in the range of 10^3 - 10^4 per g (Fig. 2). Yeasts also were responsible for most of the colonies which developed on the Plate Count agar which explains the similar configurations of the two histograms for cherries. Yeasts predominate on cherries because of the low pH of the fruit, about 3.5.

The high populations of viable organisms on vegetables as received at the processing plant appeared to be largely responsible for the high counts found on samples obtained from subsequent processing steps. The peas delivered to one cannery, for example, yielded an average viable count of 11×10^6 per gram (Table 1). This vegetable, which was essentially sterile while in the intact pod, picked up most of its initial microflora from the viner, equipment which was operated in the growing field. While the count was gradually reduced as peas passed through various processing operations such as washing and fluming, a significant portion of the original microflora apparently was retained until the blanch which, in canning, is usually one of the last processing steps before filling and the retort. The viable population on peas in the filler hopper reflected recontamination following the blanch, presumably originating from flume

TABLE 1. *Contamination of peas during different stages in their preparation for canning*

Sampling position	Viable count/g X 10^3[a]	
	Aerobes	Yeasts & molds
Grower's truck (vined)	11,000	93
Bucket elevator	10,000	89
Hopper	13,000	120
Air cleaner	5,600	120
Rod washer	3,300	40
Flume	1,200	10
Inspection belt	710	5.8
Flume to blancher	200	13
Filler hopper	830	2.8

[a]Averages of surveys conducted on 6/28, 7/6, 7/8, 7/14, and 7/19.

water.

Similar results were obtained with some of the other vegetables. Beets, a product which had been in contact with soil, were delivered with plate counts ranging from 56×10^6 to 310×10^6 per g while green beans, as received at the factory, had counts ranging from 0.5×10^6 to 16×10^6 per g. Following the blanch, cut green beans were conveyed by belt directly to the filler and thus most samples collected from filler hoppers gave counts of 10^3 per g or less. French style beans, on the other hand, went through a slicer following the blanch with the result that significant recontamination often occurred. French style beans collected from fillers usually gave counts of 10^5 to 10^6 per g.

Of the 230 samples that were examined for machinery mold, 42 were positive and most of them were green beans (Table 2). The single positive sample of peas, taken from a filler hopper, yielded 60 *Geotrichum* fragments per 500 g, while the contaminated beets, a sample of scraps which were to be discarded, gave a count of 15 fragments per 500 g. The five bean processing factories which yielded positive samples were canneries; the negative line was a freezing operation. About 49% of the bean samples from the five canneries were contaminated with *Geotrichum*. The average count was 130 fragments per 500 g with a range of 20 to 600 per 500 g.

Factory B, the freezing line, made a special effort to produce a low count product by conducting frequent clean ups and by maintaining a residual of free chlorine in wash and flume water. Bacterial counts were done routinely on line samples. Although we believe that this processor was more concerned about contamination by vegetative microorganisms than were most of the canneries, we should point out that only one line survey was made and, therefore, machinery mold might also have been uncovered here had green beans been collected on other days.

TABLE 2. *Incidence of* Geotrichum *fragments on fruits and vegetables obtained from processing lines*

Product	Factory	No. samples	*Geotrichum*-positive samples	
			No.	Per cent
Peas	A	45	1	2
	B	9	0	
Cherries	C	30	0	
	D	21	0	
Green beans	E	14	8	57
	F	12	9	75
	C	21	6	28
	G	10	4	40
	B	13	0	
	H	25	13	52
Beets	I	19	1	5
Corn	E	12	0	

The source of *Geotrichum* contamination in the bean canneries was difficult to pinpoint. Most of the samples were taken from conveyors following passage of the beans through machinery such as cluster busters, snippers, size graders, and unsniped bean removers. Since, in general, belts and vibrating conveyors were not slimy, it was believed that most *Geotrichum* originated from the interior surfaces of other equipment. This was proven in Factory H where beans picked up an average machine mold count of 415 fragments per 500 g from a French-style slicer. Product collected just before the slicer was negative for the mold.

The viable microbial populations of green beans contaminated with *Geotrichum* have been compared with samples taken from the same areas that were negative for the mold (Table 3). No correlation was observed; the *Geotrichum*-positive and *Geotrichum*-negative samples yielded comparable geometric means for both aerobic bacteria and fungi.

TABLE 3. Geotrichum *contamination of green beans versus viable populations of aerobic organisms, yeasts, and molds*

Sample source	No. samples	*Geotrichum*[a] fragments (per 500 g)	Viable counts per g[a]	
			Aerobes	Yeasts & molds
Conveyor and inspection belts	29	90	3.3×10^6	2.8×10^4
	34	negative	1.6×10^6	1.9×10^4
Vibrating conveyor	1	570	2.5×10^6	2.4×10^4
	2	negative	2.2×10^6	1.5×10^4
Slicer, French style	7	160	7.0×10^5	2.5×10^3
	3	negative	2.1×10^6	1.0×10^4
Belts, post-blanch	2	45	8.0×10^3	$<1.0 \times 10^2$
	9	negative	1.0×10^4	1.0×10^2

[a]Geometric means

The reason that machinery mold was a more common contaminant of green beans than of the other vegetables is not well understood. In general, the viable counts on this product were not higher than on peas, corn, and many beet samples (Fig. 1 and 2), and our visual inspection of the different factories failed to indicate that bean processors paid less attention to sanitation. In fact, some of the factories also processed peas or corn, products which were essentially negative for *Geotrichum*. Thus, the same management and clean-up crews were involved.

The explanation may be in the types of machinery that are used. Bean lines use numerous size grading and cutting devices, machines that might provide better surfaces for mold build up. Another possible explanation, although unlikely, is that the solubles from green beans provide a better medium for growth of

Geotrichum than do the juices of the other products. This is something that might be studied in the future. A third possibility is that *Geotrichum* was a contaminant of beans in the growing fields and was introduced into the factory on the raw product. Our attempts to substantiate this hypothesis were not successful, however, as no machinery mold was found on bean samples collected from the grower's trucks.

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Journal of Food Protection Vol. 40, No. 6, Pages 406-410 (June, 1977)
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Lactic Acid Production by *Streptococcus lactis* and *Streptococcus cremoris* in Milk Precultured with Psychrotrophic Bacteria

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(Received for publication November 1, 1976)

ABSTRACT

Psychrotrophic organisms isolated from raw milk were inoculated into fresh raw milk and incubated at 7 C for 5 days. Half of each sample was autoclaved, the other half, pasteurized. *Streptococcus lactis* and *Streptococcus cremoris* were individually inoculated into the milks and incubated at 21 C for 20 h. Acid production was measured at 2-h intervals. Lactic acid production was greater in milks precultured with added psychrotrophs or the normal flora than in pasteurized control milks. In most instances, pasteurized precultured milks had more acid than did autoclaved precultured milk. More titratable acid was produced in autoclaved rather than pasteurized control milks. Milks with the psychrotroph and lactic streptococci added simultaneously had more titratable acid than did control milk. When cell-free filtrates of psychrotrophic cultures were used instead of cells of psychrotrophs, results resembled those obtained with the bacteria. Simultaneous inoculation of milk with lactic acid bacteria and cell-free filtrate gave results comparable to those from milks precultured for 5 days with the cell-free filtrate.

Processors of cultured dairy products are concerned about the quality of milk used to manufacture products which depend on acid production by lactic starter cultures. Since conditions of milk production and handling have changed markedly in recent years, some milk may be 5 or more days old before it is pasteurized and processed into dairy products. This extended refrigerated storage of milk enables psychrotrophic bacteria to grow and thus allows the bacteria or their products to cause irreversible changes in milk before it is processed.

There are conflicting reports on how growth of psychrotrophic bacteria in milk affects subsequent activity of lactic acid bacteria in the same milk. Claydon and Fryer (*3*) reported that increased lactic acid production in refrigerated stored milk was a seasonal phenomenon. Several investigators (*8,13,14,15*) noted that the presence in milk of psychrotrophic bacteria or their enzymes can stimulate production of acid by lactic cultures. Other researchers found that acid production was slow after psychrotrophic bacteria grew in milk (*1, 10*).

This research was initiated to determine if growth of psychrotrophic bacteria in milk alters the milk sufficiently to affect subsequent acid production by *Streptococcus cremoris* and *Streptococcus lactis.*

MATERIALS AND METHODS

Cultures

Psychrotrophs used in this study were randomly isolated from Plate Count Agar (PCA-Difco) or Trypticase Soy Agar (TSA-BBL) inoculated with raw milk (from University of Wisconsin Dairy Plant immediately after delivery and from dairy farms located northwest of Beloit, Wisconsin a few hours after milking in the morning) held at 7 C for 1 to 5 days. The isolated psychrotrophs were characterized by the following tests done according to the *Manual of Microbial Methods* (*17*) and *The Genera of Bacteria* (*16*): gram stain, sugar metabolism, pigment production, litmus milk, growth on triple sugar iron agar, motility, gelatin liquefaction, catalase reaction, starch hydrolysis, O/F reaction, oxidase reaction (Kovac's), proteolysis, lipolysis, and growth in broth (sediment or pellicle formation). Isolates were classified into genera according to the diagnostic tables of Cowan and Steel (*4*), Harrigan and McCance (*9*), and *Bergey's Manual of Determinative Bacteriology* (*2*).

S. lactis (4175) and *S. cremoris* (C13) were obtained from the Marschall Division of Miles Laboratories, Inc., Madison, Wisconsin.

Milk samples

Raw whole milk was obtained immediately after it was received by the University Dairy Plant. This milk was divided into four portions for subsequent experiments as shown in Fig. 1.

Total aerobic (PCA at 30 C for 48 h) and psychrotrophic (PCA at 7 C for 10 days) plate counts were made daily during the 5-day incubation. One-fourth of a percent of an 8- to 10-h-old milk culture of either *S. lactis* or *S. cremoris* was inoculated into each of the eight samples of milk. Milks were then incubated at 21 C for 10 h. Acid production was measured at 2-h intervals until milks were coagulated; 9-ml milk samples were titrated with 0.1 N NaOH to pH 8.6 (Corning pH meter, Model 10 with a Sargent-Welch combination electrode No. S-30070-10).

Cell-free filtrates

Psychrotrophic isolates were grown in Brain Heart Infusion Broth (BHI-Difco) for 5 days at 21 C. Cell-free filtrate was obtained by centrifigation (Beckman Model L-2 Ultracentrifuge, 10,000 rpm for 15 min) of a broth culture followed by filtration of the supernatant fluid through a Seitz filter (Hercules Filter Corp., Paterson, N. J.). Ten percent of the filtrate was inoculated into pasteurized milk as shown in Fig. 2. Milks were incubated and titrated as just described for samples with psychrotrophic bacteria.

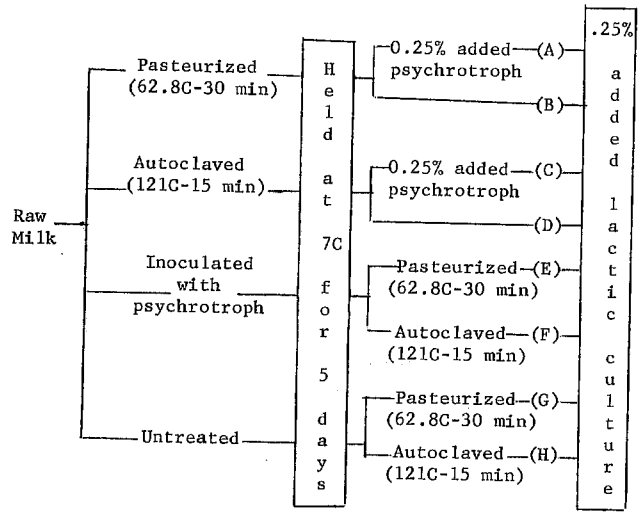

Figure 1. *Schematic for experiments with intact psychrotrophic bacteria.*

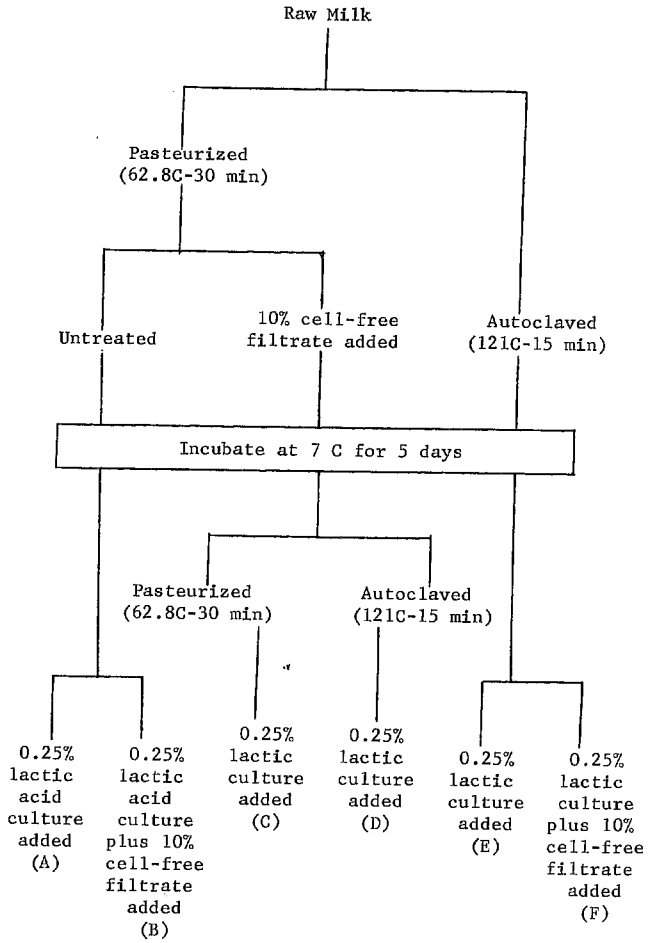

Figure 2. *Schematic for experiments with cell-free filtrate.*

RESULTS

Cultures

The psychrotrophic bacteria used in this study were: *Flavobacterium* sp. (No. 26), *Lactobacillus* spp. (No. 29 and 34), *Micrococcus* sp. (No. 32), and *Pseudomonas* spp. (No. 1,10,13,31, and 36). Selection was based on biochemical properties, especially lipolysis and proteolysis. *Micrococcus* sp. (No. 32) and *Pseudomonas* spp. (No. 10,13,31, and 36) were lipolytic and proteolytic. *Flavobacterium* sp. (No. 26) and *Lactobacillus* sp. (No. 29) were only proteolytic, and *Lactobacillus* sp. (No. 34) and *Pseudomonas* sp. (No. 1) were neither proteolytic nor lipolytic by agar plate methods.

