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## Cranberry Pigments as Colorants for Beverages and Gelatin Desserts

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### ABSTRACT

A dry-powdered pigment extract was prepared from dried cranberry pomace by methanol extraction, concentration and spray drying. The extract was added to a cherry-flavored beverage mix and a strawberry-flavored gelatin dessert mix. The former was packed in foil/paper pouches and the latter in polyethylene/paper pouches. Storage studies were conducted at 40, 75, and 100 F with relative humidity (RH.) values of 10, 40, and 90%, respectively. All were evaluated for color, flavor, and pigment stability versus samples containing Red No. 2. Samples stored at temperatures up to 75 F had considerable breakdown after 12 weeks of storage but still showed acceptable color. Pigment stability was greatly increased with nitrogen flushing, and decreased with increasing temperature of storage, reaching a maximum at 100 F at 90% RH. The pigment extract imparted an astringent flavor which could be detected by triangular panels, and indicated the need for some type of "clean-up" procedure.

Nature's insistence on coloring natural foods has created a learned response in the consumer to judge the quality of food, to a large degree, on the basis of color.

Recent regulatory decisions along with increased demands for the abstract quantity called "absolute safety" have either removed or placed at risk many of the colorants which have been commonly used. This phenomenon has led to an increased interest in other colorants, particularly those from natural sources.

The American cranberry (*Vaccinium macrocarpon*, Ait.) has undergone extensive investigation in terms of the anthocyanin pigments present (8,12), extraction from pomace (3) and many studies involving degradation and stabilization in cranberry products, as reviewed by Francis (5). However, little work has been carried out to investigate the potential of cranberry anthocyanins as colorants in foods.

This study was initiated in an attempt to define the stability characteristics of cranberry anthocyanins in actual food products. Certainly, any colorant from a natural source has theoretical potential in to-day's environment; however, it is hoped that this study will

define better, actual behavior in high-acid foods, such as gelatin and beverage base mixes, during production, storage and use.

### MATERIALS AND METHODS

Cranberry anthocyanin pigments obtained from dried pomace have previously been used as a possible source of increased color in cranberry cocktail (2).

In this study two 50-lb. bags of dried pomace were obtained from the Ocean Spray Co., Hanson, Mass. and stored at -20 F until needed. The pomace was ground in a Fitzpatrick Mill (Fitzpatrick Co., Chicago, IL) using a 10-mesh screen to increase the surface area for extraction, which was carried out with an ethanol-citric acid (0.01%) solvent in a 3:1 solvent to pomace ratio and pumped in a continuous circulatory fashion for 2 h resulting in a percolation solvent extraction system. The solvent extract was drawn off and the pomace remaining was pressed under 35,000 lbs. (Wabash hydraulic press, Wabash Products, Inc., IN). This press extract was added to the original solvent extract, filtered through No. 1 Whatman filter paper and concentrated under vacuum using a pilot-plant-scale vacuum pan (Hamilton Kettle, Division of Brighton Corp., Cinn., OH). The vacuum pan concentrate was spray dried using Morrex 1918 (10-13 D.E.) as a carrier agent, as described by Main et al. (9).

#### *Product, package, and storage studies*

Two foods used in this study consisted of a colored and colorless low-acid beverage mix and a powdered gelatin dessert mix. The ingredient line for the commercial beverage mix was sugar, citric acid, monocalcium phosphate, artificial flavor, vitamin C, with or without artificial color, vitamin A and BHA. The ingredient line for the commercial gelatin dessert mix was sugar, gelatin, adipic acid, sodium citrate, fumaric acid, artificial flavor, and with or without artificial color. The control products were colored with Red #2. The colorless bases used in the study omitted Red #2. The actual bases used in this study were obtained from a commercial source and the actual ingredient proportions are unknown to the authors.

Spray dried powder containing cranberry anthocyanins was used to color the colorless bases. The level of spray dried product was determined visually such that upon rehydration, the most visually acceptable color, in comparison to the FD&C Red #2 control, was selected.

The sample variables chosen for the study were as follows: (a) cherry beverage mix colored with cranberry anthocyanin (Acy) packed in foil/paper under ambient conditions; (b) gelatin dessert mix colored with cranberry Acy packed in pouch paper under ambient and nitrogen atmospheres; and (c) gelatin dessert mix colored with FD&C Red #2 packed in pouch paper under ambient conditions.

<sup>1</sup> General Foods Corporation, Technical Center, Tarrytown, New York.

To produce a manageable number of variables only one sample was packed under nitrogen to evaluate the atmospheric effect.

The cranberry color base was mixed at the selected concentrations (9.51 g of cranberry powder/100 g of colorless strawberry gelatin base and 16.6 g of cranberry powder/100 g of colorless cherry beverage base) and dry-blended until a uniformly colored powder was obtained (9).

After mixing, the dry-powdered batches were weighed and placed into separate packages (12.00 g of cherry beverage/pouch and 21.25 g of strawberry gelatin dessert/pouch; one pouch makes 118 ml of final product). Packages used for the cherry beverage were foil (0.285 mil) pouch paper laminates. The strawberry gelatin (except for nitrogen packs) used pouch paper laminated with 1.5-mil polyethylene (Reynolds Metals Co., Richmond, VA). The packaging material was selected to simulate commercial packaging of dry beverage mixes and gelatin dessert mixes, respectively. Ambient pouches were simply heat sealed while the nitrogen packs were flushed with nitrogen gas by inserting a hypodermic needle into the corner of the pouch. After allowing 5-10 sec for nitrogen flushing, the needle was removed and the pouch was quickly heat sealed. All nitrogen packs were packed in 1-mil foil pouches.

Control samples were frozen and stored at -20 F while other samples were stored at 40, 75, 100 F with various external relative humidity values. Storage studies at 100 F and 90% R.H. were conducted in an environmental chamber (Hotpack, Phila. PA) to evaluate accelerated degradation. Studies at 75 F were conducted in a room which was equipped with positive pressure and produced a R.H. of 40% to simulate more normal storage conditions. Samples at 40 F were stored in a refrigerator with a R.H. of 10% to simulate refrigeration storage, while the 100-F samples were held in an oven with R.H. of approximately 20-30%. Degradation of pigment and subsequent changes in color were monitored by chemical and instrumental means as well as by a panel of trained judges.

#### Flavor evaluation

Although this study was primarily concerned with color, simple flavor evaluations were made. Triangle tests are useful in determining flavor differences and can be applied in situations where a product has been modified or altered by ingredient substitution (10).

In this study a modification of the triangle test, better known as the Duo-Trio Test, was used. In addition to obtaining information on flavor differences, a question of the flavor acceptability of the chosen odd sample as compared to the reference was included.

The sensory evaluation test was aimed at determining if there was a detectable difference in samples colored with cranberry anthocyanins and if their acceptability is better, comparable or poorer than that of the control.

#### Refrigeration and light stability study

A short-term color-stability study was done on the effects of light and length of refrigeration storage time on both of the reconstituted prepared powdered products. These tests were deemed necessary because any powdered product must be stable for a reasonable time after reconstitution with water. The effect of light is of major interest with gelatin desserts which sometimes are sold in restaurants from a display case which is refrigerated but open to light. Also, beverages may be exposed to sunlight if consumed outdoors. It should be noted that the light stability tests are very limited, but were deemed to be of value as a preliminary screening. If the product was unstable to this degree of light stress, then it would be pointless to investigate more fully.

To evaluate these parameters, the dry packaged materials were prepared according to consumer directions, placed in window light for 1 day and the color measured after 1 and 3 weeks of refrigeration. Two-mm cells were used to obtain color data on the General Electric Recording Spectrophotometer and Gardner XL-10.

#### Determination of total anthocyanin content and degradation index

In this study, pigment analysis was to be done on stored samples which may have appreciably degraded. For this reason the pH differential method for total anthocyanins developed by Fuleki and Francis (7) was used. This method involves measurement of optical density (O.D.) at 510 nm (Hitachi Perkin-Elmer Model 139 Spectrophotometer) of

samples at pH 1.9 and pH 4.5. This, in turn, allows not only calculation of total anthocyanin but also calculation of the Degradation Index (DI). The DI measures the percentage of degraded anthocyanin pigment in the sample and thus as degraded pigment increases the DI increases.

#### Colorimetric analysis

Colorimetric data were obtained by use of the following instruments: General Electric Recording Spectrophotometer (GERS) (Diano Corp., Foxboro, MA) equipped with a tristimulus integrator (Davidson and Hemmendinger, Inc., Easton, PA). All samples were measured in 2-mm cells at 30 C and raw data were converted to the following functions:  $(X^2 + Y^2)^{1/2}$ ,  $(X^2 + Z^2)^{1/2}$ ,  $X/Y$ ,  $(Y^2 + Z^2)^{1/2}$ , dominant wavelength and purity. A Gardner XL-10 Colorimeter (Gardner Laboratories, Bethesda, MD) was used to obtain data for the following functions:  $L$ ,  $a$ ,  $b$ ,  $Y$ ,  $a/b$ , arctangent  $a/b$ ,  $(a^2 + b^2)^{1/2}$ , and the Scofield-Hunter color difference (6).

#### Visual color evaluation

Storage samples were refrigerated overnight before visual assessment. Sample sets consisted of a frozen control (-20 F) and four samples representing various storage temperatures and relative humidity. The reference sample (-20 F) was initially presented alone to the panelists in a consumer type clear drinking glass or clear dish. The panelists were asked to evaluate the overall color quality of the sample as a cherry-colored beverage or a strawberry gelatin dessert and to check the appropriate scale (1-9) from very poor to excellent. This provided eight replications of the assessment of the color quality of the products with grape anthocyanin versus those with Red No. 2. Following this the four other coded samples representing the four conditions of storage, were randomly placed next to the reference control one sample at a time. The panelists were asked to note the color difference on a scale from 0 corresponding to no change up to 10 corresponding to an extremely large difference. The bigger the difference, the less acceptable is the product.

Seven judges with normal color vision were used throughout the study. Viewing was conducted with the MacBeth Lablite (MacBeth Daylighting Corp., Newburgh, NY) to obtain controlled daylight illumination.

All results were analyzed statistically by the use of a computerized analysis of variance (ANOVA) and Duncan's New Multiple Range Test (11).

## RESULTS AND DISCUSSION

### Correlation of visual versus objective techniques

The initial data analyses of this study were concerned with identifying colorimetric or chemical functions which correlated highly with visual assessment of color during storage. Although this was not a prime objective of the study, it was felt that such information would be valuable to establish objective techniques for the analysis of natural pigments in food products and is therefore included.

Results indicated that in the cherry-beverage system, ambient packs, all physical variables except for  $Y$ ,  $DI$ , and  $(a^2 + b^2)^{1/2}$  had correlations of  $r = 0.82$  or greater with visual assessment and with each other. Under nitrogen in the same pack these correlations decreased dramatically to  $r$  values ranging from 0.28 to 0.78. This same trend was observed in an earlier study with grape anthocyanins (4) and it was postulated that under nitrogen there was less pigment degradation and, therefore, less accuracy in differentiating samples.

Some 21 color functions were correlated with visual assessment and with each other but for the sake of brevity further detail will be omitted.

These results, along with earlier work (4), point out an interesting and important fact about choosing colorimetric techniques for quality assessment. It is essential to reevaluate the correlation between visual and physical color assessment when changing a product line and utilizing colorimetric techniques.

#### Assessment of color acceptability

To evaluate the cranberry anthocyanins it was necessary to establish, first, a final product color which was acceptable to the consumer. That is, the stability of the anthocyanins should be judged at a concentration level which is similar to that which might be used at the consumer level. Results of a visual panel color quality evaluation are shown in Table 1. By means of eight replications and seven judges a mean score was established which indicates that the anthocyanin pigment allows a product to be made which, although not as good as Red No. 2, is acceptable.

#### Visual assessment of storage samples

Analysis of variance (Table 2) of cherry beverage, colored with cranberry anthocyanin shows that the storage treatments, weeks of storage and their interactions were all significant. Duncan's Multiple Range

Test on the storage conditions demonstrate that the high temperature, high humidity storage condition is significantly different from all other storage conditions. The significant visual color changes (Table 2) seem to occur first at week 6 and 8 which were significantly different from all other weeks of storage and second at week 12 and 16, which were also significantly different from all other weeks.

Table 3 shows the results of analysis of strawberry gelatin dessert colored with cranberry anthocyanin packaged under ambient conditions. These results indicate that storage conditions, weeks of storage, and their interactions were all significant. Duncan's Multiple Range Test reveals that the high temperature, high humidity storage condition again was the only treatment which was significantly different from all other storage treatments. Duncan's analysis of time is more complex and shows that color changes seemed to occur gradually throughout storage instead of at a particular time. This was indicated by the many similar groupings of mean values for each week which overlap into other groups of similar means. The only obvious point of visual color change was between week 4 and week 6. This can be seen by Duncan's test where the break in overlapping lines occur.

TABLE 1. Visual panel color quality evaluation of reference samples judged on a (1-9) scale over the storage period.

Sample	Number of Judges	Number of replications	Mean color <sup>a</sup> quality score	Std deviation
Cherry beverage with cranberry Acy - ambient	7	8	7.32	1.18
Strawberry gelatin dessert with cranberry Acy - ambient	7	8	7.00	1.44
Strawberry gelatin dessert with grape Acy - nitrogen	7	8	7.20	1.34
Cherry beverage with Red No. 2- ambient	7	8	7.89	1.02
Strawberry gelatin dessert with Red No. 2 - ambient	7	8	7.55	1.08

<sup>a</sup>1 - very poor, 3 - poor, 5 - fair, 7 - good, 9 - excellent.

TABLE 2. Analysis of variance of cherry beverage mix colored with cranberry anthocyanin packed under ambient conditions. (Results of visual scoring test for degree of color difference).

Source of variation	df	SS	MS	F ratio test (from EMS)	F value	Significance
Treatment	3	125.51	41.84	T/R:T	15.27	.5%
Weeks	7	170.55	24.36	W/Error	13.46	.5%
Replication: treatment	24	65.79	2.74	No Test	—	
Treatment: weeks	21	242.06	11.53	TW/Error	6.37	.5%
Error (WR:T)	168	303.64	1.81			
Total	223	907.55				

Duncan's Multiple Range Test - among the storage treatment means.<sup>a</sup>

100 F Ambient		40 F Ambient		75 F Ambient		100 F-90% Relative humidity	
9.5		9.63		10.25		21.88	
Week 1	Week 2	Week 3	Week 4	Week 6	Week 8	Week 12	Week 18
1.0	1.0	1.0	1.29	2.86	3.71	5.71	8.43

Variables (temperature, humidity, time) which are not significantly different from one another are underlined.

<sup>a</sup>Tested at the 1.0% level of significance.



TABLE 3. Analysis of variance of strawberry gelatin dessert mix colored with cranberry anthocyanin packed under ambient conditions. (Results of visual scoring test for the degree of color difference.)

Source of variation	df	SS	MS	F ratio test (from EMS)	F Value	Significance
Treatment	3	720.77	240.26	T/R:T	210.75	.5%
Weeks	7	174.28	24.90	W/Error	9.02	.5%
Replication: treatment	24	27.25	1.14	No Test	—	
Treatment: weeks	21	208.34	9.92	TW/Error	9.92	.5%
Error (WR:T)	168	463.75	2.76			
Total	223	1594.39				

Duncan's Multiple Range Test - among storage treatment means.<sup>a</sup>

75 F Ambient		40 F Ambient		100 F Ambient		100 F-90% Relative humidity	
10.75		11.13		13.75		40.75	
Week 2	Week 1	Week 3	Week 4	Week 6	Week 8	Week 16	Week 12
1.14	3.43	3.86	4.86	7.29	8.14	8.43	9.43

Variables (temperature, humidity, time) which are not significantly different from one another are underlined.

<sup>a</sup>Tested at the 1.0% level of significance.

Results of strawberry gelatin dessert mix colored with cranberry Acy packed under nitrogen exemplify the finding that moisture pick-up of dry products is the most detrimental factor in degradation of color.

Table 4, Analysis of Variance of visual color differences shows, again, that storage treatment, weeks of storage, and interaction during storage are all significant from the standpoint of visual color changes. Duncan's Multiple Range Test, which is used to separate the interactions, shows that the high temperature, high humidity storage condition was the only treatment which was significantly different from all other treatments, which were all similar to one another. Duncan's test on storage week means indicates that a number of groups of means are not significantly different from one another. This makes it apparent that a slower, more uniform rate of color change was occurring. This indicates better stability of the foil-packaged, nitrogen-swept product and suggests that the type of package and/or nitrogen

make a substantial difference in stability and product shelf life.

#### Objective assessment of storage samples

In general, objective color measurements confirmed results of visual assessment as discussed previously. However, an interesting phenomenon occurred which should be pointed out because it illustrates some of the problems which might be encountered if colorimetric data are not examined by both visual and chemical means.

During degradation of anthocyanins browning compounds are formed which affect a number of colorimetric functions such that they first decrease with a loss of pigment and then increase due to formation of browning compounds. This was apparent with the following color functions: Gardner L which increases with initial loss of pigment and increased %T, and then decreases with browning as %T is decreased. C.I.E. X and Z decrease

TABLE 4. Analysis of variance of strawberry gelatin dessert mix colored with cranberry anthocyanin packed under nitrogen. (Results of visual scoring test for the degree of color difference.)

Source of variation	df	SS	MS	F ration test (from EMS)	F value	Significance
Treatment	3	89.73	29.91	T/R:T	13.98	.5%
Weeks	7	127.53	18.22	W/Error	7.29	.5%
Replication: treatments	24	51.29	2.14	No Test	—	
Treatment:weeks	21	75.09	3.58	TW/Error	1.43	.5%
Error(WR:T)	168	419.38	2.50			
Total	223	763.02				

Duncan's Multiple Range Test - among storage treatment means.<sup>a</sup>

40 F Ambient		75 F Ambient		100 F Ambient		100 F-90% Relative humidity	
10.75		12.0		14.0		22.13	
Week 3	Week 4	Week 1	Week 6	Week 8	Week 2	Week 12	Week 16
.71	1.86	2.29	2.59	3.43	4.0	5.0	5.43

Variables (temperature, humidity, time) which are not significantly different from one another are underlined.

<sup>a</sup>Tested at the 1.0% level of significance.

on pigment loss and then increase on browning, which may be explained by the increased color intensity at the lower end of the visible spectrum, 400nm - 460nm, due to browning. This increased intensity would be reflected in an increased response in the  $\bar{z}$  and  $\bar{x}$  standard observer curves and thus an increase in X and Z values. The y chromaticity coordinate which is a function of lightness also follows the increase with loss of color then the decrease with browning and decreased lightness. Purity which is a measure of color intensity shows the color reversal effect very well; purity decreases on pigment loss early in storage, then reverses itself and increases with formation of browning compounds. The tristimulus X value and chromaticity coordinate x would be expected to increase on browning, but do not in this instance. This could mean that loss of red has a greater effect than occurrence of browning.

The color measurements which do not show any trends in reversal due to browning are Gardner a which continues to decrease with loss of redness. The  $\Delta E$  color difference continues to increase. The DI which increases with loss of pigment, browning formation, and decrease in total anthocyanin content continues to decrease until all pigment is degraded.

It becomes apparent from these results that care must be taken in adopting a colorimetric function without first insuring that it measures the desired visual or chemical parameters.

Since the most significant changes occurred at high-temperature high-humidity storage (100 F-90% R.H.) it was decided to discuss the anthocyanin degradation pattern in detail only under these conditions.

Figure 1 shows the effect of storage at 100 F and 90% R.H. on total anthocyanin concentration in cherry beverage mix packed under ambient conditions and strawberry gelatin dessert packed under ambient conditions and nitrogen. It is apparent from Fig. 1 that

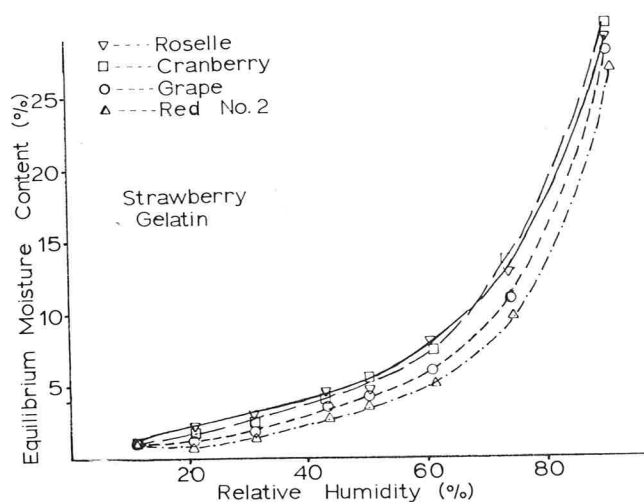


Figure 1. The effect of storage at 100 F-90% relative humidity on the total anthocyanin concentration of cherry beverage mix and strawberry gelatin dessert mix colored with cranberry anthocyanins and packed under ambient and nitrogen conditions.

stability of the anthocyanins was much greater in the nitrogen pack. This result was also obtained by Clydesdale et al. (4) in an evaluation of Concord grape pigment and is not unexpected when the ingredient line of the product is examined. The presence and subsequent effect of reducing sugars or anthocyanins would be expected to be different under ambient conditions than under nitrogen and even without other ingredients, an inert environment would be expected to confer greater stability. It may also be seen (Fig. 1) that the stability of the anthocyanins was very similar in both the beverage and the dessert under ambient conditions, thus showing that in this instance the product line was much less important than the environment.

Studies of the degradation index confirmed the trends found for total anthocyanins. However, it should be noted that the DI did not correlate well with visual assessment in this study and, therefore, would not be a good multi-purpose physical parameter.

#### Flavor difference evaluation

Table 5 gives results of the flavor difference evaluation. Judges consistently ranked the cranberry anthocyanin products poorer than the Red #2 control. This ranking was due to a strong dislike of the astringent note imparted by the cranberries. This points out the need for some type of flavor "clean-up" before consideration of astringent materials as natural colorants.

#### Effect of refrigeration and light on color stability

The stability of cranberry anthocyanins in the beverage mix and the gelatin dessert was excellent after 1 day of sunlight and 1 week of refrigeration as judged by colorimetric analysis. It was found that only minimal degradation of color occurred after 3 weeks in the refrigerator, at which time mold became the problem, not color. In all areas evaluated Red No. 2 remained virtually unchanged from the initial sample.

TABLE 5. Flavor difference evaluation (duo-trio) for experimental cranberry anthocyanin colored food products versus Red No. 2 colored products.

Sample	No. of judges	No. of correct responses	Significance	Flavor acceptability comments <sup>a</sup>
Cherry flavored beverage mix - cranberry Acy	12	12	0.1%	B = 0 C = 1 P = 11
Gelatin dessert mix cranberry Acy	12	10	5%	B = 0 C = 0 P = 10

<sup>a</sup>B = The number of responses rating the experimental sample better than Red No. 2.

C = The number of responses rating the experimental sample comparable to Red No. 2.

P = The number of responses rating the experimental sample poorer than Red No. 2.

## CONCLUSIONS

This study was initiated due to interest in use of natural materials as possible sources of food colorants.

The investigation involved evaluation of cranberry anthocyanins as potential colorants in dry-pack food products such as dry-mix beverages and gelatin dessert mix. As well, several physical functions were evaluated as possible quality control tools for such products.

Some 21 colorimetric functions were evaluated as predictors of visual assessment. All functions, with the exception of Y, DI, and  $(a^2 + b^2)^{1/2}$  showed high correlations under ambient environment. These correlations decreased, however, under nitrogen environment possibly because there was less pigment degradation and, therefore, less color difference which affected accuracy of measurement.

Color stability of the cranberry pigments was less than that of Red No. 2, as judged by subjective visual means as well as by objective chemical and colorimetric methods. It was found that significant changes occurred first at between 6 and 8 weeks of storage and secondly at between 12 and 16 weeks of storage in the beverage while the gelatin product showed a more gradual degradation of pigment. However, increases in stability were obtained with a nitrogen environment.

In an earlier study with anthocyanins from Concord grapes (4), similar results were noted but the grape pigments seemed to possess greater stability on storage.

A limited evaluation of the final reconstituted product indicated that light and refrigeration did not adversely affect the product. However, use of cranberry anthocyanins produced a generally unacceptable flavor, indicating a need for "clean-up" before use as a food colorant.

Results of this study indicate that the cranberry anthocyanin extract, as prepared for this study, possess a limited potential for use in dry-pack food products.

#### ACKNOWLEDGMENTS

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## *A Research Note*

# Chlorinated Pesticide Residues in Butter from the Tehran Region

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### ABSTRACT

A survey conducted during 1976-1977 on chlorinated pesticide residues in 105 samples of butter from the Tehran region revealed the presence of lindane, DDT with its metabolites DDE and DDD, and dieldrin in 100-93-100-100 and 73 samples, respectively. The means and ranges in ppm were .11 (.041 to .24), .037 (.011 to .82), .053 (.019 to .45), .034 (.001 to .11) and .015 (.001 to .09) for lindane, DDT, DDE, DDD and dieldrin, respectively. The mean concentration of chlorinated pesticide residues were lower than tolerances established by the World Health Organization for dairy products which are in ppm 1.25 for DDE, DDD and DDT singly or in combination, 0.15 for dieldrin and 0.2 for lindane. The reason for the low values might be zero grazing, use of partly dehydrated feeds and recent curtailment in use of these pesticides.

For many years chlorinated pesticides have been used in Iran for agricultural purposes and to control insect-borne diseases of man. These pesticides can contaminate milk and milk products through direct application to dairy herds and barns; spraying of pastures, feeds, silage, water, air, soil and through drift from neighboring farms. In some instances fodder and soil are probably the most important sources of contamination. Though the import and use of many chlorinated pesticides has been restricted since July 1976, specific uses in agriculture and in vector control programs is continuing. Owing to the persistence of organochlorine pesticides in the environment it is expected that they will be present for many years.

The purpose of this study was to determine the presence and amounts of these pesticides in butter and thus to learn how this food contributes to the total dietary intake of these residues by man.

### MATERIALS AND METHODS

One hundred and five samples of butter were purchased from various stores and retail shops throughout the Tehran area and brought to the laboratory. The samples were analyzed by the method of the A.O.A.C. (7). Five microliters of an extract obtained with either 6 or 15% diethyl ether in petroleum ether were injected into a Varian Aerograph series 1400 gas chromatograph equipped with <sup>63</sup>Ni electron capture detector and a 1518 × 3.2 mm O.D. pyrex glass column packed with 5% DC-200 on Chromasorb W 60/80 mesh and a Perkin-Elmer gas chromatograph 910 with <sup>3</sup>H electron capture detector and pyrex glass column 1518 × 6.25 mm O.D. packed with 10% OV-1 on Varaport 30 for validation of chromatogram peaks. (6,9,13). Operating conditions for the Varian Aerograph were 220, 180, 250 C and for the Perkin-Elmer

gas chromatograph 200, 180, 200 C for injector, column and detector, respectively. In both gas chromatographs nitrogen flow rate was 40 ml/min and recorder speed adjusted to 0.5 cm/min. The residues in the samples were identified by reference to retention times of chromatographic peaks of standard organochlorine pesticides supplied by Polyscience Inc. Because all analyses were done at isothermal and isobaric conditions, peak heights alone were used for quantitation. The average recoveries of DDT, DDE, dieldrin and lindane were 83, 87, 85 and 82%, respectively. The data do not include corrections for percentage recovery.

### RESULTS AND DISCUSSION

Sources of contamination of milk and milk products by chlorinated pesticides are: control of parasites on the animal, insect control in stables, fodder (pasture, forage and supplement) contamination from the environment (water, air, soil) and accidents and negligence (12). Use of persistent organochlorine compounds in a region can be and often is reflected in contamination of the milkfat produced in that region. Hence, when use of pesticides is restricted, this is likewise reflected by contaminants in milkfat (3). During this study chlorinated pesticides were in use in Iran. However, due to restriction in use and total ban on import of DDT it is expected that the residue problem will decrease. It is too early to determine how rapidly the recent curtailment in use of DDT will be reflected in reduction of residues in milk products because the persistence of chlorinated pesticides in the environment means that some residues will be found for many years. Long persistence of many of these pesticides in soil results in forages grown on land after properly treated crops to have an unacceptably high residue level; a more serious situation results from improper use of these pesticides on the dairy herd or from use of treated seeds as feedstuffs (4).

In this study lindane and DDT and its metabolites DDE and DDD were detected in approximately all, and dieldrin in 71% of samples tested (Table 1). The range of residue concentrations (ppm) was lindane, .046 to 0.24 (mean .11); DDE, .019 to .45 (mean .053); DDD, .001 to .11 (mean .034); DDT .011 to .82 (mean .037); and dieldrin .001 to .09 (mean .015) (Table 1). The mean concentration of total DDT compounds (.124 ppm), dieldrin (.015) and lindane (.11 ppm) were far less than tolerances accepted by the World Health Organization, which are 1.25 ppm for DDT compounds, 0.15 ppm for

TABLE 1. Ranges and mean concentrations of organochlorine pesticide residues in the 105 samples of butter.

Pesticide	Positive samples (Number)	Range (ppm)	Mean (ppm)
lindane	100	.047 - .24	.11
DDE	100	.009 - .45	.053
DDD	100	.001 - .11	.034
DDT	93	.011 - .82	.037
Dieldrin	71	.001 - .09	.015

dieldrin and 0.2 ppm for lindane, respectively (11).

The concentration of DDT compounds in 48% of samples was less than 0.1 ppm. After DDT is applied dermally to a dairy herd, which occasionally is done, its concentration in the milk and hence in butter is approximately equal to that of DDD. However, after ingestion the concentration of DDD is much greater than that of DDT; this undoubtedly results from metabolism of microorganisms in silage and in the rumen and is useful in predicting the source of residues in milk and milk products (8). In the present study, metabolites of DDT constituted approximately 70% of total DDT compounds in butter with the level of DDD being slightly less than that of DDT (Table 1). DDD never occurs as a single contaminant; the more slowly removed compounds, DDE and DDT, will, in effect, control the rate and the time at which milk and milk products become marketable.

Lindane is present in some powders or sprays employed for exoparasite control on cows and its use is not restricted in Iran. Dieldrin is used most effectively against malaria. It has been found that a given intake of DDT from environmentally contaminated feeds resulted in larger fractions of DDT and its metabolites in milk than when DDT in oil solution was fed. This difference has been attributed to such factors as a longer retention time in the rumen for DDT from environmental sources. The most important milk contaminant is DDE when exposure is from an environmental source while DDD and DDT are more important when DDT is fed directly (5). The concentration of DDT, DDE and dieldrin decline in the milkfat at a rate of about 1% per day (10). The concentration of DDT compounds in butter was far less than that in human milkfat in Iran (7).

The organochlorine pesticides will co-distill with water and therefore one might expect removal of some of these pesticides either in the drying process or by washing with various solvents. In general, it has been found that drying a wet material will remove some of the residues. Under

realistic conditions removal of 50% of DDT residues by commercial dehydration is about what can be expected (4). The low concentration of organochlorine pesticide residues in butter from the Tehran Region in comparison with results from other regions (6,13) reflects the lower contamination from environmental and feed sources. In this respect, recent restriction in use of DDT, zero grazing, hot climatic conditions, use of dehydrated feeds such as dried beet cake and left over dried bread crumbs as part of the feed for dairy herds may be the principal factors involved.

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## Roselle (*Hibiscus sabdariffa* L.) Anthocyanins as Colorants for Beverages and Gelatin Desserts

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### ABSTRACT

A dry powdered pigment extract was prepared from a Roselle liquid concentrate obtained from Trinidad. The pigment base was added to two dry pack foods, a beverage mix and a gelatin dessert, and evaluated under various temperature and humidity conditions. During a 16-week storage period, color, flavor, and pigment stability were evaluated versus those of Red No. 2. All samples were less stable than those with Red No. 2 but showed good stability up to 4 weeks of storage, after which, the rate of pigment degradation accelerated. Reconstituted products showed good light and refrigeration stability but the roselle powder imparted an unacceptable flavor to the products.

Roselle (*Hibiscus sabdariffa* L.) is an annual shrub 1.5-2.5 m in height, which produces fleshy, red, edible calyces and fiber. It belongs to the family *Malvaceae* (4,11) and is related to okra and cotton (3).

Roselle has been used not only as a fruit for making jelly, jams, preserves, chutnies, flavor extracts, sauces, beverages and wine (1,4,6) but also as a source of plant fiber known as "roselle hemp" (1), "India rosella hemp", "rosella fiber", "rosella hemp" and "pusa hemp" (3) for use in making clothing, linen, fishing nets, ropes, and the like. Also, the leaves may be used in salads and the seeds as a source of oil (3).

Due to its widespread use, interest developed in the potential of roselle as a food colorant. Du and Francis (5) investigated the pigment composition of roselle. They also found the pigment content to be quite high, 1.5 g of anthocyanin per 100 g of dry weight roselle calyces. Esselen and Sammy (6) pointed out that the spectral transmission curves for roselle are very similar (Fig. 1) to the curves of Red No. 2 (amaranth). The reason for this is that the major pigments in roselle are cyanidin and delphinidin derivatives which produce this particular hue (5).

Recent studies (2,3) have investigated the stability of both grape and cranberry anthocyanins in dry-pack

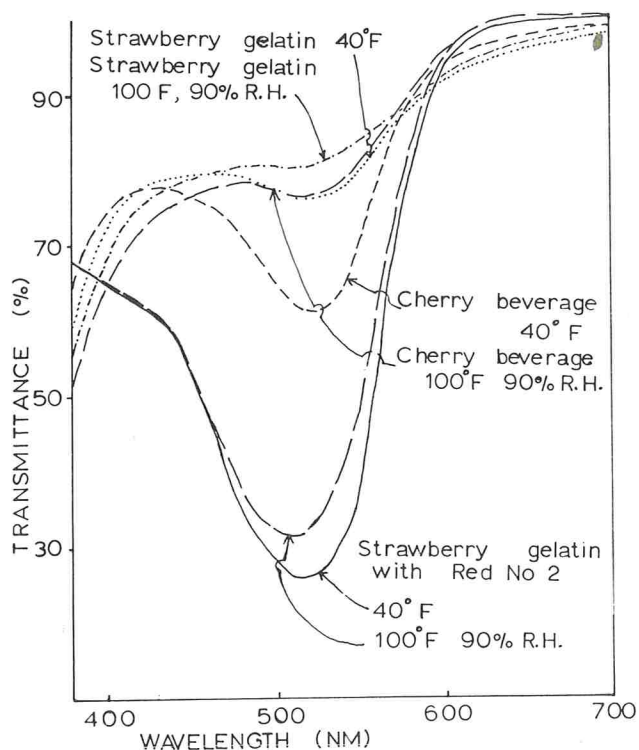


Figure 1. Spectral curves of cherry beverage and strawberry gelatin desserts colored with Roselle pigments and stored at 40 F and 100 F/90% R.H. for 16 weeks. Curves of strawberry gelatin colored with Red No. 2 are included for comparison.

foods. It was found that grape anthocyanins had greater potential than cranberry anthocyanins based on stability and flavor characteristics. This study was initiated to extend the evaluation of anthocyanin sources in such foods. Roselle was chosen because of its former food uses, high pigment content and spectral similarity to Red No. 2.

### MATERIALS AND METHODS

The Roselle pigments used in this study were obtained from Trinidad as a liquid concentrate<sup>2</sup>. The extraction and concentration process

<sup>1</sup>General Foods Corporation, Technical Center, Tarrytown, NY.

<sup>2</sup>Courtesy of Dr. George Sammy, University of West Indies, Trinidad.

involved extraction of 1 lb. of fresh Roselle calyces with 1 gal. of hot water producing a "single strength" extract. This extract was concentrated under vacuum to approximately a 10 to 1 liquid concentrate (7). A total of 3,000 ml of Roselle concentrate was obtained for spray drying as described by Main et al. (10).

The foods used in this study were a colored (with Red No. 2) and colorless low-acid beverage mix and a powdered gelatin dessert. The colorless bases used did not contain Red No. 2 and their ingredient line was reported previously (2,3).

The spray dried Roselle powder was used to color the colorless bases. The amount of powder required to produce a product comparable to the control was determined by a visual panel.

The sample variables chosen for the study were as follows: (a) cherry beverage mix colored with Roselle anthocyanin (Acy), packed in foil and held under ambient condition, and (b) gelatin dessert mix colored with Roselle Acy, packed in pouch paper and held under ambient condition.

Previous studies (2,3) indicated the added stability which a nitrogen environment confers upon anthocyanin pigments. Therefore, it was decided to omit this variable since the effect of nitrogen in a dry-pack system had been established previously.

The Roselle powder was mixed at the concentrations established by the visual panel (8.46 g of Roselle powder/100 g of colorless strawberry gelatin base and 14.81 g/100 g of colorless cherry beverage base) and dry blended until a uniformly colored product was obtained (10).

After mixing, the dry-powdered batches were weighed and placed into separate packages (12.00 g per cherry beverage and 21.25 g per strawberry gelatin dessert pouch). One pouch makes 118 ml of final product. Packaging material was selected to simulate commercial practices [cherry beverage package - foil (0.285 mil) pouch paper laminates, strawberry gelatin package - pouch paper laminated with 1.5 mil polyethylene].

Control samples were frozen and stored at -20 F while other samples were stored at 40, 75 or 100 F with varying external relative humidity. Storage studies at 100 F and 90% R.H. were conducted in an environmental chamber (Hotpack, Philadelphia, PA) to evaluate accelerated degradation. Studies at 75 F were conducted in a room which was equipped with positive pressure and produced a R.H. of 40% to simulate more normal storage conditions. Samples at 40 F were stored in a refrigerator with a R.H. of 10% to simulate refrigeration storage, while the 100-F samples were held in an oven with R.H. of approximately 20-30%.

Degradation of pigment and subsequent changes in color were monitored by chemical and instrumental means as well as by a panel of trained judges. Anthocyanins were analyzed by the pH differential method (9) which also allows calculation of the degradation index. Further details have been discussed in other related studies (2,3).

Colorimetric data were obtained by use of the following instruments: General Electric Recording Spectrophotometer (GERS) (Diano Corp., Foxboro, MA) equipped with a tristimulus integrator (Davidson and Hemmendinger, Inc., Easton, PA). All samples were measured in 2-mm cells at 30 C and raw data were converted to the following functions:  $(X^2 + Y^2)^{1/2}$ ,  $(X^2 + Z^2)^{1/2}$ ,  $X/Y$ ,  $(Y^2 + Z^2)^{1/2}$ , dominant wavelength and purity. A Gardner XL-10 colorimeter (Gardner Laboratories, Bethesda, MD) was used to obtain data for the following functions: L, a, b, Y, a/b, arctangent a/b,  $(a^2 + b^2)^{1/2}$ , and the Scofield Hunter color difference (8).

Visual color assessment was carried out with a panel of seven judges, who possessed normal color vision, utilizing a MacBeth Lablite (MacBeth Daylighting Corp., Newburgh, NY) as discussed in a previous study (3). All results were analyzed statistically by use of a computerized analysis of variance (ANOVA) and Duncan's New Multiple Range Test (12). Limited flavor evaluations and refrigeration and light stability studies were also conducted as described by Clydesdale et al. (3).

## RESULTS AND DISCUSSION

### *Efficacy of objective techniques for visual assessments*

One of the objectives of this study was to evaluate some

21 color functions as predictors of visual assessment of dry-pack products colored with Roselle. As in previous studies (2,3), it was found that correlations of objective techniques with visual assessment were variable, further stressing the importance of re-evaluation of objective color parameters with visual judgements when changing product lines or product ingredients.

In the cherry beverage all color functions with the exception of X, Z, D.I., purity and  $(X^2 + Z^2)^{1/2}$  had correlations of  $r = 0.75$  or greater with visual assessment. However, evaluating the same functions with strawberry gelatin, it was found that these correlations decreased dramatically and only a measure of total anthocyanins (T Acy) had a high correlation ( $r = 0.779$ ).

Optical characteristics of product may change with formulation or ingredient line and these results point out the need for close scrutiny of quality control variables.

### *Visual assessment of storage samples*

Results of the analysis of variance of visual assessment of the strawberry gelatin dessert are in Table 1. Storage treatment, time and their interaction are all significant (0.5%). Further statistical treatment (Table 1) by Duncan's Multiple Range Test, which was used to separate treatment and time effects, showed that both the high temperature, high humidity storage treatments were significantly different from all other storage treatments and that the high temperature storage under ambient humidity was also significantly different from all other storage conditions. Duncan's test on the means obtained at each week of storage revealed a break point between week 6 and week 8 and another between week 8 and weeks 12 and 16.

Table 2 shows results of analysis of variance for the cherry beverage. Duncan's Multiple Range test on storage treatment means identified the high temperature, high humidity (100 F-90% R.H.) storage condition to be significantly different from all other storage conditions, which were not significantly different from one another. Duncan's test on weeks of storage means showed that weeks 12 and 16 were significantly different from all other weeks of storage and in fact the greatest color difference occurred at this time.

These results are generally consistent with those obtained with anthocyanin from other sources, such as grapes and cranberries, in dry-pack systems (2,3).

### *Objective assessment of storage changes*

Spectral curves of the gelatin colored with Roselle (Fig. 1) showed a decrease in absorption at 520 - 525 nm with increased temperature of storage. The greatest change occurred under the high temperature, high humidity (100 F-90% R.H.) storage condition. The increased absorption at 400-440 nm indicated that browning was occurring.

The Gardner a value and T.A. both decreased with storage, as expected. Also, the E values and D.I. increased with loss of pigment and browning on storage. Both C.I.E. Y and Gardner L values increased which is

TABLE 1. Analysis of variance of strawberry gelatin dessert mix colored with Roselle anthocyanin packed under ambient conditions. (Result of visual scoring test for the degree of color difference.)

Source of variation	df	SS	MS	F ratio test (from EMS)	F value	Significance
Treatment	3	189.89	63.30	T/R:T	72.76	0.5%
Weeks	7	261.07	37.30	W/Error	12.86	0.5%
Replication: treatment	24	20.82	.87	No Test	—	
Treatment: weeks	21	376.25	17.92	TW/Error	6.18	0.5%
Error(WR:T)	168	487.47	2.90			
Total	223	1335.50				

Duncan's Multiple Range Test - among storage treatment means.\*

75 F-Ambient		40 F-Ambient		100 F-Ambient		100 F-90% Relative Humidity	
<u>13.0</u>		<u>13.3</u>		<u>17.4</u>		<u>28.9</u>	
Week 3	Week 1	Week 2	Week 4	Week 6	Week 8	Week 12	Week 16
0.86	1.29	1.71	2.29	3.14	6.57	8.43	8.71

Variables (Temperature, Humidity, Time) which are not significantly different from one another are underlined.

\* Tested at the 1.0% level of significance.

TABLE 2. Analysis of variance of cherry beverage mix colored with Roselle anthocyanin packed under ambient conditions. (Results of visual scoring test for degree of color difference.)

Source of variation	df	SS	MS	F ratio test (from EMS)	F value	Significance
Treatment	3	244.34	81.45	T/R:T	290.89	0.5%
Weeks	7	127.14	18.16	W/Error	8.04	0.5%
Replication: treatment	24	6.75	.28	No Test	—	
Treatment: weeks	21	338.00	16.10	TW/Error	7.12	0.5%
Error (WR:T)	168	380.23	2.26			
Total	223	1096.46				

Duncan's Multiple Range Test - among storage treatment means.\*

100 F-Ambient		75 F-Ambient		40 F-Ambient		100 F-90% Relative humidity	
<u>11.3</u>		<u>11.8</u>		<u>11.9</u>		<u>28.5</u>	
Week 3	Week 2	Week 4	Week 1	Week 6	Week 8	Week 12	Week 16
9.5	10.3	13	13.5	15.3	17.3	23	25

Variables (Temperature, Humidity, Time) which are not significantly different from one another are underlined.

\* Tested at the 1.0% level of significance.

evidence that the sample was getting lighter even though browning compounds were being formed. The X tristimulus value and x chromaticity coordinate decreased with loss of redness, and C.I.E. X/Y steadily decreased throughout storage.

Other color functions (C.I.E. X, Z, purity and y) showed a variable response throughout storage. This was probably due to an initial response to pigment loss which was then masked by the response of these variables to the browning compounds formed later in storage. Since the change in pigment is not a mole to mole relationship with formation of browning compounds, difficulties often arise using colorimetric techniques to follow chemical changes or visual assessment as indicated previously.

Spectral curves of the cherry beverage stored under all conditions showed that the 100 F-90% R.H. storage condition caused the greatest overall color changes. A

large decrease in absorption occurred at 525 nm due to loss of pigment and the absorption increased in the 400-440 nm range caused by the formation of browning products.

In general, the individual colorimetric responses were similar to those found in gelatin.

Total anthocyanins (TAc), and all other objective parameters, were analyzed in all samples during storage. However, due to the large amount of data generated and the fact that the greatest changes occurred at 100 F-90% R.H., only those results will be presented. Figure 2 shows the effect of storage at 100 F-90% R.H. on the total anthocyanin content of the two products. Degradation proceeded at a similar rate in both products with the greatest change beginning after 4 weeks of storage. Due to the results obtained in previous studies (2,3), it was assumed that this degradation could be slowed by packing under nitrogen. During the storage study, the



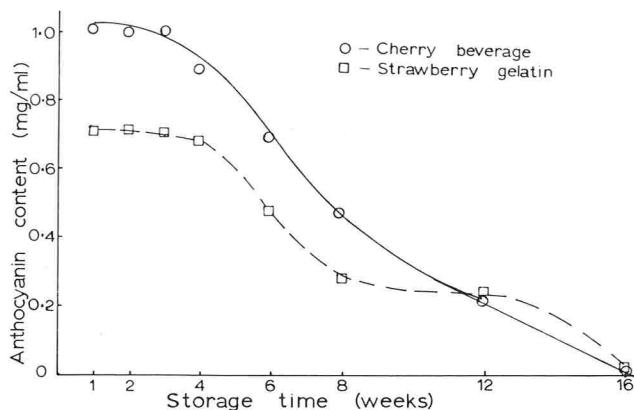


Figure 2. The effect of storage at 100 F/90% R.H. on cherry beverage mix and strawberry gelatin dessert mix colored with roselle anthocyanin and packed under ambient conditions.

color of the Red No. 2 control remained virtually unchanged.

#### Product flavor and stability

Flavor analyses showed that the flavor imparted by the Roselle powder at the levels used was detectable and disliked by the panelists. Similar results were obtained with cranberry anthocyanin preparations (3) but it was found that grape anthocyanin preparations produced an acceptable flavor (2).

As with grape and cranberry anthocyanins (2,3), stability in these products after reconstitution and storage for 1 day of sunlight and 1 week of refrigeration was excellent. Minimal pigment loss was noted even after 3 weeks in the refrigerator when mold became a problem.

It was evident from this study and preceding studies (2,3) that anthocyanins from various plant sources have

limited potential in dry pack foods unless flavor problems were solved and more importantly packaging conditions were chosen to prevent moisture transfer and high humidity conditions to exist.

#### ACKNOWLEDGMENTS

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## Evaluation of a Modified Sina/Beckman Hygrometer

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### ABSTRACT

A modification of the SINA/Beckman recording electric hygrometer is described which permits this instrument to be calibrated and used with greater ease. This increase in convenience is attained without sacrificing either accuracy or precision.

The term water activity ( $a_w$ ) specifies the water condition within a system. This parameter, which is the ratio of the vapor pressure of pure water to the vapor pressure of a solution at a given temperature, is useful in predicting microbial growth in foods and in other environments (4). Water activity also may indicate the potential for other deleterious changes in foods such as browning reactions, autoxidation of lipids, enzymatic reactions, and degradation of nutrients (7).

Various instruments and procedures are available for measurement of  $a_w$ . One of the most frequently encountered  $a_w$ -measuring devices is the Sina Hygrometer, distributed in the U.S. by Beckman Instruments, Inc. This instrument measures the passage of an electrical current through a proprietary formulation of hygroscopic electrolytes contained in a sensor element which is exposed, at equilibrium, to the sample to be measured. As the electrolyte-containing element hydrates or dehydrates, conductivity increases or decreases, respectively. These changes are read directly in percent equilibrium relative humidity which is converted to  $a_w$  simply by dividing by 100. The Sina/Beckman hygrometer uses range plugs which permit selection of ranges of  $a_w$  values that can be determined. These plugs may be fixed resistance types or adjustable resistance units. In the former, a standard isotherm must be established using various saturated salt solutions and all readings must be referred to the isotherm to obtain actual  $a_w$  values. Calibration of adjustable resistance plug-in units is done by adjusting small potentiometers within the range plugs to the  $a_w$  value of the standard solution. Because these plugs are calibrated internally, usually it is

not necessary to construct a standard isotherm. The procedures for calibrating this instrument are described in greater detail in an article by Troller (6).

