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Research Papers

General Interest

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Analyst Variation in Doing the Standard Plate Count as Described in *Standard Methods for the Examination of Dairy Products*[1,2]

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ABSTRACT

Five analysts participated in a study to evaluate the following aspects of the 13th edition of *Standard Methods for the Examination of Dairy Products* (*SMEDP*): (*a*) analyst variation in overall Standard Plate Counts (SPC), and (*b*) analyst duplication of bacterial colony counts on agar plates. Each analyst prepared 24 samples of pasteurized, homogenized milk during a successive 8-day period (i.e., 3 samples/day), and then the analysts estimated the numbers of bacterial colonies for these, as well as other analysts' plates, initially after 48 h of incubation, and then 1 h later and 24 h later. Statistically significant differences in colony enumerations were found between analysts in preparation of agar plates on 3 days. Significant differences were also noted between analysts for bacterial counts of agar plates. Mean bacterial estimates of certain analysts ranged between 565 and 948, and fluctuated greatly between the initial, 1-h, and 24-h determinations. These results indicate that the "standards of accuracy" currently specified in *SMEDP* are not realistic, i.e., (*a*) among-analyst variation of 18.2% compared to 10%, and (*b*) within-analyst variation of 7.7% compared to 5% in *SMEDP*.

Recent editions of *Standard Methods for the Examination of Dairy Products* (*SMEDP*) (*1, 2, 3*) state that laboratory analysts should be able to duplicate their own bacterial counts for a sample of milk within 5%, and counts of other analysts within 10%. To date, however, empirical verification of these "standards of accuracy" has been lacking. Errors of measurement (e.g., pipetting, counting) and procedure (e.g., blending, incubation) are only a few of the potential sources of variation which affect the reliability of the standard plate count (SPC). It is questionable whether or not these 5% and 10% standards are realistic performance criteria for analyst reproducibility with the SPC.

In one of the few systematic studies of analyst reliability for SPC, Donnelly et al. (*6*) reported that 7 of 21 analysts who prepared and counted duplicate agar pour plates of single milk samples showed a consistent bias in their bacterial estimates. Similarly, Courtney (*4*), in a descriptive study of SPC steps believed important to the accuracy of enumeration (e.g., pipetting, counting, etc.), concluded that: (*a*) accuracy was directly related to the magnitude of SPC ratios, with average ratios ≥ 2.0 between duplicate plates judged to be unreliable, and (*b*) pipetting was the single most important operation capable of producing unreliable ratios.

Although these studies are the basis for current *SMEDP* "standards of accuracy," Donnelly et al. (*6*) were unable to provide standardized instruction and supervision to analysts (i.e., the study was conducted as a survey in which samples were shipped to a number of widely separated laboratories); and Courtney (*4*) failed to use a systematic research design and analysis for his data. Additionally, in neither study (*4, 6*) did the analysts recount bacterial colonies, a straightforward technique of assessing the stability of analysts' counts across time. These omissions, and the likelihood that analysts influence SPC, warrant further evaluation of analyst reliability under controlled instruction, preparation, and counting of split milk samples.

This study was designed to assess the among- and within- analyst variability associated with SPC determinations when a split milk sample procedure is used. Specifically, estimates of analyst variation in (*a*) plating split-sample aliquots of homogenized, pasteurized milk according to *SMEDP* guidelines (*3*) and (*b*) counting duplicate agar plates after 48 h of incubation, 1 h later, and 24 h later, were sought.

MATERIALS AND METHODS

Analysts

Relevant experience of the five analysts is as follows: (*a*) Analyst 1-BS in microbiology; approximately 2 years of experience in food microbiology; (*b*) Analyst 2-over 10 years of experience in regulatory microbiology; (*c*) Analyst 3-approximately 3 years of experience in microbiology; (*d*) Analyst 4-professional degree; approximately 2 years

[1] *The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.*
[2] *Reprint requests to: Commander, LAIR, ATTN: Medical Research Library, Presidio of San Francisco, CA 94129.*
[3] *Present address: P.O. Box C, Fort Benning, GA 31905.*
[4] *Present address: American Dry Milk Institute, 130 N. Franklin St., Chicago, IL 60606.*

of experience in food microbiology; and (*e*) Analyst 5-approximately 6 months of experience in food microbiology. All analysts had thoroughly reviewed Chapter 5, *SMEDP* (*3*) and had been observed for proper laboratory techniques associated with SPC procedures before the start of the study.

Sample preparation

Three one-half gallon cartons of whole homogenized, pasteurized milk were purchased daily from a local retail store. The contents of each container were mixed in accordance with paragraph 5.062, *SMEDP* (*3*), and five aliquots of approximately 160 ml were prepared. One aliquot of each of the three samples was then plated by each analyst. The experiment was conducted daily for eight consecutive days. Each analyst plated three different samples of milk each day. Therefore, 24 samples were plated by each of the five analysts during the course of the study. Laboratory procedures using the 11-99 ml dilution scheme were followed (*3*). Duplicate plates of dilutions 10^{-1} 10^{-2}, and 10^{-3} were prepared for each sample; incubation of agar plates was at 32 ± 1 C for 48 ± 3 h.

Determinations

Agar plates were counted following incubation at 32 C for 48 h, 1 h later, and 24 h later. Plates were in refrigerated storage during the 1-24-h interval. Recounting at 1 h was used to measure short-term reliability indices. The 24-h recounting period was included to provide information about the question of valid counting after a 24-h refrigeration period as allowed by paragraph 5.111, *SMEDP* (*3*).

Randomization of agar plates

To minimize bias among analysts, each was assigned a code letter to identify plates and date throughout the study. Before counting agar plates, the experimenter coded plates of each analyst. Except for recognizable bacterial patterns or pour characteristics of plates, dilution and pourer identification was "blinded" within the experiment. Each analyst was given a sheet numbered 1-90 to record actual colony counts; numbers were converted back to the original coding by the experimenter. Colony counts reported by analysts were converted to SPC by the experimenter. Analysts were not allowed access to these data until the entire experiment was completed.

Experimental design and statistical analyses

Data were analyzed by use of BMDO8V (*5*) as a four factor experiment (day × pourer × counter × determination) with repeated measures on two factors (counter and determination) (*8*). The day factor was analyzed as a random factor while the other factors were assumed fixed. The mean SPC value for duplicate pour plates served as the dependent variable. When a significant F value for an effect was found, a posteriori multiple comparisons were used to test for differences among means by the Tukey honestly significant difference procedure (*8*). Additionally plate count reliabilities among- and within-analysts were assessed by obtaining coefficients of variation. The 0.05 level of significance was used with all statistical tests.

RESULTS

The analysis of variance for the SPC data is presented in Table 1. Line 1 shows a significant difference among the overall mean SPC counts for the 8 days. Although line 2 indicates that no significant difference existed among the overall SPC means for the five analysts in preparing the agar plates, line 3 shows a significant interaction between pourer and days. This relationship is depicted in Fig. 1. Multiple comparisons of the interaction means indicated significant differences between pourers on days four, six, and eight.

Lines 5 and 6 of Table 1 indicate a significant difference between counters as well as a counter × day interaction effect. The counter × day interaction is illustrated in Fig. 2. Significant differences were found among the counters' means every day. Although a significant day × time interaction occurred (line 11), no significant differences were found among mean times for any given day.

To determine the variation which existed among and within analysts, coefficients of variation were calculated. The coefficients of variation among- and within-analysts were 18.2% and 7.7%, respectively.

DISCUSSION

Many laboratory supervisors have endorsed the concept that specialized training and proper supervision of analyst will result in generation of uniform, consistent SPC data. Although the current data do not directly refute this, SPC coefficients of variation both among and within analysts (18.2% and 7.7%) exceeded prior expectations. It is acknowledged that this finding could

TABLE 1. *Analysis of variance determinations*

Line	Source of variation	Error term, Line no.	F	Sum of squares	df	Mean square
Between Plates						
1	A (Day)	4	50.41*	43581547	7	6225935
2	B (Pourer)	3	1.33	1334439	4	333610
3	AB	4	2.03*	7035502	28	251263
4	Plates within Samples			9880213	80	123503
Within Plates						
5	C (Counter)	6	49.92*	29835405	4	7458851
6	AC	9	8.01*	4183798	28	149421
7	BC	8	1.07	366794	16	22925
8	ABC	9	1.15	2402234	112	21449
9	C × Plates within Samples			5971209	320	18660
10	D (Determination)	11	3.01	157762	2	78881
11	AD	14	5.19*	367294	14	26235
12	BD	13	1.04	46002	8	5750
13	ABD	14	1.09	309862	56	5533
14	D × Plates within Samples			809053	160	5057
15	CD	16	2.05	213049	8	26631
16	ACD	19	3.56*	726593	56	12975
17	BCD	18	.92	126504	32	3953
18	ABCD	19	1.18*	959556	224	4284
19	CD × Plates within Samples			2332458	640	3644

*Significant F values ($p < 0.05$)