All psychrotrophic isolates grew at temperatures ranging from 0 to 32 C. However, at 0 C 5 to 7 days were required before growth was noticeable.

Plate counts

The initial psychrotrophic counts for inoculated milks for experimentation with *S. lactis* ranged from 3.1×10^5 to 5.6×10^6/ml. After 5 days at 7 C counts ranged from 1.8×10^7 to 1.0×10^8/ml. Similar psychrotrophic counts were observed when *S. cremoris* was evaluated: 3.5×10^3 to 6.9×10^6/ml initially which increased to 6.1×10^7 to 3.3×10^8/ml after 5 days at 7 C. Uninoculated milks had inital psychrotrophic counts in the range of 1.3×10^3 to 9.0×10^4/ml which rose to 4.2×10^6 to 2.4×10^8/ml. Plate counts were generally higher than psychrotroph counts (data not given).

Experiments with psychrotrophic bacteria

When *S. lactis* was inoculated into the milks precultured with the psychrotrophic *Lactobacillus* sp. 34 or *Pseudomonas* sp. 31, more acid was produced in the precultured than in the control milk (Fig. 3 and 4). In most instances the precultured pasteurized milk also had more titratable acid than did autoclaved milks (Fig. 4).

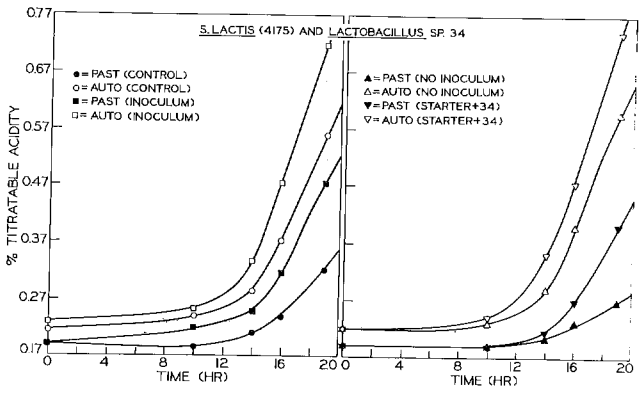

Figure 3. *Increase in titratable acid produced by* S. lactis *during growth in milks precultured with* Lactobacillus *sp. 34. Past (Control) = (B) in Fig. 1, Auto (Control) = (D), Past (Inoculum) = (E), Auto (Inoculum) = (F), Past (No inoculum) = (G), Auto (No inoculum) = (H), Past (Starter + 34) = (A), Auto (Starter + 34) = (C).*

In most trials the amount of acid was slightly less when the normal flora rather than the pure culture grew in milk, showing that added psychrotrophs appeared beneficial for acid production by *S. lactis* (Fig. 4). Autoclaved milks which had supported growth of the normal flora gave variable results. In some instances more acid was produced than in the pasteurized counterpart (Fig. 3), whereas in other instances (Fig. 4) acid production was comparable in both milks.

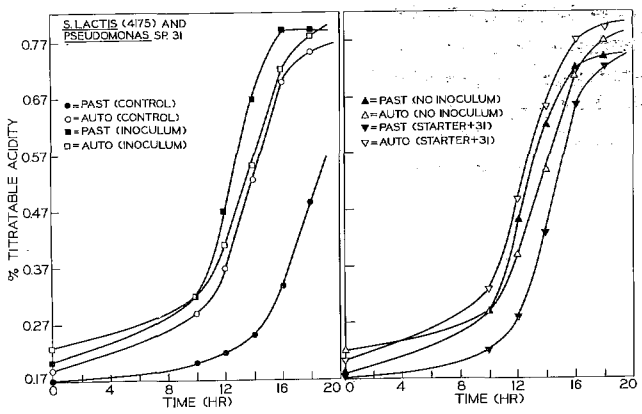

Figure 4. *Increase in titratable acid produced by* S. lactis *during growth in milks precultured with* Pseudomonas *sp. 31. See Fig. 3 for key to abbreviations.*

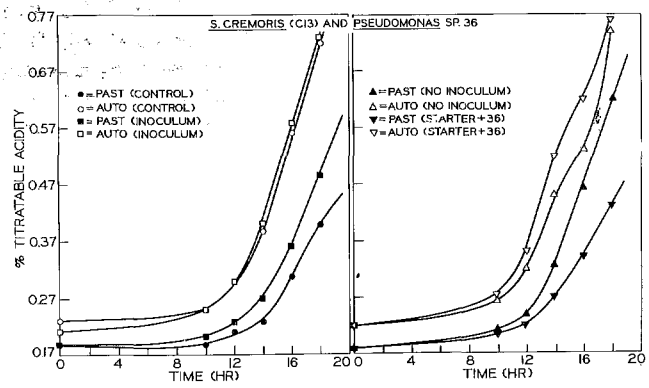

Figure 6. *Increase in titratable acid produced by* S. cremoris *during growth in milks precultured with* Pseudomonas *sp. 36. See Fig. 3 for key to abbreviations.*

Samples to which psychrotrophs were added simultaneously with starter cultures developed acid similar to that of milk in which the normal flora flourished.

Experiments with the remainder of the psychrotrophs gave results similar to those just described so the data are not presented. Milks precultured with *Micrococcus* sp. (No. 32) gave results comparable to those for *Lactobacillus* sp. (No. 34) (Fig. 3). All other milks precultured with psychrotrophs (*Flavobacterium* sp. 26, *Lactobacillus* sp. 29, and *Pseudomonas* spp. 1,10,13,36) gave results similar to those observed when *Pseudomonas* sp. (No. 31) was evaluated (Fig. 4).

Acid production by *S. cremoris* was similar to that of *S. lactis*. More acid was produced in precultured than in control milks (Fig. 5 and 6).

Milks in which the normal flora had grown developed more titratable acid than did controls (Fig. 5 and 6). In all trials where the psychrotroph and *S. cremoris* were grown simultaneously, slightly more acid was produced than in control milks (Fig. 5 and 6).

With all psychrotrophic isolates except *Pseudomonas* sp. (No. 36) pasteurized precultured milks yielded more titratable acid than did autoclaved milks.

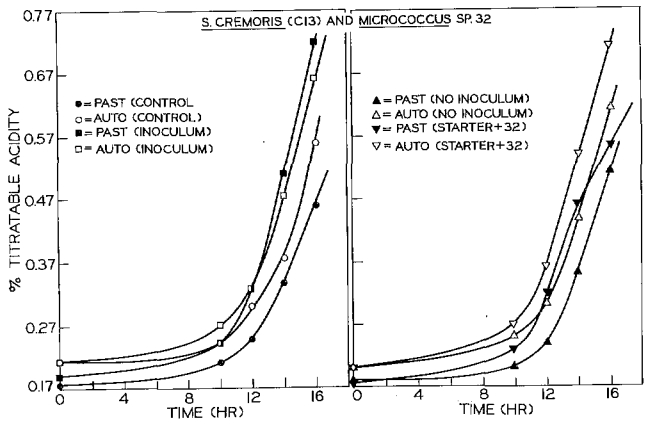

Figure 5. *Increase in titratable acid produced by* S. cremoris *during growth in milks precultured with* Micrococcus *sp. 32. See Fig. 3 for key to abbreviations.*

Experiments with cell-free filtrates

Results of these experiments mirrored those described previously when psychrotrophic bacteria were used. The major exception was that acid production in milks inoculated simultaneously with starter cultures and cell-free filtrates resembled results observed for milks precultured 5 days with the cell-free filtrate (Tables 1 and 2). Milks pretreated with cell-free filtrates permitted production of more acid than did pasteurized and autoclaved controls.

DISCUSSION

The increased titratable acid observed in precultured milks probably resulted from proteolysis caused by development of psychrotrophs in milk; this may have supplied *S. lactis* and *S. cremoris* with usable nitrogen fractions. Foster (*6*) reported that growth of natural contaminants in milk before heating produced changes which made milk a better medium for starter growth than when such growth had not occurred. The increase in acid production is attributable to products of protein degradation which have become available to lactic acid bacteria (*13*).

Other work with the psychrotrophic bacteria used in these tests demonstrated that they degraded β- and α_s-casein into peptide or amino acid fractions (*5*). *Pseudomonas* spp. caused the most and *Lactobacillus* spp. the least degradation of casein and *Micrococcus* sp. exhibited intermediate acitvity. Overcast (*15*) found that growth of psychrotrophic organisms in milk caused a decrease in the total protein content and changes in amounts of the different protein fractions. Another researcher found that after 7 days of psychrotrophic growth in milk, chromatograms showed that some β- and α_s-casein had disappeared (*19*).

Since autoclaved milks supported good lactic acid production, it is reasonable to assume that the heat treatment altered the milk in such a way that the constituents were readily available for growth of these bacteria. Various researchers have reported that there is improved growth of lactic acid bacteria in autoclaved

milk probably because readily available nitrogen compounds from breakdown of milk proteins are available to the bacteria (*6,7,11*).

Acid production in milks where the normal flora grew gave variable results which at times were similar to those obtained when milk was inoculated with a psychrotroph. At other times the results were similar to those of control milks. This probably reflects the degree of proteolysis caused by the psychrotrophs in milk. Some researchers (*12,18*) indicated that there were variations in lactic acid production in milk because cultures were reacting to inhibitory substances, growth of antagonistic bacteria, or heat treatment of milk.

Since milks to which psychrotrophs were added simultaneously with the starter cultures developed more acid than did control milks, the increased numbers of bacteria in the milk could be important. The increase in bacterial numbers could have created favorable conditions so that the lag phase for starter cultures was shortened. In most instances simultaneous inoculation of milk with psychrotroph and lactic cultures resulted in less acid than in precultured milks suggesting that previous psychrotrophic growth served to enhance acid production by the lactic acid bacteria.

Cell-free filtrates were obtained to test for extracellular enzymatic activity since it has been stated that pasteurization does not destroy metabolic products and enzymes arising from bacterial growth in raw milk (*10*). Results from our experiments suggest that extracellular enzymes produced by the psychrotrophs may have been responsible for increased acid production by lactic streptococci. Cell-free filtrates from different bacteria have been reported to stimulate production of acid by lactic starter cultures (*8,13,14*).

From our results it is evident that the presence of psychrotrophs used in this study or of their metabolic products in milk did not interfere with acid production by lactic streptococci. On the contrary, preculturing of milk with these psychrotrophic bacteria enhanced acid production by *S. lactis* and *S. cremoris*.

ACKNOWLEDGMENT

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, and by Dairy Research, Incorporated, Rosemont, Illinois.

TABLE 1. *Acid production by* Streptococcus lactis *(4175) in milks treated with cell-free filtrates*

Treatmentof milk	Identifying symbol, Fig. 2	Titratable acid (%) after incubation					
		0 h	10 h	12 h	14 h	16 h	18 h
Pasteurized							
Control	(A)	0.16	0.20	0.22	0.25	0.36	0.47
Filtrate 31[1]	(C)	0.17	0.23	0.29	0.39	0.54	0.60
Filtrate 31 + *S. lactis*[3]	(B)	0.16	0.22	0.25	0.36	0.58	0.75
Filtrate 34[2]	(C)	0.17	0.25	0.38	0.44	0.61	0.63
Filtrate 34 + *S. lactis*	(B)	0.17	0.23	0.27	0.34	0.53	0.60
Autoclaved							
Control	(E)	0.17	0.23	0.27	0.31	0.44	0.54
Filtrate 31	(D)	0.18	0.25	0.33	0.42	0.65	0.75
Filtrate 31 + *S. lactis*	(F)	0.17	0.26	0.41	0.49	0.66	0.72
Filtrate 34	(D)	0.21	0.30	0.41	0.54	0.72	0.84
Filtrate 34 + *S. lactis*	(F)	0.19	0.30	0.53	0.57	0.70	0.80

[1]*Pseudomonas* sp. (No. 31)
[2]*Lactobacillus* sp. (No. 34)
[3]Simultaneous inoculation

TABLE 2. *Acid production by* Streptococcus cremoris *in milks treated with cell-free filtrates*

Treatment of milk	Identifying symbol, Fig. 2	Titratable acid (%) after incubation					
		0 h	10 h	12 h	14 h	16 h	18 h
Pasteurized							
Control	(A)	0.16	0.21	0.26	0.41	0.57	0.74
Filtrate 32[1]	(C)	0.16	0.25	0.49	0.62	0.70	0.72
Filtrate 32 + *S. cremoris*[3]	(B)	0.17	0.23	0.38	0.55	0.65	0.72
Filtrate 36[2]	(C)	0.17	0.23	0.32	0.58	0.76	0.79
Filtrate 36 + *S. cremoris*	(B)	0.16	0.23	0.30	0.56	0.72	0.82
Autoclaved							
Control	(E)	0.17	0.25	0.38	0.56	0.73	0.80
Filtrate 32	(D)	0.19	0.29	0.44	0.62	0.81	0.86
Filtrate 32 + *S. cremoris*	(F)	0.18	0.31	0.59	0.67	0.83	0.88
Filtrate 36	(D)	0.20	0.29	0.40	0.69	0.76	0.96
Filtrate 36 + *S. cremoris*	(F)	0.18	0.28	0.52	0.71	0.82	0.86

[1]*Micrococcus* sp. (No. 32)
[2]*Pseudomonas* sp. (No. 36)
[3]Simultaneous inoculation

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Journal of Food Protection Vol. 40, No. 6, Pages 411-414 (June, 1977)
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Microbiological Standards for Cheese: Survey and Viewpoint of the Canadian Health Protection Branch

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(Received for publication November 2, 1976)

ABSTRACT

The Health Protection Branch, Health and Welfare Canada is considering proposals for microbiological standards for cheese. These proposals are based on a 2-year study (1974-1976) carried out by the Branch. The proposed standards per gram are: total coliforms m = 500, M = 1500, fecal coliforms m = 100, M = 500, and *Staphylococcus aureus* m = 100, M = 1000, for cheeses made from pasteurized milk; total coliforms m = 5000, M = 50,000, fecal coliforms m = 500, M = 1000, and *S. aureus* m = 1,000, M = 10,000 for cheeses made from heat treated or unpasteurized milk. The type of standard proposed will be based on a three-class acceptance plan as developed by the International Commission on Microbiological Specifications for Foods. Use of this plan in interpretation of the analytical results allows for the normal variation between analytical samples.

In the Canadian Food and Drug Regulations there are several microbiological standards already promulgated. Many of these standards cover dairy products and include milk for manufacture, cottage cheese, ice cream, ice milk, and flavored milk. The intent of these standards is to deal with both the elements of safety and sanitation during production and subsequent handling.