In both the model SJT non-recording and the model SMT recording instrument, the range plugs are located in the rear panel of the instrument. This arrangement requires that adjustment of potentiometers located within range plugs be done with a mirror or by constant and inconvenient referral to the indicator at the front of the panel while fine-adjusting the potentiometers at the rear. The location of the range plugs at the rear of the instrument case also makes it inconvenient to change range plugs. The recording model has all of the instrument control switches, such as sensor channel selector, off/on and sensor dwell interval controls located on the rear panel.

As a result of these shortcomings, the arrangement of this instrument was altered as shown in Fig. 1 to provide greater accessibility to the controls without altering its

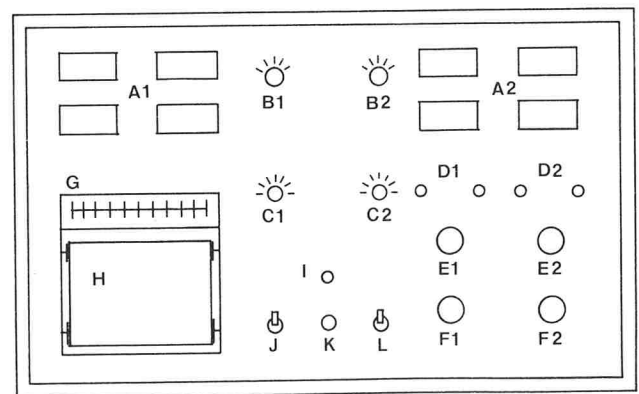


Figure 1. Schematic diagram of the modified Sina/Beckman hygrometer. A1 and A2, range plugs; B1 and B2, range selector switches; C1 and C2, recorder dwell interval switch; D1 and D2, calibration adjustment knobs; E1 and E2, calibration low/high range selector switches; F1 and F2, calibration selector switches; G,  $a_w$  indicator; H, strip chart recorder (2 Channel); I, pilot light; J, sensor selector switch; K, fuse; L, on/off switch.

excellent analytical characteristics. This alteration was facilitated by the modular construction of the model SMT hygrometer so that the chart drive module (H) could be exposed on the front panel and the potentiometer module controls could be turned 180° to similarly make them accessible from the front. In addition to providing access to the controls, range plugs (A1 and A2) were moved to the front panel and three additional plug sockets provided for switching selection through nine pole four position switches (B1 and B2). In this manner, any one of four range plugs could be selected without the inconvenience of removing and replacing range plugs. Furthermore, the front panel location of the plugs facilitates the adjustment of those units that contain adjustable potentiometers.

Another feature of the altered instrument, as shown in Fig. 1, is the front panel location of an internal calibration system consisting of low and high range dummy resistance adjustment controls (D1 and D2) and selection circuits (E1 and E2). Use and design of these circuits are described in the instruction manual accompanying the hygrometer and in the publication of Troller (6). Switching from the normal operating mode to the calibration mode is accomplished by switch (F1) for sensor 1 and switch (F2) for sensor 2. Alternatively, some workers use a completely independent dummy resistance circuit which employs a digital readout of resistance directly rather than switching the resistance through the existing panel indicator for reading. We believe that our system of internal calibration is much less complex and somewhat less costly without appreciably affecting convenience.

The chart paper transport and dial indicator, letters (H) and (G) in Fig. 1, are located in the left front corner of the instrument. The opening provided is sufficiently large to facilitate changes in the dial indicator if other  $a_w$  ranges are measured. Also, the chart transport module can be removed, as before, for convenient chart replacement or replenishment.

Finally, a sensor selector switch (J), off-on switch (L), pilot light (I), fuse access (K), and sensor dwell interval switches (C1 and C2) have been moved to the control portion of the front panel.

Temperature stabilization of the sensor assemblies (not shown) is critical to accurate  $a_w$  measurement and so sensors and sample holders normally are located in a constant-temperature box. This container may be incorporated into the same chassis as the measurement and switching modules described above although we have not chosen this design for our present instrument.

A photograph of the front panel of the modified instrument is shown in Fig. 2. This photograph corresponds to the schematic diagram shown in Fig. 1. The outside dimensions of the front panel are approximately 12 × 21 inches.

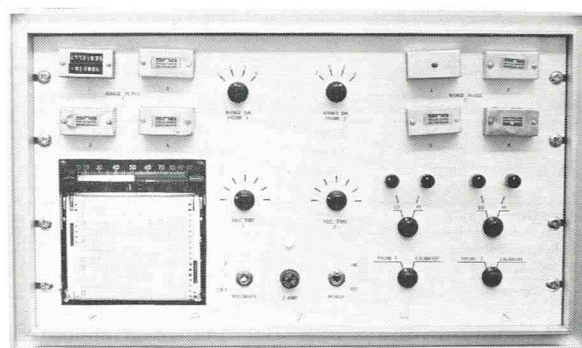


Figure 2. Front view of modified Sina/Beckman hygrometer.

## MATERIALS AND METHODS

### Standards

The standards used in these experiments were saturated, slurried solutions of NaCl, KCl,  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  and  $\text{K}_2\text{SO}_4$ . Standard  $a_w$  values were assigned to these solutions (Table 1) as listed in the publication of Stokes and Robinson (5) for  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  or the tables of Greenspan (1) for the remaining salts. All measurements were taken at  $25 \pm 1$  C.

### Instrument calibration

The instruments employed in these studies are as described above. Calibration and operation were identical for both modified and unmodified hygrometers and essentially are as described elsewhere (6). Equilibration within the sample chambers normally required 1 h for the highest  $a_w$  level measured and this interval was therefore chosen for all four standard solutions. In all instances, the measurements were approached from the low end of the  $a_w$  scale.

### Statistical analyses

Statistical data were obtained using standard procedures. Five replicates of each salt were obtained per sensor. Previous work (6) had shown that sensor-to-sensor variation was insignificant and so these data were pooled to give 10 replicates at each  $a_w$  level. The sequence of replicate testing was determined by assigning random numbers to each analysis.

## RESULTS AND DISCUSSION

The precision and accuracy of this instrument both alone and in comparison have been reported in the literature (2,3,6). These parameters have not been altered (Table 1) by the described modifications. This is not unexpected because the changes that have been made related only to switches and other features and have not involved the functional aspects of this instrument's operation nor have modifications been made to the sensor.

Neither accuracy nor precision have diminished appreciably as a result of the alterations described herein. In fact, the marginal improvement in accuracy observed (Table 1) in our tests may be the result of more precise adjustment of the range plug potentiometers. This adjustment is greatly facilitated by the frontal location of these plugs. Similarly, precision parameters have not suffered appreciably and the standard deviations for both modified and unmodified instruments are similar to those described by Karan-Djurdjic and Leistner (2) and Troller (6). In all instances the standard deviations were less than  $0.005 a_w$ .

TABLE 1. Comparison of unmodified and modified hygrometers.

Saturated salt solution	Standard $a_w$ Value	Unmodified instrument				Modified instrument			
		Accuracy		Precision		Accuracy		Precision	
		Average measured mean <sup>c</sup>	-% of Std.	Std. dev.	90% Conf. limits	Average measured mean <sup>c</sup>	-% of Std.	Std. dev.	90% Conf. limits
NaCl	0.753 <sup>a</sup>	0.749	-0.40	0.004	0.7415 - 0.7505	0.757	+0.62	0.002	0.756 - 0.758
KCl	0.843 <sup>a</sup>	0.838	-0.60	0.001	0.8376 - 0.8384	0.845	+0.24	0.004	0.843 - 0.847
BaCl <sub>2</sub> •2H <sub>2</sub> O	0.902 <sup>b</sup>	0.894	-0.55	0.001	0.8936 - 0.8944	0.901	-0.15	0.004	0.898 - 0.902
K <sub>2</sub> SO <sub>4</sub>	0.973 <sup>a</sup>	0.970	+0.31	0	0.9700 - 0.9700	0.976	+0.31	0.002	0.975 - 0.977

<sup>a</sup>Date from Greenspan (1).<sup>b</sup>Data from Stokes and Robinson (5).<sup>c</sup>Ten replicates - 2 sensors<sup>d</sup>Mean - Std.  $a_w$  Value
$$\frac{\text{Mean} - \text{Std. } a_w \text{ Value}}{\text{Std. } a_w \text{ Value}} \times 100$$

The alterations in the Sina/Beckman hygrometer that have been described in this report have improved the utility and convenience of this instrument. These improvements have been attained without sacrificing the accuracy or reproducibility.

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## Microbiology and Composition of Snack Sausages

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### ABSTRACT

Chemical, bacteriological and processing characteristics of experimental and commercial snack sausages were investigated. Snack sausages are narrow diameter (ca. 10-12 mm), all-beef products which are relatively dry and shelf-stable, and which may or may not be fermented. The bacterial flora of each product consisted of gram-positive, catalase-positive sporeforming rods (bacilli), reflecting a time-temperature effect of heating/smoking which destroyed most other organisms. The products have low moisture content (av. 20.6%), water activity (av. 0.78), and moisture/protein (M/P) ratio (av. 0.81/1). Judged by the sausage classification system of Acton and Dick relating moisture content to M/P ratio, the snack sausages are fully dry products. The yield of snack sausage prepared in our pilot plant was 39.6% but increased to 51.4% when the initial fat content of the meat mixture was increased from 7.2 to 25.7%.

Snack sausages comprise a group of narrow diameter (ca. 10-12 mm), all beef, relatively dry, shelf-stable products which may or may not be fermented. There is little in the literature about their processing, microbiology, chemistry or composition. Komarik et al. (3) described the processing of a fermented, nondried, spicy snack sausage. Previous work from our laboratory (7) described the influence of internal product temperature on destruction of salmonellae and staphylococci during thermal processing of a nonfermented snack sausage.

As a part of a continuing interest of our laboratory in the microbiology and technology of sausage products, we have investigated the general microbiology and composition of commercial and pilot-plant-produced snack sausages. The results of these investigations are presented here.

### MATERIALS AND METHODS

#### *Preparation of snack sausage*

All-beef nonfermented snack sausage was prepared in our pilot plant as described previously (7). The process consisted of heating and smoking the product in a smoke house for 3-1/2 h at an internal

product temperature of 57.8 C followed by 4 days of drying at 21 C and 50-55% relative humidity. To determine the influence of initial fat content on the finished product, snack sausage mixtures containing 7.2, 18.5, and 25.7% fat (low, medium, and high fat; designated experimental 1, 2, and 3, respectively) were prepared by mixing various amounts of beef fat and lean from the side of a choice grade beef carcass.

#### *Commercial snack sausages*

Samples of the various commercial snack sausages were purchased from retail sources. Except for product F, which was labeled "Keep Refrigerated," all were considered shelf-stable products and sold without refrigeration.

#### *Microbiology*

Microbiological analyses were carried out on the commercial and experimental snack sausages as described previously (9). The different colony types on the various media were examined by gram stain and catalase tests. These two tests were found to be extremely useful because previous experience with a dry sausage, pepperoni (6), indicated that the selective agars were not sufficiently specific for the various microbial types in sausage products.

#### *Compositional analyses*

Moisture, ash, fat, and protein content of commercial and experimental snack sausages were determined on twice-ground (1/8-inch plate) samples by standard procedures (AOAC, 2). The pH, titratable acidity, and water activity ( $a_w$ ) were determined as described previously (6).

## RESULTS AND DISCUSSION

Some physical and chemical characteristics of 10 commercial and three experimental snack sausages are given in Table 1. Starter culture was used in products A and B, and their low pH values of 4.7 and 4.8, respectively, indicate that fermentation had occurred. The product H-2 also had a low pH, 4.7; in this instance the fermentation was probably carried out by the natural flora including lactic acid bacteria. With the exception of product H-1 and G, with pH values of 5.2 and 5.4, respectively, all other products had relatively high pH values, 5.9 to 6.4. The acid content tended to agree with pH values, low pH values had higher acid levels and vice versa.

<sup>1</sup>Federal Research, Science and Education Administration, U.S. Department of Agriculture.

The moisture content of the commercial products ranged from 10.6 to 49.1% and fell into two groups: five products at 18.5% and below and five at 27.3% and above. The experimental products had very low moisture levels. The  $a_w$  and the moisture/protein (M/P) ratio tended to parallel the moisture level but not in all instances. This same pattern was observed in pepperoni (6). The M/P ratio is an indication of the shelf stability of the product. For example, the maximum M/P ratio expected for pepperoni is 1.60/1 (Laboratory Guide Book, USDA, CMS, Technical Services Division), and, as a general rule, pepperoni is considered to be shelf-stable. With the exception of Company F, which suggests that its product be refrigerated, all other snack sausage processors consider their products to be shelf-stable. Their low M/P ratios support this, with the exception of product H-2.

Product H-1 may owe its shelf stability to the fact that the products of Company H are heavily smoked. Product H-2, though it has a high moisture content, M/P ratio, and  $a_w$ , is very acidic (pH 4.7, 1.02% acid); thus its shelf

stability is probably due to the combination of smoke and acid.

Acton and Dick (1) recently proposed a classification system for dry sausages based on M/P ratio and percent moisture. We applied their system to the data given in Table 1. In their system, all experimental and most of the commercial snack sausages would be classified as fully dry, product H-2 would be semi-dry; and product F (labeled "Keep Refrigerated") and H-1 would be medium dry. Since the data for M/P ratio and moisture content of the individual snack sausages fall very close to the regression line of Acton and Dick, their classification system would also seem valid for snack sausage products.

Results of the bacteriological analysis of commercial and experimental snack sausages are in Table 2. In general, total counts (on APT agar) were low, and, except for two products (B and E), the flora detected consisted of bacilli (gram-positive, catalase-positive, sporeforming rods). Judged by colony morphology, on EMB, MSA, and especially APT agar, the organisms were *Bacillus subtilis* (4).

TABLE 1. Physical and chemical analysis of commercial and experimental snack sausages.

Commercial products	pH	Acid <sup>a</sup> %	$A_w$	Moisture %	Fat %	Ash %	Protein %	Moisture/protein ratio
Company A <sup>b</sup>	4.8	0.79	—	14.0	58.9	4.3	16.5	0.85/1
Company B <sup>b</sup>	4.7	0.88	0.66	10.6	59.4	4.4	26.5	0.40/1
Company C-1 <sup>c</sup>	5.9	0.51	—	10.9	53.3	6.6	26.5	0.41/1
Company C-2	5.9	0.29	0.69	11.4	59.3	4.4	20.5	0.56/1
Company D	6.1	0.27	0.82	18.5	48.3	5.2	21.7	0.85/1
Company E	6.1	0.25	0.86	27.3	42.2	6.2	22.2	1.23/1
Company F	6.4	0.17	0.92	34.7	37.6	3.7	19.2	1.81/1
Company G	5.4	0.45	0.87	29.0	35.6	5.6	25.5	1.14/1
Company H-1	5.2	0.57	0.83	38.7	14.6	6.8	22.9	1.69/1
Company H-2	4.7	1.02	0.88	49.1	18.8	5.8	21.8	2.25/1
Experimental-1	5.6	0.80	0.70	18.1	19.7	7.1	52.4	0.35/1
Experimental-2	5.6	0.72	0.69	16.4	37.1	5.9	40.4	0.41/1
Experimental-3	5.7	0.52	0.69	15.3	44.1	5.0	33.2	0.46/1

<sup>a</sup>Acidity was expressed as percent lactic acid.

<sup>b</sup>A lactic acid starter culture was listed on the manufacturer's label.

<sup>c</sup>This product contained soy protein.

TABLE 2. Numbers and types of viable microorganisms present in commercial and experimental snack sausages.

Commercial	Numbers/g found on				Major bacterial types found on			
	APT <sup>a</sup>	ROG <sup>a</sup>	EMB <sup>a</sup>	MSA <sup>a</sup>	APT	ROG	EMB	MSA
Company A <sup>b</sup>	$2.1 \times 10^4$	$< 1 \times 10^2$	$1.0 \times 10^3$	$2.3 \times 10^4$	1 <sup>c</sup>		1	1
Company B <sup>b</sup>	$5.4 \times 10^4$	$1.3 \times 10^5$	$5.0 \times 10^3$	$2.3 \times 10^5$	4,1	4	3	1
Company C-1	$1.5 \times 10^3$	$4.0 \times 10^2$	$< 1 \times 10^2$	$1.0 \times 10^3$	1	4		1
Company C-2	$2.0 \times 10^2$	$< 1 \times 10^2$	$< 1 \times 10^2$	$< 1 \times 10^2$	1			1
Company D	$3.4 \times 10^3$	$< 1 \times 10^2$	$4.0 \times 10^2$	$2.8 \times 10^3$	1		1	1
Company E	$1.5 \times 10^2$	$< 1 \times 10^2$	$2.0 \times 10^4$	$1.0 \times 10^5$	2,1		2	2
Company F	$2.0 \times 10^2$	$< 1 \times 10^2$	$< 1 \times 10^2$	$< 1 \times 10^2$	1			
Company G	$9.7 \times 10^3$	$< 1 \times 10^2$	$< 1 \times 10^2$	$2.3 \times 10^2$	1			1,2
Company H-1	$7.0 \times 10^4$	$< 1 \times 10^2$	$2.6 \times 10^4$	$4.0 \times 10^3$	1		1	1
Company H-2	$1.3 \times 10^3$	$< 1 \times 10^2$	$9.4 \times 10^3$	$4.9 \times 10^3$	1		1	1
Experimental-1	$4.2 \times 10^3$	$< 1 \times 10^2$	$3.7 \times 10^3$	$3.2 \times 10^3$	1		1	1
Experimental-2	$2.4 \times 10^3$	$< 1 \times 10^2$	$5.8 \times 10^3$	$2.8 \times 10^3$	1		1	1
Experimental-3	$4.7 \times 10^3$	$< 1 \times 10^2$	$2.7 \times 10^3$	$2.0 \times 10^3$	1		1	1

<sup>a</sup>(APT) Difco APT agar; (ROG) Difco Rogosa SL agar; (EMB) Difco Eosin Methylene Blue agar; (MSA) Difco Phenol Red Mannitol Salt agar.

<sup>b</sup>A lactic acid starter culture was listed on the manufacturer's label.

<sup>c</sup>1 = Catalase- and gram-positive sporeforming rods; 2 = catalase- and gram-positive cocci; 3 = catalase-positive, gram-negative rods, not typical coliforms; 4 = catalase-negative, gram-positive cocci.

Lactic acid bacteria were detected in only two products: product B (prepared with starter culture,  $1.3 \times 10^5/g$ ) and C-1 ( $4 \times 10^2/g$ ). The small numbers of lactic organisms probably resulted from time-temperature effects of heating which destroyed virtually all the microflora except bacilli.

The fat content of the three experimental snack sausages appeared to have no influence on their bacteriology. The numbers and types of organisms were similar (Table 2). This provides further support for our earlier study (8) which indicates that differing fat levels had no influence on thermal destruction of non-pathogenic bacteria in sausages. In addition, we found that differing fat levels had no influence on thermal destruction of salmonellae and staphylococci during processing of snack sausage in our pilot plant (7).

To study the influence of fat on yield of product, snack sausages were prepared with initial fat content of 7.2, 18.5, and 25.7%. The yield of these three products was 68.2, 71.6, and 75.1% after the heating/smoking step, and 39.6, 46.1, and 51.4% after the drying step, respectively. Thus, increasing the initial fat content of the sausage mixtures increased the yield of snack sausage. This same relationship had been noted during processing of pepperoni (5); when the initial fat level of the pepperoni mix was increased from 13.3 to 25.1%, the

yield of pepperoni after drying increased from 48.0 to 58.3%.

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## Fermented Mechanically Deboned Poultry Meat and Survival of *Staphylococcus aureus*

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### ABSTRACT

Mechanically deboned poultry meat (MDPM) was used to formulate a fermented sausage product prepared by a natural lactic acid fermentation (fermentation by indigenous lactic acid bacteria). Salted MDPM (3% NaCl) stored at 5 C promoted growth of lactobacilli to a level of  $10^6$  cells/g after 12 days. During the same period the population of indigenous *Staphylococcus aureus* decreased to below a detectable level but no change was observed in the population of added *S. aureus* ( $10^7$  cells/g). The MDPM attained a pH of 4.7 after 60 h of fermentation with a corresponding developed acidity of 1.6% expressed as lactic acid. The heat treatment given to the sausage (to attain an internal temperature of 60 C) brought about a reduction in the population of both the lactobacilli and *S. aureus* (4.1 and 5.6 log cycles, respectively); the latter was decreased to an undetectable level. Acid, sodium chloride and sodium nitrite in combination with a heat treatment (60 C, 60 min) gave the largest reduction of the population of *S. aureus* resulting with a D-value of 23.6 min. Succinic acid in combination with either a heat treatment (60 C, 60 min) or low temperature storage (7 C, 7 days) was the most effective treatment against *S. aureus*. Other acids active against *S. aureus* arranged in decreasing order of effectiveness were lactic, acetic and citric.

Manufacture of fermented meat products is an important and dynamic branch of the meat industry. During 1976, Federally inspected plants processed over 136 thousand metric tons of fermented (dried and semidried) sausage registering a 13% increase over the previous year's production (1).

Many of the older and smaller manufacturers of fermented sausage still prepare their product using a natural fermentation (fermentation by indigenous lactic acid producing bacteria in the meat) (7). The process includes an aging period of the salted meat to enhance growth of lactic acid producing microorganisms (9).

Mechanically deboned poultry meat (MDPM) from broilers' necks and backs has been used in the formulation of fermented sausage using a starter culture (5). However, no details are available on natural lactic acid fermentation of MDPM and the microbiological changes that occur in the various steps, including the fate of pathogenic microorganisms.

Staphylococcal food poisonings and the occurrence of *Staphylococcus aureus* in fermented sausage were recorded in 1971 following outbreaks of gastroenteritis due to *S. aureus*-contaminated Genoa sausage (2,3). The studies of Lee et al. (8) and Tatini et al. (14) showed that

*S. aureus* may grow and produce toxin in fermented and non-fermented sausage.

This work was undertaken to study the natural fermentation of MDPM and its effect on *S. aureus*. In addition, the thermal resistance of *S. aureus* in the sausage mix and the effects of different organic acids were studied.

### MATERIALS AND METHODS

#### MDPM

The MDPM was prepared from broilers' backs and necks using a Beehive Deboner Model AUX 1272 (Beehive Machinery, Inc. Salt Lake City, Utah). After deboning the MDPM was frozen (-25 C) until use.

#### Sausage formula

A semidry type summer sausage was prepared using the ingredients shown in Table 1.

TABLE 1. MDPM sausage ingredients.

Ingredient	Amount (g/kg MDPM)
Dextrose	15.00
NaCl	30.00
Black Pepper	3.00
Sweet Paprika	3.00
NaNO <sub>2</sub>	0.100
Sodium Isoascorbate	0.550
Coriander	0.300
Mustard Powder	0.150
Allspice	0.300

#### Processing

The MDPM was mixed with NaCl and aged for 12 days in trays at 5 C to promote growth of lactic acid-producing microorganisms. After aging, the meat was mixed with the remainder of the ingredients and stuffed into 45-mm diameter fibrous casings which were transferred into the smokehouse at 30 C. When the sausage attained a pH of 4.7 they were cooked to an internal temperature of 60 C by gradually raising the temperature of the smokehouse to 65 C.

#### pH Measurements

The pH was measured in a slurry prepared by blending (2 min) 30 g of sausage with 270 ml of 0.1% Peptone (Difco) water. The slurry was first sampled for bacteriological examinations before pH measurements were made.

#### Titrateable acidity

The slurry prepared for pH measurements was titrated to pH 7.0 using a standard base (0.1 N NaOH) and a pH meter. The acid was expressed as percent lactic acid.

#### Bacteriological examinations

Appropriate dilutions were prepared using 0.1% Peptone (Difco) water. Total count was estimated using APT agar (BBL) incubated at 30 C for 48 h. Lactobacilli (gram positive rods, catalase and benzidine negative) were enumerated using Rogosa SL Agar (Difco) with

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incubation for 72 h at 30 C, while coagulase-positive staphylococci were determined on Baird-Parker medium (Difco). Incubation was at 35 C for 48 h. Selected *S. aureus* colonies were confirmed by the coagulase test.

#### Inoculation

All treatments were inoculated with  $1.0 \times 10^7$  cells of *S. aureus* FRI 100/g (Food Research Institute, University of Wisconsin), an enterotoxin A producing organism that had been grown in BHI broth (Difco) (35 C, 18-20 h).

#### Heat treatments

MDPM inoculated with *S. aureus* FRI 100 ( $1.0 \times 10^7$  cells/g) was divided into several equal portions. Three such portions are given: (a) acidified to pH 4.7 using lactic acid (Mallinckrodt); (b) as (a) plus 3% NaCl; (c) as (b) plus 100 ppm  $\text{NaNO}_2$ . The MDPM sample of each treatment [(a), (b) and (c)] was divided into six 60-g subsamples which were placed each in a beaker (48-mm internal diameter) and heated in a circulating 65-C water bath. The heat treatment was timed for 60 min when the center of the meat sample attained 60 C. The coming up time was 22 to 25 min. The temperature was monitored by inserting a copper constantan thermocouple (connected to a Honeywell recorder Model 153X64 Brown Instruments Div., Philadelphia, Pennsylvania) at the center of the sample. At 10-min time intervals a beaker was removed from the water bath and chilled immediately in crushed ice (to terminate the heat treatment) and the level of *S. aureus* was monitored.

The straight line formula  $\log N/N_0 = \frac{-K}{2.303}$  was used to calculate a regression line for the results of the heat treatments (where N is the number of survivor at various heating time and  $N_0$  is the initial number of *S. aureus* cells). The slope of the calculated straight line,  $\frac{-K}{2.303}$ ,

is equal the reciprocal of the decimal reduction time (1/D) and K represents the death rate constant.

#### Effect of organic acids

Acetic, citric, lactic and succinic acids (Mallinckrodt) were used to acidify the MDPM inoculated with *S. aureus* FRI 100 to pH 4.7. The individual MDPM portions acidified with different acids were divided into two subportions. One subportion was subjected to a heat treatment (60 C, 60 min) while the second was stored at 7 C for 7 days. At the end of each treatment, the level of *S. aureus* was determined. Each experiment was done in triplicate.

## RESULTS AND DISCUSSION

The total count of the uninoculated sample of salted (3% NaCl) MDPM increased from about  $10^5$  to approximately  $10^9$  cells/g during the aging period (5 C, 12 days) (Fig. 1). Lactobacilli increased from  $10^3$  to  $10^6$  cells/g in the same MDPM sample. A similar increase in lactobacillus numbers was observed in MDPM inoculated with *S. aureus*. The inoculated *S. aureus* population showed little or no change but the population of the indigenous *S. aureus* decreased below a detectable level after 9 days of aging (Fig. 1). No change in the pH of the MDPM was observed during the aging period. So pH cannot be considered as a factor during this phase for reduction of the population of the indigenous *S. aureus*. It is well established that lactobacilli produce certain antibacterial agents as byproducts of their metabolism such as hydrogen peroxide antibiotics (13) bacteriocins (16) and other unidentified agents (10). One or more of these antimicrobial agents may have caused the reduction of the population of the indigenous *S. aureus* during aging. These results verify the finding of Raccach and Baker (11) and Raccach et al. (12) who reported that

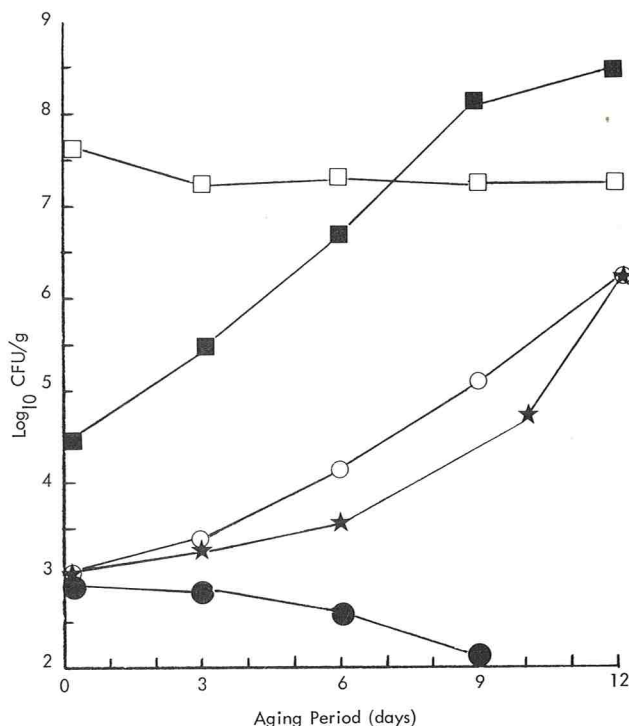


Figure 1. The effect of aging MDPM on inoculated *S. aureus* (□). Total Count (■); lactobacilli in uninoculated (○) and inoculated (★) samples and indigenous *S. aureus* (●).

the lactic acid-producing organisms should outnumber *S. aureus* population by a range of  $10^5$  to  $10^6$  to repress the pathogen. So it is not surprising that the population of the inoculated *S. aureus* ( $1.0 \times 10^7$  cells/g MDPM) was not affected by the lactobacilli during this phase. The population of lactobacilli increased about 100-fold during the first 45 h of the fermentation in samples with and without added *S. aureus* (Fig. 2) but no change was observed in the total count of the uninoculated sample. The count of the population of the *Lactobacillus* on Rogosa SL agar equalled that of the total count in APT agar after 45 h of fermentation (Fig. 2). Almost all of the bacterial population isolated in APT agar consisted of gram-positive rods, catalase- and benzidine-negative. It is probable that the lactobacilli gradually became the predominant microorganisms by producing unfavorable conditions for competing flora in the sausage mix.

A pH of 4.7 was attained after 60 h of fermentation with a corresponding developed acidity of 1.6% (expressed as lactic acid) (Fig. 3). As shown in Fig. 3, more than 80% of the acidity developed before 45 h of fermentation decreasing the pH value to 5.0. The developed acidity may have suppressed the growth of many undesirable microorganisms including inoculated *S. aureus* (Fig. 2). A pH of 4.7 is the minimum for *S. aureus* growth while a pH value of 5.3 is the minimum for enterotoxin A production (15).

The heat treatment given to the sausage (Fig. 4) brought about a significant reduction in the different bacterial groups examined. The population of *Lactobacillus* determined with Rogosa SL agar showed a decrease of 7.7  $\log_{10}$  cycles but only 4.1  $\log_{10}$  cycles when

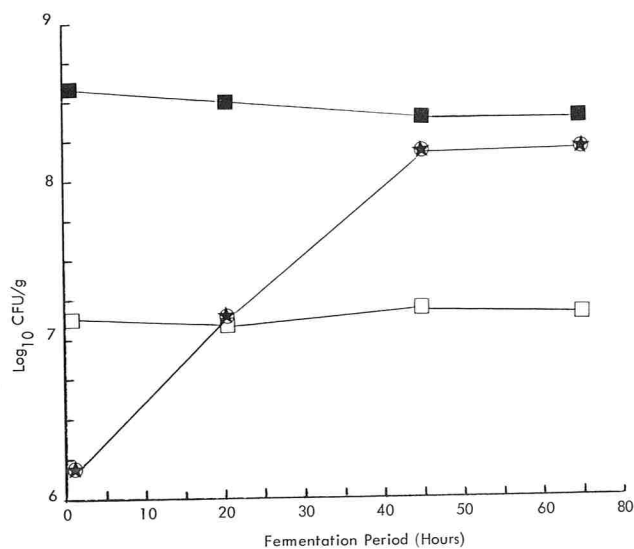


Figure 2. The effect of MDPM-fermentation on the total count (■); lactobacilli in uninoculated (○) and inoculated (★) samples with *S. aureus* (□).

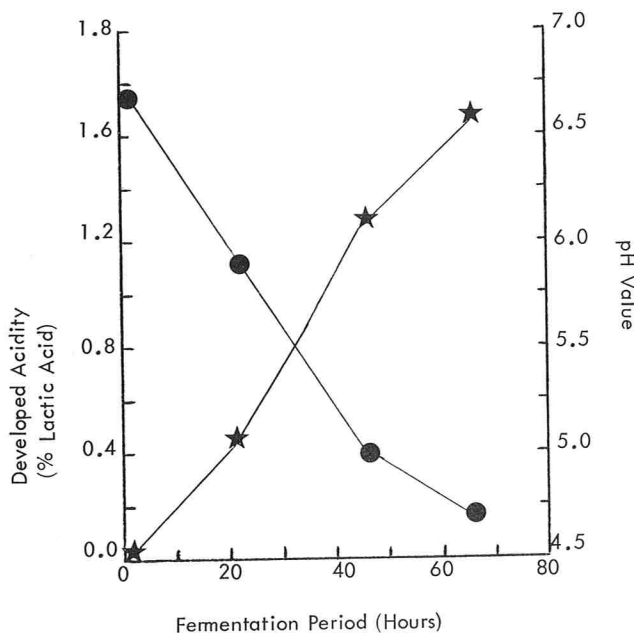


Figure 3. Development of acid (★) and changes in the pH (●) of MDPM during the fermentation period.

counted on APT agar. This difference may be ascribed to injured lactobacillus cells which lost their ability to form colonies on selective media. The population of inoculated *S. aureus* decreased to  $<10^2$  cells/g of sausage, a reduction of more than 5.6  $\log_{10}$  cycles. This reduction is very important especially from a public health aspect. In another study, at least  $4.0 \times 10^7$  *S. aureus* cells/g were required to produce detectable enterotoxin A in sausage (3).

The heat treatment experiments were undertaken to examine the effect of lactic acid, sodium chloride and sodium nitrite on thermal resistance of inoculated *S. aureus* in the sausage. For comparative purposes, the D-value of *S. aureus* in acidified MDPM (44.7 min) was considered the basal value. Table 2 shows the effect of

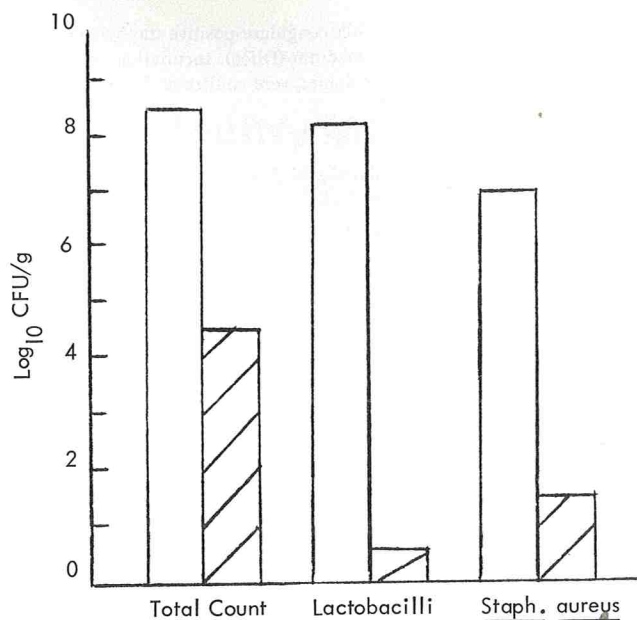


Figure 4. The population composition of the fermented sausage before (□) and after (▨) the heat treatment.

TABLE 2. The thermal resistance of *Staphylococcus aureus* in MDPM under different conditions.

Thermal resistance	Acid <sup>a</sup>	Acid & salt <sup>b</sup>	Acid, salt & nitrite <sup>c</sup>
Decimal reduction time (min), D	44.7	32.6	23.6
Death rate constant ( $\text{min}^{-1}$ ), K	0.05	0.07	0.10

<sup>a</sup>MDPM acidified with lactic acid to pH 4.7.

<sup>b</sup>Acidified MDPM (pH 4.7) + 3% NaCl.

<sup>c</sup>Salted acidified MDPM + 100 ppm  $\text{NaNO}_2$ .

the addition of sodium chloride alone or in combination with sodium nitrite to the acidified MDPM on the thermal resistance of *S. aureus*. Sodium chloride decreased the basal thermal resistance (D-value) by 37%. An additional 10% decrease was obtained by addition of sodium nitrite to the salted acidified MDPM. The D-value of *S. aureus* under these conditions was 50% of that in the acidified MDPM (Table 1). According to these results, addition of sodium chloride to the acidified MDPM promoted a greater reduction of the thermal resistance of the pathogens than addition of nitrite to the acidified salted MDPM. Goepfert and Chung (6) reported similar results. They found that a combination of acid and sodium chloride was responsible for destruction of *Salmonella* cells observed in a fermented sausage product.

Succinic acid had the greatest adverse effect on the population of *S. aureus* followed in decreasing order of effectiveness by lactic, acetic, and citric acids (Fig. 5). The heat treatment was more efficient than the low temperature storage only in combination with acetic and citric acids ( $P < 0.05$ ). The effect of each one of these two acids in combination with the heat treatment was not significantly different ( $P < 0.05$ ) than the control.

It is probable that heating at 60 C (a higher

temperature than the maximum growth temperature, 45 C, of *S. aureus*) of MDPM samples treated with acetic or citric acid enhanced the inactivation of staphylococci by these acids.

Citric acid treatment of the MDPM followed by low temperature storage did not inhibit *S. aureus* resulting with a survival three times greater than the control. No significant difference ( $P < 0.05$ ) was found between the acetic acid-treated MDPM and the control at low-temperature storage. No significant difference ( $P < 0.05$ ) was found between the heat treatment and low temperature-storage in combination with either lactic or succinic acid. Succinic acid in combination with either the heat treatment or the low temperature-storage caused about a 100-fold reduction in the population of *S. aureus*. This is comparable to the effect of lactic acid and sodium chloride in combination with the heat treatment ( $D = 32.6$  min, Table 2). Lactic acid under the same conditions caused approximately a 50-fold reduction of the pathogen population. These results suggest that for direct acidification of MDPM use of lactic acid in combination with either a heat treatment or low temperature-storage is adequate even though succinic acid was superior. Since fermented sausages are acidified mainly by lactic acid and contain a minute quantity of acetic acid, it is tremendously important, especially from the practical standpoint, that these two acids were biologically active in MDPM against the population of *S. aureus*.

The results presented in Table 2 and Fig 5 showed that neither one of the acids used in combination with either a heat treatment or low temperature storage nor the lactic acid in combination with sodium chloride and sodium nitrite caused the same extent of reduction of the population of *S. aureus* observed in the sausage after it was given a heat treatment (Fig. 4). These data may suggest that the lactic acid-producing flora played a larger role than just acidifying the meat. The lactobacilli may have formed certain antibacterial agents which in

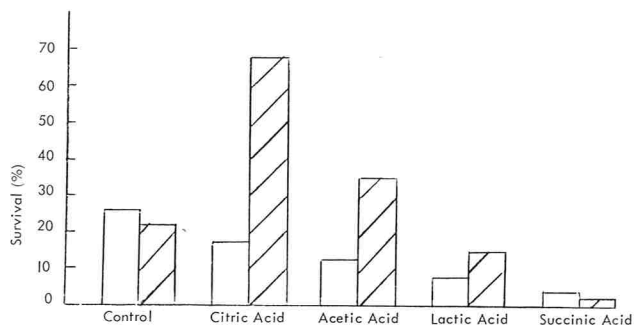


Figure 5. The effect of the heat treatment ( $\square$ ) (60 C, 60 min) and low temperature storage ( $\boxtimes$ ) (7 C, 7 days) on the survival of *S. aureus* in MDPM.

combination with the heat treatment suppressed the population of *S. aureus*.

This work showed that under commercial conditions it is feasible to use MDPM for sausage production by a natural lactic acid fermentation. Even a gross contamination with *S. aureus* can be reduced below a detectable level if a proper heat treatment is given to the sausage. Nevertheless, these results do not imply that unsanitary conditions can be used along with a proper heat treatment. One should bear in mind that the heat treatment used in this work was effective against the cells of *S. aureus* but this may not be in the case against staphylococcal enterotoxin or other pathogens not examined in this study.

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## Types of Bacteria and Shelf-Life of Evacuated Carbon Dioxide-Injected and Ice-Packed Broilers

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### ABSTRACT

Broiler carcasses were packed in low-permeability film bags that were evacuated, injected with CO<sub>2</sub>, then held at 2 C. Broilers were examined microbiologically and for off-odor to determine the shelf-life and types of bacteria. Vacuum level did not significantly affect bacterial counts. Broilers stored in 65% CO<sub>2</sub> had a 1-day longer shelf-life than those held in 20% CO<sub>2</sub> and about a 5-day longer shelf-life than ice-packed broilers. Spoiled carcasses from either 20 or 65% CO<sub>2</sub> packages had an acid-sour off-odor, and more than 90% of the bacteria present were *Lactobacillus*. Ice-packed broilers had the typical putrid off-odor at spoilage, and more than 95% of the bacteria were non-pigmented *Pseudomonas*.

Kraft (19) listed 24 genera of bacteria as isolates from poultry and Cox (9) found one additional genus. Only a few genera, however, affect the acceptability and shelf-life of poultry meat. Most observers found many different types of bacteria at the time of slaughter, but on carcasses that were held aerobically at 0-5 C the *Pseudomonas* or *Achromobacter*<sup>3</sup> groups predominated at the time of spoilage (1,3,5,15,22,23).

Coyne (12), Haines (16), Ogilvy and Ayres (22) and Scott (28) found that the two principal spoilage organisms *Pseudomonas* and *Achromobacter* were inhibited by CO<sub>2</sub>. Average generation time for *Pseudomonas* apparently was reduced as a logarithmic function of CO<sub>2</sub> level (22). Haines (16) and King and Nagel (17) found that CO<sub>2</sub> levels of 20 to 70% doubled the generation time for *Pseudomonas* if other environmental factors were constant. Thomson (32) found that psychrotrophs were inhibited by 10 and 20% CO<sub>2</sub>.

The predominant spoilage microorganisms on beef stored in air were *Pseudomonas* and *Achromobacter* (2,18,26). Gardner et al. (13) found that 96% of the

microflora on spoiled stored pork were *Pseudomonas* or *Achromobacter*. Clark and Lentz (8) found that in vacuum-packaged beef held at 5 C, the *Pseudomonas* and the *Moraxella-Acinetobacter* group were inhibited by 15% CO<sub>2</sub>. Shank and Lundquist (29) showed that the spoilage of vacuum-packaged cured meats involved lactic acid bacteria almost exclusively. Patterson and Gibbs (23) found that lactobacilli were the predominant spoilage bacteria on beef that was vacuum packaged and held at 0-2 C. Pierson et al. (24) found that 95% of the bacteria on anaerobically packaged beef were lactobacilli. *Lactobacillus* and other lactic acid bacteria whose growth is not significantly inhibited by CO<sub>2</sub> have a direct bacteriostatic effect on many gram-negative bacteria (14) and on three strains of *Pseudomonas* and one strain of *Salmonella typhimurium* (25).

In this study we aimed to determine the effect of CO<sub>2</sub> and vacuum on types of bacteria on broilers in commercially feasible, bulk shipping packs of low permeability film, and to determine the effect of this microflora on shelf-life and olfactory quality of broilers.

### MATERIALS AND METHODS

A "Conofresh 4000" packaging system (Continental Forest Industries - Corrugated, Greenwich, CT)<sup>4</sup> was used to package 560 broiler carcasses in a simulated commercial evacuated, CO<sub>2</sub>-flushed bulk pack. Chilled broilers were taken from the drip line at a commercial processing plant and placed four to a plastic film bag (7 cc/100 in<sup>2</sup>, permeability); then bags were placed into a wax coated corrugated box for insertion into the packaging chamber. The chamber was evacuated to 10, 17 or 24 inches of Hg. Carbon dioxide was injected into a film bag to either 20 or 65%, and the bag was sealed. Percentage of CO<sub>2</sub> in each bag was not measured directly, but was based on extrapolation from the known pressure applied by the CO<sub>2</sub> injection mechanism; the pressure required to produce each CO<sub>2</sub> content with this packaging equipment had been determined previously. The packs were held at 2 C and sampled for microorganisms and for off-odor after 5, 9, 12, 15 and 18 days of storage. Controls consisted of broilers that were packed in ice and held in wax-coated corrugated boxes and stored at 2 C. Controls were also sampled microbiologically and for off-odor after 5, 9, 12, 15 and 18 days of storage. All treatments were duplicated.

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<sup>2</sup> Russell Agricultural Research Center.

<sup>3</sup> The eighth edition of Bergey's Manual of Determinative Bacteriology now classifies *Achromobacter* as *Acinetobacter*.

<sup>4</sup> Mention of specific brand names does not imply endorsement by the authors or the U.S. Department of Agriculture to the exclusion of others not mentioned.

For total plate counts a 12.3-cm<sup>2</sup> area of breast skin was swabbed with calcium alginate swab which then was dissolved in a sterile 9.9-ml 1% sodium citrate blank. Appropriate serial dilutions were made, plated in duplicate on Plate Count Agar (Difco) and plates were incubated for 48 h at 30 C. Counts were expressed as log<sub>10</sub>/cm<sup>2</sup>. After sampling, carcasses were discarded and a new bulk pack was opened on each sampling day.

Carcasses were evaluated for off-odor when packs were opened for microbiological sampling. Sampling was terminated when the carcasses became spoiled, as determined by strong off-odor. Strong off-odor had become apparent on the carcass at a count of log 6.5 which, therefore, was defined as spoilage.

All data were analyzed by the Statistical Analysis System of Barr and Goodnight (6). Significance was determined by the F-test and a difference was considered significant at the 5% level of probability.

A total of 375 isolated colonies were picked from countable plates and generically classified with the identification schemes of *Biochemical Tests for Identification of Medical Bacteria* (20), and *Bergey's Manual of Determinative Bacteriology* (7). Thirty colonies were picked from two plates each day that packs were opened for evaluation. For control samples, 15 colonies were picked from duplicate plates on sampling days. A statistical randomizing procedure employing a numerical grid (10) and a random numbers table (30) was used to obtain a representative sample of the colonies from each plate.

Colonies were transferred to brain heart infusion (BHI) broth (Difco) and incubated at 25 C for 48 h. BHI plates were streaked for isolation and then incubated at 25 C for 18 to 24 h. Isolated colonies were picked, checked for purity and then tested for: gram reaction and morphology, motility, and catalase and oxidase reaction. We then did the additional tests which were necessary for generic identification. The biochemical characteristics that identified each genus were as follows: (a) *Micrococcus* - gram-positive, catalase-positive cocci, no growth in 15% NaCl, no growth in 40% bile and Voges - Proskauer-negative; (b) *Staphylococcus* - gram-positive, catalase-positive cocci, growth in 15% NaCl, growth in 40% bile and Voges-Proskauer-positive; (c) *Lactobacillus* - gram-positive, catalase-negative non-sporeforming rods, no reduction of nitrate, no true branching (a representative sample of *Lactobacillus* isolates was confirmed by use of *Lactobacillus* - 50 strips - API); (d) *Bacillus* - gram-positive, catalase-positive, sporeforming rods; (e) *Microbacterium thermosphactum* - gram-positive, catalase-positive, nonmotile rods, methyl red-positive and growth on STAA agar (14); (f) *Corynebacterium* - gram-positive, catalase-positive, non-sporeforming rods, H<sub>2</sub>S-negative, gelatin liquefaction-positive; (g) *Pseudomonas fluorescens* - gram-negative, catalase-positive rods, oxidase-positive, motile, slow growth at 37 C, no growth at 41 C, no reduction of nitrate, no fermentation of dextrose, fluorescent pigment produced; (h) non-pigmented *Pseudomonas* - gram-negative, catalase-positive rods, motile, oxidase-positive, aerobic acid production from dextrose, no anaerobic fermentation of dextrose, no pigment produced; (i) *Acinetobacter* (formerly *Achromobacter*) - gram-negative, catalase-positive rods, oxidase-negative, indole-negative, H<sub>2</sub>S-negative.

## RESULTS AND DISCUSSION

Bacterial counts on carcasses were significantly lower in vacuum-treated packages flushed with CO<sub>2</sub> than on controls at 15 and 18 days (Fig. 1). Counts on carcasses in 65% CO<sub>2</sub> were significantly lower after 15 days. Vacuum levels did not significantly affect bacterial counts throughout these experiments. At the time of spoilage, off-odor of carcasses in CO<sub>2</sub> was acid-sour. Ogilvy and Ayres (22), Shank and Lundquist (29) and Sutherland et al. (31) also found a characteristic acid-sour off-odor when lactic acid bacteria were the predominant spoilage bacteria on meats.