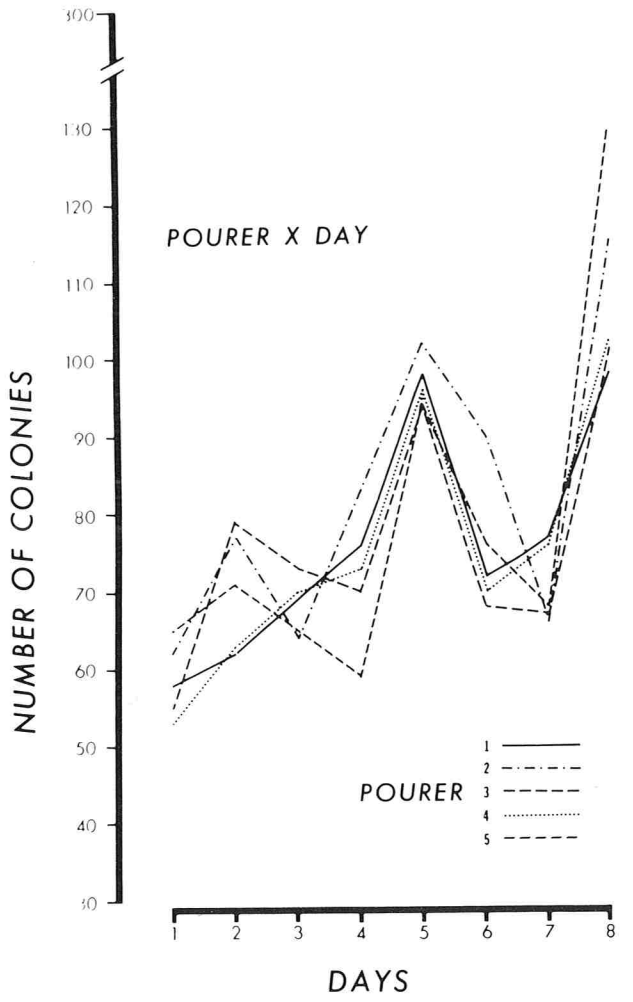

Figure 1. *Mean bacterial colony estimates for analysts in preparing agar plates (pourer) by day.*

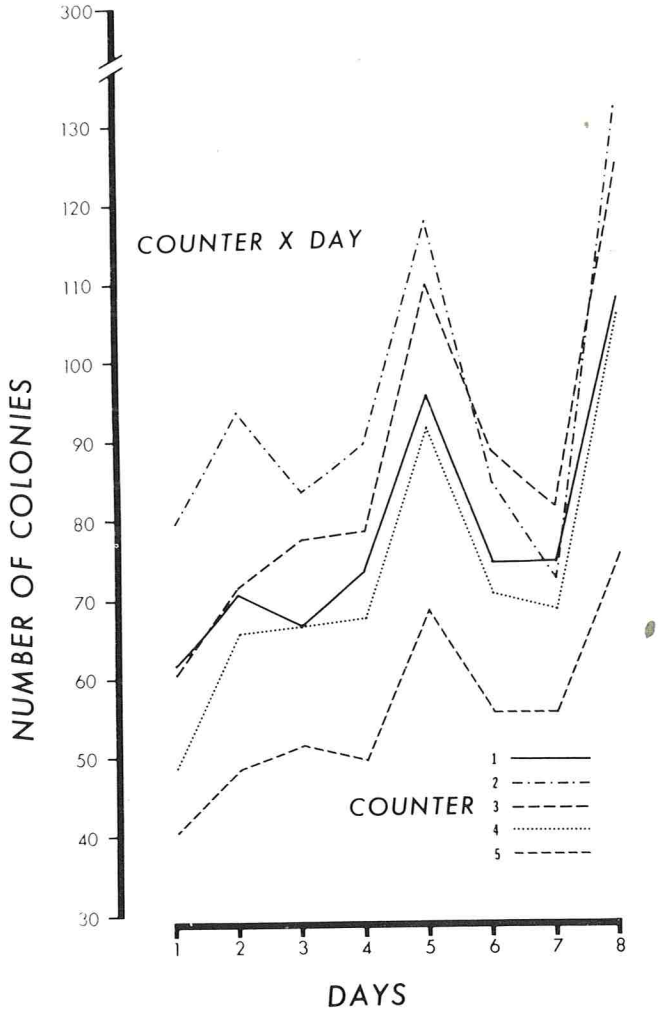

Figure 2. *Mean bacterial colony estimates for analysts counting agar plates (counter) by day.*

reflect an experience factor of the analysts studied. After receiving proper training and supervision, analysts prepared agar plates for SPC determinations in such a manner that no significant differences in mean agar plate count values were obtained overall. However multiple comparison tests on the pourer × day interaction showed significant pourer differences on 3 days. These results reflect favorably upon the detailed procedural directions presently contained in *SMEDP* (*3*), since this served as the guide for training analysts, but indicate that significant variation in preparing agar plates can exist on a day-to-day basis.

More specifically, while few statistically significant differences were present between SPC associated with the poured agar plates, there was a significant difference among analysts doing the SPC (line 5, Table 1). Such results indicate that much greater difficulty is encountered in uniformly counting rather than in preparing agar plates. This may be related directly to analyst experience, over and above training, per se.

Although the 1-h and 24-h recounting was used to measure short- and long-term reliability of the SPC, plate storage also provided information on colony stability across time. As shown in Table 1, no significant differences were found between analyst mean counts for the initial, 1-h, and 24-h counting periods. This indicates that increases in bacterial colonies are minimal for plates refrigerated 24 h. Therefore, refrigerated storage of SPC agar plates for 24 h before counting seems perfectly acceptable.

Results of this experiment did not confirm the "standards of accuracy" contained in recent editions of *SMEDP* (*3*). Our results show that agreement in counts among analysts for the experiment was 18.2%; this does not compare favorably with the figure of 10% given in *SMEDP* (*3*). Likewise, the ability of analysts to duplicate their own plate counts was found to be 7.7% as compared to 5% given in *SMEDP* (*3*). These data strongly indicate that the suggested standards regarding the counting of bacterial colonies are not realistic. Fruin et al. (*7*), in a more recent study, presented data which further question the present "standards of accuracy" in *SMEDP* (*3*). The disparity evident in such counts for respective analysts suggests experience may be a necessary consideration for analysts selected to count bacterial colonies.

In summary, the conclusions from this study are: (*a*) the

present method of preparing plates described in *SMEDP* (*3*) can result in a uniform preparation of agar plates by analysts with various levels of experience, (*b*) 24-h refrigeration of agar plates has no significant effect on the SPC, and (*c*) the present "standards of accuracy" in *SMEDP* (*3*) are probably unrealistic.

ACKNOWLEDGMENT

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REFERENCES

1. American Public Health Association. 1960. Standard methods for the examination of dairy products, 11th edition. New York, NY.
2. American Public Health Association. 1967. Standard methods for the examination of dairy products, 12th edition. New York, NY.
3. American Public Health Association. 1972. Standard methods for the examination of dairy products, 13th edition. Washington, D.C.
4. Courtney, J. L. 1956. The relationship of average standard plate count ratios to employee proficiency in plating dairy products. J. Milk Food Technol. 10:336-344.
5. Dixon, W. J. (ed.) 1973. BMD: Biomedical computer programs. University of California Press, Berkeley, CA.
6. Donnelly, C. B., E. K. Harris, L. A. Black, and K. H. Lewis. 1960. Statistical analysis of standard plate counts of milk samples split with state laboratories. J. Milk Food Technol. 23:315-319.
7. Fruin, J. T., T. M. Hill, J. B. Clarke, J. L. Fowler, and L. S. Guthertz. 1977. Accuracy and speed in counting agar plates. J. Food Protect. 40:596-599.
8. Winer, B. J. 1971. Statistical principles in experimental design, 2nd edition. Wiley, New York. pp. 198-201, 571-577.

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Tissue Distribution and Residues of Antibiotics in Normal and Emergency — Slaughtered Dairy Cows After Intramammary Treatment

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ABSTRACT

Following intramammary infusion of normal cows with a single dry cow and four lactating-cow antibiotic formulations containing penicillin G, neomycin, dihydro-streptomycin, lincomycin and framycetin, low concentrations of drug residues were detected in the kidney, urine, and for some drugs, also in blood and the liver, during the first 24 h after treatment. Drug residues were not detected in meat. In emergency-slaughtered mastitic cows, drug levels were considerably higher and persisted for a longer period than in normal cows.

Persistence of drug residues is discussed in terms of the rate and extent of drug absorption from the udder, differences between normal and diseased cows, and effect of dosage form.

Numerous intramammary antibiotic formulations are available for prevention and treatment of bovine mastitis. The infused drugs may be absorbed from the udder at various rates and to different extents chiefly by nonionic passive diffusion mechanisms (*7,15,16,20*). It is well recognized that drug absorption, independent of dosage form, is related to the physicochemical properties of the drugs (*7,21*). In addition, the composition of vehicles or bases, in which the antibiotics are suspended or dissolved, determines the pharmaceutical availability of the drug in the udder. The physiological state of the udder, either lactating or dry, also influences drug absorption rates (*3,4,5,7,8,17,20,22,*). Available evidence suggests that in the normal udder the blood-milk barrier behaves as an inert lipoid layer to these drugs, but during udder inflammation the functional integrity of the lipoid barrier is temporarily deranged, leading to accelerated leakage of the drug from the udder into the circulatory system (*5,21*). The absorbed antibiotics may result in drug residues in edible tissues of slaughtered animals and this may have consequences on the suitability of the carcass for human consumption, as judged by official meat inspection regulations.

[1]*Meat Inspection Service, The Netherlands.*
[2]*Ministry of Agriculture, Israel.*

Although the problem was clearly defined (*5,6*), the subject has received limited attention. Van Os et al. (*14*) investigated two types of mastitis formulations for lactating cows and two "dry-cow" formulations using qualitative and quantitative assay methods as well as the *Sarcina lutea* Kidney Test of Van Schothorst (*18*) which is the official antibiotic residue test prescribed in The Netherlands. A positive *S. lutea* Kidney Test was seen 42 h after treatment with four injectors of one "dry-cow" formulation containing 300,000 I.U. of procaine penicillin, and 100 mg of nafcillin. Bäckström, [1] reported residues in the liver and kidney 24 h after the intramammary infusion of a formulation containing 25,000 I.U. of benzathine penicillin G and 50 mg of dihydrostreptomycin suspended in a paraffin oil containing 3% aluminum monostearate. Gedek and Pfeiffer (*2*) investigated the relationship between drug concentrations in serum and urine after intramammary treatment with several commercial formulations and pointed to differences between the different drugs. Nouws (*9*) could not detect residues in meat, liver, and kidneys of cows after intramammary infusion of a rifamycin SV formulation. Ziv and Nouws (*24*) reported on the limited time after treatment that dapsone residues were detected in the body organs of lactating and dry cows. With the introduction of some new antimicrobial drugs for mastitis therapy it was felt that gathering of additional data on the subject is justified.