Although cheese has been shown to cause food-borne illness (*3, 4, 8, 9, 11*), epidemiological experience places cheese among the relatively infrequent vehicles. However when such outbreaks do occur they usually involve large numbers of people (*3, 4, 8, 11*). When one considers the diverse geographical areas and the wide range of different factory conditions that cheeses are made under, one cannot ignore this potential for contamination. Coupled with these facts, cheese as supplied to the consumer, is ready to eat and has been classified as having a high degree of concern as a potential health hazard (*6*). With these points in mind the Health Protection Branch is considering introducing microbiological standards for cheese.

Most people acquainted with the area of microbiological standards for foods are also aware of the problems in their development. With cheese, these problems are somewhat magnified both by the nature of the product and the methods used in its manufacture. One is faced with such problems as growth of microorganisms during preparation and curing as well as the eventual die-off during storage. This is coupled to the problem of different rates of die-off between microorganisms such as *Staphylococcus aureus* and *Escherichia coli* (*2, 14*).

It is obvious therefore that no single standard would suffice and that any proposed standards would have to take into account the technology and science of cheese manufacture. Development of standards using the format of the International Commission of the Microbiological Specifications for Foods (ICMSF) goes a long way in setting up realistic standards (*6*). In brief, ICMSF recognizes the benefits of a defined sampling plan where the producer and consumer risk has been reduced to a minimum. The plan is based on a certain number of subsamples being taken at random from any lot. This number of subsamples for analysis is denoted by "n." The other parameters used are: the number of defective subsamples permitted to exceed acceptable values (e.g. GMP level) denoted by "c;" the level of acceptable contamination denoted by "m," and the level of organisms considered entirely unacceptable denoted by "M." Use of such plans recognizes the number of samples examined based on cost, degree of hazard, and variability of bacterial population between subsamples.

METHODS

The survey was carried out between 1974 and 1976, at five Health Protection Branch regional laboratories located in Vancouver, Winnipeg, Toronto, Montreal, and Halifax. Samples of cheese (five subsamples per lot) were obtained at import level and at domestic plants. The domestic lots were taken, wherever possible just before shipment to the retail trade. The number and types of cheeses analyzed was based on availability and consumption rates in Canada. All subsamples were analyzed for total coliforms, fecal coliforms, and coagulase positive staphylococci. Bacteriological examinations were done for coliforms and faecal coliforms using the 5-tube MPN

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procedures while coagulase-positive staphylococci were enumerated on Baird-Parker agar, according to acceptable methods (5) of the Health Protection Branch.

RESULTS AND DISCUSSION

Cheddar cheese

For the sake of ease of tabulation, the survey results were broken down into cheese types. In the case of Cheddar, a further division of results was necessary to include Cheddar made from pasteurized milk, according to the definition in the Food and Drug Regulations (143 F for a period of not less than 30 min or equivalent) and heat treated or unpasteurized milk. Thus both raw and heat treated milk used for making Cheddar cheese would be termed unpasteurized.

TABLE 1. *Distribution of total coliforms in Cheddar cheese prepared from pasteurized or unpasteurized milk*

Arbitrary grouping, No. per gram	Pasteurized		Unpasteurized	
	No. of subsamples[a] in each group	Percentage of subsamples in each group	No of subsamples[b] examined	Percentage of subsamples in each group
<20	71	47.3	42	28.0
>20-100	20	13.3	29	19.3
>100-500	20	13.3	38	25.3
>500-1600	4	2.7	8	5.3
>1600	35	23.4	33	22.1

[a]Total of 150 subsample (30 lots each of 5 subsamples)
[b]Total of 150 subsamples (30 lots each of 5 subsamples)

The total coliform results for Cheddar cheese made from pasteurized and unpasteurized milk are given in Table 1. The overall microbiological quality with respect to total coliforms for both types of Cheddar appears to be the same. Taking an arbitrary cut off point of 500 coliforms per gram, the percent subsamples above this figure in both instances is about 25%. A further point is that the percent subsamples exceeding 1600 per gram is also about the same (23%). A closer comparison of Table 1 shows that the distribution of total coliforms between samples is more even in the unpasteurized milk Cheddar. The total coliforms show a "tailing off" between 500-1600, and then rise at >1600. A similar pattern emerges if one examines the faecal coliform levels shown in Table 2. There appears to be no obvious significant

TABLE 2. *Distribution of fecal coliforms in Cheddar cheese prepared from pasteurized or unpasteurized milk*

Arbitrary grouping, No. per gram	Pasteurized		Unpasteurized	
	No. of subsamples[a] in each group	Percentage of subsamples in each group	No. of subsamples[b] examined	Percentage of subsamples in each group
<20	70	46.6	66	44.3
>20-100	37	24.6	37	24.6
>100-500	2	1.3	10	6.6
>500-1600	6	4.0	9	6.0
>1600	35	23.5	28	18.5

[a]Total of 150 subsamples (30 lots each of 5 subsamples)
[b]Total of 150 subsamples (30 lots each of 5 subsamples)

difference in microbiological quality between the two cheeses. Such a result is disturbing especially when one notes the percent subsamples containing fecal coliforms >1600 in the Cheddar cheese made from pasteurized milk. This result suggests a lack of control over sanitation especially when most subsamples had 100 or less fecal coliforms. The survey data also show that when total coliforms were reported, in about 50% of the cases, the fecal coliforms were also found at the same level.

Levels of coagulase-positive *S. aureus* found in Cheddar cheese from pasteurized or unpasteurized milk

TABLE 3. *Levels of* Staphylococcus aureus *found in Cheddar cheese made from pasteurized or unpasteurized milk*

Arbitrary grouping, No. per gram	Pasteurized		Unpasteurized	
	No. of subsamples in each group	Percentage of subsamples in each group	No. of subsamples examined	Percentage of subsamples in each group
<100	140	93.4	130	86.6
>100-500	8	5.3	8	5.2
>500-1,000	2	1.3	2	1.4
>1,000-10,000	0	0.0	5	3.4
>10,000	0	0.0	5	3.4

are shown in Table 3. Comparison of the pasteurized and unpasteurized milk Cheddar reveals the type of difference one would expect; the levels being much lower in the pasteurized milk cheese. The levels shown for the Cheddar cheese prepared from unpasteurized milk indicate an improvement from earlier reported Canadian studies (*12, 13*).

These results also compare well with those studies reported by Sharpe and Jackson (*10*) and Donnelly et al. (*1*). Some of the high coagulase-positive *S. aureus* results reported by these authors were not found in this study.

TABLE 4. *Distribution of total coliforms in hard cheeses[a] made from pasteurized milk*

Arbitrary grouping, MPN per gram	No. of subsamples in each group	Percentage of subsamples within each group
<20	74	72.5
>20-100	4	3.9
>100-500	20	19.7
>500-1600	4	3.9
>1600	0	0.0

[a]Includes Asiago, Cheshire, Colby, Danbo, Edam, Emmentaler, Fynbo, Gouda, Gruyere, Havarti, Provolone, Romano, and Tilsiter.

Varietal cheeses

The results in Table 4 indicate the distribution of total coliforms between samples of hard cheeses. Most of these cheeses had 100 or fewer coliforms.

The fecal coliforms were found to be low and most cheeses had fewer than 20. Comparison between the domestic product and imports showed no significant difference.

The problems of coliforms associated with Cheddar were not observed in these other hard cheeses. This point is difficult to explain especially when one considers the similarity in making Colby cheese and Cheddar.

Total coliforms and fecal coliform levels in semisoft cheese are shown in Table 5. Most subsamples had >500 total coliforms and >100 fecal coliforms. Similar results are shown in Table 6 for soft cheeses. The coliform problem associated with soft and semi-soft cheeses is clearly shown by the percentage of subsamples which exceeded

TABLE 5. *Distribution of total and fecal coliforms in semi-soft cheeses*[a] *made from pasteurized milk*

Arbitrary grouping, MPN per gram	Total coliforms		Fecal coliforms	
	No. of subsamples in each group	Percentage of subsamples in each group	No. of subsamples	Percentage of subsamples in each group
<10	142	62.6	167	87.9
>10-100	30	13.2	20	10.3
>100-500	14	6.2	2	1.0
>500-1600	10	4.4	0	0.0
>1600	31	13.6	2	0.8

[a]Includes Blue, Belpaese, Brick, Caerphilly, Munster, Danablu, Esrom, Gorgonzola, Jack, Limburger, Mycella, Scamorza, Port Salut, Roquefort, and St. Paulin.

TABLE 6. *Distribution of total and fecal coliforms in soft cheeses*[a] *made from pasteurized milk*

Arbitrary grouping, MPN per gram	Total coliforms		Fecal coliforms	
	No. of subsamples in each group	Percentage of subsamples in each group	No. of subsamples examined	Percentage of subsamples in each group
<10	119	63.3	155	82.4
>10-100	12	6.4	24	12.9
>100-500	16	8.5	5	2.7
>500-1600	7	3.7	0	0.0
>1600	34	18.1	4	2.1

[a]Includes Brie, Brynza, Camembert, Carre de l'Est, Coulommiers, Neufchatel, and Reblochon.

1600 per gram. In comparison with the hard cheeses (Table 4) there is an increase from 0 to 18.1% in the subsamples of soft cheese which exceeded 1600 total coliforms (Table 6).

The problem of coliforms in soft cheeses is not new (*15*); however it was apparent from this survey that this problem was not due to any particular soft cheese. The high coliforms reported in this survey were due to variations within samples. The extent of the variation is shown in Table 7 using Camembert as an example. From

TABLE 7. *Variation of total coliform counts among samples*[a] *of Camembert cheese*

Arbitrary grouping, MPN per gram	No. of samples in each group	Percentage of samples within each group
<10	18	56.3
>10-100	3	9.3
>100-500	2	6.3
>500-1600	6	18.8
>1600	3	9.3

[a]There is a total of 5 subsamples for each sample. The mean value of the 5 subsamples was used to determine the variation.

these results it was possible to obtain Camembert with fewer than 10 coliform per gram while on the other hand some samples had greater than 1600 per gram. This variation may be due to the age of the cheese when analyzed since during the aging period there is a decline in the numbers of coliforms. This explanation is not however complete especially when one analyses such cheeses made a day apart and still finds this wide variation. Closer studies at the manufacturing level suggest that conditions of sanitation during manufacture as well as initial contamination rates play an important role in the final levels of total and fecal coliforms.

The results of this survey suggest a need for some improvement in sanitation practices. One way to accomplish this is to apply microbiological standards for cheeses. This suggestion is not new and was suggested both by Thatcher et al. (*12*) and Jones et al. (*7*).

Analysis for *S. aureus* revealed that hard cheeses had a count of 100 or less per gram. This was also true for soft or semi-soft cheeses analyzed.

Proposed standards

In line with our concern over the high levels of both total and faecal coliforms found in some cheeses some standards were proposed. These proposals are given in Table 8. The proposals are based on the format presented by the ICMSF. They advocate use of the three-class acceptance plan to accomodate the variability associated with microbiological standards. The parameters of the three-class plan are given in Table 9. It has been determined that about 85% of the cheeses imported or produced in Canada would be able to meet these standards. These proposed standards appear to be realistic in light of other recent surveys available to the Health Protection Branch. When similar proposals were issued as guidelines to the cheese industry in 1975 a separate survey of the Ontario cheese industry by the University of Guelph revealed an 83% compliance rate. A further survey by the Health Protection Branch (Toronto regional laboratories) in 1976 of Ontario cheese showed that over 95% of the cheeses sampled were able to meet the proposed standards.

TABLE 8. *Three class plan for proposed Canadian standards for cheese made with pasteurized and unpasteurized (heat treated) milk*

Test	n	c	m	M	n	c	m	M
Total coliforms	5	2	500	1500	5	2	5000	50,000
Fecal coliforms	5	2	100	500	5	2	500	1,000
S. aureus	5	2	100	1000	5	2	1000	10,000

TABLE 9. *Definitions of parameters for three class plan for cheese*

LOT:	All packages of a product that can be produced, handled, and stored within a limited period under uniform conditions.
SAMPLE:	The portion of a lot which is taken for analysis. A sample shall consist of a predetermined number of subsamples.
n:	No. of subsamples (packages) to be examined.
m:	Maximum No. of bacteria per g that are of no concern.
c:	Maximum No. of subsamples that can have bacterial concentrations higher than m without rejection of the lot under examination.
M:	Maximum No. of bacteria per g in any one subsample above which causes rejection of the lot under examination.

Several criticisms have been raised to these proposals in early discussions with the industry and with other regulatory agencies. The generous level for total coliforms and *S. aureus* for cheeses made from unpasteurized or heat treated milk has been questioned especially in light of the survey finding. It has to be pointed out that under the current Canadian Regulations

no distinction is made nor can one effectively be made between raw milk or heat treated milk cheese. Both types of products would come under the unpasteurized proposals. It has been our experience that raw milk cheeses tend to be higher in total coliforms and *S. aureus* and allowances for this would have to be made in the proposed standards. Another criticism has been that these standards should have been developed for cheese at the manufacturing level and not at the retail level. Such a standard would place the Health Protection Branch in the role of a quality control agency for the cheese manufacturer. The manufacture of good wholesome cheese is the responsibility of the manufacturer and not the Health Protection Branch. It has been our experience that any cheese that is below the M values given in Table 8 at the manufacturing level can usually meet the proposed standards once the cheese reaches the retail level.

It is hoped that after final discussion, these proposals will be found acceptable by the cheese industry.

ACKNOWLEDGMENT

Presented in part at the 62nd Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Toronto, August 10-14, 1975.

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Journal of Food Protection Vol. 40, No. 6, Pages 415-422 (June, 1977)
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Microbiology of Mayonnaise and Salad Dressing: A Review

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(Received for publication November 11, 1976)

ABSTRACT

Mayonnaise and salad dressing commercially produced in the United States are defined in accordance with the Food and Drug Administration Standard of Identity. The microbiological content of these products is dictated primarily by the high acetic acid concentration found in their aqueous phase. The overall microbiological content of mayonnaise and salad dressing is low with a very low incidence of spoilage. Lactobacilli, yeasts, and bacilli are the organisms commonly found. The organisms most frequently isolated from spoiled products are yeast and, to a lesser extent, lactobacilli. The major preservative effect is from the acetic acid content with a minor influence from salt or sugar concentration. Mayonnaise and salad dressing produced in the United States are inimical to bacteria, especially food pathogens. The acetic acid levels used by the major producers, 0.31-0.32% for mayonnaise and 0.90-0.928% for salad dressing, are effective in destroying salmonellae and staphylococci. Salad dressing and mayonnaise used to prepare salads and sandwiches have an inhibitory effect on pathogenic bacterial growth in these products, which is attributed to the acetic acid from the mayonnaise and salad dressing. Contrary to popular opinion, mayonnaise and salad dressing when added to salads or sandwiches will not increase spoilage or public health hazards, but actually retard spoilage and growth of pathogenic microorganisms.