Control birds had the highest counts on all sampling days and spoiled in 14 days, which was about 4 days

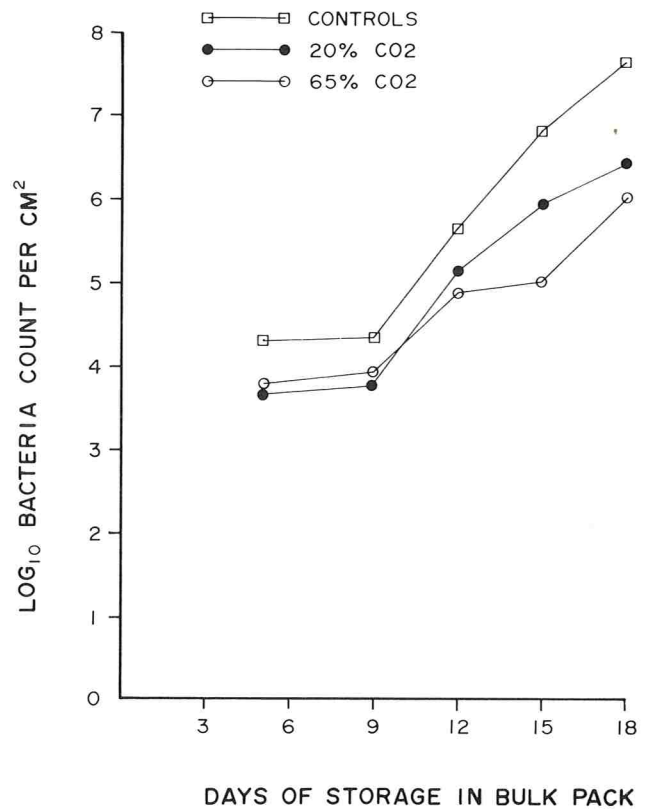


Figure 1. Total aerobic bacterial counts of broilers packaged in vacuum in bulk packs containing 20 and 65% CO<sub>2</sub> and of ice-packed controls. All birds were stored at 2 C.

before birds stored in 20% CO<sub>2</sub> and about 5 days before birds stored in 65% CO<sub>2</sub>. At the time of spoilage, control carcasses had the typical putrid off-odor that was characterized by Ayres et al. (3).

The bacterial population of evacuated packaged broilers with CO<sub>2</sub> injected into packages was predominantly *Lactobacillus* (Tables 1, 2). Packs containing 20% CO<sub>2</sub> showed a mixed microflora and *Lactobacillus* constituted 53% of the bacteria on day 5 of storage; *Lactobacillus* constituted not less than 97% of the microflora thereafter (Table 1). After 18 days of storage, broilers were spoiled and 100% of the bacterial

TABLE 1. Percent of isolates recovered from broilers held in vacuumized shipping packs containing 20% CO<sub>2</sub> and stored at 2 C.

Isolates	Days of storage				
	5	9	12	15	18
<i>Micrococcus</i> sp.	7	—	—	—	—
<i>Staphylococcus</i> sp.	7	—	3	—	—
<i>Lactobacillus</i> sp.	53	97	97	100	100
<i>Bacillus</i> sp.	—	3	—	—	—
<i>Microbacterium thermosphactum</i>	—	—	—	—	—
<i>Coryneform</i> sp.	17	—	—	—	—
<i>Pseudomonas fluorescens</i>	13	—	—	—	—
Non-pigmented <i>Pseudomonas</i>	—	—	—	—	—
<i>Acinetobacter</i> sp.	3	—	—	—	—
Gram-negative species	—	—	—	—	—
Mold sp.	—	—	—	—	—

TABLE 2. Percent of isolates recovered from broilers held in vacuumized shipping packs containing 65% CO<sub>2</sub> and stored at 2 C.

Isolates	Days of storage				
	5	9	12	15	18
<i>Micrococcus</i> sp.	13	7	—	—	—
<i>Staphylococcus</i> sp.	10	13	—	—	—
<i>Lactobacillus</i> sp.	47	77	97	83	90
<i>Bacillus</i> sp.	—	—	—	—	—
<i>Microbacterium thermosphactum</i>	—	—	3	10	—
<i>Coryneform</i> sp.	10	3	—	7	10
<i>Pseudomonas fluorescens</i>	13	—	—	—	—
Non-pigmented <i>Pseudomonas</i>	—	—	—	—	—
<i>Acinetobacter</i> sp.	—	—	—	—	—
Gram-negative species	3	—	—	—	—
Mold sp.	3	—	—	—	—

population sampled were *Lactobacillus*. Packs with 65% CO<sub>2</sub> also showed a mixed microflora on day 5 of storage, and 47% of the bacteria were *Lactobacillus* (Table 2). On day 9 of storage, 77% were *Lactobacillus*, and this proportion remained high until 18 days of storage, when birds were spoiled and 90% of the bacteria were *Lactobacillus*. These findings confirmed other studies (14,22,27) in which lactobacilli predominated on poultry or meat stored in CO<sub>2</sub>.

The microflora was mixed on control carcasses through day 12 of storage, when non-pigmented *Pseudomonas* accounted for 40% of the bacteria present (Table 3). The non-pigmented *Pseudomonas* made up 93% of the bacterial population by day 15 of storage when the carcasses were spoiled and 100% by day 18 when a slime layer had formed on the surface of the carcasses. McMeekin (21) also showed that group II non-pigmented *Pseudomonas* (odor producers) outgrew group I *Pseudomonas* (fluorescent) when chicken meat was held at 2 C, and Barnes and Impey (4) and Cox et al. (11) found that non-pigmented *Pseudomonas* produced a more intensive off-odor than other bacteria present and were the predominant microorganism present when aerobically-stored poultry reached spoilage.

Packaging environments, whether ice-packed or vacuum-packed, determined the types of bacteria that

TABLE 3. Percent of isolates recovered from ice-packed broilers held at 2 C.

Isolates	Days of storage				
	5	9	12	15	18
<i>Micrococcus</i> sp.	20	13	27	—	—
<i>Staphylococcus</i> sp.	13	27	20	—	—
<i>Lactobacillus</i> sp.	33	20	7	—	—
<i>Bacillus</i> sp.	—	—	—	—	—
<i>Microbacterium thermosphactum</i>	—	—	—	—	—
<i>Coryneform</i> sp.	13	33	7	7	—
<i>Pseudomonas fluorescens</i>	—	—	—	—	—
Non-pigmented <i>Pseudomonas</i>	20	7	40	93	100
<i>Acinetobacter</i> sp.	—	—	—	—	—
Gram-negative species	—	—	—	—	—
Mold sp.	—	—	—	—	—

grew on our stored broiler carcasses. The predominant spoilage bacteria on aerobically stored ice-packed broilers were non-pigmented *Pseudomonas* species and on vacuum-packaged, CO<sub>2</sub>-injected packs of broilers were *Lactobacillus* species. This difference resulted in an extended shelf-life and an acid-sour type of spoilage, rather than the putrid type of spoilage in broilers that were ice-packed. As an alternative to the commonly used ice-pack system of packing broilers for transport, the bulk vacuum-CO<sub>2</sub> packaging system offers advantages that include longer product shelf-life and greater economy, the ability to ship mixed meat loads, reduction of the short weight problem and elimination of the use of ice in shipping.

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## Effect of Garlic Oil or Onion Oil on Toxin Production by *Clostridium botulinum* in Meat Slurry

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### ABSTRACT

Garlic oil (or onion oil) when used in the proportion of 1500 µg per g of meat slurry inhibited toxin production by *Clostridium botulinum* type A (strain 73A). The inhibition, however, was not complete. Toxin production by *C. botulinum* type B (strain RIV 1) and type E (strain RIV 2) was not inhibited. It is not recommended that these oils be used for inhibiting toxin production by *C. botulinum*, as meat and meat products can contain several types of *Clostridium* sp. and not just type A.

Several factors can be considered for preventing growth and toxin production of *Clostridium botulinum* in meat and meat products, i.e. heating, lowering pH, lowering water activity, and use of sodium chloride with sodium nitrite. The greatest effect is achieved by a combination of these factors (8).

The disadvantage of nitrite is that in the presence of secondary and tertiary amines, carcinogenic nitrosamines might be formed (4). Consequently it is important to find alternatives to nitrites.

Since garlic and onion extracts have bacteriostatic activity (1,2,3,5) and as apparently there is no objection to addition of these to meat products (Dutch Food Act, Meat and Meat Products Regulation), garlic oil and onion oil were separately tested in meat products to determine whether they would prevent toxin production by *C. botulinum* types A, B and E.

### MATERIALS AND METHODS

#### Oil solutions

Garlic oil and onion oil were donated by International Flavours and Fragrances (IFF), Tilburg, The Netherlands. Miglyol 812-Neutralöl (Dynamit Nobel Chemicals, West Germany) was used as solvent. This is a triglyceride of saturated vegetable fatty acids of 8 to 12 carbon chain-length.

The total sulfur content of the oils was determined by the Element Analysis Section of the Institute for Organic Chemistry TNO, Utrecht, The Netherlands. The method used was a Schröniger-type oxygen-flask combustion followed by a titration of sulfate with Ba<sup>2+</sup> using a thiorin/methylene-blue mixture as indicator. The proportions of sulfur were as follows: garlic oil 44.64 mg per 100 mg, onion oil 50.51 mg, Miglyol 0.18 mg. The error of this method was 0.2 mg per 100 mg absolute, thus the sulfur content of Miglyol was practically zero. This was supported by the fact that Miglyol lacked any odor of sulfur

compounds.

Garlic oil (45 mg), or onion oil (45 mg) were separately dissolved in Miglyol to a final volume of 1.0 ml to give the respective oil solutions.

#### Meat slurry

The meat slurry was prepared according to methods of Rhodes and Jarvis (8). Lean pork meat was trimmed of fat and connective tissue, sliced and minced twice in a mincing machine whose sieve plate had pores of 4.5-mm diameter.

Meat slurry (100 g) was prepared of equal parts, by weight, of minced meat and sodium chloride solution (12 g per liter) and 3.3 ml oil solution (or Miglyol alone as a control), and blended for 30-45 sec. The pH was 6.4 - 6.5. The concentration of oil in the final volume was 1500 µg per g of meat slurry (1500 ppm).

#### Spore inoculum

Spores of *C. botulinum* type A (strain 73A), type B (strain RIV 1) and type E (strain RIV 2) originated from stock cultures and were inoculated separately in the medium of Tsuji and Perkins (9) (5% trypticase and 1% ammonium sulfate pH, 7.1). For production of *C. botulinum* type E 0.5% glucose was added to this medium. This was incubated at 30 C for 7 days after which the spores were harvested by centrifugation at 10,000 × g for 20 min at room temperature. The sediment was rinsed twice with physiological saline solution. The spore suspension was heated at 70 C for 20 min (heat shock treatment) and stored at 4 C (Spore inoculum).

#### Enumeration of *C. botulinum*

Numbers of spores of *C. botulinum* in the spore inoculum were determined using freshly prepared Brain Heart Infusion Egg Yolk Agar (Oxoid). After inoculation, plates were incubated in anaerobic jars for 2 days at 30 C.

#### Meat slurry system

The meat slurry system was obtained by inoculation of meat slurry with spore inoculum. Universal bottles (30 ml) were separately filled with meat slurries containing garlic oil, onion oil, or Miglyol alone (control), and inoculated with approximately 10 spores of *C. botulinum* type A, approximately 30 spores of type B, or approximately 20 spores of type E, respectively. The bottles were closed with screw caps, heated for 30 min at 70 C and incubated at 20 C. The amount of botulinum toxin was determined after various periods. The examinations were carried out in duplicate.

#### Determination of botulinum toxins

The quantity of toxin produced by *C. botulinum* in the meat slurry system was determined with a mouse-bioassay. To do this the meat slurry system was suspended with an equal part of 0.07 M phosphate buffer pH 6.5 containing 0.1% gelatin (PBG) and centrifuged at 10,000 × g for 10 min at room temperature. Two mice of 18-20 g were intraperitoneally (i.p.) injected with 0.5 ml of serial five-fold dilutions of the supernatant fluid in PBG, to determine the titer of the toxin (7). The mice were observed for 5 days for death with typical symptoms of botulism. The results were expressed as log<sub>10</sub> i.p. mouse LD<sub>50</sub> per gram. Furthermore, the type of toxin was confirmed by neutralization tests in

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mice, using monovalent antitoxins from the Institute Pasteur, Paris.

For the meat slurry system with spores of *C. botulinum* type A, the presence of toxin in the previously mentioned supernatant fluid was also detected with the Enzyme-linked Immunosorbent Assay (ELISA). The method used was the same as described by Notermans et al. (6).

RESULTS AND DISCUSSION

The concentrations of toxin from *C. botulinum* type A in the meat slurry system after incubation at 20 C for 7, 14 and 21 days are recorded in Fig. 1. Each point represents the average of two samples.

Figure 2 is as Fig. 1, but the toxin was determined with the ELISA-method instead of the mouse-bioassay. There is satisfactory agreement between the results of both methods. Thus the ELISA-method, formerly only used in artificial media, was not disturbed by the meat slurry. Therefore it may be useful for screening meat slurry systems.

After observing Fig. 1 and 2, it may be stated that garlic oil as well as onion oil inhibited production of toxin by *C. botulinum* type A. Probably the inhibition is not caused exclusively by the oils but depends also on the incubation temperature since *C. botulinum* type A has its optimum temperature at 37 C. An incubation temperature of 20 C is suboptimal for *C. botulinum* type A.

Figure 3 is as Fig. 1, but records toxin production

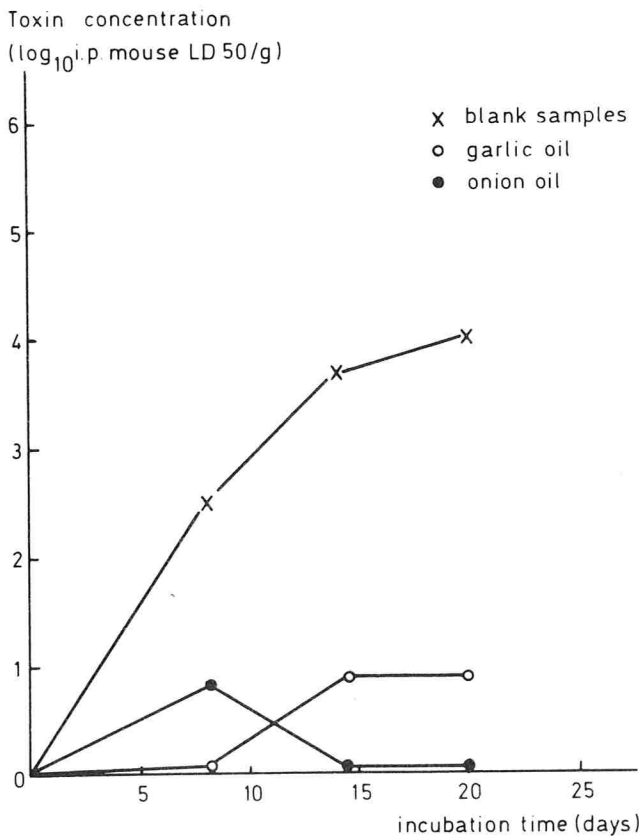


Figure 1. Toxin concentration produced in meat slurry systems by *C. botulinum* type A in the presence of garlic oil, onion oil, or Miglyol (control), after incubation at 20 C.

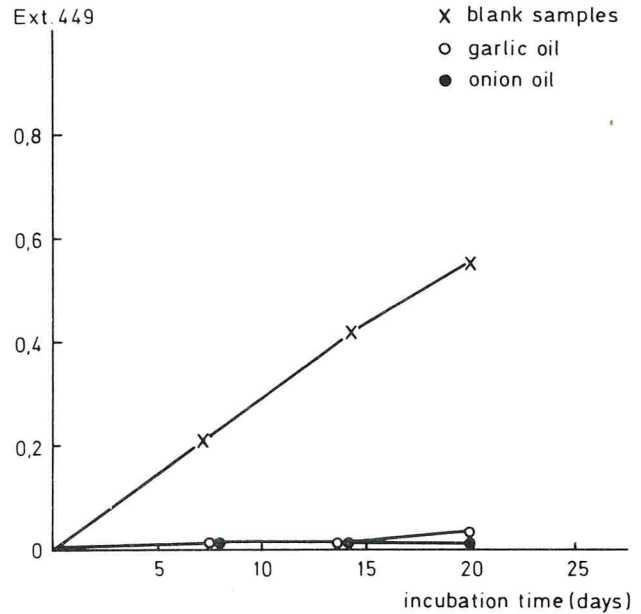


Figure 2. As Fig. 1, but toxin concentration was determined by the Enzyme-linked Immunosorbent Assay instead of the mouse-bio-assay.

from *C. botulinum* type B, after two periods of incubation (7 and 14 days). During the first 7 days there was a difference in toxin production between the experimentals (systems with garlic or onion oil) and the control. However, after 14 days incubation the difference was negligible.

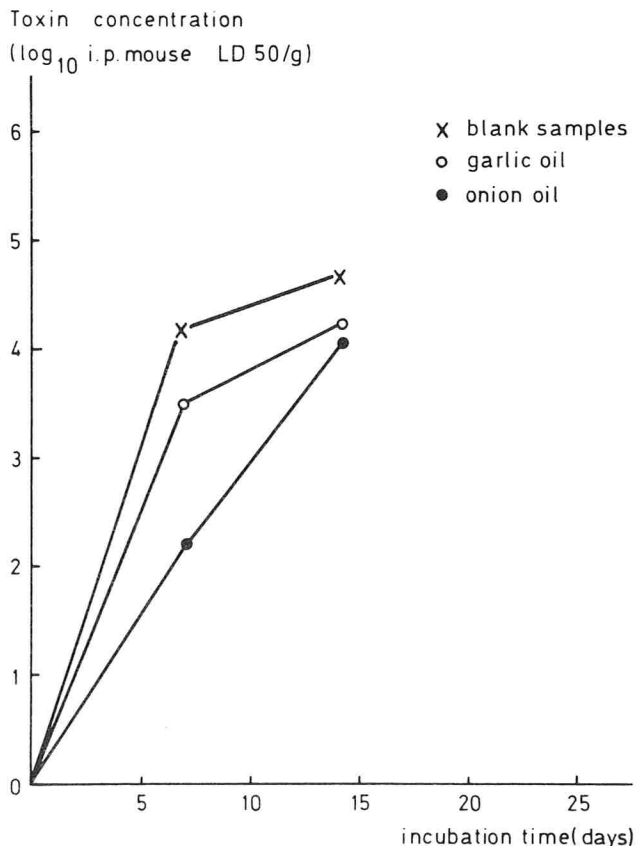


Figure 3. Toxin concentration produced in meat slurry systems by *C. botulinum* type B in the presence of garlic oil, onion oil, or Miglyol (control), after incubation at 20 C.

Figure 4 is as Fig. 3, but for *C. botulinum* type E. It is clear that both oils had no effect in inhibiting toxin production by type E.

It may be concluded that garlic oil (as well as onion oil) can not be applied in the meat industry because these preparations would only inhibit toxin production by type A. In industrial meat products several types of *C. botulinum* can be present and not only type A.

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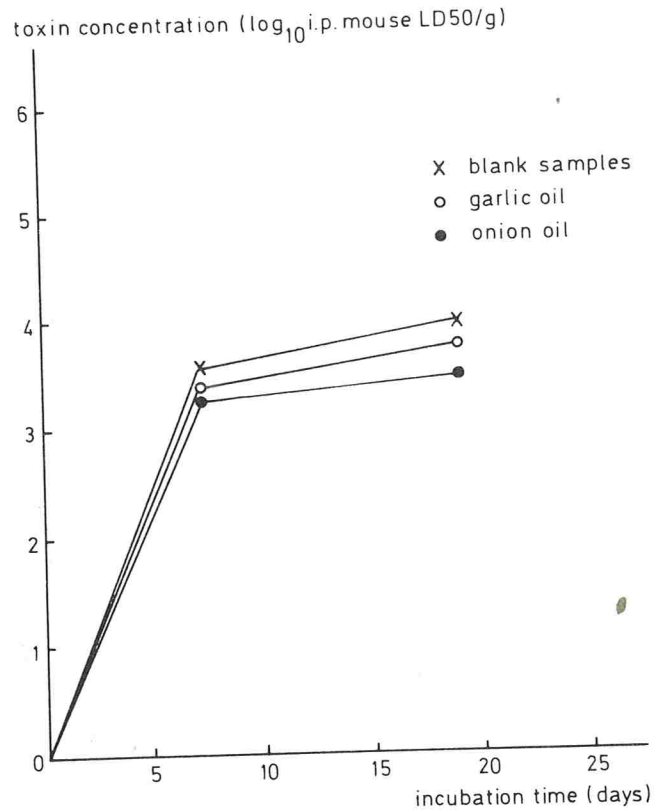


Figure 4. Toxin concentration in meat slurry systems produced by *C. botulinum* type E in the presence of garlic oil, onion oil, or Miglyol (control), after incubation at 20 C.

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## Effect of Anthocyanin Preparations as Colorants on Hygroscopicity of Dry-Pack Foods

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### ABSTRACT

The equilibrium relative humidity isotherm of a cherry beverage base and a strawberry gelatin dessert mix colored with anthocyanins from grape skins, cranberries and roselle was evaluated versus a Red No. 2 control. As well, the water vapor transmission rate of the packaging material was evaluated to evaluate probable shelf-life. Results indicated that physical parameters, such as hygroscopicity, must be considered in estimating effects of the use of natural colorants in foods as well as the color and chemical stability normally investigated.

One of the most important factors involved in preventing degradation of dry-pack powdered foods is the package. It must act as a barrier to moisture to prevent physical, as well as chemical changes from occurring.

The importance of packaging becomes more acute when consideration is given to use of natural pigments as food colorants due to their greater inherent chemical and physical instability as compared to synthetic colorants.

Chemical instability of anthocyanins in wet-pack products has been well documented and has been discussed in recent reviews (1,6). Carotenoids in the natural form also have stability problems (1) which are lessened to some extent when synthesized (7). Von Elbe (14) has pointed out that the stability of betalains in a gelatin dessert powder can be increased by packing in aluminum pouches or storing at a low relative humidity to reduce the water activity. Furthermore, Pasch and von Elbe (12) have shown that a four-fold increase in stability of betalaine may be attained when the water activity is reduced from 1.0 to 0.37. They postulate that this may be due to reduced mobility of reactants or limited oxygen solubility. In a study of physical stability of natural pigments, Soukup and Maing (13) noted that colors derived from dried aqueous extracts or juices are typically hygroscopic since other water-soluble materials are present. They qualitatively evaluated dried beet juice,

dried grape skin and turmeric versus synthetic dyes for stability as measured by several physical parameters, one of which was hygroscopicity.

With beet juice and grape skin extracts, hygroscopicity was rated as high and extreme, respectively, versus synthetic dyes rated as none while turmeric was rated as moderate (13). Thus it may be seen that hygroscopicity is a serious problem with natural pigments and in particular with anthocyanins from grape-skin extracts.

Until recently, little work has been done on evaluation of anthocyanins in dry-pack systems. However, several recent studies (2-4) evaluated the chemical stability of grape, cranberry and roselle anthocyanins in a cherry beverage base and a strawberry gelatin dessert. During these studies it was noted visually that hygroscopicity was a serious problem which would decrease the shelf-life of such products. Thus in estimating the product's shelf-life, the degree of hygroscopic activity and the rate of water vapor transmission through the packaging material are of great interest.

Therefore, this study was initiated to evaluate quantitatively the equilibrium relative humidity isotherm (ERHI) of cherry beverage base and strawberry gelatin dessert mix colored with anthocyanins from grape skins, cranberries and roselle, respectively, versus a Red No. 2 control. It was also necessary to calculate the water vapor transmission rate (WVTR) of the packaging material used in each instance to estimate the probable shelf-life of such materials. Such data provide further information on the potential of natural colorants in foods which must be evaluated not only on the basis of chemical or color stability but also on the basis of important physical parameters such as hygroscopicity.

### MATERIALS AND METHODS

The sources of anthocyanin pigment used in this study were cranberry pomace (Ocean Spray Cranberries, Inc., Hanson, MA), Concord grape filter trim (tartrate sludge, Welch Foods, Inc., Westfield, NY), and an extract of the calyces of roselle (*Hibiscus sabdariffa* L.) which was obtained as a liquid concentrate from Trinidad (5). The first two were

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extracted with a mixture of 95% ethanol and 0.01% citric acid while the third was obtained as a liquid extract. All three were concentrated (10:1) in a vacuum pan and chilled before filtering. A carbohydrate carrier (Morrex 1918) was added to obtain a 30% total solids mixture and the product was spray dried according to procedures established by Main et al. (10).

Amounts of spray dried powder required to color the dry-pack products to approximate the desired color achieved with Red No. 2 were established by visual panels (2-4).

The pigment powders were blended with colorless bases obtained from a supplier in the following proportions: (a) cherry beverage mix: 4.93 g of grape powder/100 g, 16.6 g of cranberry powder/100 g, 14.81 g of roselle powder/100 g and (b) strawberry gelatin dessert: 2.54 g of grape powder/100 g, 9.51 g of cranberry powder/100 g, 8.46 g of roselle powder/100 g.

The blended mixes were then packaged. The cherry beverage mix contained 12.00 g per cherry beverage pouch which produced 118 ml of final product. Packages used were foil (0.285 mil) pouch paper laminates. The strawberry gelatin dessert mix contained 21.25 g/pouch which also produced 118 ml of final product. Packages used were pouch paper laminated with 1.5 mil polyethylene (Reynolds Metals Co., Richmond, VA). The packaging material was selected to simulate commercial packaging of dry beverage mixes and gelatin dessert mixes (2-4).

#### Equilibrium moisture isotherms

Relative humidity (E.R.H.) data were obtained by use of the simplified E.R.H. apparatus described by Levine and Fagerson (9) as follows: (a) five-gram samples of dry product were dried in the vacuum oven at 70 C and weighed; (b) dried samples were placed in glass jars of selected atmospheres of saturated salt solutions at increasing relative humidities as follows: 11.1%, 20.4%, 31.9%, 43.4%, 50.0%, 66.8%, 75.1%, and 91.1%; (c) jar's lids were tightly closed and wrapped with tape; (d) they were placed in a constant temperature storage cabinet at 100 F and weighed every day until a constant weight was reached; (e) gain in weight was calculated as percent moisture pick-up; and (f) the E.R.H. absorption isotherm was plotted as percent moisture content vs. relative humidity; and (g) initial moisture content and the critical moisture content (C.M.C.) (where the product is unsaleable) were noted for shelf life calculations (11).

#### Moisture determination

Five- and 10-gram samples were placed in aluminum weighing dishes and their weights recorded. The dishes were placed in a vacuum oven at 70 C for 4 h. They were then removed and placed in a desiccator and allowed to attain room temperature after which they were weighed. From the change in weight, the moisture content was calculated as follows:

$$\% \text{ moisture content} = \frac{\text{weight change (water)} \times 100}{\text{weight of sample}}$$

#### Water vapor transmission rate (W.V.T.R.)

Because many packages have weak seals and may be folded or tightly creased, it is desirable to determine the (W.V.T.R.) by using the entire package (8). For determination of the W.V.T.R. the following procedure was used (8): (a) Foil and pouch paper packages used in the storage study were both tested. A quantity of desiccant was placed in the packages and heat-sealed in the same manner as the storage packs. At the same time, packages were filled with the product colored with powdered spray-dried anthocyanin and also sealed. (b) Packs were weighed and then placed into a controlled chamber set at 90% relative humidity and 100 F and were weighed for the first time in no later than 24 h. (c) Weighings were continued on a day-by-day basis until there was no change in the rate of gain. (d) The weight gain in water was then plotted against time and from the slope the W.V.T.R. was determined. From both the moisture equilibrium data and the W.V.T.R. rates, an estimation for shelf-life was calculated.

## RESULTS AND DISCUSSION

E.R.H. isotherms were plotted as shown in Fig. 1 and 2. These studies indicated that the natural colors

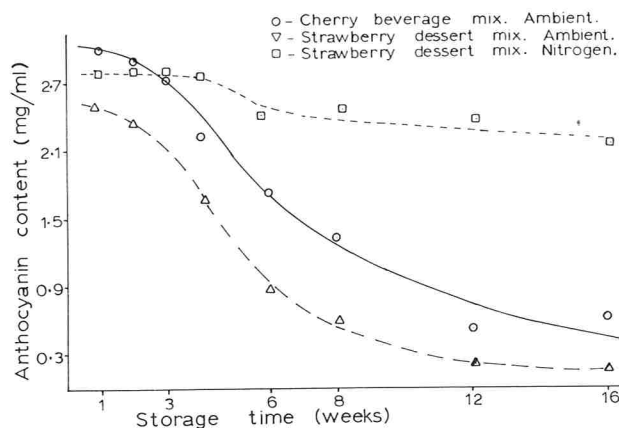


Figure 1. E.R.H. isotherm for strawberry dessert mix with different powdered colorants at 100 F.

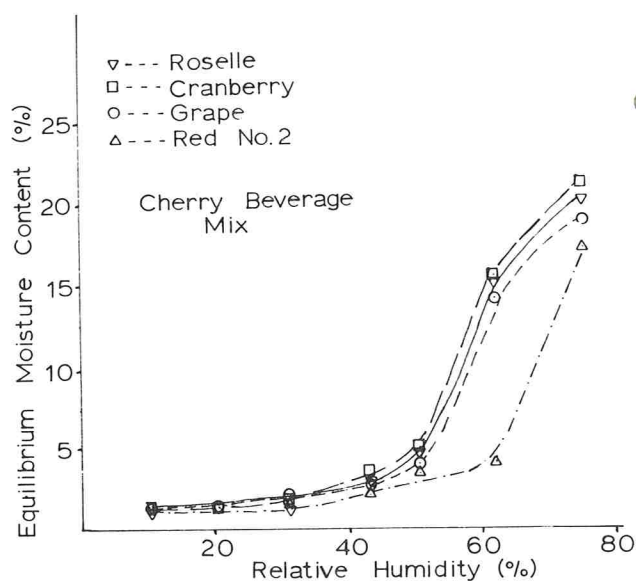


Figure 2. E.R.H. isotherm for cherry flavored beverage mix with different powdered colorants at 100 F.

increased the hygroscopic behavior of the food products and that a shortened shelf-life can be expected unless packaging is improved to compensate for the change. The Critical Moisture Content (CMC) for strawberry gelatin with Red #2 was 3% moisture, whereas with natural colors the CMC was 1.5-2% moisture. With cherry beverage mix the CMC for the Red #2 control was 2% moisture and 1-1.5% moisture for the anthocyanin colors. All critical moisture contents were calculated at the point where products caked up, which was not related to chemical changes. Caking occurred in all dry gelatin products colored with natural anthocyanin powders at 31.9% relative humidity at 100 F and in the cherry beverage mixes caking occurred at 20.4% relative humidity at 100 F.

Water vapor transmission rates (W.V.T.R.) were calculated as shown in Fig. 3 and 4, by plotting weight gain ( $H_2O$ ) versus time (days) for the two packages and their respective products. The amount of water absorbed

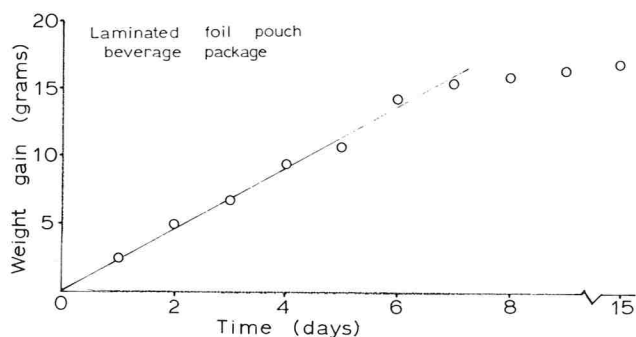


Figure 3. The W.V.T.R. of a laminated pouch beverage package, determined at 100 F and 90% R.H. with  $CaCl_2$  desiccant.

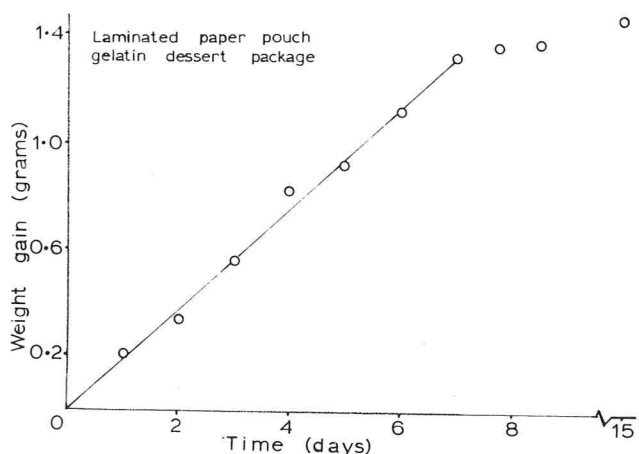


Figure 4. The W.V.T.R. of a laminated pouch paper gelatin dessert package, determined at 100 F and 90% R.H. with  $CaCl_2$  desiccant.

per day can be calculated (8), once the E.R.H. isotherm, critical moisture content (Fig. 1 and 2) and W.V.T.R. (Fig. 3 and 4) have been calculated, as follows:

- E = P.A. t. sP
- E = weight of water absorbed
- P = permeability of package to water vapor (W.V.T.R.)
- A = area of package
- t = time

These calculations showed the estimated shelf-life of the natural colored cherry beverage to be 200 days, and that of the natural colored strawberry gelatin to be 56 days. The amount of water absorbed by the product through the package (Fig. 3 and 4) is controlled by the W.V.T.R. (Fig. 3 and 4) of the particular package. The shelf-life is determined by this rate, and by obtaining the difference in moisture between the original moisture content, and the C.M.C. This figure divided by the daily uptake rate (Fig. 1 and 2) provides an estimated shelf-life

for a particular package and product under the conditions used. As storage temperature and humidity are lowered, shelf-life would be extended. Or by using a package with a lower W.V.T.R., shelf life of natural colored products could be increased. This is shown by the difference in the estimated shelf life of the two test products where package construction resulted in different rates of water adsorption.

Results of this study indicate that physical parameters such as hygroscopicity must be considered in estimating effects of the use of natural colorants in formulation as well as the color and chemical stability normally investigated.

ACKNOWLEDGMENTS

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## Attachment of Bacteria to Teats of Cows

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### ABSTRACT

The mechanism of attachment of bacteria to meat skin is of great importance in slaughter hygiene and sampling methods. In this study teats of cows were chosen as the meat surface for attachment experiments. Both the attachment rate and strength of attachment were determined. Results show that bacteria attached themselves readily to the surface of the teat. After the initial attachment the strength of attachment increased. This increase appeared to be faster at higher storage temperatures, and an optimum was reached after 2 h at 20 C and after 3 h at 12 C. After longer periods of storage the strength of attachment decreased, probably due to formation of colonies.

Attachment of bacteria to solid surfaces has been studied extensively. These investigations involved both biological surfaces (9,12), and non-biological surfaces (11,13-15,20) and were usually done to elucidate the predomination of some specific groups of bacteria on certain surfaces (1,7,8,10). Besides the just mentioned studies there have also been studies regarding the contribution of bacterial appendages, like flagella, fimbriae and spinae (15,19), and bacterial polymers (5) to the attachment.

In spite of all these investigations there has only been a small number which deals with attachment of bacteria to surfaces of meat, which is of great importance in improvement of slaughter-hygiene. In this respect the mechanism of attachment and detachment is of special interest, but knowledge about this is also of value as regards sampling methods for estimation of bacterial counts from meat. Studies by Notermans et al. (18) and Notermans and Kampelmacher (16), on attachment of bacteria to the skin of broiler chickens show this clearly. This attachment caused an important difference between bacterial counts determined with rinsing broiler carcasses with peptone-saline solution, and counts determined by macerating a portion of the carcass.

Blankenship (3) demonstrated that spores of *Clostridium perfringens* attach to skin of broiler chickens. He

also stated that knowledge of microbial attachment characteristics may be useful in devising processing decontamination techniques. Up to now it was not known whether the attachment of bacteria and bacterial spores, as studied by Notermans and Kampelmacher (16), and by Blankenship (3), occurred on substances other than chicken skin. Therefore another kind of meat skin (teats of cows) was used for these investigations to study aspects of bacterial attachment. Besides the attachment rates of different bacteria to the skin, studies were also made of the strength of the attachment.

### MATERIALS AND METHODS

#### Bacterial strains and their counting media

In these experiments different bacteria were used. Some of them were the same as used in an earlier study by Notermans and Kampelmacher (16). (a) *Escherichia coli* K12 - naladixic acid-resistant, peritrichous flagella. Growth medium: Brain-Heart-Infusion broth (Oxoid) with incubation at 37 C for 23 h. Counting medium: Violet-Red-Bile agar (Oxoid) +200 ppm naladixic acid, incubation for 20 h at 37 C. (b) *Klebsiella* sp. Non-motile, slime-producing, resistant to naladixic acid. The same growth and counting media as for *E. coli* were used. (c) *Pseudomonas* EBT/2/143 - Lophotrichous polar flagella, arginine-positive, non-pigmented, growth at 1 C, isolated from the skin of a turkey. Growth medium: Brain-Heart-Infusion broth (Oxoid) with incubation at 18 C for 18 h with rotary shaking (150 rpm). Counting medium: Brain-Heart-Infusion agar (Oxoid) with surface inoculation and incubation for 14 days at 1 C. (d) *Staphylococcus aureus* - Produces enterotoxin type C, isolated from skin of teats. Growth medium (Brain-Heart-Infusion Broth (Oxoid) with incubation for 20 h at 37 C on a rotary shaker (200 rpm). Counting medium: Baird-Parker (Oxoid) agar, surface inoculated, incubated for 24 h at 37 C. (e) *Salmonella typhimurium* II-505 and x-201 - Two strains isolated from different sources. Growth medium: Brain-Heart-Infusion broth (Oxoid), incubation for 20 h at 37 C. Counting medium: Brilliant-Green agar (Oxoid), with surface inoculation and incubation at 37 C for 20 h.

#### Teats

Teats of cows were obtained from a local slaughter-house. They were cleaned and deep-frozen till experimentation. The teats were thawed by holding them at 4 C for 15 h before attachment experiments.

#### Attachment suspension

Teats were dipped in physiological saline solution (8.7 g of NaCl/liter) containing phosphate buffer (0.01 M) of pH 7.2. The

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attachment suspension contained ca  $10^5$  bacteria/ml for those bacteria counted by plate pouring and ca  $10^6$  bacteria/ml for bacteria counted by surface inoculation.

#### Counting methods

From every teat two skin samples (each with a surface area of  $4.83 \text{ cm}^2$ ) were cut with a sterile cutting cylinder. One sample was counted using the blending method while the other was counted by the rinse method.

The blending method as described by Avens and Miller (2) was used. For this purpose the skin sample was cut, using scissors, into a laboratory blender containing 100 ml of 0.1% peptone water, and then the blender was operated for 1 min. Fluid was then diluted and/or plated in the appropriate medium. It was assumed that using this method all the bacteria present on the skin tissue were counted.

Using the rinse method, the skin sample was introduced to a sterile glass-jar containing 100 ml of 0.1% peptone water. The sample was shaken on a rotary shaker (250 rpm) for 1 min. The fluid was diluted and plated in the appropriate medium. This method enumerated only bacteria which were not attached or which are attached insecurely.

#### Attachment to skin of bacteria in the suspension

The teats were dipped in a bath containing 12 liters of attachment suspension. The attachment suspension was mixed by forced aeration and maintained at 20 C. After holding the teats for the appropriate time, they were removed from the bath. To count only attached bacteria, teats were washed by gently moving them in sterile physiological saline. This washing was repeated three times using fresh physiological saline.

Attached bacteria were regarded as those which were transferred from the attachment suspension to the skin, and which remained on the skin after washing.

The attachment rate was the number of bacteria which attached per minute to the skin with a surface area of  $4.83 \text{ cm}^2$ .

The difference between the logarithm of bacterial numbers obtained by the blending method and those obtained by the rinse method is expressed as the S-value.

#### Incubation after attachment

Teats were dipped in the attachment suspension for 20 min and then washed in sterile physiological water, as described above. They were then transferred to sterile glass jars which were firmly closed to prevent the skin from drying out. Jars were transferred to an incubator at either 20 or at 12 C. After various storage times the numbers of bacteria were estimated by using the blending method and the rinsing method.

## RESULTS

#### Attachment of bacteria to teats in a suspension

The attachment rates of five different strains of bacteria to teat skin were determined by the blending method and are reported in Fig. 1. From the results it becomes clear that all the bacterial strains tested show a marked attachment. *Ps. EBT/2/143* showed the fastest rate of attachment, and *S. typhimurium* II-505 the slowest rate. This slow rate was shared by another *Salmonella* strain (*S. typhimurium* x-202).

The attachment of some bacterial strains (*S. aureus*, *E. coli* K12, *S. typhimurium* II-505) was slow at the beginning, but reached a constant value after the teats had been in the attachment suspension for a few minutes.

To determine the strength of attachment, the number of bacteria was determined using both the rinse, and the blending methods. The numbers of *Ps. EBT/2/143* on the teat skin after immersion in the bacterial suspension for various periods of time are shown in Fig. 2. From data in

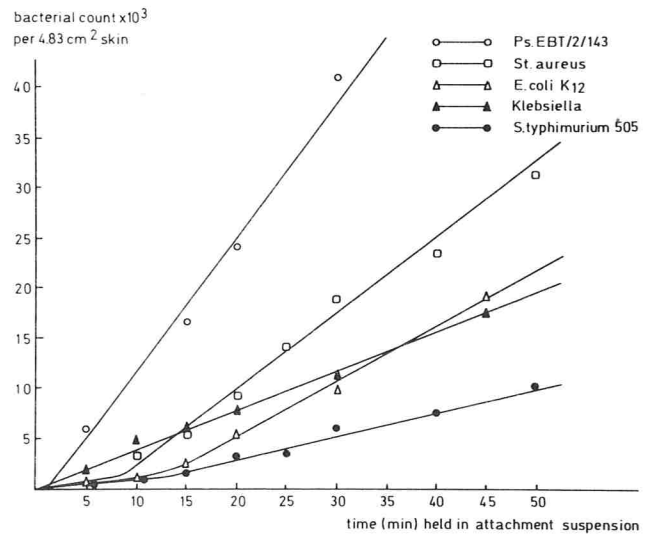


Figure 1. Attachment rates of different bacteria to teat skin at 20 C, based on a bacterial concentration of  $10^5$  /ml of attachment suspension.

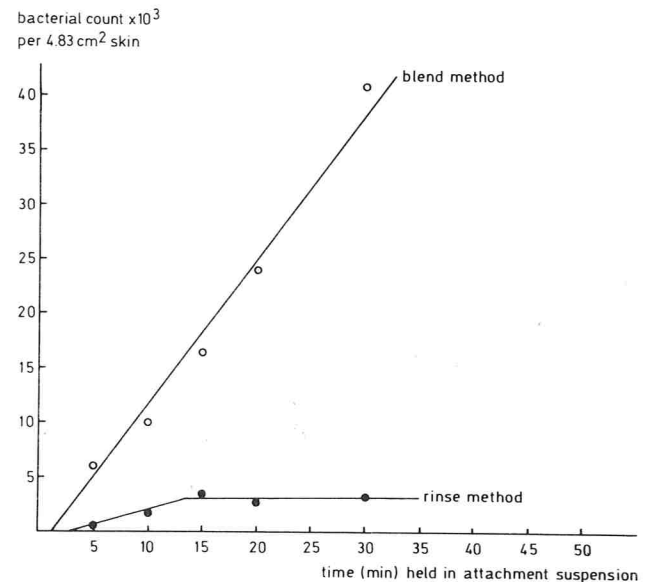


Figure 2. Comparison of rinse and blend methods to estimate the numbers of *Ps. EBT/2/143* attached to teat skin at 20 C, based on a bacterial concentration of  $10^5$  /ml of attachment suspension.

this graph it is evident that the numbers obtained by the blending method rose with time, whereas those obtained by the rinse method showed hardly any increase. For *E. coli* K12 (Fig. 3), however, a different type of behavior was observed. In this instance the numbers of *E. coli* K12, determined with the rinse method, rose in a slow but constant rate.

The attachment rate as well as the S-values of different bacterial strains are summarized in Table 1. From the results it is evident that a high attachment rate did not always result in a high S-value. Furthermore the S-value increased considerably after a longer immersion time of the teats in the bacterial suspension.

#### Incubation after attachment

In these experiments bacteria were allowed to attach to

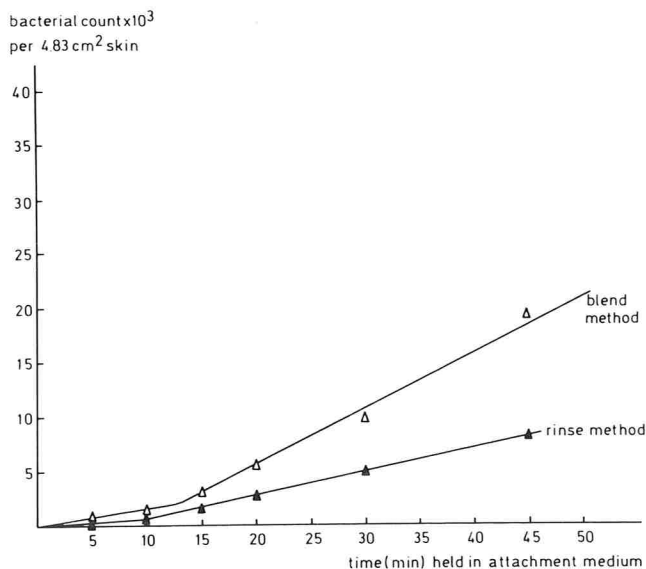


Figure 3. Comparison of rinse and blend methods to estimate the numbers of *E. coli* K12 attached to teat skin at 20 C, based on a bacterial concentration of  $10^5$  /ml of attachment suspension.

teat skin in a bacterial suspension at 20 C for 20 min. After washing the teats in sterile physiological saline, the teats were stored at 20 or at 12 C. The numbers of bacteria present after different storage times were estimated using both the blending and the rinsing methods. Results are in Fig. 4 and 5, and show clearly that the S-value increased during the first hours of storage, although the actual numbers of bacteria increased only in a moderate way during this time. Differences between the counting methods have a maximum after 1.5-3 h of storage at 20 C, and after 2.5-3.5 h of storage at 12 C. After longer storage times, the S-value decreased and at the same time the number of bacteria was higher for both methods. One-way analysis of variances was done to test whether the differences between the S-values were significant (6). The null-hypothesis in this case was that there is no difference in S-value obtained after different storage times. The results of this test are in Table 2. The differences were significant.

### DISCUSSION

From earlier investigations by Notermans and Kampelmacher (16), it was already clear that bacteria

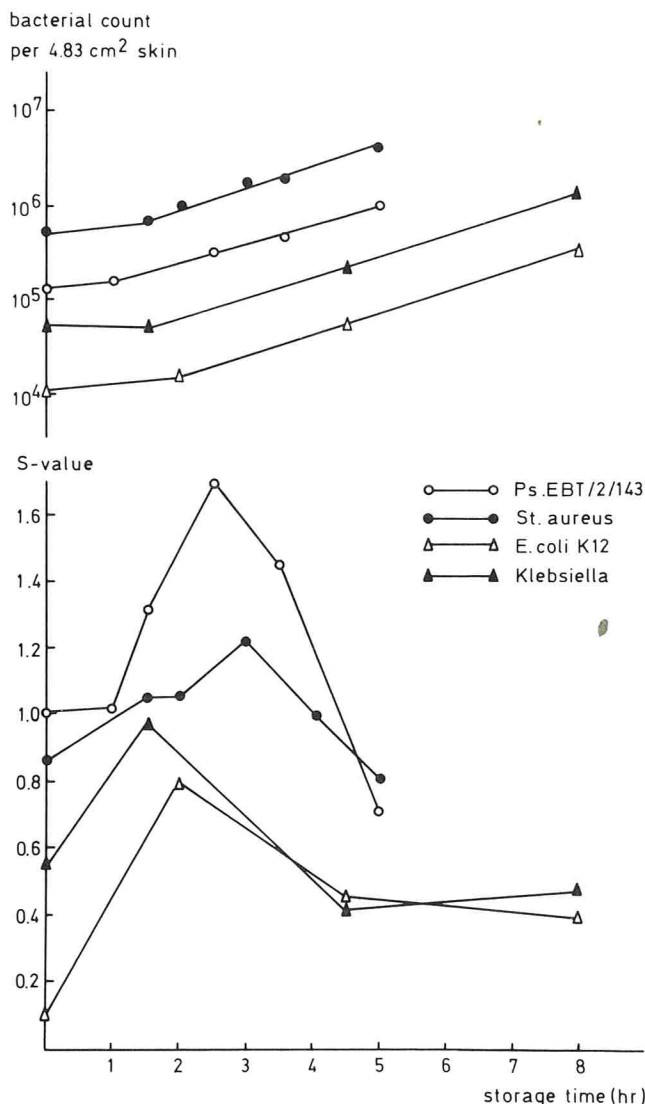


Figure 4. Effect of storage time at 20 C on growth and on the difference between counting methods. S-value =  $\log_{10}$  (bacterial count blend method) -  $\log_{10}$  (bacterial count rinse method).

can attach to skin of broiler chickens. From the results described here it is evident that this can also happen on the skin of cows' teats. The observed attachment rates of the different bacteria are in the same order as those obtained with the skin of broiler chickens. This fact underlines the dominant role of the bacteria in the mechanism of the attachment. The experiments of Notermans and

TABLE 1. Attachment of different bacterial strains at 20 C, based on  $10^5$  bacteria/ml of attachment suspension.