This report describes experiments on antibiotic residues in tissues of normal and emergency-slaughtered dairy cows after eight different antibiotic formulations were infused intramammarily.

MATERIALS AND METHODS

Normal cows

Nineteen clinically normal Meuse-Rhine-Yssel and Holland Friesian dairy cows (400 to 600 kg each) which were at the end of their lactation period were used. Animals were located in a slaughterhouse and were treated once in two, three, or four quarters of the udder, as indicated in Tables 1 and 3. At intervals after treatment with products A, B, and C,

blood samples were collected from the jugular vein and serum was separated by centrifugation. Animals treated with products A, B, C, and G, were slaughtered 22½, 4½, 5, and 17.5 h after infusion, respectively. Kidneys were collected and tested with the *S. lutea* Kidney Test. Kidneys and the meat from the diaphragm muscle were processed (*11*) and tested with the *Bacillus subtilis BGA*[3] method at pH 6 and 8. After treatment with product B, the kidney was also tested with the *S. lutea* test method at pH 8.0 (*11*).

Emergency-slaughtered cows

Procedures used for selecting organs from emergency-slaughtered dairy cows were previously described (*12*). In brief, reports from veterinary practitioners submitting cows for emergency slaughter were reviewed, the clinical diagnosis and type of treatment were established, and cows receiving intramammary treatments only were selected. From these cows, samples of blood, urine, meat, and kidney were collected. The *S. lutea* Kidney Test was conducted and from cows giving positive test results to this, and the other antibiotic residue kidney tests, samples of kidney tissue were sent to the National Institute of Public Health, Bilthoven, The Netherlands, for electrophoretic identification and confirmation of the type of antibiotic involved. Altogether, findings from eight emergency-slaughtered cows, which were treated by the intramammary route and in which drug residues were identified electrophoretically were available and were included in the present report. Procedures used to prepare samples, and assay methods, were described (*10,11,12*). Samples were kept at −20 C and assays were conducted within 2 weeks of sample collection. In the assay procedure for the aminoglycoside antibiotics in tissue fluids, possible activity of benzylpenicillin was eliminated by penicillinase.

RESULTS

Results of qualitative antibiotic residue tests on samples taken from experimentally infused normal cows are in Table 2 and show that the *S. lutea* Kidney Test was consistently negative, even for cows infused 4½ and 5 h before slaughter. After treatment with product A, B, or C. (Table 1), antibiotic residues were detected in kidney medulla using the *B. subtilis* BGA test at pH 8.0. Four and a half hours after infusion of product B, which contained lincomycin and neomycin, residues were detected in the kidneys of three of the four cows. Lincomycin, however, was identified electrophoretically in all the four kidneys and neomycin in three of them. In the kidneys of cows treated with product A and C, both penicillin and dihydrostreptomycin were similarly identified, thus complementing the microbiological assay results (Table 2). Kanamycin was not identified in the kidneys of cows treated with product C. Following treatment with products F and G, drug residues were not detected in kidneys of any of the treated cows. However, urine of cows treated with these products contained measurable quantities of benzylpenicillin and cephacetrile (Table 3).

Quantitative antibiotic residue test data (Table 3) indicate that after products A and C were infused, penicillin levels were highest in urine, averaging 25.1, 2.47, and 5.69 μg/ml in samples collected 5,17½ and 22½ h later. High penicillin levels were also found in bile, and the kidney medulla contained higher drug levels than the kidney cortex. Penicillin was detected in the meat of two cows treated in three and four quarters of the udder but only in samples collected 5 h after treatment. Serum penicillin levels in cows treated with the "dry-cow" product A were generally lower than those seen after treatment with product C intended for lactating cows (Fig. 1). Residues of the aminoglycoside antibiotics could be measured quantitatively in the urine, bile, serum and kidneys but not in the muscle.

Four hours after treatment with product B, lincomycin was measured in the serum and the liver but the neomycin component of this product was undetected in these organs. Liver and kidney cortex lincomycin levels were similar but the drug was not detected in the meat.

Tissue distribution of antibiotics in the emergency-slaughtered cows is given in Table 4 according to the type of clinical and pathological findings and the table also gives the ratios of kidney cortex-to-serum, muscle drip-to-serum, and kidney cortex-to-muscle drip con-

[3]*BGA = Bundesgesundheitsamt, Berlin.*

TABLE 1. *Intramammary antibiotic products involved*

Product	Name of product	Manufacturer	Antibacterial agent(s) per injector	Treatment period	Number of cows treated
A	Mascodry[R]	Aesculaap, Boxtel	300,000 I.U. Procaine penicillin G 300 mg Dihydrostreptomycin sulphate	Drying-off	4[a]
B	Lincocin Forte[R]	Upjohn s.a., Ede.	200 mg Lincomycin HCL monohydrate 200 mg Neomycin sulphate	Lactation	4[a]
C	Kanamycen Injektor[R]	Centrafarm, Rotterdam.	200,000 I.U. Procaine penicillin G 35 mg Kanamycin sulphate 225 mg Dihydrostreptomycin sulphate 550 mg Dapsone[c]	Lactation	5[a]
D	Leo Yellow[R]	Leo, Denmark.	150,000 I.U. Penethamate hydriodide[d] 150 mg Dihydrostreptomycin sulphate 50 mg Framycetine	Lactation	2[b]
E	Mypenzal 72[R]	Mycofarm, Delft.	300,000 I.U. Sodium Penicillin G 100 mg Dihydrostreptomycin sulphate	Lactation	2[b]
F	Penzal N 300[R]	Mycofarm, Delft	300,000 I.U. Procaine penicillin G	Drying-off	2[a]
G	Celofor[R]	Ciba-Geigy, Switzerland.	Sodium cephacetrile in sterile saline	Experimental	4[a]
H	Orbenin[R] longacting.	Beecham, U.K.	200 mg Sodium cloxacillin	Lactation	1[b]

[a]Experimental treatments
[b]Mastitis treatment (emergency-slaughtered)
[c]Diamino-diphenylsulphone
[d]An ester of benzylpenicillin

centrations. Qualitative and quantitative antibiotic residue test findings are presented. Intramammary application of 1 million units of procaine penicillin or 2 million units of sodium penicillin resulted in positive qualitative test results in the kidneys but not in the meat. Sixty hours after treatment with penethamate hydriodide (a derivative of benzylpenicillin), penicillin could not be detected in the serum, meat, or kidney. A cow with amyloid nephrosis treated with a "dry-cow" product (A) 144 h later contained penicillin in the kidney in excess of

TABLE 2. *Results of several qualitative residue tests for intramammary antibiotic products*

Product	Hours after treatment	No. of cows	No. of quarters infused	S. lutea Kidney Test[a]	Muscle			Kidney cortex			Kidney medulla		
					Test methods			Test methods			Test methods		
					A	B	C	A	B	C	A	B	C
											(Diameter of inhibition zone, mm)[b]		
A	22½	4	16	—[c]	—	—	X[d]	—	—	X	4 (1) n = 3	2 (1)	X
B	4½	4	16	—	—	—	—	—	—	4 (3)	—	3 n = 1	6 (3) n = 2
C	5	1	4	—	—	—	X	—	3	X	—	7	X
	5	1	3	—	—	—	X	—	3	X	—	9	X
	5	1	2	—	—	—	X	—	—	X	—	4	X
	17½	1	4	—	—	—	X	—	6	X	—	3	X
	17½	1	2	—	—	—	X	—	4	X	—	2	X
F	144	2	8	—	—	—	X	—	—	X	—	—	X
G	17	4	16	—	—	—	—	—	—	—	—	—	—

[a]The official Van Schothorst Kidney Test, including 12.7-mm diameter of the paper disc, mean ± S.D. (in brackets)
[b]Mean ± S.D. in brackets
[c]No inhibition zone
[d]Not done. Test method A = *B. subtilis* BGA at pH 6.0, Test method B = *B. subtilis* BGA at 8.0; Test Method C = *S. lutea* test at pH 8.0
X = Not done.

TABLE 3. *Concentrations of antibiotic residues in tissues of normal dairy cows after intramammary infusion*

Product	Hours after treatment	No. of cows	No. of quarters infused/cow	Antibiotic infused	Dose/cow[h]	Kidney		Muscle		Liver	Serum	Urine	Bile
						Cortex	Medulla	Homogenized	Drip				
						(Concentration, I.U. or μg/ml or g tissue)[a]							
A	22½	4	4	p[b]	1200	0.048 (0.016)	0.077 (0.039)	—	—	X	0.013 (0.003)	5.69 (2.96)	1.09 (1.75)
				dhs[c]	1200	1.23 (0.19) n = 2	0.94 n = 1	—	—	X	—	12.98 (7.32)	0.14 n = 1
B	4½	4	4	linc[d]	800	1.01 (0.43) n = 3	0.73 (0.33)	—	—	1.10 n = 1	0.13 n = 1	3.44[f] (1.04)	0.18 n = 1
				neo[e]	800	1.98 n = 1	1.05 n = 1	—	—	—	—	8.42[f] (5.42)	—
C	5	1	2	p	400	—	0.20	—	—	—	0.014	17.5	1.65
	5	1	3	p	600	0.18	1.08	—	0.011	—	0.031	34.6	1.84
	5	1	4	p	800	0.11	0.45	—	0.058	—	0.029	23.2	1.09
	17½	1	4	p	800	—	—	—	—	0.52	0.031	0.96	3.78
	17½	1	2	p	800	0.055	0.24	—	—	0.69	0.008	3.9	0.17
	5	1	2	dhs	450	0.67	0.63	—	—	—	0.17	8.9	0.13
	5	1	3	dhs	675	0.45	0.57	—	—	0.46	0.13	22.9	0.16
	5	1	4	dhs	900	0.57	0.87	—	—	T	0.16	16.7	0.26
	17½	1	4	dhs	900	1.34	1.11	—	—	—	0.17	49.3	0.69
	17½	1	2	dhs	450	0.77	0.80	—	—	—	—	11.2	0.39
F	144	2	4	p	1200	—	—	—	—	—	—	0.25 (0.28)	—
G	17	4	4	cep[g]	1000	0.84 (0.34)	X	—	—	—	—	19.9 (7.0)	—