Mayonnaise and salad dressing are remarkably stable food products. They are resistant to most microbial spoilage and are spoiled by only a few select groups of microorganisms. In addition, properly prepared mayonnaise and salad dressing will not support growth of pathogenic bacteria. Consequently, they are of little public health concern. In Europe, public health problems still persist with certain mayonnaise-like products. However, these products are not acid mayonnaise as produced in the United States and are occasionally vehicles of food poisoning. The acid mayonnaises as produced in the U.S. in accordance with the Food and Drug Administration of Standard of Identity (*59*) are inimical to bacteria and will not encourage growth of food poisoning bacteria. Indeed, both mayonnaise and salad dressing retard microbial spoilage, and prevent growth of or kill bacterial pathogens when properly mixed into salad and sandwich spreads. This paper is a review of the literature on the microbiology of these products and a report of some supporting data from our laboratory.

DEFINITIONS

The exact origins of mayonnaise are obscure. Nevertheless, this failed to stop the celebration of its 200th anniversary in 1956. The following is a short historical outline presented by McCully (*38*) for Best Foods: "The tradition is that mayonnaise was invented by a chef of the Duc de Richelieu in 1756. However, this theory does not rule out other possibilities. For instance, Careme, a famous French chef, maintained that mayonnnaise originated from the word 'manier' which means to manipulate. Perhaps the most convincing theory is that proposed by the *Dictionnaire de l'Academie des Gastronomes* which believes that it is derived from the old French word 'moyeunaise' (moyeu = egg yolks)."

Salad dressing is a more recent derivative of mayonnaise, which was first prepared in the early 1930's as its low cost substitute (*64*). In fact, it is essentially a cooked starch dressing which is mixed with mayonnaise.

Mayonnaise is defined in the U.S. Food and Drug Administration Standard of Identity (*59*) as: "The semisolid emulsion of edible vegetable oil, vinegar, lemon juice, and/or lime juice, egg yolk containing ingredients, with one or more of the following: salt, sweeteners, mustard, paprika, and other spices, monosodium glutamate. The finished product is a creamy pale yellow food with a mild flavor which has a pH range of 3.6 to 4.0. Acetic acid is the predominant acid and represents 0.5 to 0.29% of the total product. The aqueous phase contains 9.0 to 11.0% salt and 7.0 to 10.0% sugar.

Salad dressing is defined under the U.S. Food and Drug Administration Standard of Identity (*60*) as: "The semisolid emulsion of edible vegetable oil, vinegar, lemon juice and/or lime juice, egg yolk containing ingredients, a cooked starch paste that contains one or more of the following: salt, sweeteners, mustard, paprika, monosodium glutamate, and emulsifiers. The finished product contains not less than 30% of edible vegetable oil." The final product is a creamy pale yellow food with a tart flavor which has a pH range of 3.2 to 3.9. Acetic acid is generally the predominant acid and

represents 0.9 to 1.2% of the total product. The aqueous phase contains 3.0 to 4.0% salt and 20.0 to 30.0% sugar.

MICROBIOLOGICAL CONTENT AND SPOILAGE OF MAYONNAISE AND SALAD DRESSINGS

Frazier (*20*) pointed out that there are three major areas of quality problems in mayonnaise and salad dressing which are (*a*) emulsion instability, (*b*) flavor deterioration due to oxidation and hydrolysis, and (*c*) off-flavors due to microbial growth. For this review, the emphaisis will be on the microbiological aspects. The organisms most frequently found and involved in spoilage are those that are able to survive or grow at low pH values, high salt, and/or high sugar concentrations of these products.

The organisms normally encountered in these products have been characterized by Baumgart (*7*), Fabian and Wethington (*17*), and Sinell and Baumgart (*53*). The latter reported that of 43 lactobacilli-like isolates from the ingredients of mayonnaise and fresh finished mayonnaise, 28 were *Lactobacillus plantarum* and 15 were *Betabacterium binchneri.* On 15 yeasts isolated from egg yolk and finished mayonnaise, seven were *Debaryomyces kloeckeri,* six were *Pichia membranaefaciens,* and two were *Rhodotorula mucilaginosa.* In a similar survey, Fabian and Wethington (*16*) also isolated a few yeasts in the genus *Zygosaccharomyces* from mayonnaise. Furthermore, of 13 aerobic spore-forming bacteria isolated, eight were *Bacillus megaterium* and five were only partially identified. Baumgart (*7*) reported that lactobacilli, yeasts and aerobic spore-formers were the groups of microorganisms most commonly found in freshly prepared mayonnaise. Fabian and Wethington (*17*) characterized the microbial flora of 103 samples of mayonnaise, salad and French dressing. They found that generally the microbial content was very low (< 10/g) and that the predominant flora were sporogenic rods, gram-positive cocci, diplococci, and asporogenic bacilli. Tanner (*56*) reviewed the work of earlier investigators and reported that mayonnaise and salad dressing generally contained a low number of microorganisms. When the normal flora of these products was characterized, the predominant organisms were bacilli. Many were identified as *Bacillus subtilis* and *Bacillus mesentericus.* These observed low levels and frequency of contaminants and normal microbial flora are consistent with the fine product stability record of mayonnaise and salad dressing.

The usual spoilage organisms of mayonnaise and salad dressings are lactobacilli, bacilli, and yeasts (1,11,16, *19,31,46,53,56,62,66,68*). In a survey of spoiled mayonnaise and salad dressing, Kurtzman et al. (*31*) found that 13 of 17 samples contained yeast and were all identified as *Saccharomyces bailii.* This was in agreement with earlier investigators, in that yeast in the genus *Zygosaccharomyces* (*Saccharomyces*) will spoil salad dressings (*1,16,66*). Williams and Mrak (*66*) identified their spoilage yeast as *Zygosaccharomyces globiformis.* Furthermore, they traced the source of contamination to a transfer pump. *Lactobacillus fructivorans* spoiled three of 17 samples and was first described as a salad dressing spoilage organism in 1934 (*11,31*). In two samples, a number of bacilli were isolated, of which *B. subtilis* and *Bacillus pumitism* were common to both (*31*). Tanner (56) in a summary of earlier investigations reported that *Bacillus petasites* and *Bacillus lekitosis* were the cause of mayonnaise spoilage in several instances. *Bacillus megaterium, B. subtilis, B. pumitism, Bacillus polymyxa,* and *Bacillus vulgatus* have all been isolated from spoiled mayonnaise or salad dressings, and appear to have a minor but significant role in their stability (*20,31,46*).

The pH range for spoiled mayonnaise and salad dressings in the study by Kurtzman (*31*) was from 3.6 to 4.1 which was very similar to the 3.7 to 4.2 pH range for the unspoiled products. These values were consistent with the results of Wethington and Fabian (*65*) who found that mayonnaise has a pH range of 3.0 to 4.1 with a titratable acidity range expressed as percent acetic acid of 0.30 to 0.78, and salad dressing had a pH range of 3.0 to 3.9 with a titratable acid range expressed as percent acetic of 0.82 to 1.41.

Good manufacturing practices must be followed to protect these products from microbial spoilage. This includes strict microbiological control of raw ingredients, critical control points, and careful cleaning and sanitizing of manufacturing utensils and equipment (*20,31*). With salad dressings, the critical control point is the proper heating and handling of the starch paste.

MICROBIAL SURVIVAL IN MAYONNAISE AND SALAD DRESSING

Preservative factors of mayonnaise and salad dressing

The most important antimicrobial preservative substances fund in mayonnaise and salad dressing are acetic and/or citric acid. The bactericidal acitivity of acetic acid on typhoid and colon bacilli was reported by Winslow and Lockridge (*67*). They, in addition to later workers, speculated that the antimicrobal effect was not solely due to pH, but was probably a result of undissociated acetic acid molecules (*33, 67*). Work by Hentges (*24*) and Minor and Marth (*42*) supported their observations and hypothesis. Hentges (*24*) found that formic and acetic acid demonstrated inhibitory acitvity for *Shigella* that could not be attributed to pH. Minor and Marth (*42*) observed the same phenomenon with acetic, lactic, and citric acid against *Staphylococcus aureus.* They concluded that undissociated acid molecules were responsible for the toxic properties of organic acid to bacteria.

The relative merits of organic acids compared to one another and to mineral acids have been reported (*22,24,33,42,44,52,67*). Nunheimer and Fabian (*44*) compared the inhibition of staphylococcal growth by organic acids, and found that acetic> lactic > citric > malic > tartaric > HCl. However, in terms of

bactericidal properties, the order of effectiveness was acetic > citric + lactic + malic + tartaric > HCl. Levine and Fellers (*33*) found that acetic was more toxic than lactic or HCl acid against *Salmonella aertrycke, Saccharomyces cerevisiae,* and *Aspergillus niger. Salmonella aertrycke* growth in dextrose broth was completely inhibited for 48 h at pH 4.9 and the organism was killed after 48 h at pH 4.5. At a concentration of 0.02 N the order of effectiveness of various organic acids for inhibiting growth of *E. coli* at 30 C was tartaric > glycolic > phosphoric > lactic > acetic > citric (*52*). The order of effectiveness was different from other studies because these investigators were concerned with addition of acid to beverages for their preservative properties, consequently pH was not considered. Goepfert and Hicks (*22*) noted a decrease in the toxicity of organic acids to *Salmonella typhimurium* as the chain length increased. As a result of this observation, the order of effectiveness was formic > acetic > propionic > butyric > HCl. The sensitivity of *S. typhimurium* to these acids was dependent on their concentration, temperature of storage, composition of medium, and a_w (*22*). The survival of *S. aureus* in the presence of organic and mineral acids has been evaluated (*42*). Acetic, lactic, and phosphoric were more effective than HCl and citric which were equivalent. Other factors important in viability and sensitivity to acids were incubation temperature, cell concentration, and age of culture.

The second preservative system depends on the dissolved solids that bind up the available water (a_w), namely NaCl, with mayonnaise, and sugars and NaCl for salad dressing. For example, mayonnaise has a water activity of 0.925 which is equivalent to 12% NaCl, and salad dressing has a water activity of 0.929. These a_w values demonstrate that there is a limited amount of water available for growth of most food spoilage organisms and food pathogens (*51,58*). For a more complete description of the a_w and growth relationships, the review of Scott (*51*) on food spoilage organisms and of Troller (*58*) on foodborne pathogens should be consulted.

Mayonnaise and salad dressings are complicated chemical systems. Consequently, the effect of only one component controlling microbial growth is probably remote. In the following sections the interaction of acid concentration, pH, and a_w are discussed in more detail.

Fate of foodborne pathogens in mayonnaise and salad dressing

The popular opinion of the public and unfortunately of a few public health officials and physicians is that mayonnaise and salad dressing can cause food poisoning and infection. This belief is perpetuated by the news media in such articles as "The Perils of Summer" by *Newsweek* on July 25, 1972 (*2*). In this country, there has not been a scientifically documented case of food poisoning or infection due to undiluted commercially prepared mayonnaise or salad dressing for over 25 years. Although there has been some vague reference to mayonnaise being involved on at least one occasion, this was not conclusively substantiated (*3*). However, there are some recent examples of food infections resulting from mayonnaise and salad dressing in Europe (*41,47*). More recently, an outbreak of salmonellosis occurred aboard a Spanish airline from the Canary islands, where a non-acid mayonnaise was the vector (*4, 18*). Several hundred people became ill and two died.

Mayonnaise and salad dressing are unfavorable for growth and survival of most bacteria, especially pathogens, which is primarily due to the low pH of the foods (*5,6,9,10,13-15,22-24,26-28,30,32,33,37,41-45,50, 52,55,57,61,65,67*).

Salmonella. In mayonnaise and salad dressings, the principal potential source of *Salmonella* contamination is from egg yolks and/or whole eggs. These ingredients, when unpasteurized, are occasionally contaminated with *Salmonella.* Therefore, most research has been concerned with their survival or growth potential in mayonnaise and salad dressing. The scientific literature available on this subject is voluminous (*9,10,14,15,23,28,30,32,43,50, 61,65*). In addition, Krause (*30*) recently reviewed the literature on *Salmonella* survival and infections from mayonnaise and dressings. Generally, salmonellae die in a matter of days in mayonnaise or dressings where the acetic acid concentration is adequate. The results of these investigations are summarized in Tables 1 and 2. All serotypes have approximately the same survival rates in these products.

Chung and Geopfert (*13*) found that pH 5.4 was the minimum pH at which salmonellae would initiate growth in tryptone-yeast extract-glucose broth with acetic acid used as the acidulant. The lowest HCl-adjusted pH for growth was 4.05. This appears to be a stable characteristic of salmonellae in general, although recently Huhtanen (*26*) reported that the acid tolerance of salmonellae could be increased for HCl and lactic acid by subculturizing on pH gradient plates. He did not investigate acetic acid tolerance. Levine and Fellers (*33*) found that the inhibiting pH was 4.9 for *S. aertrycke,* but the lethal pH was 4.5 in broth. Meyers and Oxhoj (*41*) found *S. typhimurium* inoculated into mayonnaise at pH 4.9 and stored at 30 C would grow. Other factors of importance in limiting *Salmonella* growth in mayonnaise and salad dressing were a_w or NaCl and sugar concentrations (*37*). Troller (*58*) reported that the lowest a_w for *Salmonella* growth was 0.94 when NaCl was used. Since mayonnaise and salad dressing have an a_w of 0.93 (equivalent to 12% NaCl), obviously salmonellae will not grow.

In summary, it appears that to maintain a *Salmonella*-free product, the pH should be 4.1 or less (0.25% acetic acid), and the product should be held at 18-22 C for at least 72 h unless pasteurized eggs are used. This is consistent with provisions for mayonnaise and salad dressing manufacture as outlined in Title 21, *Code of Federal Regulations* (*59,60*). Furthermore, salmonellae

inadvertently introduced into these products will not grow or survive.