Bacterial strain	Attachment rate <sup>a</sup>		S-value <sup>b</sup>	
	Bacterial number attached/min.	Regression coefficient	After 10 min.	After 30 min.
Ps. EBT/2/143	1450	0.988	0.78	1.07
<i>S. aureus</i>	720	0.996	0.59	0.99
<i>E. coli</i> K12	550	0.998	0.10	0.35
<i>Klebsiella</i>	390	0.998	0.55	0.67
<i>S. typhimurium</i> II-505	230	0.988	0.14	0.70

<sup>a</sup>Number of bacteria which attach per minute to the skin with a surface area of 4.83 cm<sup>2</sup>.

<sup>b</sup>S-value =  $\log_{10}$  (bacterial count blending method) -  $\log_{10}$  (bacterial count rinse method).



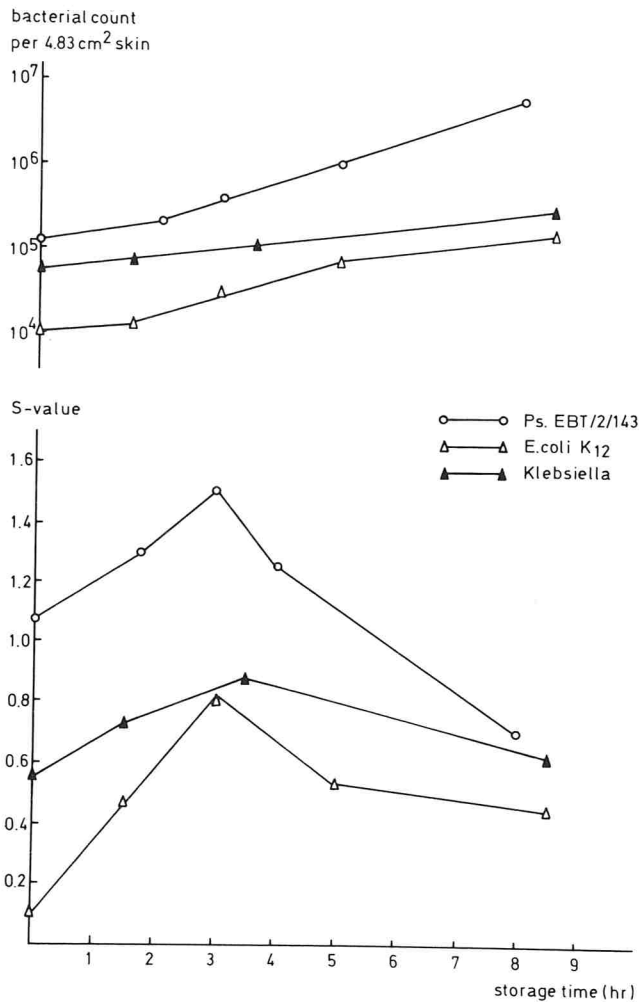


Figure 5. Effect of storage at 12 C on growth and on the difference between counting methods.  $S\text{-value} = \log_{10}(\text{bacterial count blend method}) - \log_{10}(\text{bacterial count rinse method})$ .

Kampelmacher (16) implicated flagella in the attachment of microorganisms to the skin of broiler chickens. The flagellated *E. coli* K12 had a higher attachment rate than *Klebsiella*. The same was found in these experiments. Now it becomes clear in using the rinse method that relatively fewer *Klebsiella* were determined than *E. coli* K12. This could mean that *Klebsiella* are attached more strongly to the surface than *E. coli* K12. In all probability this is due to slime production by the *Klebsiella* sp.

When the teats were stored after contamination in a

bacterial suspension, the difference in the counts obtained by the blending method and the rinsing method increased with length of storage. This increase in difference could serve to indicate the strength of bacterial attachment.

This increase also appears to be faster at higher storage temperatures, and is probably due to formation of extra-cellular substances produced earlier when the bacteria have a higher rate of metabolism (4,13). These substances, often composed of acidic polysaccharides (5), can play a role in the strength of attachment. The decrease in the difference between the blending and the rinsing methods after longer storage times could be due to formation of colonies by the bacteria. As the numbers of bacteria increase, more and more are probably attached to each other and not to the skin, and these may be easier to remove by the rinse method. It would be valuable to gain more information about this by using a scanning electron microscope.

From the results described here it is evident that attachment is a time-dependent process, and bacteria once attached are difficult to remove. This has important consequences for improving both hygiene and determination of bacterial counts in meat processing plants. For example, it will be important to prevent fecal contamination during slaughtering, and should it occur the bacteria should be removed as quickly as possible, by say, spray-cleaning (17).

From the results described here it can be seen that bacterial counts of surface contamination (e.g. from meat tables etc.) could be significantly different depending on the sampling method used.

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TABLE 2. Variance analysis of  $S\text{-value}^a$  after different times.

Bacterial strain	Storage temperature (C)	Number of time point	Total number of samples	F value <sup>b</sup>
<i>Klebsiella</i>	12	4	44	6.6
	20	4	34	14.2
<i>E. coli</i> K12	12	5	48	18.1
	20	4	37	25.8
<i>Ps. EBT/2/143</i>	12	5	21	12.1
	20	6	18	23.8
<i>S. aureus</i>	20	6	36	7.5

<sup>a</sup> $S\text{-value} = \log_{10}(\text{bacterial count blending method}) - \log_{10}(\text{bacterial count rinse method})$ .

<sup>b</sup>All F values in this table are above 0.1% level of significance.

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## Interim Report Farm Methods Committee

(Editor's note: Several subcommittees of the Farm Methods Committee provided few details for this report, as detailed material has either been contained in previous reports or will appear in future reports. Thus, these reports will not be provided here. These subcommittees are: Antibiotics, Pesticides, and other Adulterants Subcommittee, Cleaning and Sanitizing of Farm Milk Equipment Subcommittee, Education Subcommittee, Plastics Task Subcommittee, Animal Waste Management Task Subcommittee, Standardization of Milking System Installations Subcommittee, Standardization of Procedures for Uniform Inspection and Recommendation for Mastitis Prevention and Control Subcommittee, and Farm Sanitation Chemical Advisory Subcommittee.)

### Precooling Raw Milk on the Dairy Farm Subcommittee

In many dairy operations the milk cooling equipment is inadequate because of changes in cooling requirements, increased milking rates, loss of efficiency in the condensing unit(s) and/or milk pick up schedules that no longer allow sufficient time to cool milk in refrigerated milk tanks. Consequently, the dairyman is faced with a decision to install a complete new milk cooling system or to improve his existing system.

Every year an increasing number of dairymen choose the latter alternative and install a precooler. As the name implies, a precooler cools the milk before it enters the milk tank. Sanitary heat exchangers designed to clean in-place with the milking system cool the milk in the portion of the pipeline used to convey the milk to the tank. The heat exchanger may be designed to remove a small portion of the heat from the milk or to cool it to a safe storage temperature. The amount of

cooling accomplished is usually determined by the need (how bad is the existing system) and/or the amount of coolant available.

Precoolers are not limited to retro-fitting. Large producers find it possible to reduce the operating cost of milk cooling systems if part of cooling is accomplished in a precooler. The reduced load on the milk tank condensing unit(s) permits the use of smaller condensing units. The reduced cost of the smaller condensing unit(s) and/or the reduced running time will, in part, offset the cost of the precooler.

Although a precooler cannot improve the quality of the milk being cooled, two problems associated with refrigeration milk tanks, churning and rancidity, are minimized because cooling is rapid and without agitation.

Sanitary heat exchangers using well water as the cooling medium serve a dual purpose. The well water is "preheated" enroute to conventional water heating equipment. The resulting cost reduction is partially responsible for the increasing popularity of precooling milk and "preheating" of water on dairy farms.

In an ideal situation, no water is wasted. The heat exchanger is sized to gain maximum cooling based on the normal water flow during the milking.

### 3-A Approved Equipment Only Guidelines for Precooler Installation

1. Openings to the heat exchanger should be in the milkroom for adequate environmental protection for cleaning, sanitizing and drainage.
2. Heat exchangers should be installed so that adequate space is provided for disassembly and inspection.
3. Milkways shall be self-draining following the cooling cycle.
4. Waterways in tubular heat exchangers shall be self-draining following the cooling cycle to prevent lowering of cleaning solution temperatures.

5. Milk filtering shall be between the milk receiver group and the heat exchanger.
6. Tubular or plate heat exchangers must be installed in a manner that permits ease of disassembly for visual inspection of the milkways. (Milkways designed for cleaning in-place are not required to be accessible for visual inspection if the milkway is cleaned as a continuous tube.)
7. The refrigeration unit(s) used on tanks in connection with a precooler may be sized so that the *combined* cooling effect of the precooler and the refrigeration unit(s) meets or exceeds applicable cooling requirements.
8. It is recommended that recording thermometers be used with refrigerated milk tanks that are equipped with minimum refrigeration and with holding tanks that have no refrigeration. (It is recommended that all raw milk holding tanks have sufficient refrigeration for maintaining the milk at safe storage temperatures.)
9. In lieu of built-in refrigerated surface in milk holding tanks, it is recommended that means be provided for recirculating milk through the precooler.

### Sampling of Milk in Transport Tanks Subcommittee

Some progress has been noted in the sampling of milk in transport tanks. Jay Boosinger, Florida Department of Agriculture and Consumer Services, reported at the Farm Methods Committee Meeting held in conjunction with the National Mastitis Council on February 20, 1978, in Louisville, Kentucky, on trials conducted by Elbert Cammack, Chief, Bureau of Dairy Laboratories, Tallahassee, Florida. High speed agitation in the over-the-road tanker for five minutes was adequate in obtaining a representative sample. The interval fat testing was used as an index for getting a representative sample that then could be used for compositional, bacteriological and other testing.

## Physical and Sensory Characteristics of Beef Packaged in Modified Gas Atmospheres

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### ABSTRACT

One hundred and ninety-six boneless beef roasts (*longissimus* muscles) were vacuum packaged. Twenty-eight roasts remained vacuum packaged to serve as controls while the remaining packages were injected with one of six gas mixtures: (a) 100% O<sub>2</sub>, (b) 20% CO<sub>2</sub> + 80% N<sub>2</sub>, (c) 50% CO<sub>2</sub> + 50% O<sub>2</sub>, (d) 20% CO<sub>2</sub> + 80% O<sub>2</sub>, (e) 25% CO<sub>2</sub> + 25% O<sub>2</sub> + 50% N<sub>2</sub> or (f) 51% CO<sub>2</sub> + 30% O<sub>2</sub> + 18% N<sub>2</sub> + 1% CO. Five cuts from each packaging treatment were stored in a 1-3-C cooler for 7, 14, 21, 28 or 35 days and subsequently evaluated for relative percentages of various gases in the intact packages, off-odor, surface discoloration, overall appearance, metmyoglobin percentages and palatability. Packages initially injected with modified gas atmospheres containing O<sub>2</sub> increased in relative percentages of CO<sub>2</sub> with advancing storage times. Roasts stored in modified atmospheres initially containing high levels of O<sub>2</sub> exhibited a greater incidence of off-odor, more surface discoloration, lower overall appearance ratings, shorter retail caselife and lower overall palatability ratings than vacuum-packaged roasts or roasts stored in an atmosphere initially containing 20% CO<sub>2</sub> + 80% N<sub>2</sub>.

Although vacuum-packaging is the predominant method for the distribution of beef cuts, problems including leaker rates, deformation of cuts and purge loss still exist. Use of modified gas atmosphere packaging offers a possible solution to these problems.

Walters (21) claimed that in gas mixtures for meat packaging, the carbon dioxide will inhibit spoilage bacteria while the oxygen will keep the meat surface pigments oxygenated. Nitrogen is used primarily to dilute the concentration of carbon dioxide and oxygen.

Clark and Lentz (5) and Taylor and MacDougall (20) reported that optimum results, relative to meat color, were obtained when low concentrations of carbon dioxide (10-15%) were used in conjunction with high concentrations of oxygen. Partmann et al. (14) compared meat samples stored in 20% carbon dioxide + 80% nitrogen and those stored in 20% carbon dioxide + 80% oxygen with samples stored in air. After six weeks of storage, samples stored in the two modified atmospheres were relatively fresh in appearance, whereas the samples stored in air were spoiled as confirmed by bacteriological counts (14). Since previous research on the use of gas mixtures is not at all extensive and often is contradictory, this research was initiated to study the effects of various gas mixtures on the physical characteristics and palatability of beef cuts.

### EXPERIMENTAL

#### Packaging

One hundred and ninety-six boneless beef roasts (*longissimus* muscles) were cut (6.3-cm thick, approximately 530 g) from 30 U.S. Choice loins six days postmortem. All roasts were vacuum-packaged by use of a chamber-type, heat-seal vacuum-packaging machine (Maxi-Vac) at the maximum capacity of the machine (747 mm of Hg). Barrier bags with the following characteristics were used: Oxygen Transmission Rate (OTR) = 32 cc/m<sup>2</sup>/24 h/23.9 C/50% RH, Moisture Vapor Transmission Rate (MVTR) = 0.8 - 1.8 g/m<sup>2</sup>/24 h/37.7 C/70% RH and Carbon Dioxide Transmission Rate (CTR) = 47 cc/m<sup>2</sup>/24 h/23.9 C/50% RH. All vacuum-packaged roasts were randomly assigned to one of seven groups. Cuts assigned to group A remained vacuum-packaged to serve as controls for comparisons with other packaging treatments. Packaged roasts assigned to groups B, C, D, E, F and G were vacuum-packaged and subsequently injected with various gases (treatments), as shown in the experimental design (Table 1), with a head-space to meat ratio (vol/vol) of 1 to 1. The gases were introduced with a needle through a layer of dried silicone glue. Three packages containing the meat samples and gas mixture from each treatment were immediately subjected to gas analysis. Roasts assigned to each of the packaging treatments were then randomly assigned to one of five storage intervals (7, 14, 21, 28 or 35 days) and stored at 1-3 C.

#### Gas analysis

At the termination of each storage interval, vacuum packages were injected with 50 cc of helium to create sufficient gaseous volume to facilitate sampling of the contents. After a 5-min equilibration time, an 18-gauge needle was inserted through a layer of dried silicone glue. The needle was connected to a 5-cc sampling loop by means of a 1.6-mm (O.D.) stainless steel tubing. The gaseous contents were then purged through a sampling loop for 30 sec at which time the loop contents were injected on the gas chromatographic column. The permanent gases (CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub>) were separated on 80-100 mesh Porapak-Q (1.52 m × 6.35 mm) and 80-100 mesh molecular sieve 5-A (1.52 m × 6.35 mm) in series in a Gow-Mac Series 550 gas chromatograph equipped with a thermal conductivity detector.

#### Subjective evaluations

At the termination of the appropriate storage periods, individual packages were opened and roasts were stripped of packaging material. After a 30-min bloom period (time from opening the package until evaluation) roasts were subjectively evaluated under 970 lux of incandescent light by two trained evaluators for detectable off-odor, using a 4-point scale (4 = no off-odor; 1 = extreme off-odor), surface discoloration by visual evaluation, employing a 7-point scale (7 = 0% surface discoloration; 1 = 100% surface discoloration) and overall appearance, according to an 8-point scale (8 = extremely desirable; 1 = extremely undesirable). Cuts with coded identity were evaluated in completely random sequence; evaluators had no knowledge regarding treatment of a cut when they evaluated it.

#### Spectrophotometry

The surface concentration of metmyoglobin (metmb) was determined

on three samples per treatment per storage interval using a Bausch and Lomb Spectronic 505 Spectrophotometer equipped with a reflectance attachment and employing calculation procedures described by Broumond et al. (3).

#### Retail caselife evaluations

At the termination of each storage interval (7, 14, 21, 28 or 35 days), roasts were divided into two portions (3.2-cm thick). One-half of each roast was placed in a STYROFOAM tray with the freshly cut surface against the tray, overwrapped with PVC film (Choice Wrap) and placed under simulated retail display conditions (1-3 C, 970 lux of incandescent light). After 1 and 4 days of retail display, subjective evaluations were made by a 5-member trained panel for surface discoloration, employing a 7-point scale (7 = 0% surface discoloration; 1 = 100% surface discoloration) and overall appearance, using an 8-point scale (8 = extremely desirable; 1 = extremely undesirable). Cuts with coded identity were evaluated in completely random sequence.

#### Palatability evaluations

The second steak from each roast was wrapped in freezer paper and frozen at the termination of each storage interval. After the 21-day storage period, steaks were randomly assigned to a cooking sequence. Steaks were broiled in an electric oven (180 C) to an internal temperature of 75 C; copper constantan thermocouples were used to monitor oven and meat temperatures. An 8-member trained sensory panel evaluated each steak for flavor desirability and overall palatability, employing an 8-point scale (8 = like extremely; 1 = dislike extremely). Steaks were evaluated in a room with fluorescent lights. Each panel member received two pieces of each cooked steak. Five samples were evaluated

at each session. Only steaks from storage intervals of 7, 14 and 21 days were submitted for palatability evaluations.

#### Microbiological

Data regarding the microbial flora of cuts from this study are included in the companion report of Christopher et al. (4).

#### Statistical analysis

Analyses of data were accomplished using one-way analysis of variance comparing vacuum-packaging to each of the other treatments, independently, at each of the five storage intervals. One-way analysis of variance was also done on data within each treatment over the five storage intervals. When significant ( $P < .05$ ) main effects were observed in the analysis of variance, mean separation was accomplished by the Student-Newman-Keuls' test (18).

## RESULTS AND DISCUSSION

#### Gas analysis

The relative weight percentages of the major gases (Table 2) represent the summation of the gases (carbon dioxide, oxygen and nitrogen) comprising the headspace (in packages having a gaseous atmosphere) and possibly those gases that were previously dissolved in the purge and/or muscle tissue. Analysis of the gaseous contents from vacuum packages showed a rapid increase in the weight percentages of CO<sub>2</sub>, concomitant with a decrease in O<sub>2</sub> level. However, since the N<sub>2</sub> concentration is

TABLE 1. Experimental design for number of cuts per treatment per storage interval.

Storage interval (Days)	A	B	C	D	E	F	G
	Vacuum packaged	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
0	3	3	3	3	3	3	3
7	5	5	5	5	5	5	5
14	5	5	5	5	5	5	5
21	5	5	5	5	5	5	5
28	5	5	5	5	5	5	5
35	5	5	5	5	5	5	5

TABLE 2. Relative weight percentages of carbon dioxide, oxygen and nitrogen from packages of beef stratified according to treatment and storage interval.

Storage interval (days)	Type of gas	Packaging treatment						
		Vacuum packaged	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
Initial	CO <sub>2</sub>	33.10 <sup>d</sup>	0.00 <sup>e</sup>	19.00 <sup>b</sup>	41.25 <sup>b</sup>	13.67 <sup>d</sup>	20.59 <sup>b</sup>	43.47 <sup>b</sup>
	O <sub>2</sub>	8.40 <sup>a</sup>	100.00 <sup>a</sup>	0.74 <sup>a</sup>	55.02 <sup>b</sup>	84.77 <sup>a</sup>	25.40 <sup>a</sup>	32.63 <sup>a</sup>
	N <sub>2</sub>	58.50 <sup>a</sup>	0.00 <sup>f</sup>	80.26 <sup>a</sup>	3.73 <sup>b</sup>	1.56 <sup>b</sup>	54.01 <sup>cd</sup>	23.90 <sup>b</sup>
7	CO <sub>2</sub>	70.64 <sup>c</sup>	10.47 <sup>d</sup>	16.47 <sup>b</sup>	30.54 <sup>c</sup>	16.67 <sup>d</sup>	21.07 <sup>b</sup>	39.03 <sup>b</sup>
	O <sub>2</sub>	1.12 <sup>b</sup>	87.67 <sup>b</sup>	0.37 <sup>b</sup>	65.72 <sup>a</sup>	77.79 <sup>a</sup>	19.98 <sup>b</sup>	32.07 <sup>a</sup>
	N <sub>2</sub>	28.24 <sup>bc</sup>	1.86 <sup>e</sup>	83.16 <sup>a</sup>	3.74 <sup>b</sup>	5.54 <sup>b</sup>	58.95 <sup>cd</sup>	28.90 <sup>b</sup>
14	CO <sub>2</sub>	68.03 <sup>bc</sup>	36.74 <sup>c</sup>	17.20 <sup>b</sup>	34.05 <sup>bc</sup>	23.18 <sup>d</sup>	29.23 <sup>b</sup>	41.07 <sup>b</sup>
	O <sub>2</sub>	0.69 <sup>b</sup>	59.26 <sup>b</sup>	0.28 <sup>b</sup>	59.47 <sup>ab</sup>	72.28 <sup>a</sup>	3.01 <sup>c</sup>	25.75 <sup>a</sup>
	N <sub>2</sub>	31.28 <sup>b</sup>	4.00 <sup>d</sup>	82.52 <sup>a</sup>	6.48 <sup>b</sup>	4.54 <sup>b</sup>	67.76 <sup>c</sup>	33.18 <sup>ab</sup>
21	CO <sub>2</sub>	76.81 <sup>abc</sup>	63.63 <sup>b</sup>	21.30 <sup>a</sup>	39.37 <sup>b</sup>	41.45 <sup>c</sup>	53.73 <sup>a</sup>	56.62 <sup>a</sup>
	O <sub>2</sub>	0.92 <sup>b</sup>	29.94 <sup>c</sup>	0.30 <sup>b</sup>	51.95 <sup>b</sup>	53.19 <sup>b</sup>	0.36 <sup>c</sup>	14.49 <sup>b</sup>
	N <sub>2</sub>	22.27 <sup>bcd</sup>	6.43 <sup>c</sup>	78.40 <sup>b</sup>	8.68 <sup>b</sup>	5.36 <sup>b</sup>	45.91 <sup>d</sup>	26.89 <sup>b</sup>
28	CO <sub>2</sub>	82.17 <sup>a</sup>	77.40 <sup>a</sup>	22.34 <sup>a</sup>	53.92 <sup>a</sup>	56.98 <sup>b</sup>	35.18 <sup>b</sup>	48.27 <sup>ab</sup>
	O <sub>2</sub>	0.88 <sup>b</sup>	2.26 <sup>d</sup>	0.56 <sup>a</sup>	30.99 <sup>c</sup>	23.44 <sup>c</sup>	1.25 <sup>c</sup>	7.92 <sup>bc</sup>
	N <sub>2</sub>	16.95 <sup>d</sup>	20.34 <sup>b</sup>	77.10 <sup>bc</sup>	15.09 <sup>a</sup>	19.58 <sup>a</sup>	63.57 <sup>c</sup>	43.81 <sup>a</sup>
35	CO <sub>2</sub>	80.85 <sup>ab</sup>	73.31 <sup>a</sup>	21.53 <sup>a</sup>	59.79 <sup>a</sup>	68.43 <sup>a</sup>	34.41 <sup>b</sup>	54.71 <sup>ab</sup>
	O <sub>2</sub>	1.09 <sup>b</sup>	3.59 <sup>d</sup>	1.87 <sup>a</sup>	26.63 <sup>c</sup>	12.76 <sup>c</sup>	1.47 <sup>c</sup>	2.63 <sup>c</sup>
	N <sub>2</sub>	18.06 <sup>d</sup>	23.10 <sup>a</sup>	76.60 <sup>c</sup>	13.58 <sup>a</sup>	18.81 <sup>a</sup>	64.12 <sup>c</sup>	42.66 <sup>a</sup>

abcd<sup>ef</sup>Means of the same gas type in the same column bearing a common superscript do not differ ( $P > .05$ ).

relatively low compared to that which would be present due to the entrapment of air and since the concentration of CO<sub>2</sub> is relatively high, another source of CO<sub>2</sub> other than that from respiration of bacteria and/or meat tissue may be present. Perhaps some of the CO<sub>2</sub>, which had previously been dissolved in the fluid of the muscle tissue, was released from the purge into the headspace upon injection of helium into the packages before gas analysis.

The gas analyses of packages initially containing high levels of O<sub>2</sub> showed a gradual reduction in the concentration of O<sub>2</sub> and a subsequent increase in the concentration of CO<sub>2</sub> as storage time increased. Johnson (10) determined that not only meat tissue, but also bacteria, respire and convert O<sub>2</sub> to CO<sub>2</sub>. Taylor and MacDougall (20) reported that both O<sub>2</sub> and CO<sub>2</sub> dissolve in meat tissue, while respiration consumes O<sub>2</sub> and produces more CO<sub>2</sub>. Packages initially containing high concentrations of O<sub>2</sub> tended to collapse or draw more tightly to the meat surface with increased storage. This phenomenon was most evident in packages initially injected with 100% O<sub>2</sub>, such that after 35 days of storage, these packages nearly resembled vacuum packages and were also similar in gas composition to vacuum-packaged roasts that had been stored for 7 days. It may be possible for the O<sub>2</sub> in the initial headspace to be converted to CO<sub>2</sub> by respiration of the meat tissue and/or bacteria. The CO<sub>2</sub> thus produced may then become dissolved in the purge or meat tissue, thereby reducing the gaseous headspace. Jaye et al. (8) noted an increased headspace in packages of meat with a modified atmosphere. They (8) demonstrated that the gas was primarily CO<sub>2</sub> which resulted from the heterofermentative metabolism of lactic acid bacteria.

It was also noted that packages initially injected with 20% CO<sub>2</sub> + 80% N<sub>2</sub> had smaller amounts of purge at storage intervals of 7 and 14 days as compared to packages from all other treatments. This could suggest that purge losses could be minimized by the use of a 20% CO<sub>2</sub> + 80% N<sub>2</sub> modified atmosphere. Meat stored in high nitrogen atmospheres has been reported to exhibit a more swollen ultrastructure, less destruction of myofibrils, increased water-holding capacity and less extractability of sarcoplasmic and myofibrillar proteins (1).

### Subjective evaluations

Roasts packaged in modified atmospheres initially containing 100% O<sub>2</sub> or 80% O<sub>2</sub> had a significantly higher incidence of off-odor than vacuum packaged roasts after 7 and 14 days of storage, respectively (Table 3). Odor ratings of roasts packaged in modified atmospheres initially containing high concentrations of CO<sub>2</sub> (i.e., 50% CO<sub>2</sub> + 50% O<sub>2</sub> or 51% CO<sub>2</sub> + 30% O<sub>2</sub> + 18% N<sub>2</sub> + 1% CO) usually were similar to those of corresponding vacuum-packaged roasts. Roasts packaged in modified atmospheres initially containing 20% CO<sub>2</sub> + 80% N<sub>2</sub> did not significantly increase in the incidence of off-odor until after 28 days of storage. Ingram (7) reported that when O<sub>2</sub> is lacking, gram-negative meat spoilage bacteria are inhibited and therefore, only a low rate of proteolysis (responsible for subsequent off-odors) takes place. Ingram (7) further suggested that lactic acid bacteria which lower the pH cause the fixing of amino compounds that are the main putrefactive agents. However, Joseph (11) noted that vacuum-packaged meat acquired a cheesy, sour odor after 4 1/2 to 5 weeks.

The percentage of surface discoloration (evaluated subjectively) was significantly higher on those roasts stored in O<sub>2</sub>-containing modified atmospheres after 7 (100% O<sub>2</sub>), 14 (80% O<sub>2</sub> and 25% O<sub>2</sub>) or 21 (50% O<sub>2</sub>) days of storage than vacuum packaged roasts (Table 4). These results appear to partially contradict other researchers (15,13,19) who reported that high concentrations of O<sub>2</sub> could be used to delay discoloration of fresh meats. Vacuum-packaged roasts and roasts stored in a 20% CO<sub>2</sub> + 80% N<sub>2</sub> atmosphere were seldom significantly different in surface discoloration scores over the 35-day storage period; however, roasts stored in O<sub>2</sub>-containing atmospheres usually increased significantly in the percentage of surface discoloration between 7 and 21 days of storage. Although Ledward (12) indicated that high concentrations of CO<sub>2</sub> discolored meat, Taylor (19) reported that 50-80% CO<sub>2</sub> was often found in the residual air spaces in vacuum-packaged meat with no detrimental effect. The microbial flora on surfaces of roasts are believed to be primarily responsible for the discoloration ratings. *Pseudomonas* spp. have been reported to discolor meat (16), whereas, lactic acid bacteria are not generally responsible for discoloration (2,16).

TABLE 3. Mean values for odor<sup>a</sup> evaluation of beef cuts stratified according to packaging treatment and storage interval.

Storage interval (days)	Packaging treatment <sup>b</sup>						
	Vacuum packaged	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
7	4.0 <sup>c</sup>	<u>2.9</u> <sup>c</sup>	4.0 <sup>c</sup>	4.0 <sup>c</sup>	3.9 <sup>c</sup>	3.8 <sup>c</sup>	3.8 <sup>cd</sup>
14	3.9 <sup>cd</sup>	<u>1.6</u> <sup>c</sup>	3.8 <sup>c</sup>	3.8 <sup>c</sup>	<u>2.7</u> <sup>d</sup>	<u>2.8</u> <sup>d</sup>	3.9 <sup>c</sup>
21	3.7 <sup>d</sup>	<u>2.0</u> <sup>de</sup>	3.6 <sup>c</sup>	<u>2.4</u> <sup>e</sup>	<u>1.8</u> <sup>f</sup>	<u>3.3</u> <sup>d</sup>	3.9 <sup>c</sup>
28	3.4 <sup>e</sup>	<u>1.8</u> <sup>de</sup>	3.5 <sup>c</sup>	3.0 <sup>d</sup>	<u>2.0</u> <sup>ef</sup>	<u>3.9</u> <sup>c</sup>	3.4 <sup>d</sup>
35	3.0 <sup>f</sup>	<u>2.1</u> <sup>d</sup>	3.0 <sup>d</sup>	2.9 <sup>de</sup>	<u>2.2</u> <sup>e</sup>	3.1 <sup>d</sup>	<u>3.4</u> <sup>d</sup>

<sup>a</sup>Means based on a 4-point scale (4 = no detectable off-odor; 1 = extreme off-odor).

<sup>b</sup>Means within a common storage interval that are underlined are significantly different (P < .05) from means obtained from vacuum packaged cuts.

<sup>cdef</sup>Means in the same column bearing a common superscript do not differ (P > .05).

Roasts from O<sub>2</sub>-containing modified atmospheres were significantly lower in overall appearance ratings than vacuum-packaged roasts after 7 (100% O<sub>2</sub>), 14 (80% O<sub>2</sub> and 25% O<sub>2</sub>) or 21 (50% O<sub>2</sub>) days of storage (Table 5). Roasts stored in modified atmospheres initially containing 20% CO<sub>2</sub> + 80% N<sub>2</sub> were significantly more desirable in appearance than vacuum-packaged roasts at a storage interval of 35 days. Partmann et al. (14) reported that meat stored in 20% CO<sub>2</sub> + 80% N<sub>2</sub> for 6 weeks was very fresh in appearance as compared to meat samples stored in air. Overall appearance ratings decreased at earlier periods of storage for roasts stored in O<sub>2</sub>-containing modified atmospheres as compared to roasts stored in vacuum packages or in a modified atmosphere initially containing 20% CO<sub>2</sub> + 80% N<sub>2</sub>.

#### Spectrophotometry

At storage intervals greater than 7 days, the percentages of metmyoglobin (metmb) on surfaces of roasts

stored in vacuum packages or in a modified atmosphere initially containing 20% CO<sub>2</sub> + 80% N<sub>2</sub> remained substantially lower than that of roasts stored initially in atmospheres containing 25, 50, 80, or 100% O<sub>2</sub> (Table 6). Roasts stored in an atmosphere initially containing 1% CO usually had lower metmb values than roasts stored in O<sub>2</sub>-containing modified atmospheres. Metmb values for those roasts stored in O<sub>2</sub>-containing modified atmospheres increased to a maximum at 14 or 21 days and then decreased again. Metmyoglobin percentages closely paralleled data for surface discoloration (Table 4) and for overall appearance (Table 5), suggesting that most of the color deterioration of cuts was that associated with conversion of muscle pigments to their oxidized form.

#### Retail caselife evaluations

Mean values for surface discoloration of steaks after 1 and 4 days of retail display are shown in Table 7. After

TABLE 4. Mean values for surface discoloration<sup>a</sup> of beef cuts stratified according to packaging treatment and storage interval.

Storage interval (days)	Packaging treatment <sup>b</sup>						
	Vacuum packaged	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
7	6.3 <sup>c</sup>	<u>3.9<sup>c</sup></u>	6.1 <sup>c</sup>	5.5 <sup>c</sup>	6.6 <sup>c</sup>	4.6 <sup>cd</sup>	5.6 <sup>c</sup>
14	6.0 <sup>c</sup>	<u>1.9<sup>d</sup></u>	6.0 <sup>c</sup>	5.3 <sup>c</sup>	3.0 <sup>d</sup>	2.6 <sup>e</sup>	5.1 <sup>c</sup>
21	6.3 <sup>c</sup>	<u>2.0<sup>d</sup></u>	5.7 <sup>c</sup>	<u>1.6<sup>d</sup></u>	<u>1.1<sup>f</sup></u>	<u>2.8<sup>e</sup></u>	4.4 <sup>c</sup>
28	6.1 <sup>c</sup>	<u>2.1<sup>d</sup></u>	6.3 <sup>c</sup>	<u>1.9<sup>d</sup></u>	<u>1.8<sup>ef</sup></u>	5.2 <sup>c</sup>	<u>4.5<sup>c</sup></u>
35	4.8 <sup>d</sup>	<u>2.6<sup>d</sup></u>	5.6 <sup>c</sup>	<u>1.6<sup>d</sup></u>	<u>2.1<sup>e</sup></u>	3.3 <sup>de</sup>	5.9 <sup>c</sup>

<sup>a</sup>Means based on a 7-point scale (7 = 0% surface discoloration; 1 = 100% surface discoloration).

<sup>b</sup>Means within a common storage interval that are underlined are significantly different (P < .05) from means obtained from vacuum packaged cuts.

<sup>cde</sup>Means in the same column bearing a common superscript do not differ (P > .05).

TABLE 5. Mean values for overall appearance<sup>a</sup> of beef cuts stratified according to packaging treatment and storage interval.

Storage interval (days)	Packaging treatment <sup>b</sup>						
	Vacuum packaged	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
7	7.4 <sup>c</sup>	<u>3.4<sup>c</sup></u>	6.9 <sup>c</sup>	5.7 <sup>c</sup>	7.5 <sup>c</sup>	5.5 <sup>c</sup>	6.5 <sup>c</sup>
14	6.5 <sup>d</sup>	<u>1.3<sup>d</sup></u>	6.8 <sup>cd</sup>	5.2 <sup>c</sup>	2.3 <sup>d</sup>	1.6 <sup>d</sup>	5.4 <sup>cd</sup>
21	7.3 <sup>c</sup>	<u>1.5<sup>d</sup></u>	6.3 <sup>d</sup>	1.1 <sup>d</sup>	1.0 <sup>e</sup>	2.9 <sup>d</sup>	3.8 <sup>d</sup>
28	6.4 <sup>d</sup>	<u>2.1<sup>d</sup></u>	6.7 <sup>cd</sup>	1.6 <sup>d</sup>	1.3 <sup>e</sup>	5.6 <sup>c</sup>	3.8 <sup>d</sup>
35	4.7 <sup>e</sup>	<u>2.0<sup>d</sup></u>	5.4 <sup>e</sup>	1.1 <sup>d</sup>	1.5 <sup>de</sup>	3.1 <sup>d</sup>	5.3 <sup>cd</sup>

<sup>a</sup>Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

<sup>b</sup>Means within a common storage interval that are underlined are significantly different (P < .05) from means obtained from vacuum packaged cuts.

<sup>cde</sup>Means in the same column bearing a common superscript do not differ (P > .05).

TABLE 6. Mean metmyoglobin values of packaged beef stratified according to packaging treatment and storage interval.

Storage interval (days)	Packaging treatments <sup>abc</sup>						
	Vacuum packaged	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
7	17.3	30.3	27.0	24.0	15.3	26.7	23.0
14	29.3	50.0	15.7	30.0	48.0	61.3	0.0
21	10.0	60.0	19.3	64.0	73.3	35.0	14.3
28	13.0	46.7	10.0	41.3	58.7	21.7	12.7
35	26.5	39.7	20.6	62.0	44.3	26.7	5.5

<sup>a</sup>Each value represents the mean percentage of 3 samples.

<sup>b</sup>Spectrophotometry readings taken 30 min after opening packages.

<sup>c</sup>Statistics were not performed on these data.

1 day of display, steaks from roasts stored in modified atmospheres initially containing 50% or more O<sub>2</sub> and stored for 14 days or more, consistently had significantly greater amounts of surface discoloration than steaks from vacuum-packaged roasts, however, these differences were not as apparent after 4 days of retail display. After 1 day of retail display, steaks from roasts stored in modified atmospheres containing 50, 80 or 100% O<sub>2</sub> tended to increase in surface discoloration at earlier periods of storage and remained discolored. However, with steaks from vacuum-packaged roasts or from roasts stored in a modified atmosphere initially containing 20% CO<sub>2</sub> + 80% N<sub>2</sub>, surface discoloration generally tended to increase at a slower rate or to decrease as storage interval increased, respectively.

Mean values for overall appearance of beef steaks after 1 and 4 days of retail display are shown in Table 8. After 1 day of retail display, steaks derived from roasts that had been stored for 14 or more days in modified atmospheres containing 50, 80 or 100% O<sub>2</sub> were significantly lower in overall appearance than steaks from vacuum-packaged roasts, however, these differ-

ences were not as apparent after 4 days of retail display. The overall appearance ratings of steaks from roasts stored in a modified atmosphere initially containing 20% CO<sub>2</sub> + 80% N<sub>2</sub> tended to increase as the storage interval increased.

*Palatability evaluations*

Flavor desirability ratings were usually significantly lower for steaks derived from roasts stored in O<sub>2</sub>-containing modified atmospheres as compared to steaks from vacuum packaged roasts (Table 9). Steaks from roasts stored in O<sub>2</sub>-containing modified atmospheres tended to decrease in flavor desirability ratings between 7 and 14 days of storage, whereas this decrease in flavor desirability usually occurred between 14 and 21 days for steaks from vacuum-packaged roasts or from roasts stored in a modified atmosphere initially containing 20% CO<sub>2</sub> + 80% N<sub>2</sub>. Shank and Lundquist (17) suggested that vacuum packaging prevents fat oxidation (by the exclusion of O<sub>2</sub>) and reported a mild acid taste after 21 days of vacuumized storage. Hodges et al. (6) and Jeremiah et al. (9) also reported increased incidence of off-flavor of vacuum-packaged meat as

TABLE 7. Mean values for surface discoloration<sup>a</sup> of retail beef steaks stratified according to packaging treatment and storage interval of beef steaks in atmosphere packages and day of retail display.

Day of display	Storage interval (days)	Packaging treatment <sup>b</sup>						
		Vacuum packaged	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
Day 1	7	5.5 <sup>c</sup>	2.7 <sup>c</sup>	5.3 <sup>d</sup>	5.1 <sup>c</sup>	5.7 <sup>c</sup>	3.8 <sup>cd</sup>	6.2 <sup>c</sup>
	14	6.4 <sup>c</sup>	1.3 <sup>d</sup>	6.5 <sup>c</sup>	4.1 <sup>c</sup>	1.9 <sup>d</sup>	2.5 <sup>d</sup>	4.7 <sup>cd</sup>
	21	5.7 <sup>c</sup>	1.6 <sup>d</sup>	5.9 <sup>c</sup>	1.8 <sup>d</sup>	1.1 <sup>d</sup>	2.4 <sup>d</sup>	4.4 <sup>d</sup>
	28	5.4 <sup>c</sup>	1.5 <sup>d</sup>	6.1 <sup>c</sup>	1.7 <sup>d</sup>	1.9 <sup>d</sup>	5.0 <sup>c</sup>	5.5 <sup>cd</sup>
	35	3.3 <sup>d</sup>	1.8 <sup>cd</sup>	6.1 <sup>c</sup>	1.3 <sup>d</sup>	1.7 <sup>d</sup>	3.7 <sup>cd</sup>	6.1 <sup>c</sup>
Day 4	7	1.4 <sup>d</sup>	1.1 <sup>f</sup>	1.8 <sup>d</sup>	3.1 <sup>c</sup>	2.1 <sup>c</sup>	1.5 <sup>d</sup>	3.9 <sup>c</sup>
	14	1.9 <sup>d</sup>	2.5 <sup>cd</sup>	1.8 <sup>d</sup>	1.5 <sup>d</sup>	1.5 <sup>c</sup>	1.5 <sup>d</sup>	2.9 <sup>c</sup>
	21	2.9 <sup>c</sup>	2.3 <sup>cd</sup>	4.5 <sup>c</sup>	1.2 <sup>d</sup>	1.6 <sup>c</sup>	1.7 <sup>d</sup>	3.4 <sup>c</sup>
	28	2.9 <sup>c</sup>	3.1 <sup>c</sup>	3.7 <sup>c</sup>	1.2 <sup>d</sup>	1.5 <sup>c</sup>	2.9 <sup>c</sup>	4.0 <sup>c</sup>
	35	1.8 <sup>d</sup>	2.9 <sup>cd</sup>	3.9 <sup>c</sup>	1.3 <sup>d</sup>	1.3 <sup>c</sup>	1.9 <sup>d</sup>	3.3 <sup>c</sup>

<sup>a</sup>Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration).

<sup>b</sup>Means within a common storage interval and day of retail display that are underlined are significantly different (P < .05) from means obtained from cuts from vacuum packages.

<sup>cdef</sup>Means in the same column and same day of display bearing a common superscript do not differ (P > .05).

TABLE 8. Mean values for overall appearance<sup>a</sup> of retail beef steaks stratified according to packaging treatment and storage interval of beef steaks in atmosphere packages and day of retail display.

Day of display	Storage interval (days)	Packaging treatment <sup>b</sup>						
		Vacuum packaged	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
Day 1	7	6.3 <sup>cdde</sup>	1.7 <sup>c</sup>	5.2 <sup>d</sup>	5.2 <sup>c</sup>	6.1 <sup>c</sup>	3.4 <sup>d</sup>	6.5 <sup>c</sup>
	14	7.1 <sup>c</sup>	1.1 <sup>c</sup>	6.9 <sup>c</sup>	3.9 <sup>c</sup>	1.3 <sup>d</sup>	1.7 <sup>e</sup>	5.1 <sup>c</sup>
	21	6.5 <sup>cd</sup>	1.1 <sup>c</sup>	6.9 <sup>c</sup>	1.3 <sup>d</sup>	1.0 <sup>d</sup>	2.2 <sup>de</sup>	4.2 <sup>c</sup>
	28	5.8 <sup>ef</sup>	1.2 <sup>c</sup>	6.6 <sup>c</sup>	1.2 <sup>d</sup>	1.5 <sup>d</sup>	5.1 <sup>c</sup>	5.5 <sup>c</sup>
	35	3.1 <sup>g</sup>	1.4 <sup>c</sup>	6.3 <sup>c</sup>	1.1 <sup>d</sup>	1.2 <sup>d</sup>	3.5 <sup>d</sup>	6.2 <sup>c</sup>
Day 4	7	1.0 <sup>d</sup>	1.0 <sup>d</sup>	1.3 <sup>e</sup>	3.1 <sup>c</sup>	1.7 <sup>c</sup>	1.1 <sup>d</sup>	4.4 <sup>c</sup>
	14	1.2 <sup>d</sup>	2.3 <sup>cd</sup>	1.3 <sup>e</sup>	1.3 <sup>d</sup>	1.1 <sup>d</sup>	1.1 <sup>d</sup>	2.6 <sup>c</sup>
	21	2.6 <sup>c</sup>	1.9 <sup>cd</sup>	4.8 <sup>c</sup>	1.1 <sup>d</sup>	1.2 <sup>d</sup>	1.2 <sup>d</sup>	3.6 <sup>c</sup>
	28	2.8 <sup>c</sup>	2.6 <sup>c</sup>	3.3 <sup>d</sup>	1.1 <sup>d</sup>	1.1 <sup>d</sup>	2.7 <sup>c</sup>	3.6 <sup>c</sup>
	35	1.4 <sup>d</sup>	2.9 <sup>c</sup>	4.1 <sup>cd</sup>	1.0 <sup>d</sup>	1.0 <sup>d</sup>	1.8 <sup>d</sup>	2.8 <sup>c</sup>

<sup>a</sup>Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

<sup>b</sup>Means within a common storage interval and day of retail display that are underlined are significantly different (P < .05) from means obtained from cuts from vacuum packages.

<sup>cdefg</sup>Means in the same column and same day of display bearing a common superscript do not differ (P > .05).

storage increased.

Overall palatability ratings were significantly lower for steaks from roasts stored in high O<sub>2</sub>-containing modified atmospheres (100% O<sub>2</sub> and 80% O<sub>2</sub>) after 14 days of storage and for all O<sub>2</sub>-containing modified atmospheres (100, 80, 50 and 25% O<sub>2</sub>) after 21 days of storage as compared to steaks from vacuum packaged roasts (Table 10).

Data presented in a companion paper (4) on the microbiological characteristics of roasts stored in vacuum-packaged and in modified gas atmospheres are useful to explain some of the changes in quality attributes of these samples during storage. A higher incidence of off-odor, surface discoloration and lower overall appearance ratings of roasts stored in high oxygen-containing atmospheres probably is related to a greater persistence of *Pseudomonas* spp. on these meats during storage and the relatively high initial count and percentage of gram-negative bacteria on the roasts. *Pseudomonas*, *Moraxella* and *Acinetobacter* spp. comprised 85.4% of the initial count. After 21-35 days of storage, *Pseudomonas* spp. made up 19.9 to 52.2% of the microbial population of roasts initially stored in 100% O<sub>2</sub> but only 8.7 to 18.2% on vacuum-packaged roasts. Although lactobacilli were the dominant species on all roasts after 28 to 35 days of storage, their dominance was somewhat less complete on the roasts initially stored in atmospheres containing 50 to 100% O<sub>2</sub>.

### CONCLUSIONS

Results of the present study suggest that use of gas mixtures containing high levels of O<sub>2</sub> do not create an atmosphere conducive for long-term storage of fresh beef. The use of a gas mixture of 20% CO<sub>2</sub> + 80% N<sub>2</sub> was at least equal, if not superior, to vacuum packaging. Further research is needed in such areas as CO<sub>2</sub>

evolution from meat tissue, effect of gas atmosphere on the ultrastructure and water-holding capacity of meat and on various combinations of CO<sub>2</sub> and N<sub>2</sub> that will not detrimentally affect the physical characteristics or palatability of fresh meats during storage.

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TABLE 9. Mean values for flavor desirability<sup>a</sup> of beef steaks stratified according to packaging treatment and storage interval.

Storage interval (days)	Packaging treatment <sup>b</sup>					
	Vacuum packaged	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>
7	4.6 <sup>c</sup>	3.9 <sup>c</sup>	4.9 <sup>c</sup>	4.0 <sup>c</sup>	4.3 <sup>c</sup>	4.5 <sup>c</sup>
14	4.7 <sup>c</sup>	<u>2.2<sup>d</sup></u>	4.3 <sup>c</sup>	<u>3.6<sup>d</sup></u>	<u>3.2<sup>d</sup></u>	<u>3.3<sup>d</sup></u>
21	3.5 <sup>d</sup>	<u>1.8<sup>d</sup></u>	3.1 <sup>d</sup>	2.6 <sup>d</sup>	<u>2.1<sup>d</sup></u>	<u>2.8<sup>d</sup></u>

<sup>a</sup>Means based on an 8-point scale (8 = like extremely; 1 = dislike extremely).

<sup>b</sup>Means within a common storage interval that are underlined are significantly different (P < .05) from means obtained from vacuum packaged cuts.

<sup>cd</sup>Means in the same column bearing a common superscript do not differ (P > .05).

TABLE 10. Mean values for overall palatability<sup>a</sup> of beef steaks stratified according to packaging treatment and storage interval.

Storage interval (days)	Packaging treatment <sup>b</sup>					
	Vacuum packaged	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>
7	4.6 <sup>c</sup>	3.9 <sup>c</sup>	4.8 <sup>c</sup>	4.2 <sup>c</sup>	4.1 <sup>c</sup>	4.6 <sup>c</sup>
14	4.2 <sup>c</sup>	<u>2.3<sup>d</sup></u>	4.1 <sup>c</sup>	4.5 <sup>c</sup>	<u>3.2<sup>d</sup></u>	3.4 <sup>d</sup>
21	3.4 <sup>d</sup>	<u>1.7<sup>d</sup></u>	<u>2.8<sup>d</sup></u>	<u>2.5<sup>d</sup></u>	<u>1.9<sup>d</sup></u>	<u>2.8<sup>d</sup></u>

<sup>a</sup>Means based on an 8-point scale (8 = like extremely; 1 = dislike extremely).

<sup>b</sup>Means within a common storage interval that are underlined are significantly different (P < .05) from means obtained from vacuum packaged cuts.

<sup>cd</sup>Means in the same column bearing a common superscript do not differ (P > .05).