[a]Mean ± s.d. (in brackets)
[b]p = Procaine penicillin G
[c]Dihydrostreptomycin
[d]Lincomycin HCl
[e]Neomycin sulphate
[f]Lincomycin and neomycin present
[g]Sodium cephacetrile
[h]Dose in I.U. (× 1000) or mg per cow
T = trace
X = Not done;
— = Not detectable

TABLE 4. *Tissue distribution of antibiotics after intramammary infusion in emergency-slaughtered dairy cows*

Cow No.	Clinical and pathological findings	Antibiotic infused	Dose per cow	Time after treatment[h]	*S. lutea* kidney Test[b]	*B. subtilis* BGA (pH 6)			*B. subtilis* BGA (pH 8)			Antibiotic concentration				Penicillin concentration ratios[c]
						Kidney cortex	Kidney medulla	Meat	Kidney cortex	Kidney medulla	Meat	Kidney cortex	Muscle drip	Serum	Urine	
						Inhibition zone, in mm.						Conc. in I.U. or μg/ml or g.				
1	Mastitis; normal kidney. Intramammary (mam): Na-Penicillin G	p[d]	2.5[a]	30	40	X	X	—	X	X	—	1.83	0.021	X	X	C=88
2	Mastitis; dead; Intramammary: 1 × Leo Yellow[R]	p	150 mg	8	43	14	13	7	12	12	6	7.87	0.037	1.64	1.05	A=4.8;
												12.8	—	1.52	13.1	B=0.023;
	(Penethamate Hydriodide)	dhs[e]	150 mg													C=213.
3	Parturient; acute mastitis 3 quarters.	p	0.9[a]	9½	39	10	18	9	15	17	11	2.02	0.34	1.04	X	A = 1.9;
	3 × Mypenzal-72[R]	dhs	300 mg									3.7	—	—	—	B = 9.3;
																C=5.9
4	Abortion; antepartum mastitis; pH meat was 6.8; Intramammary: 1 × Leo Yellow[R]	p	150 mg	60	—	4	5	—	4	6	—	—	—	—	0.6	
	(Penethamate Hydriodide)	dhs	150 mg									12	—	—	3.2	
5	Parturient; acute mastitis 1 quarter;	p	7.5[a]	10½	58	18	21		4	7		18.02	0.96	1.76	X	A=10.5;
	dead; septichaemia; meat pH shoulder: 6.1							6			7		(homoge-)			B=0.55;
	meat pH hind quarter: 6.8							—			—		(nate)			C=19.0
	Intramammary: Procaine Penicillin G Kanamycin	kana	2.5 g									103.1	1.25	5.01	X	(Expressed as
	Dihydrostreptomycin	dhs	2.5 g													kanamycin con.)
6	Acute mastitis; "blue udder"; T 41 C; Lying down; somnolent. Intramammary:	p	0.3[a]	9	55	17	18	7	17	20	5	12.78	0.298	0.49	234	A=26.5 B = 1.1
	1 × inj. Mypenzal-72[R]	dhs	100 mg									18.5	—	—	X	C=25.8.
7	Hydraemic cow; amyloid nephrosis: Intramammary: 4 × inj. Mascodry[R]	p	1.2[a]	144	27	11	14	—	8	6	—	1.03	—	X	9.5	C>103
	(drying-off)	dhs	1200 mg									27.2	—	—	X	
8	Acute mastitis; lying down; ; meat pH 6.8; Orbenin longacting[R] Sodium cloxacillin		200 mg	6	25	—	3	—	—	3	—	0.152	—	0.039	X	

(—) No inhibition zone or antibiotic not detectable. (X) = Not done.

[a]Approximate does in I.U. (times 10^6)

[b]The official Van Schothorst Kidney Test, including 12.7 mm diameter of the paper disc.

[d]p = Penicillin G (Procaine or Sodium salt) or Penethamate Hydriodide. e.

[e]dhs = Dihydrostreptomycin

[c]Concentration ratios: $A = \frac{\text{Kidney Cortex;}}{\text{Serum}}$ $B = \frac{\text{Muscle drip}}{\text{Serum}}$; $C = \frac{\text{Kidney cortex}}{\text{Muscle drip}}$

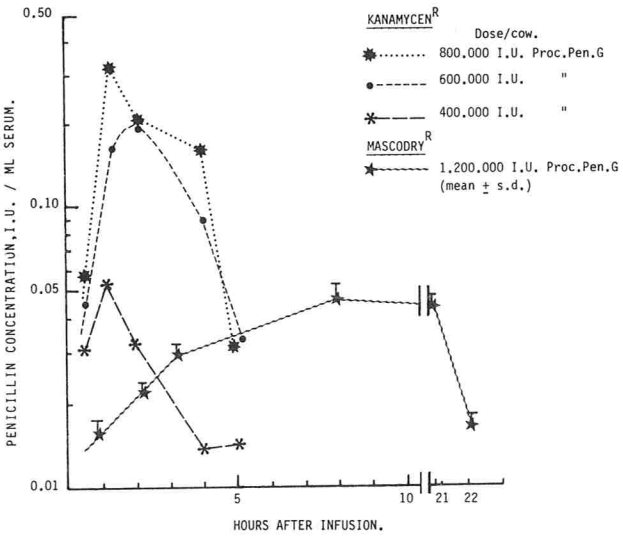

Figure 1. *Penicillin serum concentrations after intramammary infusion of Kanamycen*^{R} *(mastitis formulation) and Mascodry*^{R} *(drying-off formulation) in dairy cows.*

1.0 I.U./g and very high kidney dihydrostreptomycin levels were found (cow No. 7). These drugs, however, were not found in the meat. A cow with acute mastitis (cow No. 4) contained 12 μg of dihydrostreptomycin/g of kidney cortex 60 h after treatment with 150 mg of the drug and the concentrations of aminoglycosides in the kidney cortex were equivalent to 103.1 μg/g 10 h after a cow with acute mastitis (cow No. 5) was treated with 5 g of these drugs. Six hours after cloxacillin was infused into the udder of a cow with acute mastitis (cow No. 8), the *S. lutea* Kidney Test was positive but the drug was not detected in the meat.

DISCUSSION

Results of the present investigation can be analyzed in terms of (*a*) differences among drugs with respect to their distribution and persistence in the body, (*b*) differences between normal and emergency-slaughtered animals in the distribution and persistence of a given drug, and (*c*) the effect of dosage form. Previous studies have shown that when antibiotics are readily available for distribution, i.e. after intravenous and intramuscular administration, they differ in their persistence in the various organs of the body and the state of residues depends on their kinetic properties and the sensitivity and specificity of the assay methods (*11,12,13*). After intramammary treatment, differences in drug availability for distribution throughout the body, i.e. absorption from the udder, assume an additional major role which influences the extent of drug residues. Among the antibiotics investigated, lincomycin and penethamate hydriodide were most readily absorbed (*20*), followed by cephacetrile (*23*), cloxacillin, and benzylpenicillin (*20,21*). The aminoglycoside antibiotics were absorbed at a considerably slower rate than the former antibiotics (*20,21*). Following intramammary infusion of dapsone to goats (*25*), urinary dapsone recovery was equal to that of a similar intramuscular dose. On the other hand, only a small fraction of the intramammarily administered dose of sulfadiazine was recovered in the urine of goats, compared to recovery of a similar dose of the drug administered intramuscularly (*26*). Since the amount of drug given by the intramammary route is generally much smaller than the dose applied intravenously or intramuscularly, even extensively absorbed drugs are likely to persist in the body organs only for short periods after their intramammary infusion. Data in Table 2 support this conclusion. The *S. lutea* Kidney Test was negative 5 h after treatment with conventional intramammary doses of lincomycin, neomycin, kanamycin, penicillin G, dihydrostreptomycin, and penethamate hydriodide. Residues of these drugs were detected in the kidney with *B. subtilis* BGA test method but actual concentrations measured were very low indeed, and the drugs were not detected in the meat (Table 3), except after 600.00 I.U. and 800.000 I.U. of penicillin G were infused. In this respect, distribution of the drugs in the body organs after intramammary treatment was very similar to that observed after intramuscular administration (*11,12,13*).

These drugs generally persisted in the body of emergency-slaughtered dairy cows for a longer period than in normal cows (Table 4). Thus, penicillin G was detected in the meat 8 h after the penethamate form was administered to a cow with acute mastitis (Cow No. 2). Penicillin G was also detected in the meat 9½ and 30 h but not 60 h after treatment of cows with acute mastitis Breakdown in the integrity of the blood-milk barrier resulting in increased drug absorption is expected to occur in cows with acute mastitis. In addition, it was already shown that drug persistence in the body is prolonged in cases of severe toxemia (*12,13*). These two factors, therefore can account for the increased duration in persistence of the drug residues in the kidney and the meat of cows during severe disease conditions even though the total dose of the antibiotic administered by the intramammary route is rather low.

It is noteworthy that penicillin G was not detected in the muscle of one cow (No. 5) when the pH of the muscle at one region was 6.8. The drug was detected qualitatively in another piece of muscle from the same cow with pH 6.1. This observation may suggest that the pH of the meat can influence the sensitivity of the antibiotic test methods. For acidic drugs like penicillin, better sensitivity can be expected at lower pH values. Moreover, the quantity of muscle drip available from a piece of meat is lower at pH 6.8 compared to the quantity obtained at pH 6.1. The effect of dosage form on rate of drug absorption from the udder is illustrated in Fig. 1. Pencillin G was absorbed very slowly from the udder after intramammary infusion of the slow release "dry-cow" product. Although very low blood levels were detected with this product, the drug persisted in the serum during at least 22 h after treatment. Serum

penicillin concentrations were clearly dose-dependent after progressively greater doses of the drug in a more rapid-release formulation were infused (Fig. 1). The three factors, i.e. nature of the drug, disease state, and type of formulation, all markedly influence persistence of antibiotic residues in the carcass after intramammary infusion.