Staphylococci. Due to the moderately high NaCl concentration (9.0 to 12.0%) of mayonnaise, conditions in terms of NaCl alone could be selective for staphylococcal growth. This, however, is not true because of the low pH. Troller (*58*) briefly summarized the literature with regard to pH and NaCl a_w. It appears as if the lower limit for enterotoxin production in the presence of 10% NaCl is pH 5.45 (*21*). According to Iandolo et al. (*27*) growth of *S.*

TABLE 1. *Survival of salmonellae in mayonnaise and mayonnaise-like products*

Investigators	pH	Acetic acid concentration (%)	Storage temperature	Inoculum size	Maximum survival
Wethington and Fabian (*65*)	5.0	0.15	Room temp.	8 (log)/ml	156 h
	5.0	0.15	37 C	8 (log)/ml	156 h
	3.8	0.48	Room temp.	>7 –<9(log)/ml	14 h
	3.8	0.48	37 C	>7–<9(log)/ml	14 h
Lerche (*32*)	4.2		4 C		8 days
	4.0		Room temp.	0.1 ml of broth	6 days
	4.0		4 C	culture	11 days
	3.95		Room temp.		1 day
	3.95		4 C		7 days
Corretti (*14*)		0.3	5 C	10^5/g	14 days
(Egg yolk)		0.4	5 C	10^5/g	5 days
		0.5	5 C	10^5/g	7 days
		0.3	15 C	10^5/g	5 days
		0.4	15 C	10^5/g	1 day
		0.5	15 C	10^5/g	3 days
Corretti (*14*)		0.3	25 C	10^5/g	5 days
(Egg yolks/		0.4	25 C	10^5/g	3 days
whole eggs)		0.5	25 C	10^5/g	1 day
Burditt (*10*)	4.30	0.16	7 C	10^6/g	144 h
(Kraft, Exhibit 9)	3.90	0.31	7 C	10^6/g	72 h (<0.04/g)
	4.30	0.16	22 C	10^6/g	120 h
	3.90	0.31	22 C	10^6/g	48 h (0.04/g)
Burditt (*10*) (Henningsen, Exhibit 6)	4.05	0.224	26 C	0.5 ml. of 24 h broth culture/ 394.7g	72 h
	3.90	0.320	26 C	4.5 g of 24 h egg yolk culture/ 390.7g	72 h
Burditt (*10*) (Best Foods, Exhibits, 11a, 11b, 12)	3.9	0.31	29 C	4.0×10^5/g	24 h
Smittle (*55*) (Best Foods)	3.90	0.31	31 C	10^7/g	24 h

TABLE 2. *Survival of salmonellae in salad dressing*

Investigators	pH	Acetic acid concentration (%)	Storage temperature	Inoculum size	Maximum survival (h)
Wethington and Fabian (*65*)	4.4	0.4	Room temp.	>7–<8 (log)/ml	144
			37 C	>7–<8 (log/ml	144
	3.3	1.02	Room temp.	>8–<9 (log)/ml	10
			37 C	>8–<9 (log)/ml	10
	3.2	1.10	Room temp.	>7 (log)/ml	8
			37 C	>7 (log)/ml	8
Kintner and Mangel (*28*)	4.56		Room temp.	1 ml of 24 h culture/30g	24
	3.95		Room temp.		24
	3.40		Room temp.		2
Burditt (*10*)	4.25	0.13	7 C	10^6/g	18
(Kraft, Exhibit 9)	3.70	0.45	7 C	10^6/g	48
	3.40	0.90	7 C	10^6/g	48
	4.25	0.13	22 C	10^6/g	192
	3.70	0.45	22 C	10^6/g	96
	3.40	0.91	22 C	10^6/g	48
Burditt (*10*) (Henningson,	3.56	0.516	26 C	0.5 ml of broth culture/399.0 g	72
Exhibit 6)	3.3	0.928	26 C	0.5 ml of broth culture/0.5 pt	48
	3.3	0.928	35 C	1.0 ml of broth culture/0.5 g	72
Smittle (*55*)	3.2	0.9	10 C	10^7/g	48
(Best Foods)	3.2	0.9	31 C	10^7/g	24

aureus MF 31 was retarded by a change in pH and temperature above or below the optima which were pH 7.5 and 37 C. This effect was intensified by addition of NaCl.

In terms of pH, as adjusted with acetic acid, Levine and Fellers (*33*) found that the inhibiting pH for *S. aureus* was 5.0 and the lethal pH was 4.9. Since pH and NaCl do have interacting effects on one another, the best data for growth inhibition and lethality for *S. aureus* are from studies using salad dressings and mayonnaise. Properly prepared mayonaise and salad dressing are bactericidal to staphylococci. The results of some investigations are summarized in Tables 3 and 4.

In summary, these data demonstrate that staphylococci are as sensitive as salmonellae to a low pH or acetic acid. Since staphylococci die in mayonnaise and salad dressing, a pH of 4.1 or less (0.25% acetic acid) insures a safe product, as with *Salmonella.*

Other pathogens. Undiluted mayonnaise will not support growth of the anaerobic spore-forming food pathogens. *Clostridium botulinum* and *Clostridium perfringens* cannot grow at a pH of less than 4.7 or a water activity of 0.95 (equivalent to 8% NaCl) (*6,45,57,58.*). Not only are these factors important singly, but they have strong interacting effects on growth and germination of spores. Increase or decrease in pH from the optimum increases the minimum a_w at which germination and growth occurs. The limiting a_w for spore outgrowth of *B. cereus* is around 4 to 5% NaCl (*58*). However, the exact minimum a_w for growth and a_w-pH interaction has not been clearly elucidated. Mayonnaise as defined by the Standard of Identity is far below these limits with a pH of less than 4.0 and a water activity of about 0.93 (equivalent to 12% NaCl). For salad dressings, the primary factor controlling pathogen growth is the low pH (3.2 - 3.9) or high acetic acid concentration (0.9 - 1.1%) which is greater than in mayonnaise. Mayonnaise and salad dressing have also been shown to be bactericidal to *Streptococcus viridans* and *Shigella flexneri* (*10*). Both mayonnaise at pH 3.43, 0.49% acetic acid and salad dressing at pH 3.44, 0.90% acetic acid when inoculated with about 3.0×10^6 organisms/g destroyed *Streptococcus viridans* (*10* Kraft; Exhibit 7) in 2 h. A pH of 3.4 and 0.88% acetic acid in salad dressing destroyed 1.0×10^9 *Shigella flexneri*/g in 8 h when stored at room temperature (*10*; Kraft, Exhibit 7).

Conclusion. Undiluted mayonnaise or salad dressing will not support growth of *Salmonella, S. aureus, C. botulinum, C. perfringens, S. viridans, S. flexneri,* or *B. cereus.* Indeed, vegetative cells of these organisms are rapidly killed primarily by the acetic acid. Endospores of the spore formers remain viable, but in the event that germination occurs, cells will not grow.

MICROBIAL INHIBITION IN SALADS AND SANDWICHES BY ADDITION OF MAYONNAISE AND SALAD DRESSING

Numerous authors have investigated the microbial

TABLE 3. *Survival of staphylococci in mayonnaise*

Investigators	pH	Acetic acid concentration (%)	Storage temperature	Inoculum size	Maximum survival (h)
Bovre (*9*)	4.0		22 C		15
Gram (*23*)		0.2	37 C	1 loop/tube	192
Wethington and Fabian (*65*)	5.0	0.15	Room temp.	>8–<9(log)/ml	168
	5.0	0.15	37 C	>8–<9(log)/ml	168
	4.0	0.51	Room temp.	>8–<9(log)/ml	90
	4.0	0.51	37 C	>8–<9(log)/ml	90
	3.8	0.48	Room temp.	>8–<9(log)/ml	108
	3.8	0.48	37 C	>8–<9(log)/ml	108
Burditt, (*10*) (Kraft Exhibit 1)	3.43	0.49	Room temp.	$5.0 \times 10^7 \times$ /g	24
Smittle (*55*)	3.90	0.31	10 C	10^7/g	186
(Best Foods)	3.90	0.31	31 C	10^7/g	24

TABLE 4. *Survival of staphylococci in salad dressing*

Investigators	pH	Acetic acid concentration (%)	Storage temperature	Inoculum size	Maximum survival (h)
Kintner and Mangel (*28*)	4.56		Room temp.	1 ml of	> 24
	4.21		Room temp.		> 24
	4.08		Room temp.	24 hr.	> 24
	3.95		Room temp.		>24
	3.40		Room temp.	culture/30 g	24
Wethington and Fabian (*65*)	4.40	0.40	Room temp.	7–8(log)/ml	180
	4.40	0.40	37 C	7–8(log)/ml	180
	3.30	1.02	Room temp.	>8–<9(log)/ml	54
	3.30	1.02	37 C	>8–<(log)/ml	54
	3.20	1.10	Room temp.	>8–<(log)/ml	36
	3.20	1.10	37 C	>8–<9(log)/ml	36
Burditt (*10*) (Kraft, Exhibit 1)	3.44	0.90	Room temp.	5.0×10^7/g	6
Smittle (*55*)	3.2	0.90	10 C	10^7/g	24
(Best Foods)	3.2	0.90	31 C	10^7/g	6

flora of commercially prepared salads and sandwiches (*8,12,19,29,39,49,54*). Generally, in salads and sandwiches, where a low pH was encountered due to salad dressing, mayonnaise, or acetic acid, low total bacterial counts were detected (*12,29,34,39,40,49*). The reported low incidence of *Salmonella*, staphylococci, and coliforms in salads and sandwiches was thought to be a result of the low pH and acetic acid from mayonnaise or salad dressing (*29,39,40,54*). The inhibitory effect on bacterial pathogens was not only due to the low pH but to the toxic effect of acetic acid which was present in mayonnaise and salad dressing. Kahn and McCaskey (*29*), McCroan et al. (*39*) and McKinley et al. (*40*) singled out mayonnaise and salad dressing as being the most important ingredients in controlling pathogens in commercially prepared salads and sandwiches. Sinell and Siems (*54*) reported that gram-negative bacteria die off rapidly in delicatessen foods containing mayonniase followed shortly thereafter by death of micrococci and enterococci when present.

There have been many studies on salad and sandwich fillings deliberately inoculated with bacterial food pathogens (*12,25,32,36,40,48,63*). In these studies, the non-spore-forming pathogens, *Salmonella* and *S. aureus,* were inhibited or died off. The spore-forming pathogens, *B. cereus* and *C. perfingens,* did not grow but were dormant (*25*). The extent of growth inhibition or loss of viability was dependent on acetic acid concentration or pH (*8,12,25,32,34,48,63*), storage temperature (*12,25,54*), and type and number of microorganisms present (*25*). Holtzapffel and Mossel (*25*) found that *Salmonella* and *S. aureus* died off in meat and vegetable salads in pH 4.2 (0.5% acetic acid) and in shrimp salads at pH 5.3 (0.3% acetic acid). Death was at a faster rate in salads stored at 20 C (68 F) than at 9 C (48.2 F). Rappaport and Goepfert (*48*) demonstrated that pH 5.3 ham and pH 5.1 chicken salads containing mayonnaise did not enhance growth of *S. typhimurium* or *S. aureus* 196E when compared to ham and chicken without mayonnaise. Salad pH was considered as the predominant factor controlling growth in these products. Weiser et al. (*63*) found that a chicken salad at pH 5.5-5.7 adjusted with acetic acid retarded growth of *S. aureus*. They found that chicken salad could be held for as long as 12 h at 27 C (80.6 F) without growth occurring. Nevertheless, they recommended that freshly prepared chicken salad be rapidly cooled to 7 C (44.6 F) or below for prolonged storage. Similar results with the total bacterial count were reported by Beck and Schneider (*8*) for potato salads at pH 3.8, 4.0, and 4.2 and held at 20 C (68 F). Christiansen and King (*12*) inoculated commercially prepared chicken salad (pH 5.2) and ham salad (pH 4.8) with about 10^6 coagulase-positive staphylococci per gram and observed a steady decrease in counts at 37 C (98.6 F) and 4 C (39.2 F). Both salads stored at 37 C contained 100/g after 4 days, whereas chicken and ham salads stored at 4 C for 15 days contained 5.4×10^4/g and 9.0×10^4/g, respectively.

Lerche (*32*) demonstrated that meat-containing salads made with mayonnaise freshly inoculated with *Salmonella* were potentially dangerous. He found that *Salmonella* from the mayonnaise survived and grew in meat cubes where the pH was not low enough to kill them or prevent their growth. To minimize a problem of this nature, mayonnaise or salad dressing prepared from ingredients that might contain salmonellae, such as unpasteurized eggs, should be held for 72 h to insure their complete destruction.

McKinley et al. (*40*) and Weiser et al. (*63*) reported that growth of enterotoxin-producing staphylococci on cooked chicken was greatly retarded by the presence of mayonnaise or salad dressing. Longree et al. (*36*) demonstrated that growth of staphylococci was inhibited in sandwich fillings acidulated with lemon juice. Beck and Schneider (*8*) discussed the importance of concentration of acetic acid in mayonnaise in controlling pathogens in commercially prepared salads. Due to this inhibition phenomenon, Longree et al. (*35*) and McKinley et al. (*40*) recommended that salad dressing or mayonnaise be added to the meat portion of salads or sandwich fillings soon after cooking to aid in protecting the product. However, Longree et al. (*35*) cautioned that sandwiches using meat fillings must also be refrigerated properly to insure a safe product. Christensen and King (*12*) were in agreement. They maintained that although addition of acetic acid to meat sandwiches would be a definite advantage, the heterogenous sandwiches would contain areas where a low pH or a high enough acetic acid concentration would not be achieved, thus allowing areas where pathogens could grow.

A prevalent consumer view exists that mayonnaise or salad dressing added to salad or sandwiches will lead to rapid spoilage and cause food related illness. However, the opposite is true. Vegetables, and especially meats, are excellent media for growth of pathogenic bacteria and must be refrigerated for preservation. Because mayonnaise and salad dressings retard growth of *Salmonella* and staphylococci and prevent germination and growth of *B. cereus* and *C. perfringens* when mixed with meat and vegetables, they should be mixed as soon as possible, then refrigerated.

In terms of public hazards, a meat and vegetable salad and sandwich filling with a pH of 5.3 appears to be safe, providing the acidulant; is acetic acid as found in salad dressings and mayonnaise, is mixed uniformly into the product, and is refrigerated at 45 F or below. With reference to sandwich fillings, they should not be placed on bread until they are ready to be consumed. This, of course, would eliminate any dilution effect from the bread. In conclusion, all possible means should be taken to protect these products. An easy precaution to take is the addition of mayonnaise or salad dressing and other acid ingredients such as pickles or lemon juice.

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Journal of Food Protection Vol. 40, No. 6, Pages 423-428 (June, 1977)
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Guidelines for a Dynamic Quality Control Program in a Changing Market

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(Received for publication December 6, 1976)

ABSTRACT

The importance of "Plant Quality Controls" have never been greater than they are today. Product Quality Assurance and consumer satisfaction are the two essential ingredients for success in today's competitive market place.