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#### FARM METHODS REPORT, con't from p. 232

Dr. Edward P. Glass, Department of Food Sciences at the Pennsylvania State University, is conducting tests on the use of an automatic sampler that shows promise in obtaining representative samples to perform fat, bacteriological and other tests on tank truck milk.

The subcommittee received a communication from R. R. Johnson, Cedar Rapids, Iowa, on a patented instrument, the "Pro Rata Line Sampler" (operating on a principle similar to a diaphragm fuel pump) for obtaining a representative sample from a tank truck.

A nationwide survey of current tank truck sampling practices is to be conducted using a questionnaire that will be sent to milk cooperatives, proprietary milk plants and state milk sampling surveillance officers. This information will be helpful in preparing a guideline for sampling tank trucks prior to or during the unloading of the milk from the tank truck.

#### Water Treatment and Protection Subcommittee

Properly treated water continues to be very important for use on the dairy. It is essential to proper cleaning and sanitizing of equipment and tankage and for udder washing, as well as for drinking for the herd and for humans. These various uses and the impact of government regulations means the dairyman must give his water supply careful attention.

#### The New PMO

The committee has been advised that the new Pasteurized Milk Ordinance has been completed and approved by the National Conference of Interstate Milk Shippers and should be printed and distributed by the U.S. Government Printing Office by the end of the summer.

The committee understands that the new PMO will require sampling and bacteriological testing of water supplies every three years instead of only when the supply system is built or changed. This is a major improvement which should improve water quality and public

health. The basic policy on water system construction is understood to be the same, though expanded, and in close agreement with EPA "Manual of Individual Water Supply Systems." This manual, of course, requires that all well casings extend above ground level. The PMO will be reviewed completely by the subcommittee when it is published.

The FDA position on buried well casings remains the same. Their position is to allow wells with casings below ground level to be used if constructed before 1965, but they are to be sampled and must pass the bacteriological test every six months. If the water system is ever changed or it fails the bacteria test, it must be built with the casing terminating above ground.

#### Water Supply Manuals

The committee remains in support of the EPA manuals EPA-430/9-74-007 "Manual of Individual Water Supply Systems" and EPA-430/9-73-002 "Cross-connection Control Manual." These two booklets form a comprehensive guide to water supplies and cover construction, bactericidal treatment, mineral content control and prevention of contamination by non-potable waters. The recommendations in these booklets are well worth following by the dairymen.

#### Water Quality Education Program

The subcommittee has been continuing its work on an education program about water quality for the dairyman. The basic idea behind the program is that the way to make sure water is safe and prepared for use on the dairy is to properly treat it before use. Proper treating methods and equipment coupled with periodic water sampling and testing will result in good water for the dairy or farm.

The committee is planning to distribute information about water treatment to the dairyman. We plan to have about 4,000 sets of brochures distributed by fall. These will be disseminated in the Midwest and West by subcommittee members. By mid 1979 we should have a sounding as to the extent the information has been accepted and put to use.

The brochures that will be used are published by the Water Quality Association,

Lombard, Illinois. These brochures are information, technically accurate, impartial and were presented to the Farm Methods Committee with last year's report. The titles are descriptive of the content:

1. "Bacteriological Safety"
2. "Water Hardness"
3. "The Stainers - Iron and Manganese"

The cost of these brochures is \$.18 per set. Funding, while not complete and finalized, has been arranged for from the I.A.M.F.E.S. members. Any of these booklets are available from the subcommittee chairman to any I.A.M.F.E.S. member.

#### Proposed EPA Municipal Water Supply Standards

The EPAs proposed water quality standards are causing a controversy with municipal water treatment facilities as is pointed out in the following excerpts from the WQA Newsletter of May 17, 1978:

The average water bill for homeowners could increase as much as 100% if drinking water quality standards proposed by the U.S. Environmental Protection Agency (EPA) are adopted. The EPA is attempting to impose standards under the Safety Drinking Water Act of 1974 on substances in treated drinking water that are believed, but not proven, to cause cancer and is soliciting cities' comments on the regulations before it adopts them.

Basically, the regulations place a limit on the amount of certain water-borne chemicals that are created when naturally occurring organic chemicals react with chlorine in the treatment process. The chemicals, such as chloroform, are believed by some to cause cancer. (Many respected authorities do not believe this and say there is no proof.) The regulations also prescribe treatment techniques using activated carbon and limit the use of Chlorine-based disinfectants. For most cities the total package would involve converting water filtration plants to an activated carbon system, construction of a regeneration plant for used carbon and higher operating costs.

Cities estimate that the costs of this type of  
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## Microbiology of Beef Packaged in Various Gas Atmospheres

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### ABSTRACT

Boneless beef roasts (*longissimus* muscles) were vacuum-packaged and then the bags were injected with one of six gas mixtures: 100% O<sub>2</sub>, 20% CO<sub>2</sub> + 80% N<sub>2</sub>, 50% CO<sub>2</sub> + 50% O<sub>2</sub>, 20% CO<sub>2</sub> + 80% O<sub>2</sub>, 25% CO<sub>2</sub> + 25% O<sub>2</sub> + 50% N<sub>2</sub> or 51% CO<sub>2</sub> + 30% O<sub>2</sub> + 18% N<sub>2</sub> + 1% CO. One group of roasts, vacuum-packaged without added gas served as controls. Roasts were stored for 0-35 days at 1-3 C. At five weekly intervals, steaks were removed from roasts in each treatment and examined after storage for 5 days under retail display conditions. Psychrotrophic plate counts of roasts stored in modified gas atmospheres were usually higher than those stored in conventional vacuum packages. Differences in lactobacillus counts between roasts stored in modified gas atmospheres and those stored in vacuum packages rarely were statistically significant. Counts of retail steaks prepared from roasts stored in various gas atmospheres were usually slightly higher than those prepared from comparable vacuum packaged roasts. In most instances these differences were not statistically significant. Initially, the microbial flora of vacuum-packaged beef roasts consisted primarily of *Moraxella-Acinetobacter* and *Pseudomonas* spp. Lactobacilli predominated on roasts at later storage intervals even on roasts stored in atmospheres initially containing 100% O<sub>2</sub> or 20% CO<sub>2</sub> + 80% O<sub>2</sub>. *Pseudomonas* spp. remained a substantial part of the microflora of roasts stored in high O<sub>2</sub>-containing atmospheres.

Several studies have shown that vacuum packaging of fresh meat can control growth of common gram-negative spoilage organisms and extend shelf-life (4,7,14,18,23). King and Nagel (15,16) showed that increased levels of CO<sub>2</sub> probably are responsible for this inhibitory activity. On vacuum-packaged meats, facultative anaerobes such as lactic acid bacteria often become predominant (11,18,19). Several reports indicate that under certain conditions losses in meat quality occur in vacuum-packaged products. For example, Cutaia and Ordal (6), Watts et al. (28) and Pierson et al. (19) reported a deterioration in color associated with vacuum-packaged meat. Jaye et al. (12), Jeremiah et al. (13) and Hodges et al. (7) reported off-flavors and -odors associated with meat after prolonged storage in vacuum packages. Recently, storage of meat in controlled gas atmospheres has been suggested as an alternative to conventional vacuum packaging (26). In theory, carbon dioxide should inhibit aerobic spoilage bacteria while the maintenance of a supply of oxygen should keep the pigments on the surface of the meat oxygenated. The effect of a "filler" gas, such as nitrogen, on bacterial development is still not certain. This paper reports on the effects of different gas atmospheres as compared with conventional vacuum

packaging on the level and type of microbial flora of beef roasts stored for up to 35 days at 1-3 C and steaks from such roasts after 5 days of storage under retail conditions.

### EXPERIMENTAL

#### Samples

One hundred and eight boneless roasts prepared from the *longissimus* muscle of 25 beef loins were randomly assigned to seven treatments. All roasts were vacuum-packaged by use of a chamber-type, heat-seal vacuum packaging machine at the maximum capacity of the machine (747 mm of Hg). Barrier bags with the following characteristics were used: Oxygen Transmission Rate (OTR) = 32 cc/m<sup>2</sup>/24 h/23.9 C/50% RH, Moisture Vapor Transmission Rate (MVTR) = 0.8-1.8 g/m<sup>2</sup>/24 h/37.7 C/70% RH and Carbon Dioxide Transmission Rate (CTR) = 47 cc/m<sup>2</sup>/24 h/23.9 C/50% RH. Samples in treatment A were vacuum-packaged in the conventional manner and served as controls. The other samples were vacuum-packaged, sealed and then injected with 500 cc (head space to meat volume ratio of 1 to 1) of a gas mixture corresponding to the following treatments: B = 100% O<sub>2</sub>, C = 20% CO<sub>2</sub> + 80% N<sub>2</sub>, D = 50% CO<sub>2</sub> + 50% O<sub>2</sub>, E = 20% CO<sub>2</sub> + 80% O<sub>2</sub>, F = 25% CO<sub>2</sub> + 25% O<sub>2</sub> + 50% N<sub>2</sub> or G = 51% CO<sub>2</sub> + 30% O<sub>2</sub> + 18% N<sub>2</sub> + 1% CO.

Bacterial counts were obtained on three roasts before packaging and were used as initial counts for roasts in all treatments. Roasts from each treatment were randomly assigned to storage periods of 7, 14, 21, 28 or 35 days (Table 1) at 1-3 C (three roasts per storage interval). At the end of each storage period, leaker packages were separated from intact packages and discarded. The intact packages first were subjected to gas analysis. They were then opened and the roasts were examined for microbiological, physical and sensory characteristics. Details of the procedures concerning injection of gases, gas analysis and measurement of physical and sensory characteristics are presented in a companion paper by Seideman et al. (21). One steak (3.2-cm thick) was removed from each roast, placed in a styrofoam tray and overwrapped with polyvinyl chloride film. The steaks were displayed for 5 days under simulated retail conditions (1-3 C with 970 lux of incandescent light).

#### Microbiological tests

Sampling for microbiological analyses was carried out by swabbing a 12.9-cm<sup>2</sup> area of the lean surface of each roast with a sterile dacron swab wetted in sterile 0.1% peptone broth. The swab was placed in 10 ml of sterile 0.1% peptone broth. The sample jar was then shaken 25 times and appropriate dilutions were made with sterile 0.1% peptone broth. Psychrotrophic bacterial counts were made by spreading 0.1-ml samples of appropriate dilutions onto Plate Count Agar (Difco) with plate incubation at 7 C for 10 days. Lactobacilli were enumerated by use of Lactobacillus MRS broth (Difco) plus 1.5% agar. These plates were incubated at 25 C for 4 days. Psychrotrophic counts of steaks after 5 days of retail storage were made in the manner described for counts of the roasts.

After the various storage periods, distribution of the psychrotrophic microbial flora was determined by picking 30-40 colonies from countable plates (representing the various colony types) and placing

them on trypticase soy agar (BBL) slants. Incubation of slants was at 25 C for 2-3 days. Diagnostic schemes and procedures to identify the isolates were those published by Vanderzant and Nickelson (27).

Bacteriological count data were analyzed using one-way analysis of variance comparing vacuum packaging (control) to each of the other treatments independently within each of the five storage intervals. One-way analysis of variance was done on data within each packaging treatment over the five storage intervals. When significant ( $P < .05$ ) main effects were observed in the analysis of variance, mean separation was accomplished by use of the Student-Newman-Keuls test (25).

## RESULTS

Psychrotrophic plate counts of vacuum-packaged (control) roasts and those stored in various gas atmospheres are presented in Table 2. Differences in psychrotrophic counts of roasts stored in various gas atmospheres and comparable vacuum-packaged (control) roasts were in most instances not statistically significant. After 7 days of storage, counts of the roasts from atmospheres initially containing 50% CO<sub>2</sub> + 50% O<sub>2</sub> and 20% CO<sub>2</sub> + 80% O<sub>2</sub> (treatments D and E) were lower than those of comparable vacuum-packaged control roasts. The same was true after 14 days of storage for roasts stored in 20% CO<sub>2</sub> + 80% N<sub>2</sub> and 50% CO<sub>2</sub> + 50% O<sub>2</sub> (treatments C and D) and after 21 days for the samples stored with 20% CO<sub>2</sub> + 80% N<sub>2</sub>. The roasts stored in an initial atmosphere of 100% O<sub>2</sub> (treatment B) had consistently higher counts than the vacuum-packaged control roasts and usually were higher than counts of comparable roasts stored in various gas mixtures. After 28-35 days, counts of roasts held in

various gas mixtures were similar to or slightly higher than those of comparable vacuum-packaged control roasts. After 35 days of storage, the log psychrotrophic counts of roasts stored in the various gas atmospheres varied from 7.72 to 8.17. During this period, the increase in count of roasts stored in various gas atmospheres ranged from 1.54 to 1.99 logs, that of the vacuum-packaged control roasts was 1.54 log.

With few exceptions, lactobacillus counts of beef roasts stored for 21-35 days in various gas atmospheres were lower than those of comparable vacuum-packaged control roasts (Table 3). Few of the differences in counts, however, were statistically significant. Over the 35-day storage period, the increase in lactobacillus count of the vacuum-packaged control roasts was 3.14 logs while the increase for roasts stored in the various gas atmospheres ranged from 1.81 to 2.87 logs.

Psychrotrophic counts of retail beef steaks after 5 days of display are shown in Table 4. The data indicate that the psychrotrophic counts of steaks prepared from roasts stored under various gas atmospheres were frequently slightly higher than those of comparable steaks derived from vacuum-packaged control roasts. This was particularly true for the steaks prepared from roasts stored in 20% CO<sub>2</sub> + 80% O<sub>2</sub> and in 100% O<sub>2</sub> (treatments B and E). However, few of these differences in counts were statistically significant.

The distribution of the psychrotrophic microbial flora of beef roasts is presented in Table 5. Initially (0-7 days), *Moraxella-Acinetobacter*, *Pseudomonas* and a few other

TABLE 1. Number of samples examined bacteriologically arranged according to packaging treatment and length of storage.

Storage interval (days) <sup>a</sup>	Packaging treatment						
	A Vacuum packaged (control)	B 100% O <sub>2</sub>	C 20% CO <sub>2</sub> 80% N <sub>2</sub>	D 50% CO <sub>2</sub> 50% O <sub>2</sub>	E 20% CO <sub>2</sub> 80% O <sub>2</sub>	F 25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	G 51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
7	3	3	3	3	3	3	3
14	3	3	3	3	3	3	3
21	3	3	3	3	3	3	3
28	3	3	3	3	3	3	3
35	3	3	3	3	3	3	3

<sup>a</sup>Initial counts (0 day) were made on 3 roasts.

TABLE 2. Mean values for psychrotrophic bacterial counts<sup>a</sup> of beef roasts stratified according to packaging treatment and storage interval.

Storage interval (days)	Packaging treatment <sup>b</sup>						
	A Vacuum packaged <sup>c</sup> (control)	B 100% O <sub>2</sub> <sup>c</sup>	C 20% CO <sub>2</sub> <sup>c</sup> 80% N <sub>2</sub> <sup>c</sup>	D 50% CO <sub>2</sub> <sup>c</sup> 50% O <sub>2</sub> <sup>c</sup>	E 20% CO <sub>2</sub> <sup>c</sup> 80% O <sub>2</sub> <sup>c</sup>	F 25% CO <sub>2</sub> <sup>c</sup> 25% O <sub>2</sub> 50% N <sub>2</sub>	G 51% CO <sub>2</sub> <sup>c</sup> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
7	6.22 <sup>e</sup>	6.63 <sup>e</sup>	6.22 <sup>g</sup>	5.85 <sup>e</sup>	5.61 <sup>g</sup>	6.28 <sup>f</sup>	6.36 <sup>f</sup>
14	6.61 <sup>e</sup>	7.73 <sup>d</sup>	6.07 <sup>fg</sup>	5.97 <sup>e</sup>	6.47 <sup>f</sup>	7.03 <sup>e</sup>	6.94 <sup>def</sup>
21	7.88 <sup>de</sup>	7.91 <sup>d</sup>	6.85 <sup>ef</sup>	7.67 <sup>d</sup>	8.09 <sup>d</sup>	7.28 <sup>de</sup>	7.79 <sup>de</sup>
28	7.14 <sup>de</sup>	8.27 <sup>d</sup>	7.57 <sup>de</sup>	8.12 <sup>d</sup>	7.67 <sup>e</sup>	7.85 <sup>de</sup>	6.93 <sup>def</sup>
35	7.72 <sup>d</sup>	7.96 <sup>d</sup>	7.88 <sup>d</sup>	7.72 <sup>d</sup>	8.17 <sup>d</sup>	8.09 <sup>d</sup>	7.76 <sup>d</sup>

<sup>a</sup>Counts (log<sub>10</sub>) per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different ( $P < .05$ ) from counts obtained on vacuum packaged control roasts.

<sup>c</sup>Initial counts (log<sub>10</sub>) = 6.18.

<sup>defg</sup>Means in the same column bearing a common superscript do not differ ( $P > .05$ ).

TABLE 3. Mean values for lactobacilli counts<sup>a</sup> of beef roasts stratified according to packaging treatment and storage interval.

Storage interval (days)	Packaging treatment <sup>b</sup>						
	A	B	C	D	E	F	G
	Vacuum packaged <sup>c</sup> (control)	100% O <sub>2</sub> <sup>c</sup>	20% CO <sub>2</sub> <sup>c</sup> 80% N <sub>2</sub>	50% CO <sub>2</sub> <sup>c</sup> 50% O <sub>2</sub>	20% CO <sub>2</sub> <sup>c</sup> 80% O <sub>2</sub>	25% CO <sub>2</sub> <sup>c</sup> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> <sup>c</sup> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
7	2.49 <sup>e</sup>	2.26 <sup>e</sup>	2.06 <sup>e</sup>	2.67 <sup>g</sup>	2.24 <sup>e</sup>	2.01 <sup>e</sup>	1.97 <sup>e</sup>
14	2.46 <sup>e</sup>	4.53 <sup>de</sup>	3.07 <sup>e</sup>	3.95 <sup>efg</sup>	3.05 <sup>e</sup>	2.46 <sup>e</sup>	3.09 <sup>e</sup>
21	5.69 <sup>de</sup>	4.88 <sup>de</sup>	4.79 <sup>d</sup>	4.42 <sup>def</sup>	2.82 <sup>e</sup>	4.86 <sup>de</sup>	4.52 <sup>d</sup>
28	5.41 <sup>de</sup>	3.94 <sup>de</sup>	5.97 <sup>d</sup>	5.15 <sup>de</sup>	4.45 <sup>d</sup>	5.51 <sup>d</sup>	5.24 <sup>d</sup>
35	6.35 <sup>d</sup>	5.10 <sup>d</sup>	6.08 <sup>d</sup>	5.29 <sup>d</sup>	5.02 <sup>d</sup>	5.40 <sup>de</sup>	5.09 <sup>d</sup>

<sup>a</sup>Counts (log<sub>10</sub>) per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different (P < .05) from counts obtained on vacuum packaged control roasts.

<sup>c</sup>Initial count (log<sub>10</sub>) = 3.21.

<sup>d</sup><sup>e</sup><sup>f</sup><sup>g</sup>Means in the same column bearing a common superscript do not differ (P > .05).

TABLE 4. Mean values for psychrotrophic bacterial counts<sup>a</sup> of retail beef steaks after 5 days of retail display stratified according to previous packaging treatment and storage interval of the roasts from which they were cut.

Storage interval (days)	Packaging treatment <sup>b</sup>						
	A	B	C	D	E	F	G
	Vacuum packaged (control)	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
7	7.65 <sup>d</sup>	8.34 <sup>d</sup>	6.86 <sup>d</sup>	7.10 <sup>e</sup>	7.87 <sup>e</sup>	7.49 <sup>e</sup>	7.83 <sup>c</sup>
14	7.28 <sup>d</sup>	7.94 <sup>d</sup>	7.56 <sup>d</sup>	7.62 <sup>de</sup>	8.07 <sup>cd</sup>	7.75 <sup>de</sup>	7.99 <sup>c</sup>
21	8.12 <sup>c</sup>	8.04 <sup>d</sup>	7.78 <sup>d</sup>	8.02 <sup>cde</sup>	8.33 <sup>cd</sup>	8.22 <sup>cd</sup>	8.12 <sup>c</sup>
28	8.28 <sup>c</sup>	8.42 <sup>cd</sup>	8.51 <sup>c</sup>	8.24 <sup>cd</sup>	8.36 <sup>cd</sup>	8.23 <sup>cd</sup>	8.15 <sup>c</sup>
35	8.24 <sup>c</sup>	8.78 <sup>c</sup>	8.54 <sup>c</sup>	8.47 <sup>c</sup>	8.47 <sup>c</sup>	8.53 <sup>c</sup>	8.24 <sup>c</sup>

<sup>a</sup>Counts (log<sub>10</sub>) per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different (P < .05) from counts on steaks (after 5 days of retail display) from vacuum packaged control roasts.

<sup>c</sup><sup>d</sup><sup>e</sup>Means in the same column bearing a common superscript do not differ (P > .05).

TABLE 5. Percentage distribution of microorganisms<sup>a</sup> on packaged beef roasts stratified according to packaging treatment and storage interval.

Storage interval (days)	Type	Packaging treatment						
		A	B	C	D	E	F	G
		Vacuum packaged (control)	100% O <sub>2</sub>	20% CO <sub>2</sub> 80% N <sub>2</sub>	50% CO <sub>2</sub> 50% O <sub>2</sub>	20% CO <sub>2</sub> 80% O <sub>2</sub>	25% CO <sub>2</sub> 25% O <sub>2</sub> 50% N <sub>2</sub>	51% CO <sub>2</sub> 30% O <sub>2</sub> 18% N <sub>2</sub> 1% CO
7	<i>Pseudomonas</i>	69.8	38.2	52.2	47.1	20.1	29.1	43.0
	<i>Lactobacillus</i>	26.1	37.7	23.4	41.1	25.8	66.9	16.5
	<i>Moraxella-Acinetobacter</i>	4.1	21.5	17.5	10.9	19.9	2.1	—
	Others <sup>b</sup>	—	2.6	6.9	0.9	34.2	1.9	40.9
14	<i>Pseudomonas</i>	37.9	47.8	17.9	31.7	36.4	36.5	14.9
	<i>Lactobacillus</i>	42.6	46.4	61.4	57.1	53.9	44.7	78.9
	<i>Moraxella-Acinetobacter</i>	10.8	—	—	—	—	—	—
	Others <sup>b</sup>	8.7	5.8	20.7	11.2	9.7	18.8	6.2
21	<i>Pseudomonas</i>	18.2	36.5	13.0	6.7	10.5	16.7	9.1
	<i>Lactobacillus</i>	60.4	63.5	68.3	93.3	85.8	83.3	90.9
	<i>Moraxella-Acinetobacter</i>	21.4	—	18.7	—	3.7	—	—
	Others <sup>b</sup>	8.7	52.2	2.7	6.7	22.7	5.2	3.7
28	<i>Pseudomonas</i>	91.3	47.8	97.3	89.0	77.3	94.8	96.3
	<i>Lactobacillus</i>	—	—	—	4.3	—	—	—
	<i>Moraxella-Acinetobacter</i>	—	—	—	—	—	—	—
	Others <sup>b</sup>	—	—	—	—	—	—	—
35	<i>Pseudomonas</i>	8.8	19.9	3.1	6.6	16.0	5.6	0.4
	<i>Lactobacillus</i>	91.2	78.6	96.9	93.4	84.0	94.4	99.6
	<i>Moraxella-Acinetobacter</i>	—	1.5	—	—	—	—	—
	Others <sup>b</sup>	—	—	—	—	—	—	—

<sup>a</sup>Each percentage is based on 3 analyses. (Initial flora: *Pseudomonas* = 26.1, *Moraxella-Acinetobacter* = 59.3, other = 14.6).

<sup>b</sup>These included species of *Flavobacterium*, *Micrococcus* and *Corynebacterium*.

genera (*Flavobacterium*, *Micrococcus* and *Corynebacterium*) predominated on vacuum-packaged control roasts. After 14-21 days of storage, lactobacilli became more evident and became dominant after 28-35 days of storage. On roasts stored in an atmosphere of 100% O<sub>2</sub>, lactobacilli also became more apparent after 7 days of storage, but *Pseudomonas* spp. remained a significant part of the microflora, even after 28 days of storage. However, after 35 days of storage, lactobacilli became predominant. On roasts stored in a mixture of gases (treatment C - G) *Pseudomonas* spp. constituted a substantial part of the microflora after 7 days but they gradually decreased upon further storage. After 28-35 days of storage, they remained relatively important (16-22.7% of the microflora) in the roasts stored in 20% CO<sub>2</sub> + 80% O<sub>2</sub>. Lactobacilli became a significant part of the microflora of these roasts after 14 days and dominated the population after 21-35 days.

#### DISCUSSION

Although several reports (1,3,9,10,17) are available on the microbiological condition of meats stored in various gas atmospheres, few have compared the physical, sensory and microbiological attributes of these meats with those of conventional vacuum-packaged products. For example, Huffman et al. (10) reported that steaks stored at 1.1 C in 100% CO<sub>2</sub> had significantly lower aerobic plate counts 16-27 days post-slaughter than those stored in 100% N<sub>2</sub>, 100% O<sub>2</sub> or air. Counts of steaks stored in a mixture of 70% N<sub>2</sub> + 25% CO<sub>2</sub> + 5% O<sub>2</sub> were also lower than those held in air, O<sub>2</sub> or N<sub>2</sub> but somewhat higher (16-27 days post-slaughter) than those of steaks stored in 100% CO<sub>2</sub>. According to Bala et al. (3), aerobic plate counts of steaks stored for up to 20 days at 1 C in 15% CO<sub>2</sub> + 85% air or in 15% CO<sub>2</sub> + 85% O<sub>2</sub> were consistently lower than those of steaks stored in air. Newton et al. (17) observed that the growth rate of the psychrotrophic microflora on lamb chops stored at -1 C decreased successively (compared to air) in O<sub>2</sub> + N<sub>2</sub> (80:20 v/v), air or O<sub>2</sub> + CO<sub>2</sub> (80:20), low O<sub>2</sub> atmospheres (N<sub>2</sub> + CO<sub>2</sub>, H<sub>2</sub> + CO<sub>2</sub>, 80:20, without O<sub>2</sub>-absorbents) and O<sub>2</sub>-free atmospheres (N<sub>2</sub>, H<sub>2</sub>, N<sub>2</sub> + CO<sub>2</sub>, H<sub>2</sub> + CO<sub>2</sub>, 80:20, with O<sub>2</sub>-absorbents).

The inhibitory effect of CO<sub>2</sub> on growth of common gram-negative aerobic meat spoilage bacteria is well established. This probably is caused by its effect on decarboxylating enzymes, especially isocitric and malate dehydrogenases (15,16). Reports on the effect of N<sub>2</sub> on bacterial growth on meats are not consistent. According to Huffman et al. (10), counts of steaks stored in N<sub>2</sub> were similar to those of samples stored in air. On the other hand, Newton et al. (17) reported that counts of lamb chops stored in N<sub>2</sub> (O<sub>2</sub>-free) were much lower than those of chops stored in air, O<sub>2</sub> + N<sub>2</sub> (80:20) or in air + CO<sub>2</sub> (80:20). Factors responsible for these differences in results may include: animal species (beef versus lamb), plating media and incubation temperature (Plate Count Agar with plate incubation for 48 h at 32 C versus APT agar at 25 and 0 C), composition of the gases (about

100% N<sub>2</sub> versus N<sub>2</sub> with O<sub>2</sub> absorbent) and method of sampling (rinse versus swab method).

In this study, differences both in psychrotrophic and lactobacilli counts of conventional vacuum-packaged beef and comparable roasts stored in six gas atmospheres usually were not statistically significant. The same was true for the psychrotrophic counts of steaks prepared from these roasts and held for 5 days in retail display cabinets. The high initial counts of the roasts (about 10<sup>6</sup>/in.<sup>2</sup>) may have diminished to some extent the selective influence that different gas atmospheres would have exerted on the development of various microbial species initially present on the meat. Although the initial counts of the roasts were relatively high, no organoleptic defects were noticeable when they were vacuum-packaged (21).

The higher psychrotrophic counts of roasts in an atmosphere of 100% O<sub>2</sub> as compared to roasts held in conventional vacuum packages most likely resulted because psychrotrophic species grow rapidly in oxygen-rich atmospheres and are inhibited by CO<sub>2</sub>. For example, data in the companion paper (21) show that the relative weight percentages of CO<sub>2</sub> and O<sub>2</sub> in the headspace after 7 days of storage were 10.47% and 87.67% for the meat packaged initially in 100% O<sub>2</sub>; for the vacuum-packaged meat the same figures were 70.64 and 1.12%, respectively.

Development of lactic acid bacteria on conventional vacuum-packaged meats and on meats stored in gases which suppress gram-negative psychrotrophic bacteria is well documented (9,17,18,19,22). No specific reason can be given for the somewhat higher lactobacillus counts of conventional vacuum-packaged roasts as compared with roasts stored for 21-35 days in six different gas atmospheres. It is possible that the gaseous environment in the conventional vacuum-packaged roasts was more desirable for development of lactobacilli. Data in the companion paper (21) show that the weight percentages of CO<sub>2</sub> of the vacuum-packaged roasts were consistently higher than those of the roasts stored in various gases. However, Roth and Clark (20) reported that growth rate and final count of lactobacilli on beef were similar on samples held in air and in vacuum packages. In addition, according to Shaw and Nicol (24), lactobacilli are not affected by either CO<sub>2</sub> or O<sub>2</sub>.

The data in this study show that the psychrotrophic counts of steaks after 5 days of display usually were related to the counts of the roasts from which they were prepared. Hudson and Roberts (8) also reported that bacterial counts of retail cuts of beef after display for 24 h showed a significant correlation with counts of the vacuum-packaged primal cuts from which they had been prepared.

The initial microbial flora of the roasts consisted primarily (85.4%) of gram-negative bacteria common to fresh raw meats. After 35 days of storage *Lactobacillus* spp. had become predominant even on the samples stored in high-O<sub>2</sub> atmospheres (100% O<sub>2</sub> and 20%

CO<sub>2</sub> + 80% O<sub>2</sub>). However, *Pseudomonas* spp. continued to be a part of the microflora of these samples. These changes in the microflora agree in many respects with previous reports on the behavior of bacterial species in selected gas atmospheres. The inhibitory effect of CO<sub>2</sub> on the growth rate of *Pseudomonas* spp. is well documented. When this occurs, lactobacilli which are not affected by CO<sub>2</sub> or O<sub>2</sub> become a more significant part of the microflora upon continued storage (20,24). According to Newton et al. (17), growth of *Pseudomonas* spp. was slower in O<sub>2</sub> + N<sub>2</sub> (80:20) and in air + CO<sub>2</sub> (80:20) than in air and the inhibitory effect was greater with 20% CO<sub>2</sub> than with 80% O<sub>2</sub>. Detection of *Pseudomonas* spp. in low-O<sub>2</sub> atmospheres (N<sub>2</sub> + CO<sub>2</sub>, H<sub>2</sub> + CO<sub>2</sub>) but their absence in O<sub>2</sub>-free atmospheres (N<sub>2</sub>, H<sub>2</sub>, N<sub>2</sub> + CO<sub>2</sub>, H<sub>2</sub> + CO<sub>2</sub> plus O<sub>2</sub> absorbents) in that study indicates the significance of O<sub>2</sub> limitation in suppressing *Pseudomonas* spp. on meat during storage. Therefore, in this study inhibition of *Pseudomonas* spp. and predominance of lactobacilli in atmospheres initially containing 20 to 50% CO<sub>2</sub> and 50% or less O<sub>2</sub> most likely were caused by the direct action of CO<sub>2</sub> on gram-negative bacteria. On meats stored in an atmosphere of 100% O<sub>2</sub> (treatment B) some inhibition of *Pseudomonas* may have occurred initially (5). It should be recognized that the composition of the gas environments mentioned in this study represents initial concentrations. Data presented in a companion paper (21) show extensive changes in the weight percentages of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> in the headspace of the packages during storage. In that report Seideman et al. (21) compared the physical and sensory attributes of vacuum-packaged beef stored in various gas atmospheres with those of conventional vacuum-packaged beef. They reported a significant decrease in overall appearance of the meat between 7 and 21 days of storage in oxygen-rich atmospheres. This observation probably was related to the presence and activities of *Pseudomonas* spp. reported in this study.

Bala et al. (2) also showed a significant correlation between activity of *Pseudomonas fragi* and loss of color in beef. Additional research is needed to determine the most effective combination of gases for inhibiting growth of meat spoilage bacteria and for preventing loss of surface color and consumer appeal. Maintenance of the desirable concentration of such gases during storage offers an additional research challenge.

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## Effect of Salt Content and pH on Toxigenesis by *Clostridium botulinum* in Caviar

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### ABSTRACT

Bottled lumpfish caviar was prepared with different salt (NaCl) concentrations and pH, and injected with spores of *Clostridium botulinum*. Under abusive storage conditions (30 C), outgrowth and toxigenesis occurred at combinations of  $\leq 3.95\%$  salt in the water phase and  $\text{pH} \geq 5.2$ , and of  $\leq 4.67\%$  salt and  $\text{pH} \geq 5.6$ . No toxin was formed at salt concentrations of  $\geq 5.56\%$  or at  $\text{pH} \leq 5.0$ . A survey of commercial caviar products showed that most of these had salt-pH combinations which would effectively inhibit *C. botulinum* at abusive temperatures during storage.

Commercial caviar receives only a mild heat process ( $< 70$  C), insufficient to control growth or toxigenesis of *Clostridium botulinum*. This control is generally obtained by a combination of adequately low levels of water activity and pH. Most caviar products contain 0.1% sodium benzoate for control of yeasts and molds, but it is unlikely that above pH 5.0 it has a significant effect on clostridia (2,5,23). The safety record of caviar is good, though not without blemish. Imported caviar was incriminated as the cause of one type B botulism outbreak in Japan (6), and an isolated case of type E botulism from caviar was mentioned by Sebal (20). In a recent report (10) we listed an incident of suspected botulism in Montreal, Canada, with imported lumpfish caviar as the most likely cause.

On the other hand, numerous incidents of botulism have been caused by raw, fermented fish eggs, a traditional Indian food on the North American West coast (4), and by home-prepared or semi-commercial raw salted fish eggs around the Caspian Sea (16).

The present study was undertaken to determine the minimum requirements of salt (NaCl) and pH for the control of *C. botulinum* in caviar.

### MATERIALS AND METHODS

One lot (C-93) of imported black lumpfish caviar in glass jars, 50-g content, was obtained directly from warehouses and kept at 4 C until used. Caviar of this lot had been the most likely cause of a suspected case of botulism (10). A few 100-g jars of the same lot were also obtained.

For toxin assays, 10-g samples were homogenized in a stomacher (J.A. Seward, London, England) with 10 ml of phosphate-gelatin (3) for 2 min. The homogenates were centrifuged at  $15,000 \times g$  for 10 min and the supernatant fluids separated from the surface layer of fat. Toxin assays and neutralization were as described (8).

The pH was measured by placing a combination electrode directly in the product. The readings were essentially identical to those obtained after addition of a minute amount of water to ensure an adequate aqueous phase for immersion of the electrode, and about 0.1 unit

higher than after grinding the product in a mortar. Sodium chloride was determined by AOAC method 18.030 (AOAC, 1975) and the water content by AOAC method 7.003 (1). The brine concentration was calculated by the equation  $\% \text{ brine} = \text{g salt} \times 100 / \text{g salt} + \text{g H}_2\text{O}$ . Water activities were measured in Lufft  $a_w$  meters (G. Lufft Metallbarometerfabrik, Stuttgart, Germany) at 25 C. The instruments were standardized with slurries of different salts (7). However, in the  $a_w$  range  $> 0.94$ , the units indicated by the instruments were not identical to the actual  $a_w$  values. Standard curves were prepared by adjusting the instruments with either  $\text{KNO}_3$  ( $a_w = 0.936$ ) or  $\text{K}_2\text{SO}_4$  ( $a_w = 0.973$ ) and plotting the  $a_w$  readings of 0.8-2.0 molal concentrations of NaCl against the known  $a_w$  values of these solutions (18).

Spores of *C. botulinum* were prepared by the method of Schmidt et al. (19) and enumerated by a pour plate method (9).

Lot C-93 was tested for its ability to allow toxigenesis by *C. botulinum*. Twelve 50-g jars each were incubated with 0.05 ml of a spore suspension containing  $6.5 \times 10^7$  spores each of type A (A-6) and Type B (13983-II B); 12 100-g jars were inoculated with 0.1 ml of the same spore mixture. The jars were heated to an internal temperature of 62 C, cooled, incubated anaerobically at 30 C, and tested for toxin after 2 weeks. In each experiment, four uninoculated jars were incubated as controls.

For adjustments of salt concentrations and pH, the contents of groups of three jars (150 g) were emptied into 1.5 liters of filter-sterilized 0.01 M Na citrate at various combinations of pH and salt concentrations. The buffer concentration was kept low to minimize its effect on the water activity. At the critical brine concentration of 5% it accounted for about 1% of the total molar salt content. The caviar-brine mixture was kept at 4 C for 48 h. The pH was adjusted to desired levels at the start; readjustments were made after 24 h and, if necessary, after 48 h. The solution was then decanted and the slurry of eggs filtered off with gentle suction and returned to the three jars. The jars were injected with 0.05 ml of one of the following spore suspensions containing (a)  $10^6$  spores of *C. botulinum* type A (A-6), (b) the same number of type B (13983-II B) and (c) of type E (Gordon strain), and (d) a mixture of  $3.3 \times 10^5$  spores each of type A strains A-6, A-62 and CK2-A and of type B strains 13983-II B, 368-B and 426-B. The jars were heated to 62 C at the center, cooled, incubated at 30 C, and tested for toxin after 2 and 4 weeks.

The products listed in Table 2 were sampled at the retail level in Ottawa. The 56 products listed in Table 3 were produced by 19 companies in eight countries. They were sampled before distribution by the Inspection and Technology Branch, Industry Services Directorate, Fisheries and Environment Canada. The analytical data were kindly supplied to us by the same agency. Some of the 56 products seemed to differ merely in size of container and/or colorant added, and a few were identical (three products with two matching jars each).

### RESULTS AND DISCUSSION

The salt content of 50-g jars of lot C-93 was  $4.08 \pm 0.17\%$  (mean of 10 jars  $\pm$  SD); the water content was  $75.7 \pm 0.3\%$ , the brine salt concentration  $5.11 \pm 0.21\%$ , and the pH  $5.7 \pm 0.1$ . Aerobic colony counts (11) were of the order of 100/g, while anaerobic counts

were  $\leq 10/g$ . Most of the viable microorganisms belonged to the *Bacillus* and *Clostridium* genera.

Six out of 12 of the 50-g jars and 5/12 of the 100-g jars inoculated with *C. botulinum* spores contained toxin after 2 weeks of incubation. The toxicities were between 4 and 200 MLD/g, the contents showed a slight discoloration, and the pH was increased by about 0.1 unit, but none of the toxic products had an offensive smell. The control jars did not contain detectable toxin and showed no noticeable changes. Botulinal toxin was confirmed in all toxic samples by neutralization with specific antisera.

Toxigenesis occurred in caviar with brine concentrations of 2.27 and 3.95% at pH levels of 5.2 and higher (Table 1), but not at pH 4.6 (data not shown) to pH 5.0. With 4.67% brine, toxin was produced at pH  $\geq 5.6$  but not at pH  $\leq 5.4$ . No toxin was produced at any pH level with brine concentrations of 5.56% or higher. The corresponding  $a_w$  values are also shown in Table 1. Most of the means were about 0.003 unit higher than the

minimum values calculated from the brine concentrations (14), and it is apparent that only the salt contributed significantly to the water activity. No toxin was produced in any of the jars inoculated with *C. botulinum* type E spores.

Brine concentrations, water activities and pH of 11 commercial products are given in Table 2. On the basis of the results in Table 1, only one of these (a lumpfish caviar from country G) appears to have a pH- $a_w$  combination that might permit toxigenesis. The mean water activity of the product would not indicate a potential hazard, but the variation in the  $a_w$  measurements was relatively large (see SD, Tables 1 and 2). Two other products (salmon and lumpfish caviar from country N) are close to the critical salt content, particularly in view of their high pH. Unfortunately, we were unable to obtain the same products later for challenge with *C. botulinum* spores.

Table 3 shows a summary of the survey data supplied by Fisheries and Environment Canada. Water activities,

TABLE 1. Effect of salt content and pH on production of toxin by *C. botulinum*.

Brine (%)	$a_w^a$	pH	No. of jars (out of 3) toxic							
			2 weeks				4 weeks			
			Type				Type			
A	B	E	A + B	A	B	E	A + B			
2.27	0.986	5.0	0	0	0	0	0	0	0	
		5.2	0	0	0	0	0	0	1	
		5.4	ND <sup>b</sup>	1	0	0	ND	2	0	1
		5.6	3	1	0	2	3	2	0	3
		5.8	3	0	0	2	3	2	0	3
3.95	0.978	5.0	0	0	ND	0	0	0	ND	0
		5.2	0	0	ND	1	0	0	ND	2
		5.4	ND	1	0	2	ND	1	0	2
		5.6	1	0	0	1	3	0	0	1
		5.8	3	0	0	1	3	0	0	3
4.67	0.974	5.4	ND	0	ND	0	ND	0	ND	0
		5.6	0	0	0	0	2	0	0	1
		5.8	0	0	0	0	2	2	0	0
5.56	0.968	5.6	0	0	0	0	ND	0	0	0
7.09	0.959	5.8	0	0	0	0	0	0	0	0

<sup>a</sup>SD of 6 measurements at each brine concentration varied from 0.002 to 0.005.

<sup>b</sup>Not determined.

TABLE 2. Brine concentration, water activity and pH of some commercial caviar products.

Country of origin	Caviar product	To be kept refrigerated <sup>a</sup>	No. jars or tubes analyzed	Salt conc. of brine <sup>b</sup>	$a_w^c$	pH <sup>d</sup>
D	Lumpfish <sup>e</sup>	yes	4	6.33	0.958	5.60
	Lumpfish <sup>e</sup>	yes	2	7.74	0.950	5.71
	Lumpfish <sup>e</sup>	yes	4	6.81	0.962	5.60
ID	Lumpfish <sup>f</sup>	no	6	12.92	0.906	5.41
	Lumpfish <sup>f</sup>	no	4	12.78	0.914	5.81
G	Lumpfish <sup>g</sup>	yes	4	4.24	0.969	5.41
	Lumpfish <sup>g</sup>	yes	4	8.62	0.937	5.32
N	Salmon	yes	3	5.36	0.964	5.60
	Lumpfish	yes	3	5.23	0.964	6.21
IN	Sturgeon	no	2	7.74	0.944	5.45
S	Cod-roe paste	yes	4	18.60	0.769	5.46

<sup>a</sup>According to label.

<sup>b</sup>SD from  $\pm 0.03\%$  to  $\pm 0.50\%$ .

<sup>c</sup>SD from  $\pm 0.001$  to  $\pm 0.009$ .

<sup>d</sup>SD from 0 to  $\pm 0.06$ .

<sup>e,f,g</sup>Each produced by the same companies, differing in added colorants.



were not included, but the lowest brine concentration of any of the 56 samples was 6.0% indicating that all had water activities of  $\leq 0.962$ . None of these products, therefore, appear to present a potential hazard.

The diversity of salt content and pH, particularly among the lumpfish and salmon caviar is striking. Many of the products had salt contents far in excess of those needed to control *C. botulinum*; the pH was often so high that it would not have contributed to product safety.

The results have shown that the suspect lot (C-93) of caviar allowed growth and toxigenesis of *C. botulinum* at an abusive temperature. On the other hand, they also indicate that this product is not particularly conducive to production of botulinal toxin. While the maximum pH for effective control of *C. botulinum* is below 5.0 in various foods (8,12,13,22), no toxin was produced at this pH in our experiments. Furthermore, no type E toxin was produced under any of the experimental conditions. Even in more suitable media, the minimum  $a_w$  required for growth of *C. botulinum* type E appears to be 0.97 (14,15,21) which corresponds to salt concentrations of roughly 4% of the product and 5% of the brine. It is unlikely, therefore, that type E, the most common botulinal type in sea foods, would develop in commercial caviar.

Another safety factor of the product is its warning label to the effect that it has to be kept refrigerated. Since only *C. botulinum* type E and nonproteolytic strains of types B and F are capable of producing toxin at 5 C (17), it is unlikely that the product under investigation (lot C-93) would have become toxic under proper refrigeration. However, it is well known that caviar products are often displayed at the retail level with inadequate or without refrigeration. Our experimental incubation at 30 C for up to 4 weeks does not seem to be unrealistic.

Lot C-93 of the lumpfish caviar was not uniform: we noticed some significant differences in pH among jars of the same cardboard containers and also discovered that the jars of one container differed significantly from the jars analyzed earlier (see Results), both in pH ( $5.45 \pm 0.2$ ) and salt content ( $4.59 \pm 0.09\%$ ). It is conceivable, therefore, that the reported illness in Montreal may have been caused by a single jar that received some temperature abuse and had, relative to the total lot, high levels of water activity and/or pH. Some heterogeneity among jars of the same lot was also noticed in some of the commercial products of Table 2, and

Fukuda et al. (6) reported variations in salt content from 3.5% to 5.4% in the batch of caviar incriminated in the Japanese outbreak.

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We thank the officers of the Inspection and Technology Branch, Fisheries and Environment Canada, in particular Mr. R. M. Bond and Mr. B. Lingeman, for the supply of lumpfish caviar and their permission to include a summary of their survey data in this paper. We also thank our colleagues at the Regional Health Protection Branch Laboratory in Montreal, namely Mrs. P. Entis, Mr. H. Boisvert and Mr. R. Dufault for their most valuable cooperation.

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TABLE 3. Brine concentration and pH of 56 caviar products (from survey data supplied by the Inspection and Technology Branch, Fisheries and Environment Canada).

Caviar product	No. of jars analyzed	No. of producing companies	No. of producing countries	Brine salt conc. (%)		pH	
				Median	Range	Median	Range
Lumpfish	31	12	4	9.5	6.0 - 20.7	5.5	4.6 - 6.8
Salmon	13	6	3	20.0	6.0 - 25.0	5.8	5.5 - 6.3
Sturgeon	5	2	2	14.0	11.2 - 17.3	5.5	4.8 - 5.8
Others <sup>a</sup>	7	5	2	17.2	13.2 - 38.8 <sup>b</sup>	5.5	4.8 - 5.9

<sup>a</sup>Including four products of whitefish caviar.

<sup>b</sup>Brine concentration of smoked cod roe.

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conversion are six to ten times what the EPA has predicted (according to the American Water Works Association). For instance, Miami has predicted it will cost \$40 million to \$45 million, while the EPA has said it will cost Miami \$3.7 million.

American Water Works Association (AWWA) has gone on record stating that "EPA is arbitrary, capricious and premature" attacking the proposed organics regulations as being pulled from a hat, based not on complete research but incomplete guesswork. It also said the National Academy of Sciences did not suggest limits for organics but did call for more research.

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## *A Research Note*

# Effect of Glucose Concentration on Recovery of Fungi from Foods<sup>1</sup>

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### ABSTRACT

Glucose was added to Standard Methods agar in amounts between zero and 2.0% to determine its effect on recovery of fungi from 49 food samples. Analysis of the data showed highly significant differences between glucose concentrations; with the best recovery of fungi occurring when the medium contained between 0.1 and 0.5% glucose.

During an earlier study (4) of media used for fungal counts in foods, one of the factors that appeared to influence recovery was glucose concentration. Of the five media investigated, the two representing the extremes in glucose concentration, zero and 2.0%, ranked lowest in recovery.

With the growing concern among microbiologists of the presence of fungi and their metabolites in foods, any factor which might enhance recovery of these organisms seemed worth pursuing. Therefore, a study was initiated in which only the glucose concentration was altered in an otherwise adequate recovery medium. Glucose concentrations between zero and 2% were selected because culture media used in fungal enumeration most often contain concentrations in this range (2), and addition of greater amounts would not have yielded any usable information.

### MATERIALS AND METHODS

The 49 food samples used in this study consisted of fish, beef, nuts, flour, water, fruits, vegetables and spices and were obtained in the Gainesville, Florida area. The basal medium was Standard Methods agar made from the separate ingredients. Only the glucose concentration was changed. A basal broth was prepared to minimize mixing errors and contained distilled water, 0.5% tryptone and 0.25% yeast extract. The broth was then divided into five portions; glucose was added; depending on the concentration desired, 0.0, 0.1, 0.5, 1.0, 2.0 (w/v) and 1.5% (w/v) agar was added to each portion. The media were adjusted to a pH of  $7 \pm .1$  and sterilized at 121 C for 15 min. Preparation and plating of the samples and addition of the antibiotic solution to give a final concentration of 100 mg each of chloram-

phenicol and chlorotetracycline/liter followed the method outlined in the *Compendium* (1). Dilutions were plated in triplicate to minimize plating errors and incubated at 25 C for five days.