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REFERENCES

1. Bäckström, G. 1974. Restförekomst av antibiotika hos kor vid sintidsbehandling med långtidsutsöndrande antibiotika. Svensk Veterinärtidning 36:103-106.
2. Gedek, W. and U. Pfeiffer. 1974. Rückstände im Tierkörper nach lokaler antibiotischer Mastitistherapie beim Rind. 16 Arbeitstagung Lebensmittelygiene, Garmisch-Partenkirchen: 35-38.
3. Jepsen, A. 1950. Penicillinterapi mod yverinfektioner. Nord. Vet.-Med. 2:447-480.
4. Mercer, H. D. 1970. Milk-out rates for antibiotics in intramammary infused products in the treatment of bovine mastitis. Relationship of somatic cell counts, milk production level, and drug vehicle. Am. J. Vet. Res. 31:1549-1560.
5. Mercer, H. D., J. N. Geleta, and G. Carter. 1974. Absorption and excretion of penicillin G from the mastitic bovine udder. J. Am. Vet. Med. Assoc. 164:613-616.
6. Mercer, H. D. 1975. Antimicrobial drugs in food-producing animals. Vet. Clin. North Am. 5:3-35.
7. Mercer, H. D. 1976. Viewpoint and current concepts regarding accepted and tried products for control of bovine mastitis. J. Am. Med. Assoc. 169:1104-1114.
8. Mol, H. 1974. Antibiotics and milk. Thesis State University of Utrecht.
9. Nouws, J. F. M. 1975. De pharmacokinetiek van Rifamycine SV in runderen na intramammaire toediening van Rifamastene[R]. Tÿdschr. Diergeneesk. 100:539-545.
10. Nouws, J. F. M., and G. Ziv. 1976. The effect of storage at 4 C on antibiotic residues in kidney and meat tissues of dairy cows. Tÿdschr. Diergeneesk. 101:1145-1153.
11. Nouws, J. F. M., and G. Ziv. 1977. Tissue distribution and residues of beta-lactam antibiotics in normal dairy cows. Tÿdschr. Diergeneesk. 102:1173-1186.
12. Nouws, J. F. M. and G. Ziv. 1977. Tissue distribution and residues of benzylpenicillin and aminoglycoside antibiotics in emergency-slaughtered ruminants. Tÿdschr. Diergeneeks. 103: (Accepted for publication).
13. Nouws, J. F. M., and G. Ziv. 1977. Tissue distribution and residues of tetracyclines in normal and emergency-slaughtered ruminants. Tÿdschr. Diergeneesk. 103:(In press).
14. Os, J. L. van, P. Van Harten, and P. Meyer. 1973. Onderzoek naar de aanwezigheid van residuen in nierschors en serum met betrekking tot de "niertest" na behandeling met penicillinebevattende preparaten. Tÿdschr. Diergeneesk. 98:881-890.
15. Rasmussen, F. 1966. Studies on the mammary excretion and absorption of drugs. Carl Fr. Mortensen, Publ. Copenhagen, Denmark.
16. Rasmussen, F. 1971. Excretion of drugs in milk. In B. B. Brodie and J. R. Gillette (eds). Handbook of experimental pharmacology. Vol. 28. Springer Publ. Berlin.
17. Rollins, L. D., H. D. Mercer, G. G. Carter, and J. Kramer. 1970. Absorption, distribution, and excretion of penicillin and dihydrostreptomycin in dairy cows following intramammary infusion. J. Dairy Sci. 53:1407-1414.
18. Schothorst, M. van. 1969. Residuen van antibiotica in slachtdieren. Thesis, State University of Utrecht.
19. Schothorst, M. van, G. Peelen-Knol. 1970. Detection and identification of some antibiotics in slaughtered animals. Neth. J. Vet. Sci. 3:85-93.
20. Ziv, G. and F. G. Sulman. 1975. Absorption of antibiotics by the bovine udder. J. Dairy Sci. 58:1637-1644.
21. Ziv. G. 1975. Pharmacokinetic concepts for systemic and intramammary antibiotic treatment in lactating and dry cows. Proc. IDF Seminar on Mastitis Control. Reading, England, pp. 314-340.
22. Ziv, G., and R. Rasmussen. 1975. Distribution of labelled antibiotics in different components of milk following intramammary and intramuscular administration. J. Anim. Sci. 58:938-946.
23. Ziv, G., and J. F. M. Nouws. 1977. Clinical pharmacology of cephacetrile in ruminants. Zbl. Vet. Med. B. (In Press).
24. Ziv. G., and J. F. M. Nouws. 1977. Dapsone residues in milk and organs of cows after intramammary and intrauterine administration. Brit. Vet. J. (In press).
25. Ziv, G., A. Dekker, and R. Hageman. 1978. Absorption, distribution and elimination kenetics of ^{35}S-dapsone in lactating goats. Res. Vet. Sci. (Accepted for publication).
26. Ziv. G., A. Dekker, and R. Hagemann. 1978. Pharmacokinetics of sulfadiazine in the lactacting goat. Zbl. Vet. Med. (Accepted for publication).

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A Research Note

A Test to Determine Microquantities of Milkfat in Cleaning Solutions by Surface Film Displacement[1]

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ABSTRACT

Determining microquantities of soil is a major challenge in evaluating cleaning systems. A highly sensitive test was therefore developed to determine trace quantities of milkfat in cleaning solutions. It involved extraction of cleaning solution with organic solvents and subsequent determination of surface film displacement. The area displaced was directly related to the quantity of milkfat in the test solution with a correlation coefficient of 0.99. Usefulness of the test in evaluating cleaning systems has been shown in other research.

Residual soil on food processing equipment is indirectly related to the effectiveness of soil removal process and quantity of soil in cleaning solutions (*2,7,8*). Each of these phenomena is important in food plant sanitation, and a highly sensitive test for their evaluation is needed.

Selecting a specific constituent or class of constituents of soil and establishing a sensitive method for its determination seemed a logical approach. The milkfat fraction is one of the most tenacious fractions of milk soil (*1,9*). This fraction was therefore chosen for further study. The following two experimental approaches were taken to increase recovery and sensitivity of quantification: (*a*) extraction of cleaning solution by certain modifications of the well-known organic solvent system of the Roese-Gottlieb method (*10*), and (*b*) surface film displacement (*3,4,5*).

MATERIALS AND METHODS

Extraction and concentration

Samples of alkaline cleaner containing added milkfat, with and without the phospholipid fraction, were used as a model system in development of this method. A 100-ml sample was used for extraction with organic solvents of the Roese-Gottlieb method (*10*), employing 30 ml of ethanol, 30 ml of diethyl ether, and 30 ml of petroleum ether for the first extraction. However, when the cleaning solution contained other organic material, it was necessary to increase the quantities of solvents to facilitate rapid phase separation. The highest quantities used for the first extraction were 100 ml of ethanol, 50 ml of diethyl ether, and 50 ml of petroleum ether. For a second extraction up to 50 ml of diethyl ether and 50 ml of petroleum ether were used. No ethanol was added for the second extraction.

Solvents were removed by complete evaporation on a rotary evaporator. Removal of alcohol and diethyl ether was necessary to prevent interference with spreading of the film on the polar solvent. The milkfat was then resuspended in a known quantity of petroleum ether for dispensing and surface film displacement.

The arrangement of equipment for making surface film displacements is shown in Fig. 1. The assembly was similar to that used by Berquist and Wells (*3*). A 0.2% solution of acetic acid served as the base in a glass pie plate (40 mm deep and 200 mm in diameter), which was painted black on the outside. Reflected light provided the best resolution of the line of demarcation between the film of milkfat and the burned oil. Air currents were disruptive, and therefore were avoided.

Burned oil was applied to a pie plate full of the acetic acid solution by means of a syringe with a 23-gauge needle, while holding the needle within a few millimeters of the surface. The endpoint of adding oil to attain a standard film tension was judged visually as a 3rd order of green, which was determined as a sequence of color changes. The first drop of oil contacting the polar solvent spread rapidly as a flash of colors. Further additions of oil gave progressions of color from purple-violet through green, which was considered the 2nd order of green. Still further addition of oil gave progressions of color from purple-violet through green, which was recognized as the 3rd order of green, the usable endpoint. Addition of excess oil through additional color cycles gave a dull green and less responsive spread.

The burned oil, while seemingly simple, must be chosen and prepared with care. Earlier literature indicated a "good" oil should be used. However, we found that a non-detergent highly saturated hydro-carbon was required. In this work a household oil (Handy Oil, Mobil Oil Co.), sometimes known as machine oil, was used. This oil was oxidized by heating for 7 h at 150 C with a stream of air bubbling rapidly through it constantly.

A sample to be tested was added, as described above, to the prepared oil film, thereby displacing the green film. A glass cover was placed over the tray containing the pie plate and a tracing was made of the displaced film. Area of displacement was measured with a planimeter.

For repeated tests, the pie plate was cleared of the used oil film by passing a straight piece of glass over the surface, followed by cleaning of the glass with a paper tissue after each passage.