It is essential for plants in the Food Industry to establish the proper priorities in their operations for the development of a practical total Quality Control Program, in order to be assured of continued Quality Product production.

In this paper are presented a viewpoint analysis of Quality Control and guidelines for the development of a dynamic Quality Control Program in our changing market place.

My company, over the years, has always been quality minded, having installed an active Quality Control group many years ago, to assure that we produce uniform Quality products regardless of the production location. In this way, our consumers come to know that they will always receive the same uniform product regardless of where it may be purchased. Kraft has long realized that quality is a much *sought after* reality with the consumer, and thus long ago, instituted measures to maintain continuous and unvarying product quality through *a standardization program.* Our leadership in the food industry has been attained and maintained through constant and continuing emphasis on the importance of quality.

In times past, for many companies, the Quality Control function was considered to be a luxury — not absolutely necessary for successful business operations. But not so today! The story of company Quality Controls is different. The government and the consumer advocates are demanding safety and quality in food and other products through vastly increased governmental activities and numerous new regulations. Now it is recognized by most companies that Quality Controls are vital and represent, in many instances, the very life blood that is necessary to compete successfully in today's competitive and changing market. In one way or another, all of us must have experienced some effect from this new evaluation and change.

In this paper I want to re-emphasize the growing importance today of *food quality* as it relates to both the food and packaging industries. This phrase *food quality* has received notable and frequent press, radio, and television media coverage. *There is no question* that methods used in food processing, packaging, and in shipping and storage of products are coming under much closer surveillance, scrutiny, and inspection than at any time before. The time has well come for all food and packaging manufacturers to recognize that an important opportunity exists in today's competitive market place, where progressive companies can, through the continued production of high quality products, establish a stronger quality image and a more potent customer appeal for their products.

With the economy playing a major role in management decisions today, many company buyers have shown keen interest in "price-quality" relationships, and as such, it becomes a must to have superiority through Quality Controls built into products manufactured if companies expect to continue to market their products successfully. Now, in the succeeding paragraphs, let's look at today's Quality Controls involved in the processing of packaged food products. Perhaps first, it would be well to define Quality Assurance — Quality Control as we know it today. "Quality Assurance," as a departmental name, in place of "Quality Control," is fast becoming very popular in many companies to describe their staff-level activities. In others, for all practical purposes, the terms "Quality Assurance" and/or "Quality Control" are used interchangeably and are considered synonymous.

We use "Quality Assurance" in my company, as an overall departmental name at both the corporate and company staff levels. At operating levels of management, Quality Control titles continue to be used at both the general office staff and plant levels. Now for the term, "Quality Control", which may be more familiar to many of you. I wonder how many of us really understand the true meaning and function of Quality Control. Using a

little imagination, one could conceive that the idea of Quality Control might well have originated at the dawn of history, when a man pointed to an object and said, "I want another just exactly like it." Needless to say, he failed to get it, and neither does anyone get it today. For it was true then, as it is now, that no one thing is precisely, exactly, like another. You've heard the common expression, "No two people are exactly alike", so it is true with material things as well. Variation inevitably exists in natural composition, in packaging materials, and even in the precise manufacturing operations known today. Strive as we may for exact duplication, we really never quite obtain it.

As far as we are concened in Quality Assurance or Controls, variation need not bother us until it reaches a degree, level, point, or an extent where it causes difficulty in a packaging operation or is otherwise harmful in any way to the product. In Quality Control work, the variables are pinpointed, highlighted, and eventually, we hope most of them are reduced or eliminated which really results in Quality product production.

So this is where a department such as Quality Control or Quality Assurance fits into a Company's operation. This function must exist at both the top management and plant levels and the responsibilities, though relative, are necessarily different. Let's first cover briefly the basic responsibilities of both levels of Quality Control or Assurance, and later go into more detail on each of the basic points mentioned

RESPONSIBILITIES

Staff Quality Control or Assurance
has the basic responsibilities for establishing:

1. Guiding principles for the quality control function
2. Complete sanitation and product standard methods and procedures
3. Procedures for handling activities associated with finished product standard quality and regulatory compliance
4. Technical training aids for plant use
5. A comprehensive plant quality control monitoring program

Plant quality control or assurance basic responsibilities are to:

1. Set up an in-plant adequate quality control personnel training program.
2. Monitor product quality from start to finish — from the initial point of packaging and edible raw materials receipts — through processing, to the final packaging of product and the ultimate distribution.
3. Check for variance from standards.
4. Determine the extent of such variation.
5. Establish the significance and/or harm caused by the variations.
6. Scrutinize laboratory and production line control data.
7. Provide technical expertise to production operating departments.
8. Make decisions relative to acceptance or rejection of products manufactured.

GUIDELINES FOR PROGRAM DEVELOPMENT

Now that we have seen this background material, let's go into the detail guidelines we should concern ourselves with in the development of a dynamic Quality Controls program. First of all, a total Quality Assurance or Quality Control program involves six principal categories. These Are:

1. Guiding principles for the quality control function
2. Product standard methods and procedures
3. Sanitation standard methods and procedures
4. Laboratory standard methods and procedures
5. Product Quality standards and regulatory compliance
6. Plant quality controls

Now, let's review these six categories one at a time.

Guiding principles for the quality control function

1. A company philosophy and commitment to quality must be established, and the policies that guide the decisions and activities relating to Quality Control must be spelled out.
2. Quality Control jobs and their relationships to other plant management jobs must be understood.
3. It must be recognized that well qualified, trained, and dedicated people are a necessary pre-requisite to controlling product quality through manufacturing and distribution; further, that dedication to quality is a way of life that must be instilled in all who are involved in any of these activities.
4. Companies shall maintain quality control laboratories and personnel to assure compliance with the companies' own standards and with government standards. Company products will at least meet, or exceed, the requirements of regulatory agencies.
5. Quality Control shall maintain a continual reporting system covering the reporting of "out of standards" results and other quality problems. To keep everyone informed, meetings shall be conducted as often as necessary on general product quality.
6. It is to be understood that high quality is essential to the best interests of the company management, employees, stock holders, and customers.

Product standard methods and procedures

1. We must have proper product formulations and detailed manufacturing procedures.
2. A positive program should be in effect on the pretesting of incoming packaging supplies and incoming raw materials.
3. A good sound in-process control laboratory testing schedule must be in constant operation.
4. A concise but practical routine finished product laboratory testing schedule must be followed.
5. A statistical weight control program must be established.
6. Product package and shipper containers should be properly code dated.
7. Shipping and storage conditions should be spelled out.

Let's dwell more on this second principle category, as it easily warrants such further discussion.

Proper product formulations and detailed manufacturing procedures. Some of the basic details involved here are:

1. Research and development project. In most all cases, a Research Project is needed. In addition where engineering work is involved, a request for engineering project must also be made.
2. Kitchen acceptance test. All products must be first passed on by the new products committee before being shown in test kitchens. Samples are shown by R & D people with the kitchen personnel assisting in the preparation of formulations for showing.
3. Marketing and production approvals. Approvals must be obtained from the company operating departments (Production and Marketing).
4. Label processing. All labels must be approved by Quality Assurance or Control, Production, Marketing, the attorneys, and the management label committee before printing.
5. Plant production trials by R & D and Production.
6. R. & D final formula and manufacturing procedure report. Final acceptance of product by Production Department.
7. Issuance of standard detailed plant formula manufacturing procedure.

Pretesting of incoming packaging supplies and raw materials.

1. Development of packaging and raw material standard specifications. Perhaps it might be of interest if I were to describe briefly what takes place in development of packaging specifications for a new product. Let's take, for an example, our package for caramel discs, better known as Kraft Wrapples. After the idea for this item was developed, R & D was apprised for the new product and was asked to develop packaging requirements for packing discs of caramel that would remain separated with no danger of spreading or flowing under various warehouse storage and store display conditions. This meant that the product in package really must be protected from outside conditions, that is, oxygen, moisture, etc., under conditions of sales promotion and use by the consumer.

R & D proposed treating paper sheets for separating the many layers of discs in package and using a multilayer outer flexible film for product package. Since a display carton was desirable, R. & D tested several types of material for this purpose, keeping in mind that product must be protected while in distribution and while displayed in the stores. All the while packages were meant for quality purposes, to stand upright in the display carton without the danger of collapsing, or destroying the display advertising and product quality characteristics. After many trials, B flute corrugated display carton was found to be acceptable by both Engineering and Production. Samples of the various proposed packaging materials were tested by R & D with Engineering's help and equipment. Only after all tests were completed and Marketing was satisfied with costs, overall appearance of the final package, and results obtained over long term storage conditions, were the tentative specifications issued. Only after plant trials were conducted and found to be satisfactory, were the permanent specifications issued.

When a new product is being launched, many of the specifications are issued as tentative so that quantities purchased *can be* held to a minimum. Too, if changes are made, the plant is not burdened with large inventories of materials to be worked off.

The packaging specifications cover all packaging items used in product, individual package container, cap, label, wrapper, shipping containers, etc. The code date area also is shown on specifications and pallet pattern specifications also are included. Pallet patterns are designed to show how the product should be palletized for best stacking in warehouses, and to give one a pallet modulus least susceptible to product damage.

2. No packaging supplies should be allowed to be used until they have been sampled, examined, or analyzed, and passed as satisfactory.
3. Approved suppliers are needed to assure manufacturers the least amount of supply problems, and to be in a position to work more closely with suppliers in solving mutual supply problems.
4. Physical inspections are to be made of incoming materials for infestation, using black lighting and visible inspection conducted for evidence of contamination or product damage. This is necessary for Quality Assurance and company self-protection, especially in view of the interest now being given to this area by regulatory officials.

In-process control laboratory testing schedule. This should be the most important single laboratory testing function. This really represents the key to successful processing operations. Laboratory control is exercised at various critical points along the processing operations. Samples are sent to the laboratory and results returned to the processing operator in time to allow him to make necessary corrections of batches based on laboratory data received. In Kraft processing operations, we use the pneumatic tube system to convey samples from the processing and receiving areas to the laboratory. It takes approximately 10 sec to send a sample from a processing area to the laboratory. Only "quickie" analytical tests should be employed to be of service to production department processing, as time is of essence.

Finished product laboratory testing schedule. Finished products should be analyzed on a regular basis (statistical if possible) for conformance to specifications. Each is checked for physical, chemical, microbiological, where necessary, and keeping quality characteristics.

Statistical weight control. In Quality Control, one should recognize that there are certain causes of process

operational variations that must be controlled to obtain uniformity. In spite of all efforts, there will still remain a certain amount of variance, "chance causes if you will," over and above those that can be identified and controlled. In package weight control, one should recognize these two types of causes and set up control measures to give the operator the proper signal when danger is imminent. This must be done to maintain proper package weights on product produced keeping the package variance as low as possible.

Product package and shipper code dating. Each package and shipper should be code dated for identification purposes. Also, each shipper should be identified with a universal product number for proper product identification and inventory purposes.

Shipping and storage temperatures. It is absolutely necessary that standard temperatures for shipping and storage of products be known to all personnel responsible for product movement and storage. A minimum and maximum temperature should be stipulated, especially for shipping, to give the traffic department a range which might be used in cases of emergency and unforseen circumstances.

Sanitation standard methods and procedures.

Each plant operation must have an organized program on over-all sanitation which should include:

1. Plant inspection program. Each plant must have a routine plant cleanliness inspection program. The building must be checked inside and out. Insect and rodent control must be in force. All in all, a regular housekeeping system must be employed.
2. Pest extermination method and procedures. These should include ready information on common pests found in and around plants, such as cockroaches, houseflies, beetles, weevils, night-flying insects, spiders, and rodents. Also, a list of approved chemicals and equipment to be used, as well as the amounts and conditions of use, shall be spelled out.
3. Plant and equipment cleanup. A scientific approach to sanitation control is required because of the many far-reaching technological changes made in packaging and food processing, in recent years. A documented program schedule for the actual equipment cleanup, using standard approved cleaners, should be available, and should point out specific areas, both inside and outside of the plant that are to be cleaned, and the persons designated who are responsible.

Labortory standard methods and procedures

Laboratories must use standard methods or methods which produce comparable results when measured against industry accepted methods, to be assured that proper laboratory controls govern all product production. Whenever possible, it is advisable to use existing standard methods for testing raw materials, in-process product control samples, and finished products, compiling statistical data where needed, to establish testing reliability.

In those instances where more than one laboratory location is using the same method to control like finished products, it would be well to do collaborative samples among the different laboratories. Collaborative sample testing is considered a most effective tool to establish standard deviation method expectancy under conditions of use, as well as a means of detecting laboratory testing errors.

Product quality standards and regulatory compliance

1. Established methods and procedures should be set-up to develop information necessary to ensure compliance with, or secure approval of, regulatory agencies in matters affecting labeling, product formulae, and production procedures and equipment.
2. It is important to set up standard procedures governing the investigation of finished product defects or problems reported by consumers, plant and sales management, or resulting from company or governmental research findings. A person should be delegated the responsibility of investigating defective or damaged finished products to determine disposition in compliance with company quality standards and government regulations.

Plant Quality controls

The last principle category "Plant Quality Controls" completes the total Quality Control or Assurance program. This one should be regarded as the very heart — and a truly vital function in a plant operation.

We all probably know about the activities in Washington centering around the Consumer Food Act (Food Surveillance), Bill S641, which in short requires the food industry to adopt a more sophisticated Quality Control program. With such knowledge, it becomes an absolute necessity that all packaging and food companies embark on a program, if they haven't already done so, to upgrade Quality Controls in their plants. In Kraft we began such a program in January, 1974.

Since the FDA's food plant inspection approach mentioned in this legislative bill placed primary emphasis on the so called Hazard Analysis — Critical Control Point (HACCP) analysis program, we have adopted this new systematic HACCP concept method of evaluating food plant production operations as the basis for our program. To date, we have not regretted our decision as this approach has improved our controls considerably. Further, there should be no doubt under today's climate of plant operations, that FDA expects packaging and food processors to adopt more comprehensive and better in-plant Quality Assurance systems to establish greater food safety and Quality Assurance. The HACCP system conceived by FDA represents an inspectional type approach where specially trained inspectors analyze and evaluate complex production operations, identifying deficiencies in manufacturers Quality Controls to prevent production and marketing of

unsafe materials and foods.

THE HACCP SYSTEM

Hazard Analysis:

an element of process equipment or environment of a process which, if not properly monitored, can result in the introduction of hazardous foreign materials into the product.

Critical Control Point:

an operation and given process which, if not maintained within certain parameters, can result in the production of food which may be unsafe primarily from the microbiological standpoint.