### RESULTS AND DISCUSSION

The fungal count (mostly yeasts) for each of the 49 samples was observed and the log averages calculated for the various treatments (Table 1). The analysis of variance revealed highly significant differences between glucose concentrations. To best determine a trend in the differences between concentrations, we performed a quadratic regression analysis using the dependent variable  $Y = \log(\text{counts})$  and the independent variable  $X = \log(1 + 10 * \text{dilution})$ . The choice of  $Y = \log(\text{counts})$  was made to equalize the variance within high and low counts. The choice of  $X = \log(1 + 10 * \text{dilution})$  was made to obtain values of X which were as equally spaced as possible and thereby to reduce the bias in the regression curve.

The analysis of variance showed significant linear and quadratic effects. A test for lack of fit of the quadratic regression was not significant at the .05 level.

The equation of the quadratic curve is

$$Y = 3.0747 + 0.0635 X - 0.0250 X^2.$$

The fact that the coefficient on X (0.0635) is positive and that on X<sup>2</sup> (-0.0250) negative indicates that the counts increase with increasing glucose concentration, reached a maximum, and then decreased as the glucose concentration was further increased. Tests of significance that

TABLE 1. *Effect of glucose concentration on recovery of fungi from 49 food samples.*

	Percent glucose				
	0.0	0.1	0.5	1.0	2.0
Log averages CFU/g	3.08	3.10	3.12	3.08	3.03

<sup>1</sup>Florida Agricultural Experiment Stations Journal Series No. 1261.

the linear and quadratic coefficients were positive and negative were conducted and were highly significant ( $p < .01$ ) for both tests. This is strong statistical evidence that, in fact, the counts increased initially, reached a maximum, and then decreased as the glucose concentration increased. Figure 1 shows a plot of the fitted curve  $Y = \log(\text{count})$  plotted against glucose concentration.

The data indicate that glucose concentration is an important variable in recovery of naturally occurring

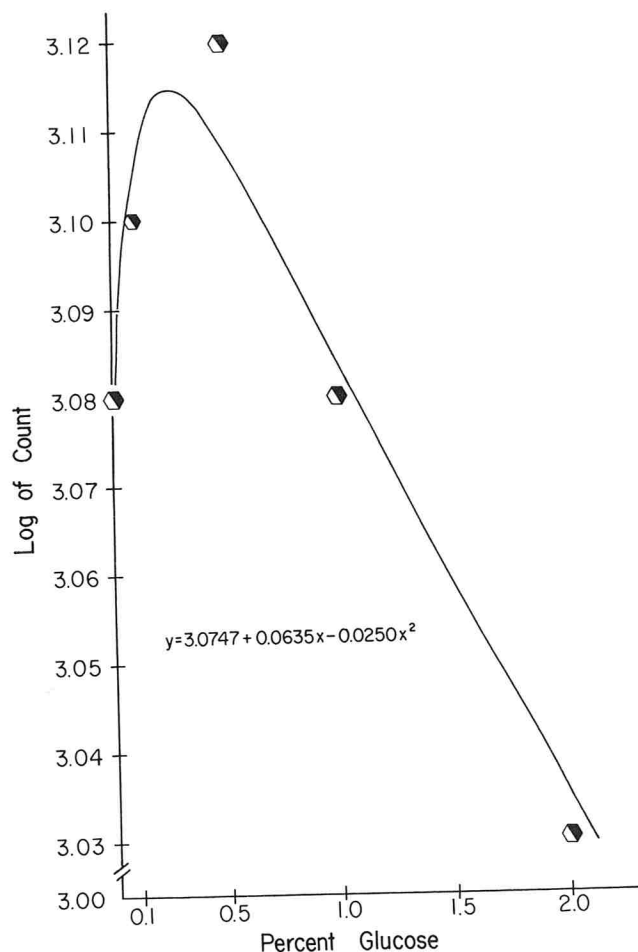


Figure 1. A fitted curve for  $Y = \log(\text{count})$  plotted against glucose concentration.

fungal populations from foods. Concentrations between 0.1 to 0.5% glucose resulted in better recovery than the more frequently used 1.0 to 2.0% glucose. Numerous factors have been shown to affect the recovery of microorganisms (3-8), however, the mode of action of glucose in this study cannot be stated with certainty. Interpretation of the data from a practical standpoint indicates some rather unexpected findings. While actual differences may seem small, recovery at the 0.5%-glucose level was approximately 20% greater than at the 2.0% level of addition; the amount found in Potato Dextrose agar. Recovery at zero and 1.0% glucose was comparable; although we tend to think of fungi as requiring carbohydrates for growth. Natural populations of fungi responded to the effect of glucose addition, indicating that the observed response may be broadly based within this group for microorganisms. This study points out the need for additional investigation into the factors affecting fungal recovery from foods.

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## Comparison of Composition and Selected Enzyme Activities in *Crassostrea virginica* and *Crassostrea gigas*, Eastern and Korean Oysters

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### ABSTRACT

Tissues of Eastern (*Crassostrea virginica*) and Korean (*Crassostrea gigas*) oysters were analyzed for proximate composition; elemental, amino acid, and pesticide contents; and selected enzyme activities. Eastern oysters contained more nitrogen but a lower ash and lipid content. In general, the amino acid and elemental contents were higher in the Korean species. Neither the Eastern nor Korean species contained pesticides or PCBs in an amount greater than 0.1 ppm. Peroxidase activity was greater in Korean oysters while lipase activity was higher in the Eastern species. Lipoxygenase was not detected in either species.

The Chesapeake Bay, bordered by Virginia and Maryland and its tributaries, is the source for half of the nation's oyster supply (24). However, the yield of the Eastern species, *Crassostrea virginica*, has declined sharply since the mid-1960's (22). Winds, sudden temperature changes, diseases and varying salinity levels caused by adverse weather conditions, as well as pollution, are factors in this decline. Concomitant with the decreasing supply has been an increase in wholesale prices. Supplementation of the Eastern oyster supply from foreign countries has been considered by seafood processors in an attempt to achieve price and market stability. The purposes of this investigation were to compare the composition and certain enzyme activities of the Chesapeake Bay (Eastern) oyster to those of the Pacific oyster, *Crassostrea gigas*, grown in Korean waters. Of primary importance are the enzymes that catalyze changes during storage or before and during processing, since adverse physical and biochemical changes of the tissue can lower acceptability and nutritional value of the product.

Peroxidase is an ubiquitous enzyme that is frequently encountered in food research. It has been suggested as causing off-flavor development in orange juice (3) and reported to exhibit significant heat stability in blue crab meat (5). Although lipoxygenase is present mostly in plant tissue (7), a lipoxygenase-type enzyme has been found in fish tissue (16). The peroxidation of lipids characteristic of lipoxygenase activity is considered a

major cause of quality deterioration in some foods. Lipases are widely distributed in nature, being found in plants, animals and microorganisms. Lipolytic enzymes are important in spoilage, since the fatty acids produced by hydrolysis of triglycerides can lead to off-flavors (7).

### MATERIALS AND METHODS

#### Oysters and reagents

Oysters of each species were shucked and frozen within 48 h after harvesting. Samples of the Eastern species were harvested from the Chesapeake Bay. The Korean samples were collected from approved waters and shipped frozen. The samples were stored at -17 C until used. Buffer salts, substrates and reagents were purchased commercially. Enzymes used as standards (peroxidase, lipoxygenase, and lipase) were purchased from Sigma Chemical Company and acrylamide gel electrophoresis reagents from BioRad. Standards and chemicals used in gas chromatographic analysis were purchased from Supelco, Inc. The oysters were freeze-dried and ground for the proximate analysis, elemental analysis, amino acid content and pesticide analysis. Tests were done in triplicate using duplicate samples for each.

#### Proximate analyses

Proximate analyses (nitrogen, ether extract and ash) were determined by the AOAC method (1).

#### Elemental analysis

The freeze-dried oysters were ground, mixed and subsampled for analysis. About 1 g of each sample was weighed into a clear polyethylene vial (1.5-cm i.d. × 2-cm high) and examined for a range of elements by nondestructive neutron activation analysis. Samples were irradiated twice, once for approximately 1 min, and again for approximately 4 h. The neutron flux was about  $10^{12}$  neutrons/cm<sup>2</sup>/sec ( $1.2 \times 10^{12}$  for the short irradiation and  $1.3 \times 10^{12}$  for the long irradiation). After the short irradiations, samples were counted within a few minutes on a Ge(Li) counting system for 8 min. An Inter-technique SA-44 4000 channel analyzer was used and the data stored on magnetic tape for later processing. For the long irradiations, samples were counted as soon as practicable considering the level of activity due to sodium. The time interval varied from 2 to 5 days after irradiation. Samples were recounted after a minimum of 10 days of decay.

#### Amino acid content

Freeze-dried tissues of the oysters were analyzed on a Beckman auto-analyzer using operating procedures recommended in the Beckman manual (2), according to the methods described by Spackman et al. (21). Samples were hydrolyzed for 24 h with constant-boiling hydrochloric acid in a nitrogen atmosphere in a sealed tube at 110 C.

#### Enzyme analysis

Extracts for the enzymatic analysis were prepared in the following

<sup>1</sup>Biochemistry and Nutrition.

manner. Approximately 70 g of each oyster species were placed in a Waring blender with 2 volumes of cold (3 C) deionized water. After homogenizing at high speed for 30 sec, the mixture was centrifuged at  $20,000 \times g$  for 5 min and the supernatant fluid filtered through Whatman No. 42 filter paper. Extracts were placed in crushed ice and analyzed within 2 min after filtration.

**Peroxidase and lipoxygenase.** Peroxidase and lipoxygenase assays were made at 25 C in a Perkin-Elmer double beam Coleman Model 124 Spectrophotometer. Peroxidase activity was measured according to a modified Nagle and Haard (15) procedure at 460 nm as described by Burnette and Flick (4). The substrate consisted of 0.1 ml of 30% hydrogen peroxide in 100 ml of 0.01 M potassium phosphate buffer (pH 6.0). A 1% (w/v) solution of o-dianisidine in methyl alcohol was used as the hydrogen donor. Into a cuvette were placed 2.8 ml of substrate, 0.1 ml of o-dianisidine solution and 0.1 ml of the oyster extract. To determine the effect of low molecular weight compounds in the extracts on the enzyme assays, the oyster homogenate was separated in an ultrafiltration cell. Peroxidase activity, however, was observed only in the filtrant containing proteins with a molecular weight of 5,000 or greater.

Lipoxygenase activity in the extracts was measured continuously at 234 nm essentially by the Worthington Enzyme Manual method (27). Phosphate and borate buffers of various pH values were used in the reaction mixture, none of which produced any detectable activity. The substrate contained 0.1 ml of technical grade linoleic acid dissolved in 60 ml of 95% ethanol and diluted to 100 ml with distilled water. This was diluted with five volumes of buffer before use. Each cuvette contained 1.0 ml of substrate, 0.1 ml of sample and 1.9 ml of buffer (pH 4.0, 6.0 and 9.0).

**Lipase.** Lipase activity was assayed by three different methods. The Worthington Enzyme Manual method employed an olive oil and gum acacia emulsion as the substrate (27). The reaction vessel contained 2.0 ml of sodium taurocholate, 5.0 ml (olive oil-gum) of emulsion, 5.0 ml of water, 2.0 ml of 3 M NaCl, 1.0 ml of 0.075 M CaCl<sub>2</sub> and 0.5 ml of sample. The rate of hydrolysis of the emulsion was determined by potentiometric titration. Lipase was also assayed by a modified Tauber method (23) employing the same substrates as the Worthington method. Tubes containing 1.0 ml of sample, 5.0 ml of emulsion and 0.5 ml of phosphate buffer (pH 6.5) were incubated for 20 h at 37 C. After incubation, 3.0 ml of 95% ethyl alcohol and 2 drops of 1% phenolphthalein were added and titrated to a deep pink color with 0.05 N NaOH. A titrimetric method from Sigma Chemical Company using an olive oil emulsion as the substrate (20) was also tested. Three ml of substrate, 2.5 ml of water, 1.0 ml of buffer (pH 6.5) and 1.0 ml of sample were incubated together for 6 h at 37 C. Three ml of 95% ethyl ethanol and 4 drops of thymolphthalein indicator were added and the solution titrated to a blue endpoint with 0.5 N NaOH.

#### Gel electrophoresis

The procedures of Nagle and Haard (15) and Lee et al. (12) were combined to prepare polyacrylamide gels used to separate peroxidase isoenzymes of the two oyster species. Each gel consisted of a stacking gel (upper) for concentrating the two samples and a separating gel (lower). The gels were immersed in a pH 8.3 buffer consisting of 0.025 M THAM [Tris (hydroxymethyl) aminomethane] and 0.192 M glycine. Samples ranging from 0.1 to 0.6 ml were applied to the gels and electrophoresis done with the application of 2 mA/tube until the marker band moved to the bottom of the tubes (approximately 4 h). The gels were stained for peroxidase by dipping in a solution containing 0.5 g of benzidine, 4.5 ml of glacial acetic acid, 18.0 ml of water and 22.5 ml of 30% hydrogen peroxide (18). Blue bands of isoenzymes were visible in 2-5 min.

#### Pesticide analysis

For each oyster species, 5 to 10 g of ground tissue were transferred to an OmniMixer stainless steel blending cup containing 25 g of anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) and ground for 4 min. The mixture was removed from the blender cup and placed in a 500-ml centrifuge bottle. The blender cup was rinsed with 100 ml of petroleum ether which was also added to the centrifuge bottle. The mixture was stirred

vigorously, centrifuged for 5 min at  $38,000 \times g$  and the supernatant fluid was decanted. The sample was extracted twice with 50 ml of petroleum ether and evaporated to dryness.

One g of fat from each sample was added to 25 ml of acetonitrile saturated with petroleum ether and shaken for 5 min in a 125-ml separatory funnel. The layers were allowed to separate and the bottom layer (acetonitrile) was transferred to a 500-ml separatory funnel containing 100 ml of petroleum ether and 300 ml of 2% Na<sub>2</sub>SO<sub>4</sub>. The extraction was repeated four times.

The 500-ml funnel was shaken gently for 1 min and layers allowed to form. The lower aqueous layer was discarded and the remaining petroleum ether was washed with two 50-ml portions of 2% Na<sub>2</sub>SO<sub>4</sub>. The aqueous washings were discarded and the petroleum ether drawn off through a 2-inch column of anhydrous granular Na<sub>2</sub>SO<sub>4</sub> into a beaker. The funnel and column were then rinsed with two 10-ml portions of petroleum ether.

The samples were evaporated to dryness on an airflow evaporator. Ten ml of petroleum ether were added and the extract was run through a 15-mm (diameter) column of Florisil (20 g), previously activated by drying overnight at 130 C in an airflow oven moistened with petroleum ether, at a flow rate less than 3 ml/min. When the sample had sunk into the column, 200 ml of a 6% ethyl ether/94% petroleum ether mixture were added. The eluant was evaporated to dryness, the residue dissolved in 10 ml of hexane and samples were injected into a gas chromatograph.

Gas chromatographic analysis was made isothermally with a Micro-Tek 220 model gas chromatograph equipped with a <sup>63</sup>Ni electron-capture detector and fitted with a 6 ft by 1/4 in. o.d. glass column, 1.5/1.95% OV-17/QF-1 on Chromosorb W HP (80/100 mesh) with a column temperature of 205 C and carrier flow of 120 ml/min. General operating conditions were: carrier gas, nitrogen; detector temperature, 340 C; injector temperature, 225 C.

## RESULTS AND DISCUSSION

The proximate compositions of Eastern and Korean oysters are listed in Table 1. Results are listed on a dry rather than wet weight basis since the moisture content of oysters is known to vary according to handling and storage conditions as well as sexual maturity and environmental factors. Additionally, it was impossible to insure the standardization of sample handling procedures.

The Eastern oysters are higher in nitrogen content than the Korean but lower in ether extract (lipid) and ash. The differences between the two species, however, were not substantial.

#### Elemental analysis

Table 2 shows the trace elemental contents of both oyster species. Both the Eastern and Korean oysters had high concentrations of calcium, chlorine, potassium and sodium. In all instances the Korean species had a considerably higher content of these elements. Values of calcium and sodium in both species of oysters were high when compared to other finfish and shellfish species (19). The 3-fold higher level of sodium and a 5-fold higher level of chlorine in Korean oysters indicate they could

TABLE 1. Composition of Eastern and Korean oysters (dry wt basis).

Component	Eastern (%)	Korean (%)
Nitrogen	9.18	7.91
Ether Extract	9.35	11.4
Ash	4.94	6.05

have come from a growing area containing a higher salt concentration. Aluminum and iron levels were similar in both species of oysters and were found to occur in substantial concentrations. Consequently, oysters could be an important source of dietary iron for individuals on restricted red meat diets, if the iron is biologically active. In comparing the elemental composition of seafood, it should be remembered that variations due to season (10,13), water salinity (26), size, geographical location and nutritional and physiological cycle can be expected to result in rather large mineral variation levels (9). It is virtually impossible to standardize conditions when analyzing oysters from a natural environment.

Levels of zinc, iron, manganese, cadmium, chromium, and cobalt have been used as a water quality index of shellfish growing areas (17). Of these, only zinc varied substantially between the two species. Eastern oysters contained 7,830 ppm while the Korean contain only 561 ppm. Oysters have the ability to concentrate zinc from sea water (26) and the level of zinc in the water will affect the amount concentrated. While the quantity found in Eastern oysters is high, it should not present a health hazard since oysters constitute a minor portion of the American diet.

#### Amino acids

The amino acid profiles in Table 3 are typical of those foods containing high moisture contents, such as oysters and other seafood. Methionine and lysine are low when compared to other animal proteins and even some vegetables. The essential amino acid levels of both varieties are too low to be nutritionally important; however, oysters are usually not a major protein source in the American diet. The Eastern oysters were higher in methionine, isoleucine, and ornithine, whereas the Korean were higher in all the other amino acids. This could be expected since the Korean species contains a higher sodium chloride content and the total free amino acid concentration in *C. virginica* is known to increase proportionally with increasing salinity (14). The free amino acids are important in osmoregulation of marine

TABLE 3. Amino acid profiles of Eastern and Korean oysters.

Amino acids	mg/g	
	Eastern	Korean
Lysine	0.082	0.102
Histidine	0.014	0.049
Ammonia	0.039	0.037
Arginine	0.327	0.571
Taurine	—	—
Aspartic Acid	0.111	0.303
Threonine	0.030	0.131
Serine	0.087	0.164
Glutamic Acid	0.338	0.464
Proline	0.016	0.049
Glycine	0.076	0.316
Alanine	0.311	0.511
Valine	0.043	0.091
Methionine <sup>a</sup>	0.101	0.093
Isoleucine	0.054	0.047
Leucine	0.073	0.095
Tyrosine	0.041	0.091
Phenylalanine	0.035	0.071
Ornithine	0.200	0.009

<sup>a</sup>Measured as methionine sulfone.

animals. Taurine was not detected. Taurine has been found in marine animals (6) and is suggested as having a sparing effect on amino acids since it is osmotically active (11). Ammonia concentrations were essentially equal in both species. Since ammonia has been proposed as a quality index, this could mean that the oysters were of similar quality.

#### Pesticides

Pesticides and PCB residues found in Eastern and Korean oysters are listed in Table 4. Oysters are able to concentrate pesticides (25) as well as other organic and inorganic compounds and elements. Both species of oysters contained low pesticide and PCB concentrations, indicating that there was little contamination of the growing waters.

#### Enzyme assays

*Lipoxygenase.* Lipoxygenase activity was not observed in either species. If the enzyme was present, it occurred in a concentration too low to be detected.

*Peroxidase.* Peroxidase activity was substantially higher in the Korean oysters than the Eastern when

TABLE 2. Trace elements in Eastern and Korean oysters (dry wt basis).

Element	ppm		Element	ppm	
	Eastern	Korean		Eastern	Korean
Al	537.	598.	Lu	0.008	0.02
As	12.3	20.6	Mg	—	540.
Au	0.05	0.02	Mn	31.7	32.2
Ba	3.1	5.6	Mo	2.0	0.7
Br	68.2	117.	Na	3790.	10740.
Ca	1130.	2000.	Rb	3.3	8.8
Cd	5.4	5.1	Sb	0.07	0.02
Cl	3120.	17000.	Sc	0.1	0.08
Co	0.8	0.6	Se	5.5	3.2
Cr	—	0.7	Sm	0.7	0.2
Cs	0.1	0.2	Ta	0.06	0.04
Dy	0.2	0.08	Th	—	0.4
Fe	490.	426.	Ti	45.	63.
Hf	0.1	0.2	V	1.3	1.8
Hg	0.16	0.2	W	0.6	0.8
I	9.2	5.4	Yb	0.08	0.08
K	8610.	14800.	Zn	7830.	561.
La	0.5	0.4			

TABLE 4. Pesticide and PCB content of Eastern and Korean oysters.

Pesticide	Eastern	Korean
		ppm
Chlordane	—	.001
DDE	—	.003
Other DDT's	< .1	Trace
PCB	< .1	—
Malathion	Trace	—
Perathane	< .1	—

compared on an equal weight basis (Fig. 1). Both species have a major isoenzyme band between 0.45 and 0.58 Rf (Fig. 2). The Eastern species had another major band near 0.95 whereas the Korean species had two additional bands. One occurred at 0.66 and the other at 0.87. The additional isoenzyme found in the Korean species suggests a possible correlation with its greater peroxidase activity. Biochemical polymorphism has been observed in the work of Fugino and Nagaya on Pacific oyster esterases (8).

**Lipase.** Lipase activity was greatest in the Eastern species (equivalent weight basis) when three methods of enzyme assay were compared (Table 5). The Tauber and Sigma method provided a greater sensitivity than the Worthington procedure. However, there were inconsistencies in relative enzyme rates between the two species. Although the Worthington and Sigma methods exhibit proportional rate differences, greater control would have to be employed with the Worthington method since the

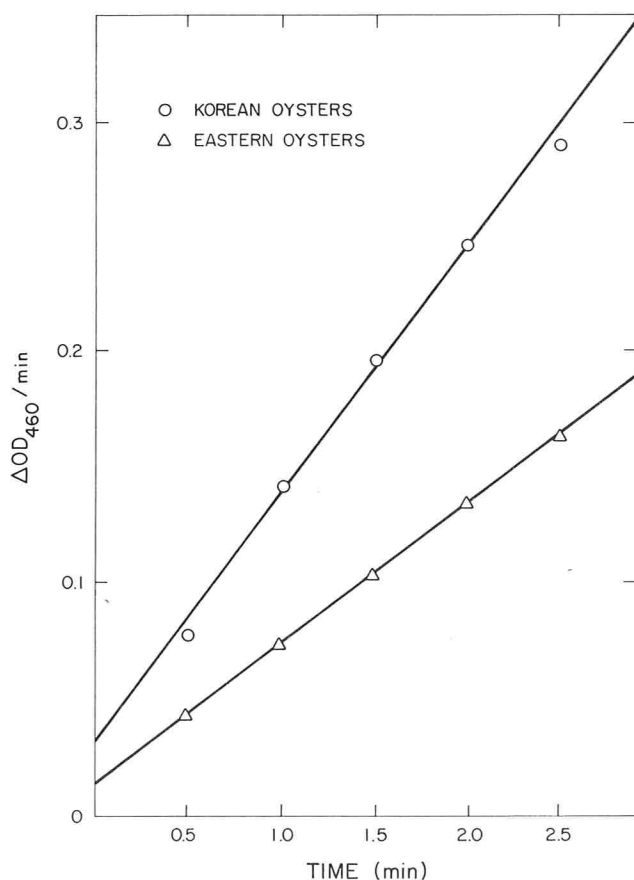


Figure 1. Peroxidase activities of Eastern and Korean oysters. Conditions as described in Materials and Methods.

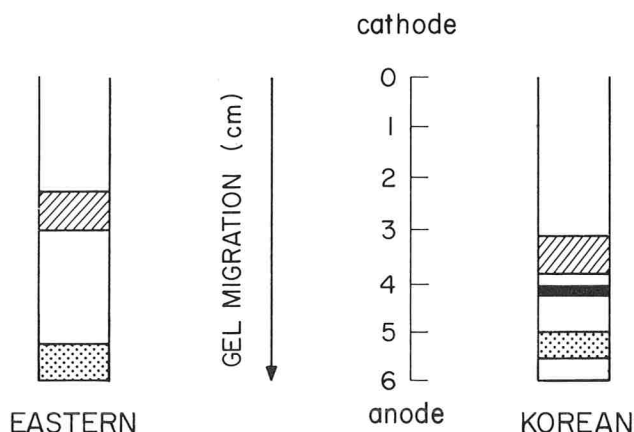


Figure 2. Polyacrylamide gel patterns for peroxidase in Eastern and Korean oysters. Conditions as described in Materials and Methods.

TABLE 5. Lipase activity in Eastern and Korean oysters.

Method	Enzyme activity	
	Eastern	Korean
Worthington	0.002 unit/mg	0.001 units/mg
Tauber	0.75 ml <sup>a</sup>	0.21 ml <sup>a</sup>
Sigma	0.99 ml <sup>a</sup>	0.51 ml <sup>a</sup>

<sup>a</sup>ml of 0.05 N NaOH/ml of oyster extract.

magnitude of the difference is quite small. If lipolysis contributes to quality degradation, it appears that lipolytic activity may be more of a problem in Eastern oysters than Korean since the enzyme activity is greater.

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## Volatile Compounds in UHT-Sterilized Milk During Fluorescent Light Exposure and Storage in the Dark<sup>1</sup>

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### ABSTRACT

When ultra-high-temperature sterilized milk (140 C for 3.5 sec) was exposed to fluorescent light over a 30-day period at 22 C, acetaldehyde, propanal, pentanal, and hexanal increased in concentration. On storage of the milk in the dark, after a 2-week period of light exposure, these same compounds decreased in concentration. No characteristic patterns were noticed in the other chromatographic peaks. When a five-fold diluted distillate of light-exposed milk was added to normal milk, a taste-panel criticized the milk as pronounced oxidation, tallowy or oily.

The adverse effect on flavor of exposing refrigerated milk to fluorescent light is well known (1,2,4,5,6). Increases in concentrations of acetaldehyde, propanal, n-pentanal, and n-hexanal in milk have recently (1) been associated with light-activated off-flavor. Samuelsson (8) reported methyl sulfide, methyl mercaptan, formaldehyde, acetaldehyde, and propanal increased on exposure of milk to light and were related to the off-flavor. Although many researchers have studied the increases in the concentration of these and other compounds when milks are exposed to fluorescent light, no one has reported changes in exposed milks after dark storage.

This paper describes changes in some volatile materials in milk exposed to fluorescent light and then placed in the dark.

### MATERIALS AND METHODS

Nine batches of raw milk with fat contents of 3.13 to 3.80% ( $\bar{x}$  = 3.50) and total solids 11.16 to 12.18% ( $\bar{x}$  = 11.80) were ultra-high-temperature (UHT) sterilized (140 C for 3.5 sec), packaged aseptically and then stored at room temperature 2 to 42 days in either aluminum foil-lined cartons or polyethylene-lined cartons before use. Samples for our light-exposure study were selected during this 42-day period. Although cartons of milk initially had different pre-trial storage periods, changes in these milks were minor compared with those that occurred when they were exposed in glass to fluorescent light. Each of these packaged UHT milks was aseptically transferred into either glass-stoppered or non-absorbent cotton-plugged, sterile liter or 2-liter glass Erlenmeyer flasks. Mouths of these flasks were covered with heavy duty aluminum foil before sterilization. The flasks, approximately half filled with sterile milk, were exposed at  $22 \pm 2$  C to 2150 lx of light from two 40-watt cool-white fluorescent tubes. Tests for microbiological spoilage were the same as those used by Mehta and Bassette (7). Aseptic

techniques were used to remove the 50-60 ml of milk necessary for testing at each time interval. Volatile materials present were determined by gas chromatography (GC) (7).

Two sets of milk samples were observed. During Phase I samples were exposed to fluorescent light in glass-stoppered Erlenmeyer flasks and examined at 0, 10, 20 and 30 days. During Phase II samples in sterile cotton-plugged Erlenmeyer flasks were exposed to light and examined at 0, 1, 2, 7, 14, 21 and 28 days. Half the samples in this second phase were exposed to light throughout a 4-week period and the other half to light for 2 weeks and then stored in the dark.

Because of the intensity of the off-flavor, no systematic organoleptic studies were conducted on these milks. Five milliliters of distillate from 50 ml of one of the milks which had been exposed to fluorescent light for 20 days, however, was added to 245 ml of pasteurized-homogenized 3.5% fat whole milk. This represented a five-fold dilution of flavor in the distillate compared with that of the light-exposed milk. This sample was evaluated according to procedures of the National Collegiate Student Judging Contest by an experienced five-member taste panel.

### RESULTS AND DISCUSSION

All the GC peaks observed previously in stored sterile milk (7) also were observed here. Changes in concentrations when light-exposed samples were stored in the dark, however, were observed only for acetaldehyde, propanal, pentanal and hexanal.

The changes in concentration of propanal with time are shown in Fig. 1. The X-axis represents the number

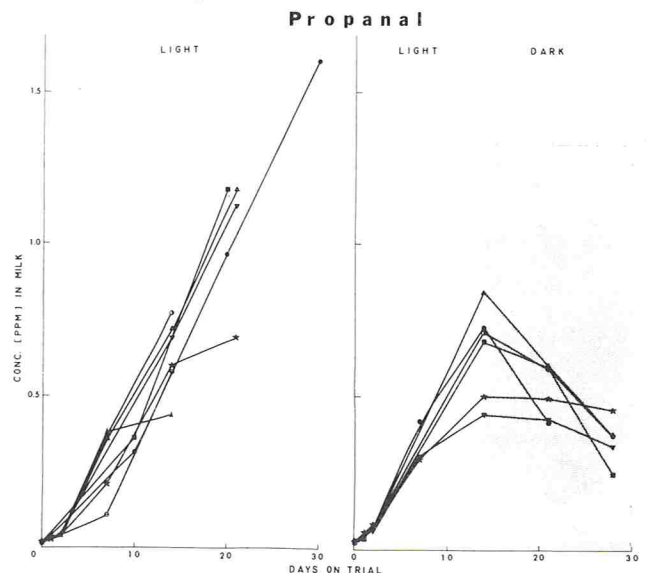


Figure 1. Changes in propanal concentrations after prolonged fluorescent light exposure of UHT milk in glass Erlenmeyer flasks and after dark storage.

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of days the sample was on trial, and the Y-axis the ppm of propanal in the milk. The graph on the left shows eight milk samples exposed to light throughout the study. Each line on the graphs designates a different sample of milk. The graph on the right shows the six milks in Phase II that were stored in the dark (see shaded area) after a 2-week period of light exposure. Upon being exposed to light, propanal increased in concentration. When the samples were removed from the light at the end of 2 weeks and stored in the dark, it decreased. Almost all samples examined showed similar trends. Day et al. (3) reported the flavor threshold level of propanal in milk to be 0.43 ppm; for most of our samples this level was passed at about 10 days. Propanal concentrations of those samples remaining in the dark decreased below this threshold value in most instances. This does not mean the flavor would have been acceptable since several of the carbonyl compounds that increase on exposure to light have additive, if not synergistic, effects on flavor (3).

Changes in pentanal were similar to those of propanal (Fig. 2); however, almost from 0 day, pentanal concentrations were above the reported threshold levels of 0.13 ppm (3) and stayed that way even when the milk was stored in the dark. Hexanal (Fig. 3) also showed similar changes and trends as pentanal. Again, the hexanal concentrations observed at 0 days were higher than the reported flavor threshold value of 0.049 ppm (3).

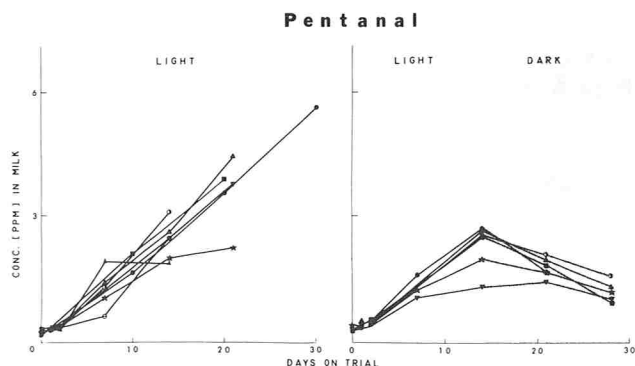


Figure 2. Changes in pentanal concentrations after prolonged fluorescent light exposure of UHT milk in glass Erlenmeyer flasks and dark storage.

Figure 4 shows changes in acetaldehyde during prolonged light exposure and dark storage periods. The results for acetaldehyde were unusual compared with the other components. As previously mentioned (7), pretreatment of milk cartons with ethylene oxide resulted in some milks having high initial acetaldehyde concentrations. The shorter the time interval between carton pre-treatment and packaging, the higher the acetaldehyde concentration. The acetaldehyde concentration decreased with the storage of milk; note on the left-hand side of Fig. 4, the five lines starting at high levels and declining rapidly. In this figure, besides observing the increase in acetaldehyde due to light, we also are observing the effects of ethylene oxide pre-treatment of the milk cartons. If we could visualize the elimination of

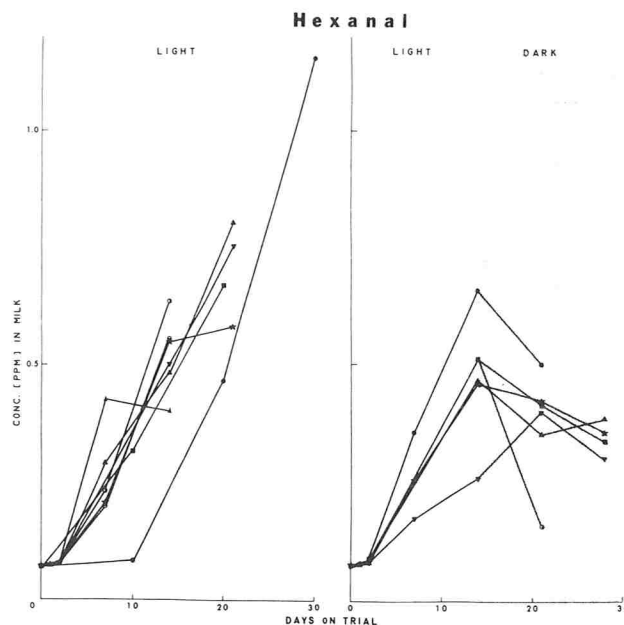


Figure 3. Changes in hexanal concentrations after prolonged fluorescent light exposure of UHT milk in glass Erlenmeyer flasks and dark storage.

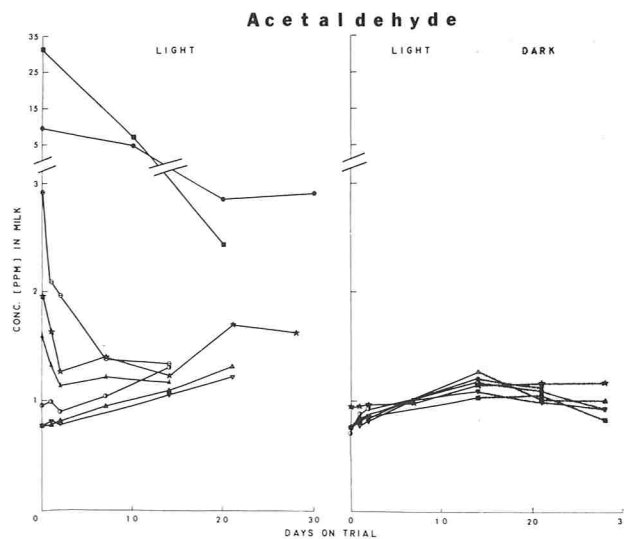


Figure 4. Changes in acetaldehyde concentrations after prolonged fluorescent light exposure of UHT milk in glass Erlenmeyer flasks and dark storage.

the effect of high initial acetaldehyde, it is likely that acetaldehyde trends are similar to those of the three other components.

In spite of the differences in the age of the UHT milks at the beginning of the experiment, the initial concentrations of propanal, pentanal, and hexanal were about the same for all milks. Although there was a considerable degree of uniformity in patterns of volatile materials throughout this experiment, there also were some deviations. Replicate analyses as shown by separate lines on the figures did not agree closely. This may have been due to the dynamic system that we dealt with, involving: (a) loss of volatiles through the mouth of the

TABLE 1. Comparing the flavor of reference milk with a reconstituted milk<sup>a</sup> made from 5 ml of distillate from 50 ml of milk exposed to fluorescent light for 20 days blended with 245 ml of reference milk.

Panelist number	Reconstituted milk <sup>a</sup>		Reference milk	
	Flavor score	Comments	Flavor score	Comments
1	32.0	pronounced oxidized, oily	38.0	less than definite cooked
2	31.0	very pronounced oxidized, slightly cooked	38.0	more than slightly cooked
3	31.0	very pronounced oily	38.0	more than slightly cooked
4	32.0	pronounced oxidized, more than slightly tallowy	38.0	less than definite cooked, very slightly oxidized

<sup>a</sup>Prepared by steam-distilling 50 ml milk exposed in glass to fluorescent light for 20 days at 22 C and adding the 5 ml distillate to 245 ml of normal milk.

flask, (b) reduction in the concentration of a volatile through its interaction with other components, and (c) increase in concentration by a chemical change of a component of the milk. Thus, the rates at which the changes in volatile compounds occur probably depend on the milk, the permeability of the closure, and the amount of radiant energy present on the surface of the milk.

These results indicate that light-induced, volatile compounds are produced constantly in large amounts only when the milk is exposed to light and not when it is in the dark. This would lead us to postulate that the reaction rate is not maintained by the process of autooxidation. The reaction apparently is photocatalyzed and requires a constant source of radiant energy to be maintained.

Results comparing the flavor of normal (reference) milk with milk with added distillate from the light-exposed milk (diluted 5×) are in Table 1. The panel judged the sample with the added distillate as strongly oxidized, tallowy or oily. Panelist 4 who criticized the control milk as very slightly oxidized may have experienced a slight carry-over of the flavor from one extremely oxidized sample. The off-flavor of the light-exposed sample was so intense that we did not include similarly prepared samples in other panel meetings. Apart from the five-member panel evaluation of milk plus sample distillate, one panel member evaluated the light-exposed milk directly (without distilling and reconstituting the distillate into regular

milk). This panelist tasted these milks at a time different from the regular panel meetings. His conclusions were: at the end of one day, the milk was slightly oxidized; at 2 days, it was definitely oxidized; at 7 days, it was strongly oxidized; and after that it progressed to tallowy.

#### ACKNOWLEDGMENT

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# Roles of Lactobacillus in the Intestinal Tract<sup>1</sup>

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## ABSTRACT

Despite the opinion of many scientists and laymen that lactobacilli benefit adult intestinal health, the medical and scientific communities do not accept this as fact. This paper offers possible beneficial and detrimental roles which lactobacilli could play; relevant literature citations also are made. Healthful roles include those exerted as a result of their presence as members of the intestinal flora as well as their benefits as culture or enzyme preparations. Speculative detrimental effects discussed are nutrient competition, carcinogen activation and detoxification interference (glucuronide hydrolysis).

In moving from the stomach through the intestinal tract, food passes through the duodenum, jejunum and ileum of the small intestine and the ascending, transverse and descending colon of the large intestine. For many microorganisms, this is a hostile environment where a number of factors or compounds, contributed in part by lactobacilli, discourage growth and in some instances survival of undesirable bacteria. These factors include gastric juice, bile, fatty acids, organic acids, hydrogen sulfide, lysozyme, lysolecithin, antibiotics and peristalsis.

In this discussion we will be concerned with bacteria of the *Lactobacillus* genus and consider the roles these bacteria might have in the intestinal tract. If they play any role at all, it follows that they would be present in a reasonable number in most humans. Savage (26) and others have indicated that this is the case. Table 1 summarizes data indicating the approximate number of lactobacilli present in the intestinal tract. Here it may be seen that they range from not detectable in some individuals, except for the feces, to from a million to a

billion per gram. Their absence in certain "healthy" persons would suggest that while their function may be desirable, it may also be dispensible. Lactobacilli are not the most numerous bacteria in the GI tract as illustrated in Table 2. This would suggest that if their intestinal metabolic activities are beneficial to man they would be useful because of what a relatively few, that is from a hundred per gram to 500 million per gram, could do. But all food microbiologists are aware that it isn't always the total number of bacteria present that is important, but the type. In this regard it is of interest that certain individuals lack or have low numbers of coliforms in stool samples due to antibiotic production by a

TABLE 1. Number of lactobacilli in the human stomach, intestinal tract and feces<sup>a</sup>.

Sample Source	Viable count (range log <sub>10</sub> /g)
Stomach	N <sup>b</sup>
Upper small intestine	N-6
Lower small intestine	N-8
Large intestine	N-9
Feces	3.6-9.3

<sup>a</sup>Adopted from Drasar (6) and Drasar and Hill (8).

<sup>b</sup>N = less than 10 per gram.

non-sporeforming variant of *Bacillus subtilis* (18). In these situations, the population of these bacteria do not exceed 10<sup>4</sup> per gram of feces (Gerhardt, personal communication) but lactobacilli are increased.

## FUNCTIONS OF LACTOBACILLI

We might speculate about functions the lactobacilli could play in the human intestinal tract and some suggested roles are shown in Table 3. Three possible

<sup>1</sup>Paper presented at 1978 annual meeting of American Society for Microbiology, May 14-19, Las Vegas, Nevada. Technical Paper No. 4932. Oregon Agricultural Experiment Station.

Table 2. Approximate numbers of various bacteria in the human stomach, intestine and feces<sup>a</sup>.

Genus	Mean log <sub>10</sub> viable count/g wet weight				
	Stomach	Upper small	Lower small	Large	Feces
<i>Bacteroides</i>	N <sup>b</sup>	2.5	3.5	8.0	10.5
<i>Bifidobacterium</i>	N	2.0	4.0	7.0	10.5
<i>Clostridium</i>	N	N	N	N	3.0
Enterobacteria	N	N	3.3	7.0	6.0
Enterococci	N	N	2.3	7.0	3.5
<i>Lactobacillus</i>	N	1.0	N	6.5	4.0
Veillonella	N	N	N	3.0	3.0
Yeasts	N	1.0	2.3	N	1.0
%lactobacilli	0	14	0	0.5	5 × 10 <sup>-6</sup>

<sup>a</sup>From Hawksworth et al. (16).

<sup>b</sup>N = less than 10 per gram.

detrimental roles, nutrient competition, carcinogen activation, and glucuronide hydrolysis are suggested while nine beneficial attributes are listed. Published research reports will support some of these roles for lactobacilli while others are speculative.

Since 1947 (15) a number of reports reviewed recently (20,27,28) have been made concerning antibiotic production by lactobacilli. These have been called by various names including acidophilin, acidolin, lactobacillin and lactocidin. Strains vary in their ability to produce these substances and cultural conditions influence the amount produced within strains. In vitro inhibitory activity has been reported against salmonellae, shigellae, staphylococci, *Proteus*, *Klebsiella*, pseudomonads, enteropathogenic *Escherichia coli*, bacilli and *Vibrio* organisms. In vivo studies with human medical and intestinal surgery patients are needed to determine if these antibiotic substances benefit intestinal health.

Organic acids such as acetic and lactic which are produced by lactic acid bacteria will inhibit growth of many bacteria, especially pathogenic gram-negative types. A number of literature reports have dealt with this phenomenon (5,13,29) and have shown that while pH per se is a factor, lower pH values also potentiate the activity of these acids since the undissociated forms are most destructive. Also these volatile acids are especially antimicrobial under the low oxidation-reduction potentials (26) which lactic acid bacteria help maintain in the intestines. Diet may be a factor determining the amount of these acids produced as, for example, with breast-fed human infants (3,4). Here, bifidobacteria, which derive growth-promoting oligosaccharides from human milk, produce acetate and lactate homofermentatively resulting in a fecal pH of about 5.0.

Apart from the growth-products influence lactobacilli likely have in suppressing undesirable bacteria in the intestinal tract, they may also out-compete other bacteria for nutrients and occupy sites as gut colonizers and make these sites unavailable to other microorganisms (10). Data supporting these possibilities are not available from research involving humans but hopefully will be forthcoming. Recent studies in animals, however, support such a model (21,23).

### BILE SALTS

Bile salts are surface-active chemicals produced in the liver from the catabolism of cholesterol. The bile acids produced in humans are chenodeoxycholic and cholic

acids (40% each) and deoxycholic acid (10%) (2). They aid in digestion by forming polymolecular aggregates (micelles) with water-insoluble lipids and fat-soluble vitamins (19) and are recycled as conjugates of glycine and taurine (3:1 ratio). Deconjugated bile acids are more inhibitory for bacteria than the conjugated acids (9) and deconjugation is one of the main microbial reactions which alter the bile acids. The small intestine contains little free bile acids but significant quantities appear in the large bowel. They may function here to control the microbial composition but no evidence proves that this is so (26). If this is important, it would be worthwhile to examine lactobacilli for their ability in this regard. Gilliland and Speck (11) have tested human fecal lactobacilli (*L. acidophilus*, *L. buchneri*, *L. casei*, *L. fermentum*, *L. leichmannii*, *L. plantarum*, *L. salivarius*) for deconjugation of glycocholate and taurocholate. Only *L. buchneri* strains were inactive on both conjugates and all of six *L. acidophilus* isolates tested deconjugated taurocholate (Table 4). Thus lactobacilli can liberate free bile acids in the intestinal tract and thereby could exert an influence on the balance of bacteria present.

TABLE 4. Ability of lactobacilli from human feces to deconjugate bile acids<sup>a</sup>.

Species	Taurocholate	Glycocholate
<i>L. acidophilus</i>	6/6	1/6
<i>L. buchneri</i>	0/3	0/3
<i>L. casei</i>	0/13	9/13
<i>L. fermentum</i>	1/5	3/5
<i>L. leichmannii</i>	3/4	3/4
<i>L. plantarum</i>	0/3	2/3
<i>L. salivarius</i>	1/2	1/2

<sup>a</sup>Data of Gilliland and Speck (11). No. positive/No. strains.

### COLON CANCER

Interactive effects between food constituents and bacteria in the intestine are believed involved in the etiology of colon cancer (7,17). Glucuronide formation in the liver is an important detoxification mechanism in humans; the glucuronides formed are excreted with bile and may be hydrolyzed by certain intestinal bacteria to release a potential carcinogen. Other enzymatic capabilities of the intestinal microflora may also be involved in carcinogen production such as reduction of azo and aromatic nitrogen compounds through azoreductase and nitroreductase activities. Steroid and protein metabolites produced by bacteria have also been shown to yield carcinogens or co-carcinogens (7). The role that

TABLE 3. Possible functions for lactobacilli in the intestinal tract.

Beneficial		Detrimental	
Natural flora	Adjunct		
1. Antibiotic production	1. Disease therapy	1. Nutrient competition	
2. Organic acid production	2. Preventative therapy	2. Carcinogen activation	
3. Lower pH and O/R	3. Enzyme source	3. Glucuronide hydrolysis	
4. Competitive antagonists			
5. Bile deconjugation			
6. Carcinogen suppression			

lactobacilli play these processes in the human intestine is difficult to access. In the rat, *Lactobacillus* species have been ranked third in  $\beta$ -glucuronidase activity behind the *Bacteroids* and bifidobacteria. However, in humans, as shown in Table 5, this activity in the intestine as contributed by the microflora is very low. In a recent study with rats, the influence of diet, advanced age and dietary supplements of *Lactobacillus acidophilus* ( $10^{10}$  viable cells per animal per day) on the bacterial enzymes  $\beta$ -glucuronidase, azoreductase and nitroreductase in the fecal microflora was measured (14). Feeding the *L. acidophilus* supplement significantly lowered the latter two enzymes in animals on a high meat diet. Lactobacilli also have been shown to be active in degrading nitrosamines (24).

TABLE 5. Bacterial  $\beta$ -glucuronidase activity of the intestinal contents from man and laboratory animals<sup>a</sup>.

Species	Activity <sup>b</sup>	
	Upper small	Lower small
Man	.02	.9
Rabbit	2.4	45.4
Guinea-pig	2.7	139
Rat	304	1341
Mouse	1200	5015

<sup>a</sup>Data of Hawksworth et al. (16).

<sup>b</sup> $\mu$ moles of phenolphthalein glucuronide degraded per h/g.

### DIETARY ADJUNCTS

The potential beneficial activities of lactobacilli in the human intestinal tract has led to their use or suggested use as dietary adjuncts for at least three purposes: (a) in intestinal disease therapy, (b) to improve or maintain intestinal health and (c) as a source of  $\beta$ -galactosidase to enable lactose-intolerant persons to consume milk (Table 3); many articles on these applications have been published including three recent reviews (1,25,30). While consumption may not increase the numbers of viable lactobacilli in the feces (12), the metabolic activity of the intestinal microflora may thereby be beneficially altered (14), especially if the organisms are consumed regularly. One recent report (22), however, indicated that commercially prepared tablets containing *L. acidophilus* and *Lactobacillus bulgaricus* were ineffective in reducing the incidence or duration of traveler's diarrhea.