RESULTS AND DISCUSSION

To determine the sensitivity and reliability of the surface film method, known quantities of milkfat were used. These were dissolved in petroleum ether for direct addition for film displacement. In addition, known quantities of milkfat were suspended in an alkaline

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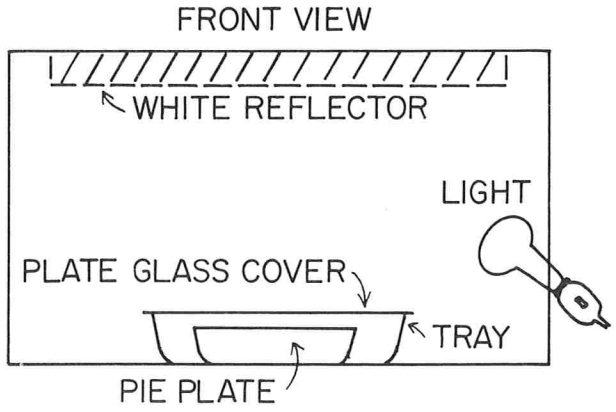

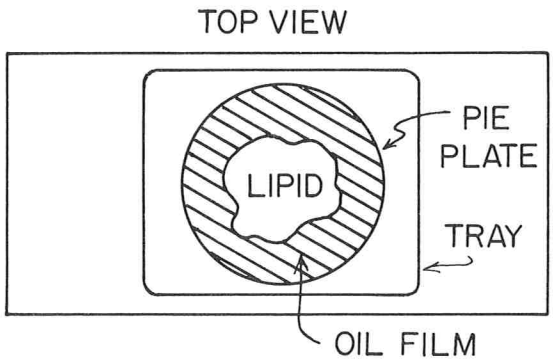

Figure 1. *Two views of equipment for making surface film displacement tests.*

cleaner, extracted with organic solvents, evaporated to dryness, resuspended in petroleum ether, and observed for film displacement. There was no apparent difference between the results with milkfat with and without phospholipids, therefore no distinction in results was made. The relation between quantity of milkfat and surface film displacement is shown in Fig. 2. Based on the method of least squares, a 0.99 correlation coefficient was obtained for known quantity of milkfat and area of surface displacement. The sensitivity was approximately 1 μg of milkfat.

Combination of an organic solvent extraction technique and surface film displacement provided a method with analytical sensitivity at least 100 times greater than our previously used gravimetric methods (*7*).

The usefulness of the test has been shown in research work relating to soil deposition, removal, and contamination of cleaning solutions. The test was used to determine

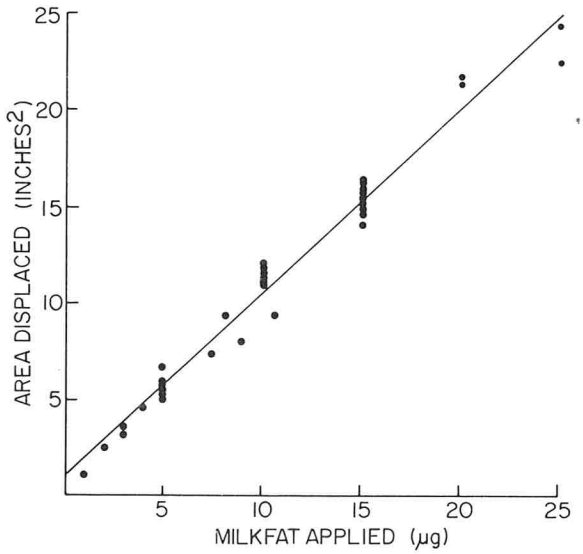

Figure 2. *The relationship between quantity of lipoidal material and area of displacement of an oil film.*

the rate of removal of soil in cleaning a bench-type heating and circulation system. It was also used to compare acid and alkaline cleaning systems as well as to study the useful life of cleaning solutions (*6*).

REFERENCES

1. Arnold, R. G., and R. B. Maxcy. 1970. Evaluating circulation cleaning by analysis of soil depletion from surfaces. J. Dairy Sci. 53:1540-1544.
2. Burton, H. 1968. Deposits from whole milk in heat treatment plant — A review and discussion. J. Dairy Res. 35:317-330.
3. Berquist, D. H., and F. Wells. 1956. The monomolecular surfaces film method for determining small quantities of yolk or fat in egg albumen. Food Technol. 10:48-50.
4. Heinemann, B., and M. R. Rohr. 1950. A micromethod for routine determinations of fat in skimmilk and nonfat dry milk solids. J. Dairy Sci. 33:703-709.
5. Jones, K. K. 1950. A micromethod for fat analysis based on formation of monolayer films. Science 111:9-10.
6. Kulkarni, S. M. 1974. Microcontaminants of food processing equipment. Ph. D. Thesis. University of Nebraska, Lincoln, Nebraska.
7. Kulkarni, S. M., R. G. Arnold, and R. B. Maxcy. 1975. Reuse limits and regeneration of solutions for cleaning dairy equipment. J. Dairy Sci. 58:1095-1100.
8. Kulkarni, S. M., R. B. Maxcy, and R. G. Arnold. 1975. Evaluation of soil deposition and removal processes: An interpretive review. J. Dairy Sci. 58:1922-1936.
9. Maxcy, R. B., and R. G. Arnold. 1974. Characterization of tenacious residue of alkaline circulation cleaning. J. Milk Food Technol. 37:469-472.
10. Newlander, J. A., and H. V. Atherton. 1964. The chemistry and testing of dairy products. 3rd ed. Olsen Publishing Co., Milwaukee, Wisconsin.

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Survival of Selected Organisms During Spray Drying of Skim Milk and Storage of Nonfat Dry Milk[1]

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ABSTRACT

Pasteurized concentrated skim milk containing 35 to 40% total solids was inoculated with cultures of *Bacillus subtilis, Micrococcus flavus,* or *Escherichia coli* to contain 1×10^6 organisms per g and spray-dried to determine the effect of exit air temperatures of 93.3, 82.2, and 71.1 C on survival of the organisms and moisture content of the finished product. The numbers of survivors increased as the drying temperature decreased. The percent survivors varied from 27.57 in the product made from milk inoculated with *M. flavus* and dried at 71.1 C to 0.02 in the product made from milk inoculated with *E. coli* and dried at 93.3 C. The organism most resistant to drying and most persistent during storage was *B. subtilis,* followed by *M. flavus* and *E. coli,* with the latter showing low survival during drying and abrupt die-off during the first 4 weeks of storage. The moisture content of the dry milks varied from 2.75 to 4.80% with low moisture associated with high drying temperature.

In 1976, 426,000,000 kg of nonfat dry milk (NDM) was manufactured in the United States (*1*). Short shelf-life in fluid dairy products fortified with NDM has created an interest in the effect of spray drying and storage on viability of organisms commonly found in dry milks.

Investigations by Crossley and Mattick (*6*) showed that the microflora of spray-dried milk was influenced by the initial population in the milk, thermal treatment before and during drying, sanitation of equipment, and post-drying contamination. Crossley and Johnson (*5*) and Findlay et al. (*8*) emphasized the desirability of using the highest drying temperature possible without causing physical damage to the dry milk to accomplish maximum microbial destruction. A decrease in microbial content normally occurs during prolonged storage of dry milks, with the rate of die-off influenced by moisture content, storage temperature, and type of organisms present (*4,5,7,8,10*). Naguib et al. (*14*) identified the predominant organisms isolated from plate counts of dry milk as streptococci, micrococci, microbacteria, sarcina, and sporeforming bacilli. Foster et al. (*9*) and Keogh (*12*) stated the microbial content of dry milks is influenced more by type of organism than by number in the original milk, as spores and thermodurics tend to survive the drying process and are resistant to die-off during storage. Higginbottom (*11*) suggested a direct relationship existed between the moisture content of dry milk, storage temperature, and microbial survival, with good product containing between 3.0 and 5.0% moisture.

The investigation reported herein was conducted to determine the survival of *Bacillus subtilis, Micrococcus flavus,* and *Escherichia coli* inoculated into concentrated skim milk which was spray dried under various conditions. These organisms were selected to include a sporeformer, a vegetative cell resistant to heat and desiccation, and a thermally sensitive vegetative cell, all of which frequently occur in raw milk.

MATERIALS AND METHODS

Preparation of cultures

Stock cultures of *B. subtilis, M. flavus,* and *E. coli* were selected from the culture collection of the Department of Food Science and Human Nutrition at Michigan State University. *E. coli* was activated in nutrient broth at 37 C while *B. subtilis* and *M. flavus* were activated in Trypticase Soy Broth at 25 C. The cultures were transferred daily and incubated on a gyrotary shaker at 150 rpm for 3 consecutive days before inoculation into the concentrated milks.

Preparation of the concentrated milk

Fifty-gallon batches of whole milk from the Michigan State University Dairy Plant were separated into cream and skim milk. The skim milk was pasteurized at 62.8 C and concentrated in a vacuum pan to a ratio of approximately 4.3 to 1 or about 10 gallons containing 35 to 40% total solids. Since it was desired to reduce the natural bacterial content as much as possible before inoculation, without altering the physical condition of the milk, a hydrogen peroxide-catalase treatment (*15,17*) was used as an adjunct to pasteurization. The concentrated milk was warmed to 48.9 C and sufficient H_2O_2 to give a 0.05% concentration was added for a contact time of 20 to 30 min. The milk was then cooled to 37.7 C and an excess of catalase was added to decompose the H_2O_2. The KI test (*17*) for presence of H_2O_2 was repeated on the milk at 5-min intervals until complete dissipation of H_2O_2 was assured. The 10-gallon batches of concentrated milk were then inoculated with 24-h-old cultures of *E. coli, B. subtilis,* or *M. flavus* to provide approximately 1×10^6 organisms/g of milk. The *B. subtilis* cultures showed approximately 10 to 20% sporulation when inoculated.

[1]*Michigan Agricultural Experiment Station Journal Article NO. 7931.*

Spray drying

The milk was dried on a small commercial vertical down-draft, direct gas fired stainless steel spray dryer with a 55-ft tower manufactured by the Marriot Walker Corporation in Detroit, Michigan. The milk was introduced at 38 C with a high pressure pump through a high pressure spray nozzle. Each batch of condensed milk was divided into three portions which were dried at exit air temperatures of 93.3, 82.2, and 71.1 C. The operating conditions are reported in Table 1. To minimize the possibility of contamination or the adventitious effect of temperature, the first batch was always dried at 93.3 C, followed by 82.2, and 71.1 C.