The specific hazards involved in our HACCP program using symbols to identify critical items are:

H —refers to those hazards relating to chemicals such as chemical toxins, aflatoxin, heavy metals (mercury, lead, etc.), PVCs, PCBs, pesticides, foreign matter (such as stones, insects, glass metal, etc.).

C — refers to critical control points, hazards that are microbiological in nature, such as bacteria and molds, including *Salmonella, Shigella, Staphylococcus, Clostridium, perfringens, Escherichia coli,* etc.

K - represents all other critical control points over and above the defined FDA hazard control and critical control points (these need to be documented and monitored, in addition to other control points to meet required overall Kraft quality of finished products and consumer acceptability.

SCOPE OF HACCP PROGRAM CONCEPT AND COVERAGE

The program entails establishment and use of Good Manufacturing and Quality Control procedures such as were discussed earlier in this paper, together with the following:

1. Establishment of a product flow diagram for each different product line operation in the plant.
2. Pinpointing the defined critical and hazardous control points on flow diagrams and separate form charts representing potential hazards to consumer safety.
3. Development of statistical methods covering laboratory sampling and testing.
4. Make OC (operational characterisitc) curves using statistical techniques to analyze laboratory data to determine reliablity effectiveness of established controls from a safety and quality standpoint.
5. Monitor, on a continuous basis, all potentially hazardous and critical control points in accordance with established procedures and maintaining adequate records.

Let's review briefly in further detail, each of the points just mentioned.

Establishment of a product flow diagram for each different product line operation in plant

It becomes necessary to develop simple block-type diagrams indicating the product-flow direction, by arrow, of each finished product process. Starting with raw material supplies at each point of use in process, the diagrams must show each in-processing step identifying the function performed and/or equipment, all the way through to the ultimate shipping and distribution records.

Pinpointing the defined critical and hazardous control points on flow diagrams and separate form charts representing potential hazards to consumer safety.

Using the abbreviated symbols for identifying each of the critical items, flag the critical areas on the flow diagrams and highlight these same critical areas in the special analysis charts used for this purpose. Items covered on the analysis chart are: product name, hazard and/or activity, frequency of testing, control used, responsibility, and location of records.

Development of statistical methods covering laboratory sampling and testing.

What is involved here is to make a complete statistical analysis of laboratory history test result data which are available. These data should cover a period of at least 9 months, and preferably a year. In this manner, it could be assumed that the standard deviations found are realistic and representative of conditions.

Make OC (operational characteristic) curve

This curve is made using statistical techniques to analyze laboratory data to determine reliability effectiveness of established controls from a safety and quality standpoint. In addition it becomes necessary to prepare an OC curve representing the specifications. This specification curve and the one representing actual laboratory data are superimposed upon one another so that a comparison can be made between: (*a*) what is required by the specification, and (*b*) what is actually encountered within the plant.

When the comparisons are made, and the process laboratory data do not agree with the standard specification data, one has but two alternatives — change the specification, or change the process itself. Changes are made so that specifications are realistic and the processes are capable of producing products meeting standard specifications. In this manner, we are assured, at all times, that the plants should be following attainable specification standard tolerances.

Monitor, on a continuous basis, all potentially hazardous and critical points in accordance with established procedures maintaining adequate records

1. Installation of program involves meetings with plant managers and all of the supervisory staff, with staff Quality Assurance and plant Quality Control personnel, so that all management personnel are fully indoctrinated on program concepts and objectives.
2. Laboratory facilities need to be reviewed to see if any adjustments or realignment of responsibilities are

necessary.

3. Training of technical personnel is required so that they are thoroughly familiar with all facets of the program.
4. Changes need to be made in plant procedures to bring them is compliance with HACCP company standards.
5. The Plant Quality Control manager is responsible for monitoring the HACCP Quality Analysis Control Program. Primary responsibility might be delegated to Quality Control supervisors to see to it that program procedures are being implemented on a daily basis in all plant areas.
6. Any plant changes in equipment use, product, flow, etc., affecting the validity of the flow diagrams and/or analysis charts shall be reported to the Plant Quality Control Manager, so that flow diagrams and analysis charts can be changed accordingly and continuously updated.
7. The program must be followed and any deviations reported to the Quality Control Manager and the Plant Manager.

SUMMARY

In summary it can be said, that once a formal Quality Control program has been established, it is impotant that the plant Quality Manager and his staff learn to:

1. Scrutinize quality data on all raw materials, in-process product mixes, finished products, product keeping-quality samples, and product complaints, to know the general quality characteristics of the operation.
2. Become involved in how to stay within procedures and specifications.
3. Identify major and minor product quality problems and learn how and why an undesirable situation occurred and how to rectify it.
4. Work out with the plant production management team, the means of providing remedies for plant quality problems.
5. Take immediate safeguard actions to prevent the same quality defect situations from recurring.
6. Consult with technical management, making the best use of their technical capabilities whenever conditions dictate.

ACKNOWLEDGMENT

Presented at the National Packaging Week Assembly, Chicago, Illinois, October 26-29, 1976.

News and Events

Dairy Film Shows Eight Steps to Better Production

The first new film in nearly 30 years dedicated solely to good milking practices is completed, and will be used in conjunction with hundred of locally sponsored dairy workshops throughout the country.

The 35 minute educational film, "Making Your Cows Worth More," sponsored by Babson Bros. Co., manufacturers of Surge dairy farm equipment, is an effort to help dairymen do an even better job of milking.

In the film, nationally noted dairy experts discuss the following eight important steps which, when done properly, give dairymen the ability to reach full potential in harvesting their milk crop:

- Milking Environment
- Proper Stimulation
- Stripping Foremilk
- Application of Milking Machine
- Machine Adjustment
- Removing Teat Cups as Quarters Milk Out
- Teat Dipping
- Importance of Equipment Maintenance

Live and animated sequences graphically explain the importance of the practices which apply as much for a herd of 20 cows milked in a stanchion barn as for a 2000 cow herd milked in an automated parlor.

The dairy workshops are sponsored by local Surge Dealers and also include presentations by Veterinarians, Sanitarians, County Agents, and University Extension people. These experts will field questions and explain how the film's content applies to local area dairy operations.

The development of the film came from studies showing that now, more than ever, good milking practices are needed to get top production with today's modern equipment and better producing cows. It is also a response to indications that most dairymen often overlook or omit some important steps in an effort to save time during milking.

Nationally noted dairy experts featured in the film are:

Dr. Allen Bringe, University of Wisconsin

Dr. L. J. Bush, Oklahoma State University

Dr. John Campbell, University of Missouri-Columbia

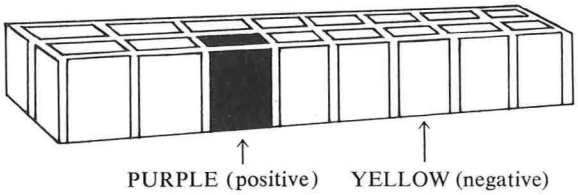
Dr. George Fisher, Ontario Ministry of Agriculture and Food

Dr. Richard Mochrie, North Carolina State University

Dr. Nelson Philpot, Louisiana State University

Film is available on a loan basis from Babson Bros. Co., Oak Brook, Illinois.

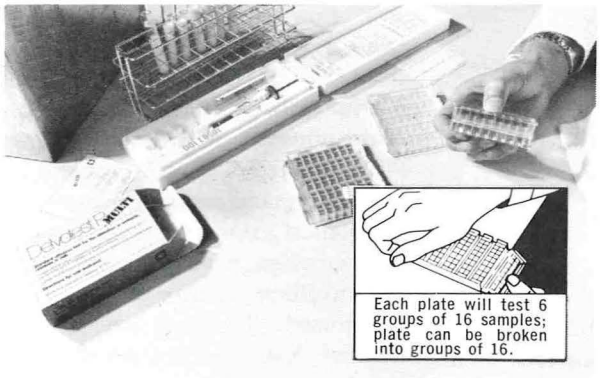
New!
For bulk tank screening.



Easy to use!
Economical!

DELVOTEST® P MULTI

A rapid, accurate screening test for Penicillin residue in milk.



Delvotest® Multi consists of 2 disposable plates, each containing **96** (6x16) cups. One plate contains agar with spores while the other plate contains nutrient tablets.

Easy to use. Plates for running **96** samples are prepared for sample addition in **less than one minute.**

It's sensitive. Penicillin concentrations of 0.003 I.U. or less per ml. of milk always yield a negative test result (entirely yellow), while levels of 0.006 I.U. or higher give a positive result (entirely purple). In-between concentrations give yellow-purple colorations.

It's diverse. Also sensitive to all antibiotics used in lactating cattle.

It's economical. Delvotest® Multi is economical enough to allow a complete antibiotic screening program for all incoming bulk tankers to assure antibiotic control also in finished products.

It's rapid. Results can be read within 2¾ hours.

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Continued Growth Predicated on Favorable Consumer Trends

Food service industry sales of $118 billion (in current dollars) are projected for 1981 by Arthur D. Little Impact Services Company. A newly completed five-year outlook study forecasts on average annual growth rate of 10 percent (including a 6 percent inflation factor). Sales for 1976 are estimated at $74 billion for combined commercial and noncommercial food service operations in the United States.

Not counting vending machines, sales of the commercial segment—restaurants, in-store eating places, recreation-area eating places, hotels and motels—will increase from $52 billion in 1976 to $87 billion (current dollars) in 1981, an average annual growth of 11 percent. Slower growth is forecast for the noncommercial segment, providing food service in plants, schools, hospitals, and other institutions. Noncommercial sales will grow from $22 billion to $31 billion (current dollars), an annual average of 7 percent. None of these figures includes food service for the armed forces.

Away-from-home eating increasingly is perceived by consumers as merely another "convenience food" option, according to William C. Hale, the Arthur D. Little food industry expert who directed the Impact study. He believes that decisions favoring a fast food or moderately priced restaurant over other convenience food alternatives are based largely on the convenience of location and amount of freedom in lifestyle.

Mr. Hale predicts that the proportion of total meals which consumers purchase away from home will increase to 35-38 percent by 1981. He bases his prediction on a continuation of the trends in consumer demographics, economics, and lifestyle, which have already increased away-from-home meals to about 33 percent. Arthur D. Little forecasts that in 1981 more than 60 percent of all households will have incomes of at least $15,000; discretionary income will reach 24 percent; and 45 percent of wives will be working. The percentage of one- or two-person households will also increase as will the number of apartment dwellers.

Mr. Hale cites population trends, more leisure time, and an increase in the number of eating establishments which are compatible with a casual lifestyle as additional factors which support further food service industry growth. Recent consumer surveys indicate that younger adults eat out more frequently than the rest of the population. The 18-44 age group is expected to experience the largest population increase between 1975 and 1980.

Fast-food chains lead growth

Fast-food chains will lead food service industry growth over the next five years with an average annual sales increase through 1981 of more than 15 percent. "Despite this overall growth, competition will be more intense," Mr. Hale observes. "There will be more emphasis on advertising and a much greater use of market research as fast-food chains attempt to diversify and expand their consumer base," he continues. "There is no clearcut nationwide answer to the saturation question because opportunities remain. For example, regional chains can expand geographically. Another example is the recent growth of Mexican and seafood operations which demonstrates that special

Indiana's Outstanding Dairyman Named

Paul Devine, Vice President and General Manager of the New Paris Creamery, Inc., of New Paris, Indiana, has been named the outstanding Dairyman of the Year 1977 in the State of Indiana, according to a recent announcement.

The annual award, which has been presented to outstanding Hoosier Dairymen for the past 12 years, was awarded to Devine in Lafayette, at a Dairy Technology Society Banquet honoring his achievement in the dairy field.

The award is co-sponsored annually by Purdue University's Animal Sciences Department, the Indiana Dairy Tech Societies, and the Midwest Dairy products Association in Indianapolis, and was presented to Devine by Robert L. Burger, President of the Burger Dairy Stores, Inc., chain and Dr. Babel, from Purdue University.

menu establishments need not be limited to hamburger and chicken."

Expansion and diversification efforts by fast-food franchisers will probably continue to follow the life-cycle pattern predicted by Mr. Hale in his 1969 analysis of the food service industry. At that time, he predicted that the first step is the shift from a one-product stand to a limited service/limited menu restaurant by expanding the menu and adding inside seating and service, thereby changing its image. "Clearly, McDonald's has already taken these steps," Mr. Hale observes. He explains that other steps in this process can be integration into food manufacturing or becoming a full-service restaurant by adding cocktail and dining rooms. A franchiser can diversify into lodging facilities or upgrade to become a theme restaurant, perhaps even launching a new generation of one-product stands along the way.

"Although it is unlikely that they will reverse the cumulative effect of positive consumer trends, there are a number of issues confronting the food service industry," Mr. Hale concedes. "For example, no one knows how gasoline prices will affect decisions to eat out. We're about to find out what President Carter's energy policy will mean for gasoline prices as well as for other energy costs.

"There are regulatory issues as well," the Arthur D. Little study director states, "having to do with such things as franchising arrangements, sanitation, and nutritional labeling. There are labor concerns, and a host of cost and supply problems ranging from the aforementioned energy to meat, coffee, and produce. Of course, some problems apply more to one segment of the industry than another. For example, skyrocketing beef prices hurt 'steakers,' or budget steak houses, the most." Regardless of the outcome of these and other issues, Mr. Hale believes that the key hurdle for chain operators will remain that of reaching the "critical mass" in the dollar sales which enables them to support strong promotion and advertising campaigns as well as to strengthen their operational procedures to contend with increased competition.

The outlook for the food service industry through 1981 is part of the continuing service available from Arthur D. Little Impact Services Company. The service covers major industries and economic issues and is provided by Arthur D. Little, Inc., the Cambridge-based management consulting, engineering, and research organization.

NIFI to Convene National Conference on Food Protection

The National Institute for the Foodservice Industry will host a National Conference on Food Protection in Foodservice, September 6-9 at Chicago's Palmer House.

Key representatives of the food-service industry, government, education, consumers, and other allied interests will take part in the meeting.

The conference will consider current and developing problems related to food served away from home and examine and plan alternative ways of solving these problems.

Focus of the working meeting will be placed on roles and responsibilities in insuring the protection of the ever-expanding "eating-out public."

Developments since the National Conference on Food Protection held in Denver six years ago will be examined. Viewpoints of all conference participants will be involved in working out action plans for the future.

For more information, contact C. Dee Clingman, Director, Food Protection Programs, National Institute for the Foodservice Industry, 120 S. Riverside Plaza, Chicago, Illinois 60606. Telephone (312) 454-1800.