On this occasion it is appropriate to read a portion of the obituary (Nature 97(2439):443, 1916) of Elias Metchnikoff (1845-1916) who, from his early studies in intestinal microbiology, suggested that *Lactobacillus* organisms were beneficial to human intestinal health. "In 1903 he found time to write a profoundly interesting popular book, *The Nature of Man* (London:Heinemann), in which, among other things, he discourses on old age, and his view that unhealthy fermentation commonly occurring in the large intestine produces poisons which are absorbed, and lead to deterioration of the tissues of the walls of the arteries, and so to senile changes and unduly early death. He satisfied himself, experimentally

and clinically, that the use of "sour milk" as an article of diet checks or altogether arrests this unhealthy fermentation in the intestine by planting there the lactic bacillus which, forming lactic acid, renders the life and growth of the bacteria of those special poisonous fermentations (which cannot flourish in an acid environment) impossible. Hence he himself daily took a pint or so of sour milk, and he recommended it to others and arranged for the commercial preparation of a particularly pure and agreeable "sour milk" from the sale of which he scrupulously abstained from deriving any pecuniary profit. This small, though valuable, adventure of his in dietetics has been-unfortunately, but perhaps inevitably — the one and only feature of his long career of vast scientific discovery which has impressed itself on the somewhat erratic intelligence of the "man in the street."

Since 1916 fascination with acidophilus milk as a health food has continued, especially the last few years when unfermented or sweet acidophilus milk has become a retail market item in the U.S. Definition of the roles that *L. acidophilus* and other lactobacilli play in intestinal health requires further research attention. But clearly, the properties of these bacteria which we have emphasized here indicate they are beneficial members of the human intestinal flora.

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## Nitrates and N-Nitrosamines in Cheese

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### ABSTRACT

Use of nitrate in the manufacture of certain cheeses is sometimes questioned because of its potential involvement in formation of N-nitrosamines. Unlike cured meats, there was not much information available, until recently, regarding the presence of N-nitrosamines in cheese and other dairy products. This paper briefly reviews the mechanism of formation of such compounds in foods and discusses the necessity of selective and sensitive methods of analysis. Factors which may possibly influence formation of N-nitrosamines in cheese as well as further areas of research are also discussed.

Cheeses of the Gouda and Edam types as produced in certain European countries are very susceptible to late blowing as a result of development of clostridia in the cheese (35,36,97,98). As a result of this defect, much research has been done on methods to prevent growth of these organisms. Techniques such as use of nisin-producing starters (37,38), hydrogen peroxide (39,105) and salting of the curd (39) have been investigated and found to have adverse effects on the structure of the cheese. In addition, the nisin-producing starters were shown to be extremely susceptible to bacteriophages and thus have limited industrial application (39). Reduction of the number of clostridial spores in the milk using methods such as bactofugation has not proven altogether successful since it is not possible to remove all the spores (55,103). Although the number of spores can be reduced by a factor of about 40, this reduction is insufficient to prevent late-blowing.

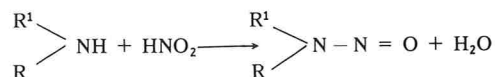
One of the most successful methods of preventing late-blowing of cheeses is addition of potassium or sodium nitrate (39,105). This additive has been shown to inhibit the development of clostridia when the water activity of the cheese is high. During this period, the salt content is low, thus permitting the most active spores to germinate. The germinating spores are very susceptible to nitrite which is produced from the added nitrate primarily by action of the milk enzyme, xanthine oxidase (35). Consequently, the number of remaining spores is so small that their germination and development can be controlled by the salt concentration that is gradually built up as a result of the salt from the brine penetrating into the cheese. This inhibitory action in cheese parallels the use of sodium nitrite in the curing of meat products, where one of its primary functions is to

prevent growth of *Clostridium botulinum* (48,81).

The practice of adding nitrate to cheesemilk is sometimes questioned because it may constitute a health hazard in that it can lead to formation of N-nitrosamines. Many of these compounds are carcinogenic and in addition some exhibit mutagenic, embryopathic and teratogenic effects (17,61,62). Since formation of N-nitrosamines in dairy products has been rarely discussed, this paper will briefly review the reactions leading to their formation as well as their presence in cheese and other food products.

### CHEMISTRY OF FORMATION OF N-NITROSAMINES

The principal mode of formation of N-nitrosamines is the reaction between secondary amines and nitrous acid.



In this reaction, R<sup>1</sup> is an alkyl group while R may be an alkyl, aryl or a wide variety of other functional groups. N-Nitrosamines can also be formed from tertiary amines, quaternary ammonium compounds and primary amines (84). The main products of the nitrosative cleavage of tertiary amines by nitrous acid include a N-nitrosamine, nitrous oxide and a carbonyl compound (1,92). Fiddler et al. (25) prepared dimethylnitrosamine (DMN) from naturally occurring quaternary ammonium compounds and related tertiary amines under conditions simulating those of comminuted meat products. More recently, Pensabene et al. (73) and Gray et al. (43) showed that DMN could be produced from lecithin-type compounds under various conditions.

The reaction of primary aliphatic amines with nitrous acid principally produces compounds other than N-nitrosamines (84). However, a recent study by Warthesen et al. (102) demonstrated that N-nitrosamines can be produced from primary amines, albeit in low concentrations. These investigators reported that formation of a stable secondary N-nitrosamine apparently requires conversion of the primary amine to a secondary amine which can then be N-nitrosated. Formation of secondary amines from aliphatic primary monoamines involves dimerization in which the unshared electrons on an unreacted amine nitrogen undergo electrophilic attack by the carbonium ion (4). More detailed discussions of these reactions are given in recent N-nitrosamine reviews (12,14,84).

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The kinetics of N-nitrosamine formation *in vitro* has been studied at length (52,67,69,80,94). Although the reaction proceeds rapidly when high concentrations of reactant are used, it is slow and therefore markedly temperature-dependent at concentrations approaching those likely to be encountered environmentally or in food. In moderately acidic media, the kinetic equation has the form,

$$\text{Rate} = k [\text{R}_2\text{NH}] [\text{HNO}_2]^2$$

that is to say, the reaction rate is proportional to the concentration of the free amine (non-protonated) and to the square of the concentration of the undissociated nitrous acid. Reactions of this type often show a bell-shaped pH dependence. Mirvish (67) reported that, for dimethylamine and nitrite under standard conditions of temperature and total species concentration, a plot of the reaction yield against pH showed a maximum at pH 3.4 (Fig. 1), close to the  $\text{pK}_a$  value of nitrous acid. The proportion of non-ionized amine present at the optimum pH value will vary with the basicity of the amine and it has been shown that an inverse relationship exists between amine basicity and N-nitrosamine formation (83). This relationship was also discussed by Mirvish (69) who reported the rates of N-nitrosation of several amines of various basicities. At the optimum pH which was about 3.0, the reaction rates increased  $1.85 \times 10^5$  times on proceeding from the strongly basic piperidine ( $\text{pK}_a$  11.2) to the weakly basic piperazine ( $\text{pK}_a$  5.57) (Table 1). In systems that approximate those of foodstuffs, the basicity of the amine is a dominant factor in determining the rate and extent of N-nitrosation (14).

#### N-NITROSAMINES IN FOODS

Since 1964, when Ender et al. (21) first reported the presence of N-nitrosamines in foodstuffs (nitrite-preserved herring meal for animal feeding), there have been many reports regarding presence of N-nitrosamines in foods including wheat products (46,63), mushrooms (20), alcoholic beverages (64,65,108) and soybean oil (45) as well as in meat (23,70,71,85,86,104) and fish products (2,22,47,89). The authenticity of some of these reports is questionable, however, because many of the analytical

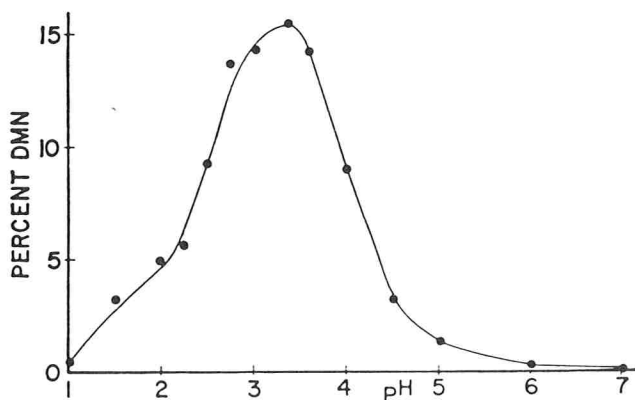


Figure 1. Dependence of DMN formation on pH (67).

TABLE 1. Rate constants for the nitrosation of amines at the optimum pH and 25 C<sup>a</sup>.

Amine	$\text{pK}_a$	Optimum pH	$k_2^b$ (M <sup>-2</sup> sec <sup>-1</sup> )
Piperidine	11.2	3.0	0.00045
Dimethylamine	10.72	3.4	0.0017
Pyrrrolidine	11.27	3.0	0.0053
N-Methylethanolamine	9.5	3.2	0.010
N-Methylbenzylamine	9.54	3.0	0.013
Proline	—	2.5	0.037
Sarcosine	—	2.5	0.23
Prolylglycine	8.97	3.0	0.25
Hydroxyproline	—	2.5	0.31
Prolylleucylglycineamide	8.97	3.4	0.38
Morpholine	8.7	3.4	0.42
Mononitrosopiperazine	6.8	3.0	6.7
Aminopyrine	5.04	2.0	80
	(5.57)		
Piperazine		3.0	83
	(9.8)		
N-Methylaniline	4.85	—	250

<sup>a</sup>Adapted from Mirvish (69).

<sup>b</sup>Rate constant for the rate equation, Rate =  $k_2$  (amine) (nitrite)<sup>2</sup>.

methods used lacked the necessary specificity for the unequivocal identification of N-nitrosamines at the very low levels normally found in foods.

Before about 1970, many of the reports of N-nitrosamines in foods were based on thin layer chromatographic procedures (15,76,90). Consequently, it is probable that many of the earlier reported findings of N-nitrosamines at the parts per million (mg/kg) level were in error. Today, gas chromatography in combination with mass spectrometry is the only reliable method for the unambiguous confirmation of the identities of N-nitrosamines in the amounts that can be isolated from foods. Even this technique can produce artifacts and it is important that only results obtained at high resolving powers are accepted as conclusive (12).

Detection and determination of N-nitrosamines in foods at the  $\mu\text{g}/\text{kg}$  level is an exacting and time-consuming process. As a result, many different techniques have been used including polarography, ultraviolet absorption, and thin layer and gas chromatography. Limitations of these methods are adequately discussed in reviews by Crosby (12), Crosby and Sawyer (14), and Fiddler (24). Since preparation of these reviews, a new specific detection system for N-nitrosamines has been developed (30). This technique, called thermal energy analysis, is uniquely selective to the N-nitroso functional group and is sensitive to picogram quantities (31). The thermal energy analyzer (TEA) has been interfaced to both a gas chromatograph (27) and a high performance liquid chromatograph (26). A recent comparison of some chromatographic detectors for analysis of N-nitrosamines showed that the TEA detection system produced quantitative chromatograms without interfering peaks on both crude aqueous distillates and in final methylene chloride extracts following extensive clean-up (28). This improved selectivity and sensitivity is essential in methods that can be used for routine screening purposes of foods such as

cheese which may contain only trace amounts of both volatile and non-volatile N-nitrosamines.

### N-NITROSAMINES IN CHEESE

Although considerable data are available on the volatile N-nitrosamine contents of cured meat products, similar data on cheese are not only few but are, at times, rather contradictory. There have been several reports before 1970 of the presence of N-nitrosamines in cheese (33,46,54,59,74,90,95). However, the validity of some of these results must be questioned because the sensitivity of the methods used was inadequate for detecting low levels ( $\mu\text{g}/\text{kg}$ ) of N-nitrosamines and no gas chromatography-mass spectrometry tests were made when seemingly positive results were obtained.

One of the most comprehensive of the early studies was conducted by Crosby et al. (13) who analyzed various types of cheese, particularly those to which nitrate had been added. Six of 11 samples contained traces (1 to 4  $\mu\text{g}/\text{kg}$ ) of dimethylnitrosamine (DMN). As these values were judged to be beyond the limit of reliability of the method (10  $\mu\text{g}/\text{kg}$ ), no definite conclusions were drawn from them. A follow-up series of experiments was conducted jointly between the Laboratory of the Government Chemist and the Netherlands Institute for Dairy Research (41). Gouda cheeses made with different amounts of nitrate were examined for the presence of six N-nitrosamines, with a detection limit of 1  $\mu\text{g}/\text{kg}$  in each instance (Table 2). Although DMN and diethylnitrosamine (DEN) may have been present in cheeses A and B just below 1  $\mu\text{g}/\text{kg}$ , they could not be confirmed by high resolution mass spectrometry. The other cheeses did not contain any of the six N-nitrosamines. Havery et al. (44) also failed to detect any of 14 N-nitrosamines in 17 samples of cheese, 10 of which were of the imported variety and which had been processed with nitrate as an additive.

On the other hand, the occasional occurrence of DMN in several cheeses was reported by Gough et al. (42) who examined 21 different varieties commonly available in the United Kingdom. Cheeses to which nitrate was added during manufacture were included in this study. However, there was no greater occurrence of DMN in these samples than in cheese made without added nitrate. Levels of DMN were similar for all the cheeses (1 to

5  $\mu\text{g}/\text{kg}$ ) except for one sample of Stilton (Table 3). When fresh, this sample contained a relatively high level of DMN (13  $\mu\text{g}/\text{kg}$ ) but within a week this had decreased to 3  $\mu\text{g}/\text{kg}$ . It is also noteworthy that when 16 other milk products having a high amine content were examined, none contained any detectable levels of N-nitrosamine.

Table 3. Occurrence of volatile N-nitrosamines in dairy products (42).

Commodity	Examined	Number of samples:	
		Containing DMN	
		1-5 ( $\mu\text{g}/\text{kg}$ )	5-15 ( $\mu\text{g}/\text{kg}$ )
Cheese	58	9	1
Yogurt	9	0	0
Dessert dishes	7	0	0

Sen et al. (87) analyzed 31 samples of cheese imported into Canada, many of which were known to have been prepared with the addition of nitrate, and an equivalent number of Canadian cheese. As can be seen from Tables 4 and 5, some cheeses from both groups contained traces of DMN and DEN. Although the incidence of occurrence of these N-nitrosamines in the imported cheese is higher than that in the Canadian cheeses, the authors were not sure whether this difference was true or apparent. They also observed that N-nitrosamines were present in both nitrate-treated and untreated samples, and concluded that there was no relationship between the occurrence of N-nitrosamines and addition of nitrate.

As a result of the findings of Sen et al. (87), the Dutch Department of Public Health carried out an investigation of the common types of cheese made in the Netherlands (19). Eighteen commercially produced cheeses of different types and in different stages of ripening were analyzed by the TEA method for the presence of several volatile N-nitrosamines. No evidence was found of the presence of the N-nitrosamines in amounts larger than 0.1 to 0.5  $\mu\text{g}/\text{kg}$ . Again, no correlations between the N-nitrosamine content of the cheeses and their nitrate or nitrite content could be established. Elgersma (19) also reported the results of a collaborative study on cheese produced in the Netherlands, in which excellent analytical agreement was obtained between three laboratories in the Netherlands, United Kingdom and Canada. Only insignificant levels of N-nitrosamines were found in these cheeses sampled at three stages in the production and distribution chain.

TABLE 2. Nitrate and nitrite contents of cheese from the same vats as those analyzed for the presence of N-nitrosamines. Figures in parentheses: contents estimated from other experiments (41).

Code	A	B	C	D	E	F	G	H	I <sup>a</sup>
g NaNO <sub>3</sub> added to 100 l cheesemilk	7.5	10	15	15	10	20	40	60	0
Nitrate content (mg/kg) of the cheese at 24 h.	25	36	(56)	(56)	29	67	137	212	< 1
Date of analysis for nitrosamines (weeks after production)	13	13	14	25	14	14	14	14	16
Nitrite content (mg/kg) of the cheese on date of analysis for nitrosamines.	(0.4)	(0.4)	(0.5)	0.5 <sup>b</sup>	0.4	0.5	0.8	1.5	< 0.2
Nitrate content (mg/kg) of the cheese on date of analysis for nitrosamines.	12	16	(22)	12 <sup>b</sup>	13	27	66	96	1

<sup>a</sup>Nisin - producing starter used.

<sup>b</sup>Nitrate and nitrite contents after 20 weeks.

These reported low levels of N-nitrosamines emphasize the necessity for a sensitive and specific means of analysis. Non-specific methods such as thin-layer chromatography and gas chromatography may give misleading results. For example, Cerutti et al. (11) analyzed 16 cheese samples and found DMN, DEN, dipropylnitrosamine and dibutylnitrosamine in a single sample at the 20 to 30- $\mu\text{g}/\text{kg}$  level. Two other compounds that give typical N-nitrosamine reactions were found in trace amounts in three samples, in two samples at the 60- $\mu\text{g}/\text{kg}$  level and in other samples at the 20 to 30- $\mu\text{g}/\text{kg}$  level. On the basis of these results, these investigators concluded that nitrate should not be added to cheese. Cantafora et al. (9) determined the concentration of N-nitrosamines in various commercial samples of cheese, butter, bread, and pasta using a colorimetric method. However, the concentrations were generally below the sensitivity of the method (60  $\mu\text{g}/\text{kg}$ ). Gas chromatographic analysis of a high colorimetric value cheese extract demonstrated an effective DMN concentration of 70  $\mu\text{g}/\text{kg}$ . Laskowski et al. (57) using similar thin-layer chromatographic procedures failed to detect any N-nitrosamines in approximately 700 samples of commercially ripened cheese. In another Polish study (77), 141 random samples of cheese were analyzed, again

by thin-layer chromatography and 31 samples gave a positive N-nitrosamine color reaction. This disparity in reported levels of N-nitrosamines is proof that thin-layer chromatography and gas chromatography (without mass spectrometry) are not suitable methods for determining N-nitrosamines at the levels in which they are present in cheese.

#### FORMATION OF N-NITROSAMINES IN CHEESE

The three main factors which may possibly influence formation of N-nitrosamines in cheese are the nitrate/nitrite and amine contents, and pH of the cheese.

##### *Nitrate/nitrite in cheese*

Although the nitrate contents of different varieties of cheeses are well documented in the literature (6,7,58,66,77,78), there have been few reports relating to the course of nitrate degradation and formation of nitrite during ripening of these cheeses. Goodhead et al. (41) studied the fate of nitrate in Gouda cheese prepared from milk containing 15 g of nitrate/100 liters of milk and reported that the nitrate content of the cheese decreased from an initial amount of 56 mg/kg immediately after preparation to about 30 mg/kg after 6 weeks. After this period, only a slight further decrease was observed (Fig. 2). The

TABLE 4. *N-Nitrosamines in cheeses imported by Canada*<sup>a</sup>.

Type	Number of samples analyzed	Number of positive samples	DMN <sup>b</sup> ( $\mu\text{g}/\text{kg}$ )	DEN <sup>b</sup>
Edam	3	0		
Spiced Gouda	1	1	2	2
Gouda	7	5	5-19	3-20
Camembert	3	2	0-2	0-4
Brie	1	0		
Havarti	1	1	11	
Havarti with caraway	2	2	0-3	2
Processed cream cheese	4	3	2-6	
Soft ripened cheese	3	2	2	
Provolone	1	1	9	
Tilsit	1	1	6	
Blue	1	1	Trace	
Feta	3	2	0-7	0-4

<sup>a</sup>Adapted from Sen et al. (87).

<sup>b</sup>Range of N-nitrosamine found in positive samples.

TABLE 5. *N-Nitrosamines in Canadian cheese*<sup>a</sup> ( $\mu\text{g}/\text{kg}$ ).

Type	Number of samples analyzed	Number of positive samples	DMN	DEN
Gouda	2	0		
Colby	2	0		
Parmesan	1	0		
Cottage	2	0		
Cheese Whiz	1	0		
Miscellaneous	6	0		
Mozzarella	1	0		
Cheddar (processed)	2	0		
Cheddar (milk)	4	1	20**	
Cheddar (medium)	2	1	9*	
Cheddar (old)	2	0		
Cream cheese	2	2 (a) (b)	17 68**	14*
Wine cheese				7*

<sup>a</sup>Adapted from Sen et al. (87).

\*Confirmed by GLC-MS,  $\text{NO}^+$  monitoring at 5,000 resolution.

\*\*Confirmed by GLC-MS,  $\text{NO}^+$  and  $\text{M}^+$  monitoring, the latter at a resolution of 10,000 in some cases.

nitrite content of these cheeses was very low, a maximum of about 1 mg/kg occurring after 2 to 3 weeks (Fig. 3). It has also been shown that nitrite, which is formed from nitrate by the action of the enzyme xanthine oxidase, is unstable due to inherent reducing properties of the cheese (35). Even cheese prepared from milk containing 60 g of sodium nitrate/100 liters of milk contained only 1.5 mg of nitrite/kg when analysed 14 weeks after manufacture [Table 2 (41)].

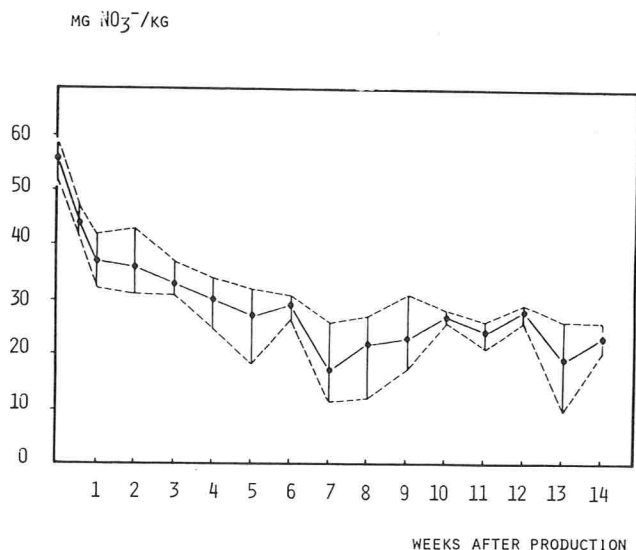


Figure 2. Effect of storage (13 C) on the nitrate content of cheese when 15 g NaNO<sub>3</sub>/100 liters milk has been added (41).

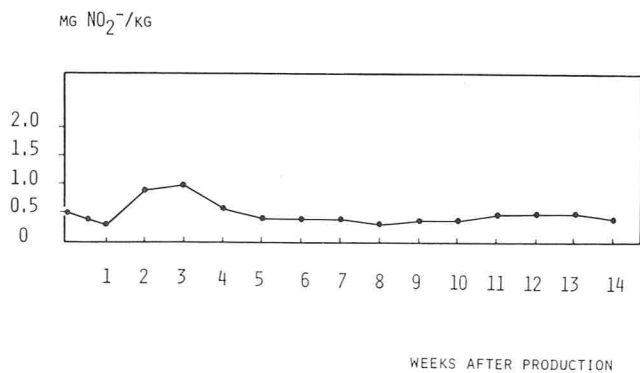


Figure 3. Effect of storage (13 C) on the nitrite content of cheese when 15 g NaNO<sub>3</sub>/100 liters milk has been added (41).

The fate of nitrite in cheese is not clearly understood, but may parallel the disappearance of nitrite in cured meats. With the exception of the cured meat pigment, nitric oxide myoglobin and the residual nitrite, knowledge of the fate of the unknown portion of the depleted nitrite until recently was minimal. Using a model system composed of myoglobin, nitrite and ascorbate, Japanese researchers (34) recovered all of the nitrogen in nitrite as residual nitrite, nitrate, denatured nitric oxide myoglobin and gaseous nitrogen compounds (nitric oxide, dinitric oxide and nitrogen gas). It has also been demonstrated (109) that myosin has the ability to bind appreciable amounts of nitrite resulting in formation of 3-nitrotyrosine, 3,4-dihydroxyphenylalanine

and several other compounds possibly including N-nitrosotryptophan. Such results indicate that C-nitrosation reactions involving protein must be considered as one of the major pathways for the loss of nitrite in cured meat. Cassens et al. (10) have generalized this loss as follows where the values are a percentage of the nitrite originally added: myoglobin, 10-20%; nitrate, 1-5%; nitrite, 5-20%; gas, 1-5%; sulfhydryl groups, 5-15%; lipid, 1-5%; and protein, 20-30%.

The disappearance of nitrite in cheese may well follow some of the above pathways. It may react with casein, for it has been demonstrated (5) that when casein was incubated with nitrite in a medium of lactic acid and lactate at room temperature, a large proportion of the nitrite reacted with the free amino groups of casein. It is also apparent from the studies of Fournaud et al. (32) that nitrite can be converted into nitric oxide, nitrous oxide or nitrogen by the reducing action of certain strains of lactobacilli.

#### Amine compounds in cheese

N-Nitrosamines may be formed in the environment, particularly food and water, whenever secondary amines, and to a lesser extent primary and tertiary amines are exposed to nitrite. Although the sources of nitrite in foods are well documented in the literature (3,100,107), information on occurrence of the individual amines in foods is sparse.

The monoamines (histamine, tryptamine, tyramine, ethanolamine) and polyamines (spermine, spermidine, putrescine, cadaverine) have been identified in fresh pork bellies (72) at concentrations per 100 g of tissue ranging from 0.03 mg for cadaverine to 8.1 mg for spermine. Similar amines were identified in fresh hams with concentrations ranging from 0.5 mg for tyramine to 189 mg for putrescine per 100 g of fresh tissue (56). The volatile amines (methylamine, dimethylamine, trimethylamine, ethylamine, n-propylamine, and isopropylamine) were detected in pork carcass meat used for Wiltshire bacon manufacture (72). The highest concentration detected was 1900 µg of methylamine per kg of fresh meat, which decreased during the curing process. Various amines (histamine, putrescine, tyramine, phenethylamine) have been detected in dry and semi-dry sausages (79).

Singer and Lijinsky (91) developed analytical procedures for determination of N-nitrosatable amines in a variety of foodstuffs and related materials including evaporated and whole milk. They concluded that morpholine and dimethylamine are ubiquitous; piperidine and pyrrolidine are found in plant-derived material while other amines are found in smaller quantities. Kawamura et al. (53) also conducted a survey of secondary amines in commercial foods including dairy products (Fig. 4). Modified powdered milk showed about five times as much dimethylamine as milk, while the contents in butter and processed cheese were trace.

Relatively little is known about the concentrations of the simple alkylamines in cheese. Ruiter (82) found

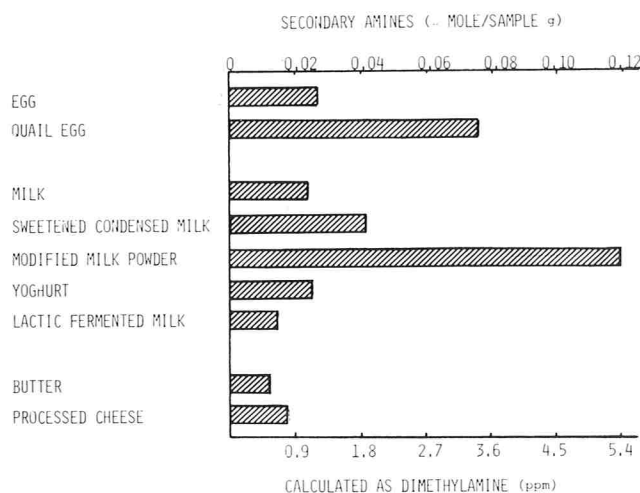


Figure 4. Secondary amines in eggs and milk products (53).

0.6 mg of dimethylamine/kg, 2.0 mg of trimethylamine/kg and no trimethylamine oxide in mature Gouda cheese. Galovnya et al. (40) detected a wide range of simple aliphatic primary, secondary and tertiary amines at the mg/kg level in mature Russian-made Gouda cheese. They also reported the presence of pyridine and piperidine. A recent *in vitro* study (106) in which several foods (pork, egg, bread, milk and cheese) were incubated under simulated gastric conditions with concentrations of nitrite similar to those occurring in foods, showed that significant quantities of N-nitrosatable amines are present in cheese. Of the foods studied, only cheese yielded detectable amounts of volatile N-nitrosamines, rather more at pH 3 than at pH 1 when concentrations up to 7  $\mu\text{mole/kg}$  were recorded.

Most of the N-nitrosamine studies in the past have centered on isolation and identification of volatile N-nitrosamines in foods. Volatility of the simple N-nitrosamines permits them to be distilled from the bulk of non-volatile interfering substances (50). The presence of less volatile and nonvolatile N-nitrosamines in foodstuffs must be investigated and this is particularly true of cheese which contains considerable quantities of biologically active amines. The recent development of

new analytical procedures (18,29,30,51,88) should provide much information about these N-nitroso compounds.

Biologically active amines are normal constituents of many foods and have been found in cheese, sauerkraut, wine and putrid, aged or fermented meats (60). These low molecular weight organic bases do not represent any hazard to individuals unless large quantities are ingested or natural mechanisms for their catabolism are inhibited or genetically deficient (79). Voight et al. (96) determined the tyramine, histamine and tryptamine contents of 156 samples of cheese purchased at retail stores in the United States. Some of their data are summarized in Table 6.

There have been few reports in the literature of N-nitrosation of such amines. However, Philpot and Small (75) observed a rapid reaction between tryptophan and nitrous acid but were unable to detect a N-nitroso derivative. Denyanov and Putokhin (16) reported formation of a brick-red powder which they regarded as N-nitroso  $\beta$ -indolylacrylic acid. Walters et al. (101) also reacted tryptophan with nitrous acid and examined the products by thin layer chromatography, but no resolution of the reaction products was achieved under conditions of the experiment. Bonnett and Holleyhead (8) showed that under mild conditions of temperatures and pH, sodium nitrite can react with simple tryptophan derivatives including an N-acetyl dipeptide to give nitroso derivatives in which the nitroso group is substituted at the N-1 of the indole system. These authors concluded that such substitution at the tryptophan units might occur when proteins are treated with nitrite, and would be expected to be accompanied by reaction at other sites such as histidine. Since tryptamine and histamine possess high  $pK_b$  values (93), rapid N-nitrosation of these amines would also be expected.

#### pH of cheese

The effect of pH on the N-nitrosation of dimethylamine has been widely studied (67,69,83), maximum N-nitrosation occurring at approximately pH 3.4. It has

TABLE 6. The average tyramine, histamine and tryptamine contents (mg/g) of various cheeses<sup>a</sup>.

Cheese	Number of samples examined	Tyramine	Histamine	Tryptamine
Cheddar				
Extra-sharp	11	0.27 (10)	0.21 (5)	0.02 (2) <sup>b</sup>
Sharp	34	0.21 (33)	0.11 (9)	0.04 (12)
Medium	18	0.24 (17)	0.14 (6)	0.02 (6)
Mild	12	0.09 (11)	0.19 (4)	0.03 (3)
Processed	7	0.11 (6)	ND <sup>c</sup>	ND
Smoked	3	0.12 (3)	ND	ND
Colby	8	0.21 (8)	0.07 (3)	0.13 (2)
Edam	2	0.13 (2)	ND	0.08 (1)
Gouda	6	0.29 (6)	0.075 (1)	0.07 (3)
Swiss	6	0.41 (5)	ND	0.19 (3)
Roquefort or Blue	7	0.36 (7)	0.50 (3)	0.20 (4)
Camembert	7	0.12 (7)	0.07 (1)	0.02 (2)

<sup>a</sup>Adapted from Voight et al. (96).

<sup>b</sup>Number of positive samples.

<sup>c</sup>ND = Not detectable at levels below 50  $\mu\text{g/g}$ .

also been observed that formation of DMN decreased sharply on the more basic side and was almost minimal about pH 5.0. Since the pH of Gouda cheese normally falls within the range 5.2 to 5.4, it appears that conditions are not optimal for the N-nitrosation reactions to occur to any great extent (39,41).

### CONCLUSIONS

Use of nitrate in the manufacture of certain types of European cheese is considered a technological necessity since it will prevent fermentative reactions in cheese by the butyric acid bacterium *Clostridium tyrobutyricum*, the spores of which survive normal milk pasteurization (39). As a consequence, Dutch cheesemakers are permitted to add 15 g of nitrate per 100 liters of milk, while a recent addition to the Canadian Food and Drug Regulations (49) permits use of 20 g of nitrate per 100 liters of milk in the manufacture of certain cheeses. The practice of adding nitrate to cheese milk is sometimes criticized on the basis that it may cause a health hazard to the consumer of the cheese. However, this is not a new situation since concern has been repeatedly expressed about the extent and effects of nitrate and nitrite in our diet. White (107), in a major investigation of US dietary habits, estimated that the average daily consumption of nitrate is 99.8 mg, four-fifths of which originates from vegetables, with less than one-sixth from cured meats (Table 7). Other sources including dairy products are not significant. The average daily nitrite intake is 11.22 mg; two-thirds of the nitrite entering the stomach originates in the saliva and slightly less than one-third comes from cured meats. Other sources of nitrite are not significant. Hence, it can be concluded that the contribution of cheese made with nitrate to the daily intake of nitrate and nitrite is small.

TABLE 7. Estimated average daily ingestion for U.S. resident (107).

Source	Nitrate		Nitrite	
	mg	%	mg	%
Vegetables	86.1	86.3	0.20	1.8
Fruits, juices	1.4	1.4	0.00	0.0
Milk and products	0.2	0.2	0.00	0.0
Bread	2.0	2.0	0.02	0.2
Water	0.7	0.7	0.00	0.0
Cured meats	9.4	9.4	2.38	21.2
Saliva	30.0 <sup>a</sup>		8.62	76.8
Total	99.8	100.0	11.22	100.0

<sup>a</sup>Not included in total.

Most of the data to date indicate that the levels of N-nitrosamines in cheese are below 10  $\mu\text{g}/\text{kg}$  and are probably more generally in the range of 1 to 5  $\mu\text{g}/\text{kg}$ . Only in a few studies have the levels been between 10 and 20  $\mu\text{g}/\text{kg}$  (9,11) and here the methods of analysis were not specific nor were they sensitive enough for the purpose. As indicated by Issenberg (50), clearly not all laboratories with an interest in determining N-nitrosamines in food possess the required equipment. Moreover, the technical expertise required for such analyses is available in only a very few laboratories. It has been reported that there does not appear to be any relationship between the

N-nitrosamine content and either the initial nitrate or the residual nitrate in the cheese (39,41,42). N-Nitrosamines have also been found in cheeses to which no nitrate was added (87). The present literature also indicates that the amounts of N-nitrosamines contributed to the diet by cheese are very small when compared to those contributed by cooked bacon.

However, there are several areas which require further definition. There is still only meagre information available on distribution of N-nitrosatable amines in cheese. It was pointed out by Walker (99) that while there appears to be a general lowering of the levels of N-nitrosamines in meat products, in vivo N-nitrosation might be the major source of N-nitrosamines. Therefore, it might be important to monitor the amine content of our foods particularly the non-volatile amines. It would also be beneficial to study the amine development in cheese throughout the ripening process. It is not known if these amines are present in the very young cheese, when the most nitrite is available (41).

Another area of potential concern is that when nitrate is used for cheesemaking, the greater part of it will be lost in the whey. This is, of course, a consequence of the use of this additive. Since whey is heat-processed such as in spray-drying, the chances of N-nitrosamine formation are greatly increased providing the precursors nitrite and amine are available for reaction. This is an area which requires further investigation.

Cheesemakers who use nitrate have to be aware that their whey has a smaller number of uses than whey without nitrate. For this reason, whey products should have their own nitrate and nitrite specifications based on acceptable daily intake.

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## Professionalism: What It Should Mean to You<sup>1</sup>

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### ABSTRACT

The sanitarian is a professional concerned with the public's health and all matters affecting it. Education in the arts and sciences, specialized training and field experience qualify the sanitarian to evaluate, plan and promote standards and changes in areas dealing with environmental health. Today, the world problems of population increases, energy availability and food supply and distribution are forcing society to re-examine its life style, which may result in changes in living habits. The sanitarian will play an important role in determining the method involved in change and the ultimate goals to be obtained. To maintain credibility and professional standing within the community and with his peers, a sanitarian must keep current by reading the literature, remaining vigilant to potential hazards, specialize, work at solving problems and not contribute to them, communicate and educate rather than police, and practice what is preached. Above all, the sanitarian should take pride in being a sanitarian.

The term professional, or professionalism can have various meanings. Synonyms are - trained, skilled, expert; antonyms are - lay, unprofessional, unskilled, amateur. Definitions may be general—"one who practices a profession as a business in contrast to amateurism." One engaged in, appropriate to or in conformity to, a profession, as professional courtesy, professional soldier, or professional job or engaged in special attainments or disciplines, or of a collective body of those following such vocation."

The Committee on Professional Education of the American Public Health Association (1) defined it as follows: "A public health sanitarian is a person whose

education and experience in the biological and sanitation sciences qualifies him to engage in the promotion and protection of public health. He applies technical knowledge to solve problems of a sanitary nature and develops methods and carries out procedures for the control of those factors of man's environment which affect his health, safety and well-being."

The work conference on Undergraduate Education in Sanitary Science (4) stated: "In the training of a sanitarian, the first objective is to produce an educated individual, a person who has developed competence in the formulation of intelligent judgements, and secondly, to provide a person qualified to enter the field of environmental health."

Another is: "One who touches on physical, biological, engineering and social problems required to interpret and enforce public health laws, ordinances and regulations; is in close contact with the farmer, food product manufacturer, food retailer, foodservices, water and sewage waste, insects, rodents, hotels, motels, trailer parks, resorts, recreation camps, swimming pools, home safety, epidemiologic investigations, civil defense planning, and evaluation of sanitation laws and regulations, industrial sanitation, urban fringe sanitation, radiological and other... ." (4).

The Proposed Model Act (5) for Registration of Sanitarians qualifies those who register as "Sanitarians whose duties in public health and environmental sanitation require a knowledge of physical, biological, and sanitary/sciences and/or environmental health, and community hygiene and whose professional pursuits and duties are necessary to the promotion of life, health and well-being of the community." The "Sanitarian," in the above, (interpreted to mean also Environmentalist, Environmental Sanitarian, etc.) is a public health professional uniquely fitted by education in the arts and sciences, specialized training and credible field experience to effectively plan, organize, manage, execute and evaluate one or more of the many diverse elements comprising the field of environmental health.

<sup>1</sup>EDITOR'S NOTE: The late Dr. K. G. Weckel presented this paper at the annual meeting of the Wisconsin Environmental Health Association at Appleton, Wisconsin on April 29, 1976. Several of Professor Weckel's colleagues felt the paper should be published because it gives views that continue to be relevant. Although the paper deals with professionalism as related to sanitarians, many of Dr. Weckel's comments are applicable to professional persons in Food Science, Food Microbiology, etc. At the time of Dr. Weckel's death, the paper lacked several essential parts: these were kindly prepared by Dr. D. A. Stuijber, Department of Food Science, University of Wisconsin-Madison. This paper is a contribution from the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

The License-Certification to act as a Registered Sanitarian by the State of Wisconsin Department of Industry, Labor and Human Relations says: "A sanitarian is a person trained in the field of sanitary science and technology who is qualified to carry out educational and inspectional duties or to enforce the law in the field of environmental health and sanitation."

Society has provided in various ways through licensing for ascertaining degrees of capability, and for compensation. In the mechanical and service trades, such as the plumber, welder, electrician, both vocational and supervised practical training for periods up to 4 - 5 years, with examination qualify for capability as apprentice, journeyman and master. The barber, for example, is required to prepare for apprentice status in a designated barber school, and subsequently training in a barber shop under supervision of a master barber for a period, in addition, to a day weekly of training in a school. Licensing is stipulated for many occupations and duties, the cheesemaker, the milk tester, the chauffeur, well driller and so forth. The Wisconsin Department of Industry, Labor and Human Relations, through its licensing and regulatory boards once oversaw 299 professions, trades and occupations, businesses and activities. Licensing involved 34 state agencies and examining boards covering 118 separate job categories. Some 377,500 persons in Wisconsin hold occupational licenses (2).

### RECOGNIZING CHANGE

If there is one guide to being a professional, it is to understand the everlasting continuing forces of change, to recognize the significance of changes that have occurred in the past, and to relate them to the future.

At the turn of the century, the high incidence of infant mortality, especially with immigrants in newly crowded but small cities, led to medical surveillance for control of causes. This led to the recommendation for boiling of milk to control transmission of human and animal diseases. Administration of milk supplies for a long time was within the surveillance of medical men; subsequently, in public health administration where supervisors were veterinarians who better understood the problems and relation of animal diseases to human health, and the acceptability of raw milk; and subsequently, the increasing role of the technologist trained in biology and engineering.

Physicians were faced with the problems of high incidence of communicable disease; vaccination had not been adopted, infant mortality was high, and there was translocation of people in great numbers across the land. The influence of Koch, Pasteur and Lister was great upon the young Americans who went to schools in Europe. The problem of animal disease was foremost. The understanding of transmissibility of animal disease to and among humans led to structuring of a system of protective procedures for raw milk and other foods.

It took the Dean of Wisconsin's College of Agriculture, Dr. Harry Russell, a bacteriologist who had studied in Europe, with the courage to prove, through a demonstration slaughter of the entire university dairy herd before a congregation of farmers invited to the event, that the tuberculin test was reliable in identifying tuberculosis-infected animals, and therefore should be compulsory by law to eradicate the bovine disease from the state. It became so and was implemented by state indemnity to farmers for loss of animals. Subsequently, Dr. F. B. Hadley, in Veterinary Science, developed the agglutination test for bovine brucellosis; this became a part of the total procedure for animal tuberculosis and brucellosis eradication programs and the concept of accredited disease-free areas.

Within the time frame of several decades, a sequence of events may be noted that continue to relate to our sanitation practices.

Occurrence of both human and animal disease epidemics focused need for improved food and water services. Severe poultry disease outbreaks in eastern markets and embargo by European countries against certain unacceptable meats exported from the United States implemented action toward enactment of national food laws, development in communities of health departments, milk and food ordinances differing extensively in form and detail, the American Medical Milk Commission certified milk programs, tuberculosis and brucellosis testing and eradication programs, and area accredited programs, and improving the nutritional value of milk by ultraviolet irradiation so milk became effective in therapeutic control of rickets.

Decades spaced developments in systems of pasteurization and types of containers; years elapsed before acceptance of half and half, 1% and 2% milks, ice milks, milk solids-fortified milk products; 25 years have elapsed and reciprocity has not been fully accepted for interstate shipments of milk and milk products.

Were these technical developments because of, or in spite of, the sanitarians? It is not clear that the technical developments ever were within the province of the sanitarian. It is clear that his capabilities did lie in implementing their adoption, within the job assignment.

The professional sanitarian must look forward to change. "The universe is change; our life is what our thoughts make it." (Marcus Aurelius Antoninus, AD 121-180). The sanitarian's objective — to sustain life, health, food.

### PROBLEMS MUST BE FACED

There are ahead of us at least five interrelated critical way-of-life problems that need the guidance of the professional sanitarian. These are population, energy, basic resources, food and communication.

#### *Population*

The population trend of the nations of the world has been given tremendous attention in the past 50 years,

and certainly since the time of Malthus. Concisely, the world population is reported now to be 4,000 million, by 1990 it is expected to be 5,500 million, and by 2000, 6,500 million. An increase of over 100 million each year. The increase of 1 billion since 1950 is equal to the world's population 150 years ago. Half of the world's present population has been born since the end of World War II.

The population of the United States, now over 220 million, may be 300 million by 2000; the birth rate has been declining since 1900 as did death rates between 1900-1950. World birth rates are declining from 34/1000 in 1965 to 30 in 1974 and projected to 20 in 1985; in the U.S., from 19 in 1965 to 15 in 1974. Even so, the population of the world increased 658 million in the last 10 years.

The population growth is essentially urban; concentrations of people do not simplify health protection problems, whatever they may be. The burden of the sanitarian, in every way, can be expected to increase. Water supply and use, water safety, waste disposal and recycling, food supply, food protection and food distribution will continue to need attention.

#### *Energy*

The energy requirements for the future at best are estimates. Seemingly reliable statements predict this nation will be out of gas by 1990, oil by 2000, and coal by 2050. Estimates, based on calculation, are that we now invest in agriculture 10 calories of fuel energy for each nutrient calorie that is produced. On the basis of calculations for "demandite", extraction of metals from less extractable ores; substitutions, alternates and recovery systems tied into population increases; and requirements for man-made energy will be 12 times that produced presently (3). This is 0.1 the solar energy absorbed and reradiated by earth.

We shall be compelled to use energy more efficiently; in obtaining and purifying water, including that for irrigation; in eliminating the extra degrees of safety in regulatory thermal processes; in available water tolerances employed in dehydrated foods; in specifications of metals or other materials in food contact surfaces, process or container; even in the selection of crops that are less energy-intensive in their production and conversion to food. Probably aesthetic purity will be abandoned for necessary safety. We surely will abandon the process of measuring many nothings to measure something.

#### *Food*

It is difficult to appraise the meaning of food in a way of life unless actually faced with hunger, starvation, illness or death. In times past, 1000 - 1500 A.D., extensive disease outbreaks and pestilence decimated populations on the order of each 10 years. Man slowly learned by trial and error and through sickness and death to avoid certain foods and the ways of handling foods. The emperor's tasters surely were the world's first sanitarians. Only in a land of plenty could the change in

the way of life as we know it occur in one generation. The family meal of bread, meat, potatoes, varied with seasonally available foods, served at regular hours, is disappearing. School lunch, adult food centers, industrial foodservice, hot-cold food dispensers, and quick foodservice centers are abundant. It is confidently expected that by 1980 50 cents of each food dollar will be spent in quick foodservice centers.

What will be the sanitarian's role in the forthcoming area of changes? He must understand changes that will be needed for increased food production and in food processing and food distribution. He must also face the problems of increased population management and greater energy cost. Can the sanitarian separate minutiae from basics?

#### *Communications*

Perhaps one of the greatest problems of our times is that of communicating—communicating adequately to establish understanding of what sanitation is all about. Although we have and now use systems of transfer of information equal to the speed of light ("about as far as we can go"), we apparently have failed through our systems of education to transfer understanding. The public, the taxpayer, the employee, the boss have much difficulty knowing, let alone understanding, what the objective of the sanitarian is, much less how it can be successfully attained.

### ON BEING A SANITARIAN

There are nine things a sanitarian can do to be successful as a sanitarian. In 1959, John Porterfield, Deputy Surgeon General said "In our fast growing world, a professional person must recognize his capabilities, and his limits. He must be flexible, cooperative and good at teamwork" (6).

#### *Read the literature*

Do read and study the literature, especially that immediately concerned with the various facets of your work. Read, evaluate, relate it to use. Make it a mandatory work project. The science in your profession, as in others, is fast expanding. The sharp and precise employment and use of the mind in this is no less exhausting, properly done, than physical work. Test your understanding through association with other information you have; repeat your understanding by writing, rewriting, or relating as a means of learning.

#### *Keep alert*

Participate in literature review or update seminars, even though you must create this activity to better understand the changes within your sphere of work. Participate in meetings of related professional groups to develop perspective in your work, of your own progress and that of others. Continuing education is mandatory for the true professional.

*Be a specialist*

To know everything is impossible. To know much about something, or things, is possible. Develop a select fund of knowledge on some phase of work in your profession. It will fortify your ego, develop your capability, and increase your opportunities when the occasion arrives. Make yourself an expert per subject per year.

*Participate with others*

The sanitarian works with individuals and groups. Much of the work is, and should be, educational. There are many tools to use to be effective in transfer of information. Booklets, sheets, drawings, illustrated slides, movies, demonstrations and well-organized presentations. Skill must be developed and efforts well managed. Study the methods of good teachers.

*Educate rather than police*

Teaching is successful with the majority; policing is necessary for the few. Examples of results of interdisciplinary understanding are the 3A Dairy Equipment Sanitary Standards, the Bakery Industry Equipment Sanitary Standards, and Food and Beverage Dispensing Equipment Standards. Committees of nations now cooperate on international standards for foods. These are examples of understanding between industry and government in contemporary problems.

*Evaluate your decisions*

Assess your decisions carefully, no matter whether minor or major, in terms of their impact on the larger scale, the larger community, the mass food system. Conceive, for example, the impact of an error in decision that could affect adversely even one of a chain of 4000 quick food service systems.

*Practice your image*

A sanitarian should be a *professional* Sanitarian. He should have a clean mind, personal habits, and practices. A sanitarian is under constant scrutiny and appraisal of whether he does as he says and whether he is truly professionally sincere.

*Take the bushel off the light*

Sanitarians generally are a shy, conservative lot. News articles in papers of their contributions, activities, efforts and results seldom make the front page. Reports are couched in negatives rather than positives of achievements.

Sanitarians should develop reading skills, speaking abilities, and participate in groups to make known the results of their work. Teaching is a skill by which you soon learn what you thought you knew, and what you ought to know.