Sampling and determining moisture

Representative samples of each batch of dry milk were collected in sterile glass jars and sealed to avoid change in moisture content. The amount of moisture in each sample was determined by the Karl Fischer titration using a Beckman model KF2 aquameter equipped with duo-platinum electrode. Sodium tartrate dihydrate containing 15.66% water was used as the standard for determining the titer of the Fischer reagent (*16*).

Microbiological analyses

All dry milk samples were analyzed according to procedures described in *Standard Methods for the Examination of Dairy Products* (*3*). Small glass beads in the phosphate buffered dilution blanks facilitated solubilizing the dry milk. Within 2 to 4 h after each batch was made, representative samples were plated to determine initial populations. Dry milk samples made from milk inoculated with *E. coli* were plated on Violet Red Bile Agar with an agar overlay and incubated at 35 C for 24 h. Samples inoculated with *M. flavus* or *B. subtilis* were plated on Standard Methods Agar (SMA) and incubated at 32 C for 48 h. Samples of all batches of product were stored in sterile glass jars at 25 C and the bacterial population determined at frequent intervals for 32 to 36 weeks.

RESULTS AND DISCUSSION

The total aerobic plate counts on typical batches of pasteurized concentrated milk after treatment with hydrogen peroxide and catalase were approximately 300, 290, and 240/g in the milks subsequently inoculated with *E. coli, M. flavus,* and *B. subtilis,* respectively. The above numbers of residual organisms had little effect on the population in the finished product since the inocula were approximately 1×10^6/g of concentrated milk.

Effect of spray-drying temperature on the bacterial content of the NDM

Data in Table 2 show the effect of spray-drying on bacterial content of the dry milks. The concentration ratio between the condensed milk and the NDM was determined by dividing the percent solids in the NDM by the percent solids in the concentrated milk. The population expected in the dry milk, if no destruction occurred, was calculated by multiplying the population in the condensed milk by the concentration ratio. The percent of organisms surviving spray drying was then computed by dividing the number of actual survivors by the number expected if no destruction occurred.

In all batches of NDM the percentage of survivors increased as the exit air temperature in the dryer decreased from 93.3 to 71.1 C (Table 2). The spore-forming *B. subtilis* was much more resistant to destruction during drying at 93.3 and 82.2 C than the non

TABLE 1. *Physical conditions used to prepare nonfat dry milk from concentrated milk inoculated with organisms and dried at temperatures as indicated. (Spray nozzle type SX, insert 65 and core 17A.)*

Physical condition	Concentrated milk with 37% T. S. inoculated with *B. subtilis*			Concentrated milk with 35% T. S. inoculated with *M. flavus*			Concentrated milk with 40% T. S. inoculated with *E. coli*		
Exit air (C)	93.3	82.2	71.7	93.3	82.2	71.1	93.3	82.2	71.1
Inlet air (C)	162.7	154.9	146.1	135.0	127.8	115.6	142.2	140.5	129.8
Ambient air (C)	23.9	23.9	23.9	31.1	31.1	31.1	23.9	23.9	23.9
Atomizing pressure (PSI)	1000	2100	2550	1200	2300	2600	1150	2300	3000
Pump vari-drive setting	0.9	3.2	3.5	1.8	3.2	3.3	1.0	3.0	3.6
Gas pressure (PSI)	4.4	4.6	4.4	5.0	3.0	3.9	4.0	4.0	3.5
ASME nozzle (inches)	1.3	1.3	1.3	1.3	1.3	1.3	1.5	1.5	1.5
Moisture in finished NDM (*2*)	3.06	3.70	4.70	2.75	3.18	4.68	3.00	4.60	4.80

TABLE 2. *Effect of various temperatures used in spray-drying concentrated skim milk on destruction of* B. subtilis, M. flavus *and* E. coli *when the concentrated milk was inoculated with* 1×10^6 *organisms/g.*

Culture used to inoculate	Air temp (C) Exit	Air temp (C) Inlet	% T.S. in conc. milk	% Moisture in NDM	Concentration ratio	Population expected in NDM if no destruction	Number of survivors in the NDM	% Survivors
B. subtilis	93.3	162.7	37	3.06	2.62	2.62×10^6	3.1×10^5	11.83
	82.2	154.5	37	3.70	2.60	2.60×10^6	3.8×10^5	14.62
	71.1	146.1	37	4.70	2.57	2.57×10^6	$5.7\ ^7\ 10^5$	22.18
M. flavus	93.3	135.0	35	2.75	2.77	2.77×10^6	3.1×10^4	1.12
	82.2	127.8	35	3.18	2.76	2.76×10^6	6.8×10^4	2.46
	71.1	115.6	35	4.68	2.72	2.72×10^6	7.5×10^5	27.57
E. coli	93.3	142.2	40	3.00	2.42	2.42×10^6	5.0×10^2	0.02
	82.2	140.5	40	4.60	2.38	2.38×10^6	5.1×10^3	0.21
	71.1	129.5	40	4.80	2.38	2.38×10^6	1.1×10^4	0.46

spore-forming organisms, but at 71.1 C the thermoduric *M. flavus* survived in greater numbers than did *B. subtilis*. *E. coli* was quite sensitive to the thermal exposure encountered during drying with destruction in excess of 99.54% in all cases. Another corresponding series of batches of dry milks (data not included) produced results which were markedly similar in all categories to the data in Table 2. *B. subtilis* and *M. flavus* are examples of a spore-former and none spore-former, respectively, which are common in raw milk and possess substantial resistance to the thermal process used during spray drying, as indicated by survival percentages of 22.18 and 27.57. These results substantiate previous reports (*9,12*) that the microbial content of dry milk is influenced by the type of organisms in the original milk. The substantial increase in survivors of *M. flavus* at 71.1 C compared to 82.2 C is attributed to the fact that *M. flavus* resists thermal destruction at temperatures up to about 80 C. Also, at 71.1 C, *M. flavus* has much more thermal resistance than *E. coli* and the vegetative cells of *B. subtilis*, as indicated by the data in Table 2. The inocula used in this experiment are greater than populations normally encountered in good quality concentrated milk but the numbers are reasonable for milk which has been carelessly handled. Admittedly, random contamination would not involve this magnitude of populations of pure cultures.

Bacterial standards published by the American Dry Milk Institute (*2*) permit maximum total aerobic plate counts of 50,000/g for Extra grade and 100,000/g for Standard grade NDM. The maximum permissible coliform count is 10/g. Moisture limitations are 4% for Extra and 5% for Standard grade. The only batch of NDM produced in this experiment which would comply with Extra grade standards is the one inoculated with *M. flavus* and dried at 93.3 C (Table 2). Obviously it is desirable to operate dryers at temperatures as high as consistent with good physical characterisitics in the finished dry milk product. It is also apparent that when milk with high initial populations of thermally resistant organisms is dried there may be sufficient survivors to represent a potential quality hazard when the product is reconstituted and used in food products.

Effect of moisture content and storage on the bacterial content of spray-dried NDM.

In all batches of dry milk produced in this experiment the percentage of die-off during storage at 25 C for 32 or 36 weeks increased as the number of organisms initially present increased and as the exit air temperature during drying decreased, with the amount of die-off varying amoung organisms (Table 3). *E. coli* decreased abruptly during the first 4 weeks while *B. subtilis* and *M.* flavus decreased gradually. When dry milks manufactured at exit air temperatures of 93.3 and 71.1 C are compared, the numbers of *B. subtilis, M. flavus,* and *E. coli* surviving the drying operation were 1.82, 22, and 25 times greater, respectively, at 71.1 than at 93.3 C. These results agree with a report by Keogh (*13*) indicating that the heat treatment the milk receives during drying does not eliminate all bacteria and most organisms present in dry milk are spore-formers or thermally resistant vegetative types such as micrococci or microbacteria. Also, Crossley (*4*) showed decreases of 50% in bacteria in dry milk stored 1 month in some instances, whereas in other instances there was little reduction in 6 months, thus leading to the conclusion that die-off depended on the nature of the organisms.

Data in Table 3 show the relationship between spray-drying at various exit air temperatures and moisture in the final product. The percent moisture varied from 2.75 to 4.80 with low moisture associated with high drying temperatures. All dry milk manufactured at an exit air temperature of 93.3 C and two of the three

TABLE 3. *Bacterial content of skim milk spray-dried at 93.3, 82.2 and 71.1 C during storage at 25 C for 32 to 36 weeks.*

Exit air temp (C)	93.3	82.2	71.1	93.3	82.2	71.1	93.3	82.2	71.1
% moisture	3.06	3.70	4.70	2.75	3.18	4.68	3.00	4.60	4.80
Storage time in wks at 25 C	inoculated with *B. subtilis*			inoculated with *M. flavus*			inoculated with *E. coli*		
	total count/g								
0	310,000	380,000	570,000	31,000	68,000	750,000	500	5,100	11,000
1	—	—	—	—	—	—	310	830	6,200
2	290,000	350,000	530,000	27,000	43,000	480,000	110	370	900
4	270,000	810,000	500,000	22,000	34,000	150,000	10	80	200
(% die-off after 4 wks)	(12.9)	(18.4)	(12.3)	(29.0)	(50.0)	(80.0)	(98.0)	(98.4)	(98.2)
8	260,000	270,000	470,000	19,000	28,000	95,000	10	10	200
12	250,000	270,000	430,000	18,000	26,000	32,000	10	10	10
16	230,000	240,000	370,000	—	—	—	1	10	10
(% die-off after 12 or 16 wks)	(25.8)	(36.8)	(35.1)	(41.9)	(61.8)	(95.7)	(99.8+)	(99.8+)	(99.9+)
18	—	—	—	18,000	24,000	27,000	—	—	—
20	220,000	240,000	350,000	18,000	24,000	25,000	1	10	10
24	220,000	240,000	350,000	17,000	23,000	23,000	1	1	10
28	210,000	240,000	340,000	15,000	20,000	19,000	1	1	10
32	210,000	240,000	340,000	15,000	20,000	19,000	1	1	1
36	—	—	—	14,000	20,000	19,000	—	—	—
(% die-off after 32 or 36 wks)	(32.3)	(36.8)	(40.4)	(54.8)	(70.6)	(97.5)	(99.8+)	(99.98+)	(99.99+)

manufactured at 82.2 C were within the moisture qualification for Extra grade (maximum 4.0%). All NDM manufactured at an exit air temperature of 71.1 C contained between 4.0 and 5.0% moisture, thus meeting the requirement for Standard grade. The fact that the percentage of bacterial die-off during storage increased as the moisture content of the product increased should not be misconstrued. The decrease is more intimately related to higher initial counts in the product dried at the lower temperature and the higher moisture is the result of the lower drying temperatures. Other workers have shown that when the moisture content is above 5.0%, survival during storage is favored by high moisture (*7,11*) but several reports (*7,10,11*) show the number of viable bacteria decrease consistently during storage of dry milk containing less than 5.0% moisture with the rate of die-off particularly dependent upon the nature of the microbial flora.