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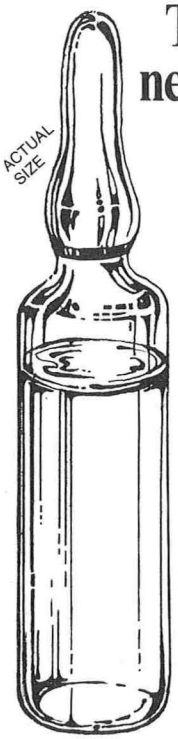

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Second Annual Toxic Substances Control Seminar to be held December 8-9 in Washington, D.C.

Government Institutes announced recently that the plans have been made to conduct the 2nd Annual Toxic Substances Control Seminar at the Shoreham Hotel on December 8-9, 1977, in Washington, D.C.

The first meeting drew over 450 leaders from the various communities concerned with toxic substances control. The focus was the new Toxic Substances Control Act (TSCA) which was passed in 1976. The 2nd seminar will analyze developments during 1977.

The new Carter appointments, EPA organizational changes, promulgations of new regulations, scientific investigation results—all will contribute to making 1977 a most eventful year for those concerned with toxic substances control. The December dates insure the timeliness of this informative seminar.

Some of the key topics that will be discussed and analyzed by leading authorities are:

- implementation of the new TSCA
- regulatory developments implementing the hazardous waste provisions of the new Solid Waste Disposal Act
- developments in other regulatory (OSHA, FDA, pesticides) areas
- organization changes and appointments of the Carter Administration
- scientific developments regarding the health effects of toxic substances

In addition, an exposition will be offered for conveying more information on the equipment and services that are presently being offered in this field.

For details on either the conference or exposition contact Government Institutes, Inc., 4733 Bethesda Ave., NW, Washington, D.C. 20014.

Petrowski Named to Kelco Research Post

Gary E. Petrowski has been appointed food section head at Kelco's research and development operations, David J. Petitt, technical director, announced.

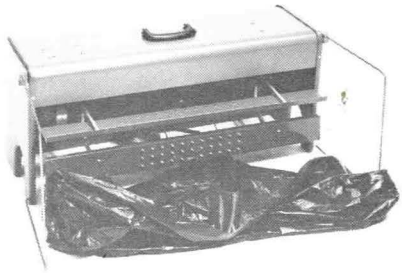
Gary E. Petrowski, Food Section Head, Research and Development Operations, at Kelco.

Before joining Kelco, he was senior research scientist for the Carnation Company. In his new post, Petrowski is responsible for developing new food applications for Kelco products. Identifying new gums for food applications, and providing technical support for the company's sales and marketing groups.

A native of LaCrosse, Wisconsin, Petrowski was graduated from Loras College in Dubuque, Iowa. He received his Ph.D. from the University of California, Los Angeles, and did post-doctoral research at the University of Colorado.

Petrowski is a member of the American Chemical Society, the American Oil Chemists Society, and the Institute of Food Technologists, and has authored several technical papers.

San Diego-based Kelco is the world's largest product of xanthan gum, and alginates, which find use as water-controlling agents in a wide variety of food, dairy, brewing, and pharmaceutical applications. Kelco is a division of Merck & Co., Inc.



Portable Rodent Electro-Exterminator Introduced by Cordell Engineering

Cordell Engineering, Inc., Everett, Mass., has developed the CE-100 Ratslayer, a portable, battery operated rodent exterminator which automatically traps and electrocutes rodents. The dead creature is ejected automatically into a heavy-duty plastic bag for convenient disposal. The unit then automatically resets itself to catch additional rodents. An insecticide strip within the bag kills all flies, lice, and other vermin the rodent may be carrying thereby eliminating the danger of contamination.

The Ratslayer is 22 inches long by 9-½ inches wide and 13 inches high. It operates from a standard 12 volt automobile battery and will kill approximately 100 rodents before recharging is required. It will function effectively in high and low temperatures and under moist conditions as well.

The device presents no hazard to pets, since access openings are designed for rat sized creatures. It eliminates the need for toxic chemicals which could contaminate food, affect the atmosphere or personnel working in the area. In addition, there is no possibility of dying rats escaping and decomposing in inaccessible locations.

In extensive field testing, the Ratslayer proved to be a highly effective means for controlling rodents and reducing damage to foods, crops, grain stores and other areas where rodents are a danger.

A dealer network is now being organized.

3-A Adopts New Standard, Amends Another

A new 3-A standard for pressure sensors and an amendment to the plastics standard were approved at the spring meeting of the 3-A Sanitary Standards Committees at Milwaukee April 5-7, 1977.

The pressure sensor standard sets sanitary guidelines for level control devices in various phases of fluid processing. The standard will become effective in mid-1978, after which time applications for use of the 3-A Symbol on equipment that complies with the standard may be filed with the 3-A Symbol Council. The amendment to the plastics standard provides criteria for the safe use of polyphenylene sulfide coating. It is effective August 6, 1977.

Highlight of the 3-A meeting was the award of the 3-A bronze plaque to Dick B. Whitehead for distinguished service to the 3-A Committees and the standards program. Whitehead has been a representative from the Mississippi Health Department for many years and the chairman of the Committee on Sanitary Procedures of the International Association of Milk Food and Environmental Sanitarians for more than 10 years. He retired from state employment in 1976.

Whitehead was cited for his successful efforts in coordinating the views of regulatory authorities in their continuing review of equipment standards. Whitehead credited the successes to the ongoing efforts of his committeemen, noting that 3-A accomplishments are "the work of many men."

In another presentation, Bruno Werra, metallurgist and engineer for the Ladish Co., Tri-Clover Division, was honored by Dairy and Food Industries Supply Association for two decades of service to the 3-A program. The special achievement award recognized his technologic counsel to the 3-A Committees in the areas of metallurgy, corrosion, and rubber, all affecting materials used in food processing contact.

Agenda items referred to the DFISA Technical Committee for additional work or future action included standards affecting cottage cheese vats; dry milk equipment for fillers, sifters, conveyors and collectors; culinary steam practices, and membrane processing equipment.

The 3-A program safeguards the public health through its standards and practices for the cleanability of dairy processing equipment to protect the product against contamination from the equipment itself or foreign elements of dust, dirt or liquids. The program is conducted through the voluntary participation of dairy processors, equipment manufacturers, public health officials and sanitarians and their trade and professional associations. In general, 3-A standards and practices are accepted in most public health jurisdictions at the federal, state and local level.

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Iowa Association Honors James Evers

James Evers of Cedar Rapids was awarded the Dr. M. P. Baker Award for being Iowa's outstanding sanitarian in 1976. The M. P. Baker Award was presented at the Iowa Association of Milk, Food and Environmental Sanitarians' (Iowa AMFES) Annual Meeting in March.

Mr. Evers is a graduate of Iowa State University and is now chief sanitarian for the City of Cedar Rapids. In addition, he is a former President of the Iowa AMFES.

Also at the Iowa meeting, Ferris Biggert received a Certificate of Recognition from the International Association of Milk, Food and Environmental Sanitarians (IAMFES) for his outstanding service in past years.

Mr. Biggert was chairman of the Local Arrangements Committee for the 1970 IAMFES Annual Meeting in Cedar Rapids. As a fieldman for Sanitary Dairies, Mr. Biggert was a pioneer in the industry's movement to bulk milk handling.

Two additional members were given the 20 year Certificate of Service by the Iowa AMFES. They were Mort Wilke from the Dubuque Department of Health and Duane Hagedon from the Sioux City Health Department.

IAMFES OFFICERS AND EXECUTIVE BOARD

President, HENRY V. ATHERTON, Dairy Building, University of Vermont, Burlington, Vermont 05401

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Future IAMFES Annual Meetings

August 14-18, 1977
Sioux City, Iowa

August 6-10, 1978
Kansas City, Missouri

August 12-16, 1979
Orlando, Florida

CLASSIFIED ADS

For Sale

Single Service milk sample tubes. For further information and a catalogue please write, Dairy Technology, Inc., P.O. Box 101, Eugene, Oregon 97401.

Index to Advertisers

1977 ANNUAL MEETING I.A.M.F.E.S.

Advance Registration Form
Sioux City, Iowa

64th Annual Meeting
August 14-18, 1977

ATTENTION: **Mr. Carl Webster, Chairman of Registration**
IAMFES
P.O. Box 284 - Waterloo, Iowa 50701

Advance Register and Save — Refundable if you don't attend
(Refund must be requested from Carl Webster before August 18, 1977.)

REGULAR REGISTRATION FEE			ADVANCE REGISTRATION FEE (If Registered prior to August 1)		
		Spouse			*Spouse*
REGISTRATION	$15.00	9.00	REGISTRATION	$12.00	$ 7.00
BANQUET	15.00	15.00	BANQUET	13.00	13.00
TOTAL	$30.00	$24.00	TOTAL	$25.00	$20.00

Name _____ (Last) _____ (First) Name _____ (Last) _____ (First)

Childrens First Names _____

Affiliate or Company _____

Address _____

City _____ State _____ Zip _____

Make checks payable to IAMFES — 1977 Meeting Fund and mail to Mr. Carl Webster

Please indicate your Professional Field

Check one
- ☐ Industry
- ☐ Regulatory
- ☐ Education
- ☐ Other _____ (Describe)

Check one
- ☐ Dairy
- ☐ Other Food
- ☐ Other _____ (Describe)

Check one
- ☐ Field Work
- ☐ Production
- ☐ Laboratory
- ☐ Other _____ (Describe)

1977 ANNUAL MEETING I.A.M.F.E.S.

Hotel Pre-Registration—Attention: Reservation Manager
Sioux City Hilton, 707 Fourth St., Sioux City, Iowa 51101
Telephone—(712) 277-4101

Reservations must be received by July 31, 1977.
Reservations will be held until 6:00 p.m. unless a later hour is specified.
Check out time is 1:00 p.m.

Arrival Date _____ Departure Date _____

Arrival Time _____ Means of Transportation _____

Name _____ (Last) _____ (First) Name _____ (Last) _____ (First)

Address _____

City _____ State _____ Zip _____

Please check type of accommodation required
- ☐ Single (one person) $20.00
- ☐ Double (two persons) $26.00

Family Plan: There is no charge for children when in same room with parents.

Free Parking when resident of hotel.
Send directly to: Reservation Manager, Sioux City Hilton.

THE ONLY *Approved*
SANITARY METHOD OF APPLYING
A U. S. P. LUBRICANT
TO DAIRY & FOOD
PROCESSING EQUIPMENT

Haynes Spray

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U.S.P. UNITED STATES PHARMACEUTICAL STANDARDS

CONTAINS NO ANIMAL OR VEGETABLE FATS. ABSOLUTELY NEUTRAL. WILL NOT TURN RANCID — CONTAMINATE OR TAINT WHEN IN CONTACT WITH FOOD PRODUCTS.

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This Fine Mist-like
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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.

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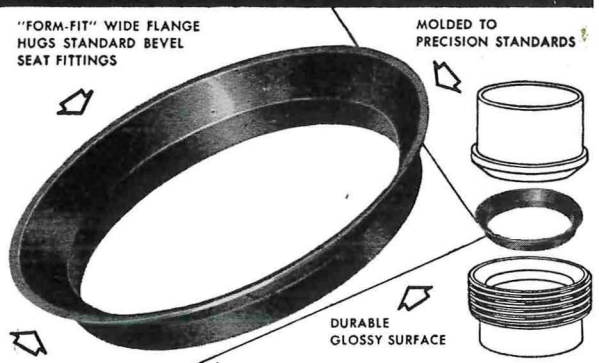
HAYNES-SPRAY INGREDIENTS ARE APPROVED ADDITIVES AND CAN BE SAFELY USED AS A LUBRICANT FOR FOOD PROCESSING EQUIPMENT WHEN USED IN COMPLIANCE WITH EXISTING FOOD ADDITIVES REGULATIONS.

HAYNES SNAP-TITE GASKETS



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Check these SNAP-TITE *Advantages*

- Tight joints, no leaks, no shrinkage
- Sanitary, unaffected by heat or fats
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- Eliminate line blocks
- Help overcome line vibrations
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Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

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SELF-CENTERING
SNAP-TITE® Gaskets

SIZES 1" - 1½"
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THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

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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...

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- *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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ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

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

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

THE HAYNES MANUFACTURING CO.
CLEVELAND, OHIO 44113

Stimulation: The First Step Toward A Better Harvest

Dr. Richard D. Mochrie
Professor, Animal Science
No. Carolina State University

It's milking time, and you are ready once more to harvest that milk crop you continue to work so hard for. You have provided the best nutrients to these dairy animals, and the best housing. You have raised them from calves and provided them with every benefit at your disposal to assure that they will be good producers. And now, they have just entered your milking parlor or are ready to be milked in the barn. Your next step, stimulation, will either make all your work worthwhile, or negate much of the effort you have put in.

Oxytocin Means Let Down

Stimulation is more than cleaning the udder before milking. Properly done, stimulation substitutes completely for the natural signal provided by the calf to tell the cow she is hungry. Oxytocin, a hormone released into the blood stream after stimulation, signals the milk making glands (alveoli) to release the milk they have produced. This squeezing out of tiny droplets of milk from each of the millions of alveoli is called "let-down." The let-down is directly related to the amount of oxytocin in the blood stream, and the amount of oxytocin present is directly related to the thoroughness of the stimulation.

Complementary Milk: Profits Left in the Udder

In tests conducted on a number of herds, we found that from three percent to twenty percent more milk was present in the udder than was being harvested, partly due to inadequate stimulation. The animals were first stimulated and milked in the normal way, by their regular milker, and production recorded just before complementary was obtained. Later, the cows were stimulated as usual and then, just before attaching the milking machine, they were injected with an adequate amount of oxytocin. The average cow gave in the area of ten percent more milk after receiving maximum stimulation with the additional oxytocin. This ten percent as complementary milk (instead of being part of the normal) represents profit lost for three reasons: First, this milk would not have been harvested during a normal milking. Second, the last of the milk is always richer in fat, and so the fat test would be lower. And last, with the complementary milk remaining in the alveoli, the cells become less active in producing milk. Over a normal lactation period, this can make a good cow produce far less than she is capable of. With proper stimulation, the amount of complementary can be reduced to about the same minimum as injecting oxytocin.

Thirty Seconds of Profitable Time

All results point to the fact that about thirty seconds is the amount of time necessary to achieve maximum stimulation and proper cleaning. This should be a vigorous massage—preferably with a disposable paper towel. Less time fails to provide the amount of needed oxytocin, and more than thirty seconds of stimulation does not increase the level. Time spent stimulating the animal will determine if she has received an adequate natural signal to allow maximum let-down. The thirty seconds you spend on each cow to assure proper stimulation may well be the most profitable time you use on the farm.

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Babson Bros. Co.,
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We make your cows worth more.