*Be proud to be a sanitarian*

Believe in yourself, your work and your contributions. Wear your pin, display your certificate, explain in clear terms the positive things you do.

## REFERENCES

1. Anon. 1956. Proposed report on educational and other qualifications of public health sanitarians. *Am. J. Public Health* 46:1149-1156.
2. Anon. 1975. Wisconsin directory of licensed occupations. Wisconsin Department of Industry, Labor and Human Relations.
3. Gollier, H. E., and A. M. Weinberg. 1976. The age of substitutability, what do we do when the mercury runs out? *Science* 191: 683-889.
4. Hanson, G. W., and J. C. Olson, Jr. 1959. On the training of sanitarians. *J. Milk Food Technol.* 22:50-56.
5. Sanitarian's Joint Council. Proposed model act registration of sanitarians.
6. Thomasson, H. L. 1959. What do I get out of the International? *J. Milk Food Technol.* 22:363-364.

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## Holders of 3-A Symbol Council Authorizations on February 15, 1979

Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y.-Treas., P.O. Box 701, Ames, Iowa 50010.

### 01-06 Storage Tanks for Milk and Milk Products

28	Cherry-Burrell Corporation (unit AMCA Int'l) 575 E. Mill St. Little Falls, New York 13365	(10/ 3/56)
102	Chester-Jensen Company, Inc. 5th & Tilgham Streets Chester, Pennsylvania 19013	( 6/ 6/58)
2	CREPACO, Inc. 100 C.P. Avenue Lake Mills, Wisconsin 53551	( 5/ 1/56)
117	DCI, Inc. St. Cloud Industrial Park St. Cloud, Minnesota 56301	(10/28/59)
76	Damrow Company 196 Western Avenue Fond du Lac, Wisconsin 54935	(10/31/57)
115	DeLaval Company, Ltd. 113 Park Street South Peterborough, Ontario, Canada (not available in USA)	( 9/28/59)
109	Girton Manufacturing Company State Street Millville, Pennsylvania 17846	( 9/30/58)
114	C. E. Howard Corporation P.O. Box 2507 City of Industry, California 91746	( 9/21/59)
127	Paul Mueller Company P.O. Box 828 Springfield, Missouri 65801	( 6/29/60)
31	Walker Stainless Equipment Co. Elroy, Wisconsin 53929	(10/ 4/56)

### 02-08 Pumps for Milk and Milk Products

214R	Ben H. Anderson Manufacturers Morrisonville, Wisconsin 53571	( 5/20/70)
212R	Babson Bros. Co. 2100 S. York Rd. Oak Brook, Illinois 60621	( 2/20/70)
29R	Cherry-Burrell Corporation (unit AMCA Int'l) 2400 Sixth St., Southwest Cedar Rapids, Iowa 52406	(10/ 3/56)
63R	CREPACO, Inc. 100 CP Avenue Lake Mills, Wisconsin 53551	( 4/29/57)
205R	Dairy Equipment Company 1919 South Stoughton Road Madison, Wisconsin 53716	( 5/22/69)
65R	G & H Products, Inc. 5718 52nd Street Kenosha, Wisconsin 53140	( 5/22/57)

145R	ITT Jabsco, Incorporated 1485 Dale Way Costa Mesa, California 92626	(11/20/63)
314	Len E. Ivarson, Inc. 3100 W. Green Tree Road Milwaukee, Wisconsin 53223	(12/22/78)
26R	Ladish Co., Tri-Clover Division 9201 Wilmot Road Kenosha, Wisconsin 53140	( 9/29/56)
303	E. R. Mitchell Co., Inc. (Mfg. by Howard Pneumatic Eng. Co. Ltd.) 576 Haddon Ave. Collingswood, NJ 08108	( 3/ 8/78)
236	Megator Corporation (Mfg. by Howard Pneumatic Eng. Co. Ltd.) 125 Gamma Drive Pittsburgh, Pennsylvania 15238	( 5/ 2/72)
241	Puriti S. A. Alfredo Noble #39, Industrial Pte. de Vigas Tlalnepantla, Mexico (not available in USA)	( 9/12/72)
148	Robbins & Myers, Inc. Moyno Pump Division 1345 Lagonda Avenue Springfield, Ohio 45501	( 4/22/64)
306	Stamp Corp. 1309 Culmen St. Madison, WI 53713	( 5/ 2/78)
72R	L. C. Thomsen & Sons, Inc. 1303 43rd Street Kenosha, Wisconsin 53140	( 8/15/57)
219	Tri-Canada Cherry-Burrell Ltd. 6500 Northwest Drive Mississauga, Ontario, Canada (not available in USA)	( 2/15/71)
52R	Viking Pump Div. Houdaille Industries, Inc. 406 State Street Cedar Falls, Iowa 50613	(12/31/56)
5R	Waukesha Foundry Company 1300 Lincoln Ave. Waukesha, Wisconsin 53186	( 7/ 6/56)

### 04-03 Homogenizers and High Pressure Pumps of the Plunger Type

247	Bran and Lubbe, Inc. 1241 Rand Rd. Des, Plaines, IL 60016	( 4/14/73)
87	Cherry-Burrell Company (unit AMCA Int'l) 2400 Sixth Street, Southwest Cedar Rapids, Iowa 52404	(12/20/57)
37	CREPACO, Inc. 100 CP Avenue Lake Mills, Wisconsin 53538	(10/19/56)
75	Gaulin, Inc. 44 Garden Street Everett, Massachusetts 02149	( 9/26/57)

- |                                                                                                                                     |                                                                                                                    |            |                                                                                                                                          |            |
|-------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|------------|------------------------------------------------------------------------------------------------------------------------------------------|------------|
| 237                                                                                                                                 | Graco Inc.<br>P.O. Box 1441<br>Minneapolis, Minnesota 55440                                                        | ( 6/ 3/72) | 79R Alloy Products Corporation<br>1045 Perkins Avenue<br>Waukesha, Wisconsin 53186                                                       | (11/23/57) |
| 309                                                                                                                                 | General Dairy Equipment<br>434 Stinson Boulevard<br>Minneapolis, Minnesota 55413                                   | ( 7/19/78) | 138 A.P.V.-Crepaco, Canada Ltd.<br>1250 Ormont Drive<br>Weston, Ontario M9L-2V4                                                          | (12/17/62) |
| 256                                                                                                                                 | Hercules, Inc.<br>2285 University Avenue<br>St. Paul, Minnesota 55114                                              | ( 1/23/74) | 245 Babson Brothers Company<br>2100 South York Road<br>Oak Brook, Illinois 60521                                                         | ( 2/12/73) |
| 282                                                                                                                                 | Knudsen Corporation<br>(Mfg. by Gaulin Inc.)<br>715 N. Divisadero Street<br>Visalia, California 93277              | (11/ 8/76) | 284 Bristol Engineering Company<br>210 Beaver Street<br>Yorkville, Illinois 60560                                                        | (11/18/76) |
| <b>05-13 Stainless Steel Automotive Milk Transportation Tanks<br/>for Bulk Delivery and/or Farm Pick-up Service</b>                 |                                                                                                                    |            |                                                                                                                                          |            |
| 131R                                                                                                                                | Almont Welding Works, Inc.<br>4091 Van Dyke Road<br>Almont, Michigan 48003                                         | ( 9/ 3/60) | 82R Hanford, California 93230<br>Cherry-Burrell Company<br>(unit AMCA Int'l)<br>2400 Sixth Street, Southwest<br>Cedar Rapids, Iowa 52406 | (12/11/57) |
| 70R                                                                                                                                 | Brenner Tank, Inc.<br>450 Arlington,<br>Fond du Lac, Wisconsin 54935                                               | ( 8/ 5/57) | 260 CREPACO, Inc.<br>100 CP Avenue<br>Lake Mills, Wisconsin 53551                                                                        | ( 5/22/74) |
| 40                                                                                                                                  | Butler Manufacturing Co.<br>900 Sixth Ave., Southeast<br>Minneapolis, Minnesota 55114                              | (10/20/56) | 304 EGMO Ltd-Isreal<br>(Martin Silver P.E.)<br>406 Kinderamack Rd.<br>River Edge, NJ 07661                                               | ( 3/16/78) |
| 66                                                                                                                                  | Dairy Equipment Company<br>1919 South Stoughton Road<br>Madison, Wisconsin 53716                                   | ( 5/29/57) | 271 The Foxboro Company<br>Neponset Street<br>Foxboro, Massachusetts 02035                                                               | ( 3/ 8/76) |
| 45                                                                                                                                  | The Heil Company<br>3000 W. Montana Street<br>Milwaukee, Wisconsin 53235                                           | (10/26/56) | 67R G & H Products, Inc.<br>5718 52nd Street,<br>Kenosha, Wisconsin 53140                                                                | ( 6/10/57) |
| 297                                                                                                                                 | Indiana Tank Co., Inc.<br>P. O. Box 366<br>Simmitt, Indiana 46070                                                  | ( 8/29/77) | 203R ITT-Grinnell Company, Inc.<br>DIA-FLO Div<br>33 Centerville Rd.<br>Lancaster, Pennsylvania 17603                                    | (11/ 7/68) |
| 305                                                                                                                                 | Light Industrial Design Co.<br>3726 Halverstick Rd.<br>Sumas, WA 98295                                             | ( 3/23/78) | 34R Ladish Co., Tri-Clover Division<br>9201 Wilmot Road<br>Kenosha, Wisconsin 53140                                                      | (10/15/56) |
| 201                                                                                                                                 | Paul Krohnert Mfg., Ltd.<br>811 Steeles Avenue<br>Milton, Ontario, Canada L9T 2Y3<br>(not available in USA)        | ( 4/ 1/68) | 287 Koltek OY<br>Kotinummentieiz<br>SF-00700 Helsinki 70<br>Finland<br>(not available in USA)                                            | ( 1/14/77) |
| 85                                                                                                                                  | Polar Tank Trailer, Inc.<br>Holdingford, Minnesota 56340                                                           | (12/20/57) | 239 LUMACO<br>Box 688,<br>Teaneck, New Jersey 07666                                                                                      | ( 6/30/72) |
| 121                                                                                                                                 | Technova Inc. Gosselin Division<br>1450 Hebert c.p. 758<br>Drummondville, Quebec, Canada<br>(not available in USA) | (12/ 9/59) | 200R Paul Mueller Co.<br>P.O. Box 828<br>Springfield, Missouri 65801                                                                     | ( 3/ 5/68) |
| 189                                                                                                                                 | A. & L. Tougas, Ltee<br>1 Tougas St.<br>Iberville, Quebec, Canada<br>(not available in USA)                        | (10/ 3/66) | 295 Precision Stainless Products<br>(Mfg. by Toyo Stainless Co. Ltd.)<br>5636 Shull St.<br>Bell Gardens, CA 90201                        | ( 8/11/77) |
| 47                                                                                                                                  | Trailmobile, Div. of Pullman, Inc.<br>701 East 16th Avenue<br>North Kansas City, Missouri 64116                    | (11/ 2/56) | 242 Puriti, S. A.<br>Alfredo Nobel #39 Industrial Pte. de Vigas<br>Tlalnepantla, Mexico<br>(not available in USA)                        | ( 9/12/72) |
| 25                                                                                                                                  | Walker Stainless Equipment Co.<br>New Lisbon, Wisconsin 53950                                                      | ( 9/28/56) | 149R Q Controls<br>Occidental, California 95465                                                                                          | ( 5/18/64) |
| <b>08-17 Fittings Used on Milk and Milk Products Equipment<br/>and Used on Sanitary Lines Conducting Milk and<br/>Milk Products</b> |                                                                                                                    |            |                                                                                                                                          |            |
| 291                                                                                                                                 | Accurate Metering Systems, Inc.<br>1731 Carmen Drive<br>Elk Grove Village, IL 60007                                | ( 6/22/77) | 73R L. C. Thomsen & Sons, Inc.<br>1303 43rd Street<br>Kenosha, Wisconsin 53140                                                           | ( 8/31/57) |



300	Superior Stainless, Inc. 211 Sugar Creek Rd. P.O. Box 622 Delavan, Wisconsin 53115	(11/22/77)	38	CREPACO, Inc. 100 CP Avenue Lake Mills, Wisconsin 53551	(10/19/56)
191R	Tri-Canada Cherry-Burrell, Ltd. 6500 Northwest Drive Mississauga, Ontario, Canada L4V 1K4 (not available in USA)	(11/23/66)	120	DeLaval Company, Ltd. 113 Park Street South Peterborough, Ontario, Canada (not available in USA)	(12/ 3/59)
250	Universal Milking Machine Div. of Universal Cooperatives 407 First Ave, So. Albert Lea, Minnesota 56007	( 6/11/73)	279	The Schluter Co. (Mfg. by Samuel Parker Ltd.) 112 E. Centerway Janesville, WI 53545	( 8/29/76)
278	Valex Products 9421 Winnetka Chatsworth, California 91311	( 8/30/76)	17	The DeLaval Separator Company 350 Dutchess Turnpike Poughkeepsie, New York 12602	( 8/30/56)
86R	Waukesha Specialty Company, Inc. Darien, Wisconsin 53114	(12/20/57)	15	Kusel Dairy Equipment Company 820 West Street Watertown, Wisconsin 53094	( 8/15/56)
<b>Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers</b>			<b>12-04 Internal Return Tubular Heat Exchangers, for Milk and Milk Products</b>		
<b>09-07 Instrument Fittings and Connections Used on Milk and Milk Products Equipment</b>			248	Allegheny Bradford Corporation P.O. Box 264 Bradford, Pennsylvania 16701	( 4/16/73)
315	Burns Engineering, Inc. 10201 Bren Road, East Minnetonka, MN 55343	( 2/ 5/79)	243	Babson Brothers Company (Mfg. by Girton Mfg. Co.) 2100 S. York Road Oak Brook, Illinois 60521	(10/31/72)
206	The Foxboro Company Neponset Avenue Foxboro, Massachusetts 02035	( 8/11/69)	103	Chester-Jensen Company, Inc. 5th & Tilgham Street Chester, Pennsylvania 19013	( 6/ 6/58)
285	Tank Mate Company 1815 Eleanor St. Paul, Minnesota 55116	(12/ 7/76)	307	G&H Products, Inc. 5718-52nd St. Kenosha, WI 53141	( 5/ 2/78)
32	Taylor Instrument Process Control Div. Sybron Corporation 95 Ames Street Rochester, New York 14601	(10/ 4/56)	217	Girton Manufacturing Co. Millville, Pennsylvania 17846	( 1/23/71)
246	United Electric Controls 85 School Street Watertown, Massachusetts 02172	( 3/24/73)	252	Ernest Laffranchi P.O. Box 455 Ferndale, California 95536	(12/27/73)
<b>10-00 Milk and Milk Products Filters Using Disposable Filter Media, As Amended</b>			238	Paul Mueller Company P.O. Box 828 Springfield, Missouri 65801	( 6/28/72)
35	Ladish Co., Tri-Clover Division 9201 Wilmot Road Kenosha, Wisconsin 53140	(10/15/56)	96	C. E. Rogers Company P.O. Box 188 Mora, Minnesota 55051	( 3/31/64)
296	L. C. Thomsen & Sons, Inc. 1303 43rd St. Kenosha, Wisconsin 53140	( 8/15/77)	<b>13-06 Farm Milk Cooling and Holding Tanks</b>		
<b>11-03 Plate-type Heat Exchangers for Milk and Milk Products</b>			240	Babson Brothers Company (Mfg. by CREPACO, Inc.) 2100 S. York Road Oak Brook, Illinois 60521	( 9/ 5/72)
316	Agric Machinery Corp. P.O. Box 6 Madison, NJ 07940	( 2/ 7/79)	11R	CREPACO, Inc. 100 CP Ave. Lake Mills, Wisconsin 53551	( 7/25/56)
20	A.P.V. Company, Inc. 395 Fillmore Avenue Tonawanda, New York 14150	( 9/ 4/56)	119R	DCI, Inc. St. Cloud Industrial Park St. Cloud, Minnesota 56301	(10/28/59)
30	Cherry-Burrell Corporation (unit AMCA Int'l) 2400 Sixth Street, Southwest Cedar Rapids, Iowa 52404	(10/ 1/56)	4R	Dairy Equipment Company 1919 South Stoughton Road Madison, Wisconsin 53716	( 6/15/56)
14	Chester-Jensen Co., Inc. 5th & Tilgham Streets Chester, Pennsylvania 19013	( 8/15/56)	92R	DeLaval Company, Ltd. 113 Park Street South Peterborough, Ontario, Canada	(12/27/57)
			49R	The DeLaval Separator Company Dutchess Turnpike Poughkeepsie, New York 12602	(12/ 5/56)

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|----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|------------|
| 10R                                                                                          | Girton Manufacturing Company<br>Millville, Pennsylvania 17846                                                     | ( 7/25/56) | 137                                                                                                                                                   | Ex-Cell-O Corporation<br>2855 Coolidge,<br>Troy, Michigan 48084                                            | (10/17/62) |
| 95R                                                                                          | Globe Fabricators, Inc.<br>3350 North Gilman Rd.<br>El Monte, California 91732<br>(not available in USA)          | ( 3/14/58) | 220                                                                                                                                                   | Hercules, Inc., Package Equipment Div.<br>2285 University Ave.<br>St. Paul, Minnesota 55114                | ( 4/24/71) |
| 179R                                                                                         | Heavy Duty Products (Preston), Ltd.<br>1261 Industrial Road<br>Preston, Ontario, Canada<br>(not available in USA) | ( 3/ 8/66) | 281                                                                                                                                                   | Purity Packaging Corporation<br>4190 Fisher Road<br>Columbus, Ohio 43228                                   | (11/ 8/76) |
| 12R                                                                                          | Paul Mueller Company<br>P.O. Box 828<br>Springfield, Missouri 65801                                               | ( 7/31/56) | 211                                                                                                                                                   | Steel & Cohen (Twin-Pak, Inc.)<br>745 Fifth Avenue<br>New York, New York 10022                             | ( 2/ 4/70) |
| 249                                                                                          | Sunset Equipment Co.<br>3765 North Dunlap Street<br>St. Paul, Minnesota 55112                                     | ( 4/16/73) | <b>19-03 Batch and Continuous Freezers, For Ice Creams, Ices<br/>and Similarly Frozen Dairy Foods, As Amended</b>                                     |                                                                                                            |            |
| 16R                                                                                          | Zero Manufacturing Company<br>Washington, Missouri 63090                                                          | ( 8/27/56) | 286                                                                                                                                                   | Alfa-Hoyer<br>Soren Nymarksvei 13<br>DK-8270 Hojbjerg, Denmark<br>(not available in USA)                   | (12/ 8/76) |
| <b>16-04 Evaporators and Vacuum Pans for Milk and<br/>Milk Products</b>                      |                                                                                                                   |            | 146                                                                                                                                                   | Cherry-Burrell Company<br>(unit AMCA Int'l)<br>2400 Sixth Street, Southwest<br>Cedar Rapids, Iowa 52404    | (12/10/63) |
| 164R                                                                                         | Anderson IBEC<br>19609 Progress Drive<br>Strongsville, Ohio 44136                                                 | ( 4/25/65) | 141                                                                                                                                                   | CREPACO, Inc.<br>100 CP Avenue<br>Lake Mills, Wisconsin 53551                                              | ( 4/15/63) |
| 254                                                                                          | Anhydro, Inc.<br>165 John Dietsch Square<br>Attleboro Falls, Massachusetts 02763                                  | ( 1/ 7/74) | <b>22-04 Silo-Type Storage Tanks for Milk and Milk Products</b>                                                                                       |                                                                                                            |            |
| 132R                                                                                         | A.P.V. Company, Inc.<br>137 Arthur Street<br>Buffalo, New York 14207                                              | (10/26/60) | 168                                                                                                                                                   | Cherry-Burrell Corporation<br>(unit AMCA Int'l)<br>575 E. Mill St.<br>Little Falls, New York 13365         | ( 6/16/65) |
| 263                                                                                          | C. E. Howard Corporation<br>240 N. Orange Avenue<br>City of Industry, California 91746                            | (12/21/74) | 154                                                                                                                                                   | CREPACO, Inc.<br>100 CP Avenue<br>Lake Mills, Wisconsin 53551                                              | ( 2/10/65) |
| 107R                                                                                         | C. E. Rogers Company<br>P.O. Box 118<br>Mora, Minnesota 55051                                                     | ( 8/ 1/58) | 160                                                                                                                                                   | DCI, Inc.<br>St. Cloud Industrial Park<br>St. Cloud, Minnesota 56301                                       | ( 4/ 5/65) |
| 277                                                                                          | ConTherm Corp.<br>P.O. Box 352<br>Newbury Port, MA 01950                                                          | ( 8/19/76) | 181                                                                                                                                                   | Damrow Company, Division of DEC<br>International, Inc., 196 Western Ave.<br>Fond du Lac, Wisconsin 54935   | ( 5/18/66) |
| 294                                                                                          | DeLaval Separator Co.<br>1600 County Rd. F.<br>Hudson, WI 54016                                                   | ( 7/19/77) | 262                                                                                                                                                   | DeLaval Company Limited<br>113 Park Street<br>South, Peterborough, Ontario, Canada                         | (11/11/74) |
| 186R                                                                                         | Marriott Walker Corporation<br>925 East Maple Road<br>Birmingham, Michigan 48010                                  | ( 9/ 6/66) | 156                                                                                                                                                   | C. E. Howard Corporation<br>240 N. Orange Ave., Box 2507<br>City of Industry, CA 91746                     | ( 3/ 9/65) |
| 273                                                                                          | Niro Atomizer Inc.<br>(Mfg. by Laguilharre's Ltd.)<br>9165 Rumsey Road<br>Columbia, Maryland 21044                | ( 5/20/76) | 155                                                                                                                                                   | Paul Mueller Co.<br>P.O. Box 828<br>Springfield, Missouri 65801                                            | ( 2/10/65) |
| 299                                                                                          | Stork-Bowen Engr. Co.<br>(Mfg. by Stork-Friesland B.V.)<br>P.O. Box 898<br>Somerville, New Jersey 08876           | (11/16/77) | 312                                                                                                                                                   | Sanitary Processing Equip. Corp.<br>Butternut Drive<br>East Syracuse, New York                             | ( 9/15/78) |
| 311                                                                                          | Wiegand Evaporators, Inc.<br>5565 Sterrett Place<br>Columbia, Maryland 21044                                      | ( 8/28/78) | 165                                                                                                                                                   | Walker Stainless Equipment Co.<br>Elroy, Wisconsin 53929                                                   | ( 4/26/65) |
| <b>17-06 Fillers and Sealers of Single Service Containers<br/>For Milk and Milk Products</b> |                                                                                                                   |            | <b>23-01 Equipment for Packaging Frozen Desserts,<br/>Cottage Cheese and Milk Products Similar to<br/>Cottage Cheese in Single Service Containers</b> |                                                                                                            |            |
| 192                                                                                          | Cherry-Burrell Corporation<br>(unit AMCA Int'l)<br>2400 Sixth St., Southwest<br>Cedar Rapids, IA 52404            | ( 1/ 3/67) | 174                                                                                                                                                   | Anderson Bros. Mfg. Co.<br>1303 Samuelson Road<br>Rockford, Illinois 61109                                 | ( 9/28/65) |
|                                                                                              |                                                                                                                   |            | 209                                                                                                                                                   | Doboy Packaging Machinery<br>Domain Industries, Inc., 869 S. Knowles Ave.<br>New Richmond, Wisconsin 54017 | ( 7/23/69) |

- 302 Eskimo Pie Corp. ( 1/27/78) 172 SWECO, Inc. ( 9/ 1/65)  
530 E. Main St.  
Richmond, Virginia 23219  
6033 E. Bandini Blvd.  
Los Angeles, California 90051
- 258 Hercules, Inc. ( 2/ 8/74)  
2285 University Ave.  
St. Paul, Minnesota 55114
- 24-00 Non-Coil Type Batch Pasteurizers**
- 161 Cherry-Burrell Corporation ( 4/ 5/65)  
(unit AMCA Int'l)  
575 E. Mill St.  
Little Falls, New York 13365
- 158 CREPACO, Inc. ( 3/24/65)  
100 CP Avenue  
Lake Mills, Wisconsin 53551
- 187 DCI, Inc. ( 9/26/66)  
St. Cloud Industrial Park  
St. Cloud, Minnesota 56301
- 177 Girton Manufacturing Co. ( 2/18/66)  
Millville, Pennsylvania 17846
- 166 Paul Mueller Co. ( 4/26/65)  
P.O. Box 828  
Springfield, Missouri 65601
- 25-00 Non-Coil Type Batch Processors for Milk and Milk Products**
- 275 Bepex Corporation ( 7/12/76)  
150 Todd Road  
Santa Rosa, California 95402
- 162 Cherry-Burrell Corporation ( 4/ 5/65)  
(unit AMCA Int'l)  
575 E. Mill St.  
Little Falls, New York 13365
- 159 CREPACO, Inc. ( 3/24/65)  
100 CP Avenue  
Lake Mills, Wisconsin 53551
- 188 DCI, Inc. ( 9/26/66)  
St. Cloud Industrial Park  
St. Cloud, Minnesota 56301
- 167 Paul Mueller Co. ( 4/26/65)  
Box 828  
Springfield, Missouri 65801
- 202 Walker Stainless Equipment Co. ( 9/24/68)  
New Lisbon, Wisconsin 53950
- 26-01 Sifters for Dry Milk and Dry Milk Products**
- 228 Day Mixing, Div. LeBlond, Inc. ( 2/28/72)  
4932 Beech Street  
Cincinnati, Ohio 45212
- 229 Russell Finex Inc. ( 3/15/72)  
156 W. Sandford Boulevard  
Mt. Vernon, New York 10550
- 173 B. F. Gump Division ( 9/20/65)  
Blaw-Knox Food & Chem. Equip. Inc.  
750 E. Ferry St., P.O. Box 1041  
Buffalo, NY 14211
- 185 Rotex, Inc. ( 8/10/66)  
(Mfg. by Orville Simpson Co.)  
1230 Knowlton St.  
Cincinnati, Ohio 45223
- 176 Koppers Company, Inc. ( 1/ 4/66)  
Metal Products Division  
Sprout-Waldron Operation  
Munsey, Pennsylvania 17756
- 27-01 Equipment for Packaging Dry Milk and Dry Milk Products
- 313 WPM Systems, Inc. (10/10/78)  
Div. of St. Regis Paper Company  
3990 Acoma St.  
Denver, Colorado 80216
- 28-00 Flow Meters for Milk and Liquid Milk Products**
- 272 Accurate Metering Systems, Inc. ( 4/ 2/76)  
1731 Carmen Drive  
Elk Grove Village, Illinois 60007
- 253 Badger Meter, Inc. ( 1/ 2/74)  
4545 W. Brown Deer Road  
Milwaukee, Wisconsin 53223
- 223 C-E IN-VAL-CO, Division of Combustion Engineering, Inc. (11/15/71)  
P.O. Box 556, 3102 Charles Page Blvd.  
Tulsa, Oklahoma 74101
- 265 Electronic Flo-Meters, Inc. ( 3/10/75)  
P.O. Box 38269  
Dallas, Texas 75238
- 226 Fischer & Porter Company (12/ 9/71)  
County Line Road  
Warminster, Pennsylvania 18974
- 224 The Foxboro Company (11/16/71)  
Neponset Avenue  
Foxboro, Massachusetts 02035
- 270 Taylor Instrument Company Division ( 2/ 9/76)  
Sybron Corporation, 95 Ames Street  
Rochester, New York 14601
- 29-00 Air Eliminators for Milk and Fluid Milk Products**
- 30-00 Farm Milk Storage Tanks**
- 257 Babson Bros. Co. ( 2/ 7/74)  
(Mfg. by CREPACO, Inc.)  
2100 S. York Road  
Oak Brook, Illinois 60521
- 31-00 Scraped Surface Heat Exchangers**
- 274 Contherm Corporation ( 6/25/76)  
P.O. Box 352  
Newburyport, Massachusetts 01950
- 290 CREPACO, Inc. ( 6/15/77)  
100 So. CP Ave.  
Lake Mills, WI 53551
- 32-00 Uninsulated Tanks for Milk and Milk Products**
- 264 Cherry-Burrell Company, ( 1/27/75)  
(unit AMCA Int'l)  
575 E. Mill St.  
Little Falls, NY 13365
- 268 DCI, Inc. (11/21/75)  
P.O. Box 1227  
St. Cloud, Minnesota 56301
- 33-00 Polished Metal Tubing for Dairy Products**
- 310 Allegheny Bradford Corporation ( 7/19/78)  
P.O. Box 264  
Bradford, PA 16701

289	Ladish Co., Tri-Clover Division 9201 Wilmot Road Kenosha, Wisconsin 53140	( 1/21/77)	292	Waukesha Foundry Div. ABEX Corp. 1300 Lincoln Ave. Waukesha, WI 53186	( 8/24/77)
308	Rath Mfg. Co. Inc. 2505 Foster Ave. Janesville, WI 53545	( 6/15/77)	<b>36-00 Colloid Mills</b>		
			293	Waukesha Foundry Div., ABEX Corp. 1300 Lincoln Ave. Waukesha, WI 53186	( 8/24/77)

**35-00 Continuous Blenders**

## News and Events

### Competition for 1979 Crumbine Award Now Open

Entries are now being accepted for the 1979 Samuel J. Crumbine Consumer Protection Award which since 1955 has honored local government public health agencies for "excellence in programs of food and beverage sanitation" in public eating and drinking establishments.

Announcement of the 1979 Award competition was made by its sponsor, the Environment & Health Committee of the Single Service Institute, the national trade association of manufacturers of single-use products for food service and packaging. The Award takes its name from the late Dr. Samuel J. Crumbine, a public health pioneer who for many years was Health Officer for the State of Kansas and also headed the American Child Health Association.

According to Charles W. Felix, Director, Environment, Health and Public Affairs of the Single Service Institute, the 1979 Crumbine Award will be given "for outstanding achievement in a comprehensive program of food and beverage sanitation at the local level in 1978." The criteria on which an independent panel of qualified jurors will judge entries include program improvement, innovative and effective use of evaluation methods, effectiveness of planning and management, and excellence of information and education activities.

The Crumbine competition is open to local government units in cities, counties, districts, towns and townships which are responsible for the design and execution of sanitation and safety programs to protect consumers in public eating and drinking places. State agencies are ineligible except for components of a state body operating under specific budgets for public health programs in local jurisdictions lacking their own local agency for food sanitation control.

The 1978 Crumbine Award was won by the Environmental Health Bureau of the Department of Human Resources, Arlington County, Virginia.

Deadline for 1979 Award entries is May 31, 1979. Submissions should be sent by first class mail to Single Service Institute, Inc., 1025 Connecticut Avenue, N. W., Washington, D.C. 20036. Applications and further information may be obtained from the institute.

### **Standard Methods 14th Edition Available**

Substantial revisions of many of the chapters, reflecting advances in scientific knowledge, have been incorporated into the 14th edition of the *Standard Methods for the Examination of Dairy Products*.

As with previous editions, the 14th edition was prepared with the guidance of an intersociety council, composed of representatives of professional societies, industry, education, government, as well as several members who served on the previous

### **Niemeyer Appointed Nasco Vice-President**

W. Phil Niemeyer has been appointed Vice President of Nasco, a major mail order supplier of products for education, agriculture, and industry.

Niemeyer was employed by Nasco in January of 1972 as Assistant Director of Agricultural Sales, and currently serves as Director of Agricultural and Distributor Sales. He will continue in this capacity as well as assume new responsibilities in his role as Vice President of the company.

The dairy industry has been one of Niemeyer's major interests, and he currently serves on the education committee of the National Mastitis Council and the government affairs and annual meeting committees of the Dairy and Food Industries Supply Association.

Council.

The revision was funded by the Food and Drug Administration as well as a contract with the American Public Health Administration (APHA). Editor and Chairman of the Council was Dr. Elmer Marth, who was selected by APHA.

More than 80 persons contributed to the 14th edition. It is available through APHA, 1015 Eighteenth Street, N.W., Washington, D.C. 20036.

## Coming Events

Mar. 9-10--SEVENTH ANNUAL FOOD INDUSTRY ASSOCIATION OF SOUTH CAROLINA CONFERENCE. Hilton Head, SC. Contact: T. C. Titus, Food Industry Association of SC, Box 708, Clemson, SC 29631.

Mar. 12-14--ANNUAL HIGHLIGHTS IN FOOD SCIENCE CONFERENCE, "Quality Control: R<sub>x</sub> for Success?" Kellogg Center for Continuing Education, Michigan State University, East Lansing, MI 48824. Contact: Jerry N. Cash, Assistant Professor, Dept. of Food Science and Human Nutrition, Michigan State University.

Mar. 19-21--KULTURES AND KURDS KLINIC. Sponsored by the American Cultured Dairy Products Institute. Hilton Inn, Columbus, OH. For additional information and/or advance registration forms, contact: Dr. C. Bronson Lane, DAFNC, P.O. Box 7813, Orlando, FL 32854 or Margie Franck, ACDPI, 910 17th Street, N.W., Washington, D.C. 20006.

Mar. 19-23--MID-WEST WORKSHOP IN MILK AND FOOD SANITATION. Ohio State University. Contact: John Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Road, Ohio State University, Columbus, OH 43210.

Mar. 20--7th ANNUAL FOOD SCIENCE SYMPOSIUM. Student Center, University of Kentucky, Lexington, KY. Sponsored by Food Science Section, Dept. of Animal Science, University of Kentucky; IFT Bluegrass Section; and Kentucky Meat Processors. Contact: J. D. Kemp, Dept. of Animal Science, Univ. of Kentucky, Lexington, KY 40506.

Mar. 20-21--NATIONAL SANITATION FOUNDATION SEMINARS, Dallas, TX. For more information, contact: Education Service, National Sanitation Foundation, NSF Building, P.O. Box 1468, Ann Arbor, MI 48106, 313-769-8010.

Mar. 21--INDIANA DAIRY INDUSTRY CONFERENCE. Purdue University. Contact: James Chambers, Assistant Professor of Animal Sciences, Animal Sciences Dept., Purdue University, West Lafayette, IN 47907.

Mar. 22-23--ENVIRONMENTAL LAWS & REGULATIONS SEMINAR, Introductory Course. Twin Bridges Marriott Hotel, Washington, D.C. Sponsored by Government Institutes. Contact: Marguerite Leishman, Government Institutes, Inc., 4733 Bethesda Ave. N.W., Washington, D.C. 20014. 301-656-1090.

Mar. 26--IOWA ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, Annual Meeting. Gateway Center Motel, Ames. Contact: Hale Hansen, Dept. of Health, Lucas Building, Des Moines, IA 50319.

Mar. 26-28--FOOD TECHNOLOGY UPDATE. Central New Jersey location. Sponsored by the Center for Professional Advancement. Fee: \$450. Contact: Mary Sobin, Dept. NR, The Center for Professional Advancement, P.O. Box H, East Brunswick, N.J. 08816, 201-249-1400.

Mar. 26-28--MISSOURI ASSOCIATION OF MILK AND FOOD SANITARIANS, Annual Meeting. Lodge of the Four Seasons, Lake of the Ozarks, MO. Contact: Erwin Gadd, Bureau of Community Sanitation, Missouri Division of Health, Box 570, Jefferson City, MO 65101.

Mar. 26-28--MICHIGAN ENVIRONMENTAL HEALTH ASSOCIATION, Annual Educational Conference. Sheraton Inn, Kalamazoo, MI. Contact: James Szejda, 414 Washington, St., Grand Haven, MI 49417.

Mar. 27--DAIRY INDUSTRY CONFERENCE. Scheman Building, Iowa State University Center, Contact: Verner Nielsen, Food Technology, ISU, Ames, IA 50011.

Mar. 27-28--WESTERN FOOD INDUSTRY CONFERENCE. University of California, Davis, CA. Contact: John C. Bruhn, Extension Food Technologist, Dept. of Food Science & Technology, University of California, Davis, CA, 916-752-2192.

Mar. 28--ONTARIO MILK AND FOOD SANITARIANS ASSOCIATION, Annual Meeting. Holiday Inn, Airport Rd., Toronto. Contact: Roger Wray, 32 Windsor St., Guelph Ont., N1E 3N2, Canada.

April 2-4--DAIRY AND FOOD INDUSTRIES SUPPLY ASSOC., 60th ANNUAL MEETING. The Breakers, Palm Beach, FL. Contact: Wes Dibbern, Dairy and Food Industries Supply Assoc., 5530 Wisconsin Ave., Suite 1050, Washington, D.C. 20015, 301-652-4420.

April 3-6--4th ANNUAL FOOD SERVICE SYSTEMS SEMINAR AND EXPOSITION. Hospitality House, Arlington, VA. Contact: G. E. Livingston, Food Science Associates, Inc., 145 Palisade St., Dobbs Ferry, NY 10522.

April 9-10--WAREHOUSE AND THRIFT STORE SANITATION COURSE. American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66502. Contact: Larry E. Wood, AIB, 913-537-4750.

April 10-11--PRESENT AND FUTURE TRENDS IN FOOD AND BEVERAGE PACKAGING, Short Course. Holiday Inn, Clemson, SC. Contact: T. C. Titus, Food Science Dept., Clemson University, Clemson, SC 29631.

April 17-19--3rd ANNUAL INDUSTRIAL RESEARCH AND DEVELOPMENT SYMPOSIUM, "Research and Development and Quality Assurance." The University of Mississippi, Oxford Campus. Contact: Wallace L. Guess, Dean, School of Pharmacy, Univ. of Mississippi, University, MI 38677.

April 19--IOWA FOOD INDUSTRY CONFERENCE. Stouffer's Hotel, Cedar Rapids, IA. Contact: Bill LaGrange, Food Technology, ISU, Ames, IA 50011.

April 22-25--4th ANNUAL TROPICAL AND SUBTROPICAL FISHERIES TECHNOLOGICAL CONFERENCE. St. Petersburg Hilton, St. Petersburg, FL. Contact: W. Steven Otwell, 325 Food Science Bldg., Univ. of Florida, Gainesville, FL 32611.

April 23-24--NSF SEMINARS, Washington, D.C. For more information, see entry for Mar. 20-21.

April 25-27--54th ANNUAL MEETING, AMERICAN DRY MILK INSTITUTE; 8th ANNUAL MEETING, WHEY PRODUCTS INSTITUTE. Chicago Marriott O'Hare, 8535 West Higgins Road (at O'Hare Airport), Chicago. Contact: Warren S. Clark, Jr., Exec. Director of both organizations, 130 N. Franklin St., Chicago, IL 60606.

April 30-May 4--APPLIED PROCESS PUMP TECHNOLOGY. Sponsored by the Center for Professional Advancement. Fee: \$580. Contact: Mary Sobin, Dept. NR, The Center for Professional Advancement, P.O. Box H, East Brunswick, NJ 08816, 201-249-1400.

May 7-10--FOOD MICROBIOLOGY LAB COURSE. American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66502. Contact: Larry E. Wood, AIB, 913-537-4750.

May 8-10--34th ANNUAL PURDUE INDUSTRIAL WASTE CONFERENCE. Stewart Center, Purdue University, West Lafayette, IN. Contact: J. D. Wolszon, Purdue Industrial Waste Conference, Civil Engineering Bldg., Purdue Univ., West Lafayette, IN 47907.

May 10-11--ENVIRONMENTAL LAWS & REGULATIONS COURSE, Masters' Seminar. Philadelphia Hilton, Philadelphia, PA. Sponsored by Government Institutes, Inc., Contact: Marguerite Leishman, Government Institutes, Inc., 4733 Bethesda Ave., N.W., Washington, D.C. 20014, 301-656-1090.

May 13-16--THE NATIONAL MEAT TRADES FAIR. Exhibition Centre, Harrogate, England. Contact: British Information Services, 845 Third Ave., New York, NY 10022.

May 14-17--DELEX, Delicatessen International Exhibition. Royal Horticultural Society's New Hall, London, England. Contact: British Information Services, 845 Third Avenue, New York, NY 10022, 212-752-8400.

May 14-19--MACROPAK 1979 and INTERNATIONAL PACKAGING CONGRESS. Utrecht, the Netherlands. Contact: Royal Netherlands Industries Fair, Special Events Dept., P.O. Box 8500, 3502 RM Utrecht, the Netherlands.

May 15-17--POWDER AND BULK SOLIDS CONFERENCE/EXHIBITION. Philadelphia Civic Center, Philadelphia, PA. Sponsored by International Powder Institute of London and Chicago. Contact: Industrial & Scientific Conference Management, Inc., 222 West Adams St., Chicago, IL 60606, 312-263-4866.

May 20-22--1979 INTERNATIONAL CHEESE & DELI SEMINAR. Madison, WI. Contact: International Cheese & Deli Seminar, 801 W. Badger Rd., Madison, WI 53713.

May 21-22--NSF SEMINARS, Seattle, WA. For more information, see entry for Mar. 20-21.

May 21-24--NALVEX, National Licensed Victuallers and Caterers Exhibition. National Exhibition Centre, Birmingham, England. Contact: British Information Services, 845 Third Avenue, New York, NY 10022, 212-752-8400.

May 22-27--INTERNATIONAL FOOD FAIR. Copenhagen, Denmark. Contact: Bella Center A/S, Center Boulevard, DK-2300 Copenhagen S., Denmark.

May 28-31--NATIONAL CONVENTION OF THE AUSTRALIAN INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY, Theme: "Food, the Consuming Interest." Adelaide, South Australia. Contact: L. Peters, Australian Institute of Food Science and Technology, 13, Bulf Parade, Brighton, South Australia 5048.

June 4-5--NSF SEMINARS, Memphis, TN. For more information, see entry for Mar. 20-21.

June 5--PENNSYLVANIA SANITARIANS ASSOCIATION, Annual Meeting. Keller Conference Center, Pennsylvania State University, University Park Campus, State College, PA 16801. Contact: Sid Barnard, Pennsylvania State University.

June 10-13--INSTITUTE OF FOOD TECHNOLOGISTS 39th ANNUAL MEETING AND FOOD EXPO. Alfonso J. Cervantes Convention and Exhibition Center, St. Louis, MO. Contact: C. L. Willey, Institute of Food Technologists, Suite 2120, 221 N. LaSalle St., Chicago, IL 60601.

June 24-27--AMERICAN SOCIETY OF AGRICULTURAL ENGINEERS, Summer Meeting. Winnipeg, Manitoba, Canada. Contact: Roger R. Castenson, ASAE, 2950 Niles Road, Box 410, St. Joseph, MI 49085, 616-429-0300

July 30-Aug. 3--ADVANCES IN FOOD AND APPLIED MICROBIOLOGY. Massachusetts Institute of Technology, Cambridge, MA 02139. Program is under the direction of Anthony J. Sinskey, MIT, Professor of Applied Microbiology. Contact: Director of Summer Session, Rm. E 19-356, Massachusetts Institute of Technology, Cambridge, MA 02139.

Aug. 13-17--WORKSHOP ON EDUCATIVE PROCESSES IN FOOD MICROBIOLOGY. Sponsored by the Joint American Society for Microbiology/Institute for Food Technologists Committee on Food Microbiology Education. Quadna Resort, Hill City, MN. Contact: E. A. Zottola, Dept. of Food Science and Nutrition, 1334 Eckles Ave., University of Minnesota, St. Paul, MN 55108.

Aug. 29-31--FOURTH INTERNATIONAL IUPAC SYMPOSIUM ON MYCOTOXINS AND PHYCOTOXINS. Co-sponsored by World Health Organization and Swiss Society for Analytical and Applied Chemistry. Lausanne, Switzerland. For participation and poster presentation, contact: Prof. P. Krogh, Dept. of Veterinary Microbiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907 or Prof. D. Reymond, IUPAC, Case postale 88, 1814 La Tour de Peilz, Switzerland.

Sept. 10-13--2nd INTERNATIONAL CONFERENCE ON FOOD SERVICE SYSTEMS DESIGN. Harrogate, England, Contact: G. Glew, Catering Research Unit, Procter Dept. of Food Science, The University of Leeds, LS2 9JT, England.

Sept- 10-14--FOOD PROCESSORS ADVANCED MICROBIOLOGY SHORT COURSE. University of California, Davis. Fee \$200. Contact: John C. Bruhn, Dept. of Food Science and Technology, University of California, Davis, CA 95616, 916-752-2192.

Sept. 18-20--WESTPACK '79. Convention Center, Anaheim, CA. Contact: Clapp & Poliak, Inc., 245 Park Ave., New York, NY 10017.

Nov. 3-6--1979 AMERICAN MEAT INSTITUTE CONVENTION. McCormick Place and The Conrad Hilton, Chicago. Contact: Judi Winslow, American Meat Institute, P.O. Box 3556, Washington, D.C. 20007, 703-841-2431.

Nov. 11-15--FOOD AND DAIRY EXPO '79. McCormick Place, Chicago, IL. Contact: Wes Dibbern, Dairy and Food Industries Supply Assoc., 5530 Wisconsin Ave., Suite 1050, Washington, D.C. 20015, 301-652-4420.

Nov. 20-23--FIE FOODPACK, International Food Industries Exhibition, Olympia, London, England. Contact: British Information Services, 845 Third Avenue, New York, NY 10022, 212-752-8400.

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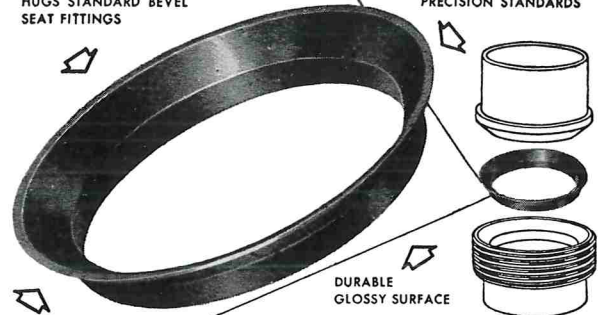
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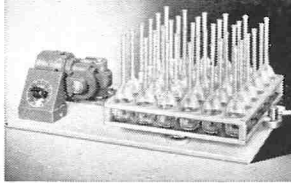
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- Dairy Industry Committee (DIC)
- International Association of Milk, Food, and Environmental Sanitarians (IAMFES)

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- Write: JOURNAL OF FOOD PROTECTION, P.O. Box 701, Ames, Iowa 50011 for a complete set of published 3A standards.
- Write Tri-Clover for a reprint of "Ten Pivotal 3A Standards" from a special report published by DAIRY & ICE CREAM FIELD.



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# Total Management Concept: The Team Working For You

*Dr. George C. Fisher, Head  
Veterinary Services Laboratory,  
Ontario Ministry of Agriculture & Food,  
Kemptville, Ontario*



There is no way the farmer today can operate as a loner. That's why more and more dairymen are looking at their total operations with an eye on Total Management. The Total Management Concept benefits the dairyman because it encourages him to identify and make use of the many

people who have been trained to offer professional advice and service. TMC is teamwork with the dairyman as head coach. He knows who his resource people on the bench are and when to call on them. And, like any winning coach, the dairyman knows the special efforts of all team members must be coordinated to succeed. These resource people are the veterinarians, equipment dealers, nutritionists, agricultural engineers, extension service people, D.H.I.A. or R.O.P., and many others who can provide current, specialized information and help with the business of dairying.

#### **Planning to Avoid Emergencies**

Through Total Management, you coordinate planning to minimize emergencies, be they in health, equipment, or production. This is done with the help of available experts in six broad areas:

1. Soil Testing
2. Nutrition
3. Cow Performance
4. Equipment Maintenance
5. Herd Health Program
6. Record Keeping

Though some areas may appear more important than others, the exclusion or neglect of any one will result in less profit, or as a sudden problem in production or herd health—a problem easily avoided with proper management and teamwork.

#### **The Team in Action**

Total Management provides the farmer with

continually updated information, and assures that all areas are working to his benefit. Since the amount of information available is more than any one person can possibly handle, specialists are essential to apply the right facts to your needs. Broadly stated then, the TMC helps you make informed decisions and to put them into practice.

#### • HERD HEALTH

Use your local veterinarian in a systematic approach to a herd health program (specifically in respect to infertility and mastitis control).

#### • PRODUCTION GOALS

Set uniform production goals designed to meet the capabilities of the herd and farm unit with the assistance of your County Agent.

#### • RECORDS

Apply a unified approach to the keeping of records relating to herd health, nutrition, reproduction, and production—(with the help of a milk recording system such as D.H.I.A. or R.O.P.). University extension people are trained to provide guidance in this area.

#### • EQUIPMENT

Have your dairy equipment dealer perform periodic checks and adjustments of the milking system through a scheduled maintenance program. This will assure proper equipment operation for better production and improved herd health.

#### • FEEDING PROGRAM

Work with your nutrition specialist to develop an in-depth feeding program using nutrient analysis to determine year-round feeding according to production. Base this program on the production, storage, and utilization of high quality forages.

Keeping pace with the times offers a rewarding challenge for the dairyman who is willing to use progressive management practices. And, if you face the future by working on Total Management with the help of the many professionals available, you will find a bright future. Today, more than ever, your future depends on having a winning team working for you.



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