The exit air temperature is the primary determinant in controlling the moisture content of dry milk, with other factors being rate of injection, residence time of droplets in the chamber, volume of hot air, and percent solids in the concentrated milk. There are economic disadvantages to drying at high temperatures but the objective is to achieve an exit air temperature which will produce low moisture without physical damage to the finished dry milk product. NDM with moisture in excess of 5.0% has a predisposition to microbial spoilage and chemical deterioration by enzymatic and non-enzymatic reactions (*7,9,11,13*), therefore, operational conditions conducive to low moisture are desirable.

REFERENCES

1. American Dry Milk Institute. 1976. Census of dry milk distribution and production trends. Am. Dry Milk Inst. Inc., Chicago, IL.
2. American Dry Milk Institute. 1973. Standards for grades of dry milks, including methods of analysis. Bulletin 916. Am. Dry Milk Inst. Inc., Chicago, IL.
3. American Public Health Association. 1972. Standards Methods for the Examination of Dairy Products. 13th ed. Edited by W. J. Hausler, Jr. Am. Pub. Health Assn., Inc., Washington, D.C.
4. Crossely, E. L. 1962. Milk hygiene—Hygiene in milk production, processing and distribution. Food and Agricultural Organization of the United Nations and the World Health Organization. Geneva, Switzerland. pp. 347-404.
5. Crossley, E. L., and W. A. Johnson. 1942. Bacteriological aspects of the manufacture of spray-dried milk and whey powders, including some observations concerning moisture content and solubility. J. Dairy Res. 13:5.
6. Crossley, E. L., and A. T. R. Mattick. 1949. Recent advances in the technique of production of whole milk powder. XII Int. Dairy Congr. 3:499.
7. Davis, J. G. 1963. Microbiological standards for dairy products. J. Soc. Dairy Technol. 16:224.
8. Findlay, J. D., C. Higginbottom, J. A. B. Smith, and C. H. Lee. 1946. The effect of the preheating temperature on the bacterial count and storage life of whole milk powder spray-dried by the Krause process. J. Dairy Res. 14:378.
9. Foster, E. M., F. E. Nelson, M. L. Speck, R. N. Doetsch, and J. C. Olson, Jr. 1957. Dairy microbiology. Prentice Hall, Inc. Englewood Cliffs, NJ.
10. Haines, R. B., and E. M. Elliot. 1944. Some bacteriological aspects of dehydrated foods. J. Hyg. 43:370.
11. Higginbottom, C. 1953. The effect of storage at different relative humidities on the survival of microorganism in milk powder and and in pure cultures dried in milk. J. Dairy Res. 20:65.
12. Keogh, B. P. 1966. Bacteriological problems in the condensing and drying of milk. Food Technol. Australia 18:126.
13. Keogh, B. P. 1971. The survival of pathogens in cheese and milk powders. J. Dairy Res. 38:91.
14. Naguib, K. H., S. Abd-El-Ghani, and S. M. Taha. 1972. Spray dried milk of the Sakha plant. II. Identification of predominating microorganisms. J. Milk Food Technol. 35:45.
15. Roundy, Z. D. 1958. Treatment of milk for cheese with hydrogen peroxide. J. Dairy Sci. 41:1460.
16. Trujillo, E. 1965. Models KF-2 and KF-3 Aquameters. Beckman Instruments, Inc., Scientific and Process Instruments Division, Fullerton, CA.
17. Walker, G. C., and L. G. Harmon. 1965. Hydrogen peroxide as a bactericide for staphylococci in cheese milk. J. Milk Food Technol. 28:36.

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Microbial Flora of Pond-Reared Shrimp (*Penaeus stylirostris, Penaeus vannamei, and Penaeus setiferus*)

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ABSTRACT

Aerobic plate counts of fresh pond-reared shrimp (*Penaeus stylirostris, Penaeus vannamei,* and *Penaeus setiferus*) ranged from 1.5×10^3 to 1.3×10^4 per gram. Coryneform bacteria and *Vibrio* spp. dominated the microbial flora of shrimp. Aerobic plate counts of pond waters ranged from 6.1×10^2 to 2.2×10^4 per milliliter. *Vibrio* spp. usually were dominant in pond waters. Enterococci and coliform levels in shrimp were low. Fecal coliforms were present in only one of 11 shrimp samples. No *Escherichia coli* was recovered from pond-reared shrimp. *Salmonella, Vibrio parahaemolyticus, Bacillus cereus,* and *Clostridium perfringens* were not recovered from either shrimp or pond waters. No relationship could be established between changes in numbers and types of microorganisms in pond-reared shrimp or pond waters and changes in characteristics of the pond waters such as temperature, salinity, dissolved oxygen, and pH.

The major effort of a mariculture project on pond-reared shrimp at Texas A&M University has focussed on factors to stabilize and improve production through improvements in pond construction, water quality and management, nutrition, and disease control. Information on quality and shelf life of pond-reared shrimp is scarce. The environmental conditions under which shrimp develop in ponds are different from those that exist in coastal waters from which shrimp usually are harvested in the United States. For example, differences may exist in water temperature, salinity, pH, available oxygen, and nutrients. Since microbial activity is one of the main causes of quality deterioration of shrimp as well as a potential cause of foodborne illness, information is needed about the microbiological characteristics of pond-reared shrimp. Little or no information exists concerning the presence in pond-reared shrimp of microorganisms of public health interest such as *Vibrio parahaemolyticus, Bacillus cereus, Clostridium perfringens,* or *Salmonella.* Preliminary studies on the microbial flora of pond shrimp were carried out at Texas A&M University 6 years ago (*12*). However, in recent years, significant changes and improvements have been made in pond construction, water management, feed characteristics, and feeding practices. These changes prompted a more detailed study of the microbiological conditions of pond-reared shrimp. This paper reports on (*a*) numbers and types of microorganisms in fresh and ice-stored pond-reared shrimp and pond waters and (*b*) presence of common food pathogens in pond-reared shrimp.

MATERIALS AND METHODS

Shrimp cultivation and sampling

0.25-acre ponds located on the Laguna Madre near Corpus Christi, Texas were stocked with post-larvae supplied by the Ralston Purina Company hatchery in Crystal River, Florida. At the time of stocking, the estimated number of postlarvae per pond was 15,000 for *Penaeus stylirostris* and *Penaeus setiferus* and 10,000 for *Penaeus vannamei.* Each species was raised in a separate pond. Each week, 20 shrimp were taken from each pond and biological measurements such as weight and length were determined. A report of these biological studies will be published elsewhere. The shrimp were placed in sterile plastic bags on ice and transported to the laboratory within 5 h. Temperature, salinity, dissolved oxygen (Winkler) and pH of the pond waters were measured twice each week according to *Standard Methods for the Examination of Water, Sewage, and Industrial Wastes* (*7*). Sampling for microbiological analyses was begun after the shrimp had been in the ponds for approximately 1 month. *P. stylirostris* was sampled in July, August, and September, *P. setiferus* in August, September, and October, and *P. vannamei* in September, October, and November. Both shrimp and water specimens were taken for bacteriological analysis.

Bacteriological methods

Shrimp were headed aseptically and 50 g were blended with 450 ml of 0.1% sterile Bacto-Peptone (Difco) in a Waring Blendor at 8000 rpm for 2 min. Aerobic plate counts were determined with the spread plate method by placing 0.1-ml aliquots of appropriate dilutions on Trypticase Soy Agar (TSA, BBL) with plate incubation at 25 C for 48 h. Aerobic plate counts of water samples were made in a similar manner. To determine the distribution of microbial types, 40 colonies were picked at random from countable plates. Identification of these isolates was carried out by procedures and diagnostic schemes reported by Vanderzant and Nickelson (*11*). Methods for the detection and

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enumeration of enterococci, coliform bacteria, *Salmonella, Vibrio parahaemolyticus, Clostridium perfringens,* and *Bacillus cereus* were those listed in the Bacteriological Analytical Manual for Foods (*8*). A similar bacteriological examination was made of *P. setiferus* and *P. vannamei* collected during the last sampling period and stored on ice for 8 days.

RESULTS

Variations in the characteristics of the pond waters during the experimental period were as follows: the water temperature was usually between 20-25 C, the pH ranged from 7.8 to 8.5, salinity varied from 29-50 ppt, and available oxygen from 1.0 to 6.0 mg/liter.

The aerobic plate count of fresh pond-reared shrimp ranged from 1.5×10^3 to 1.3×10^4 per gram (Table 1). Samples of *P. setiferus* and *P. vannamei* obtained from the last sampling period and stored on ice for 8 days had somewhat lower aerobic plate counts (5.1 to 9.4×10^2 per g) than the fresh samples. Aerobic plate counts of the pond waters ranged from 6.1×10^2 to 2.2×10^4 per milliliter.

Although variations in the distribution of microbial types were noted, coryneform bacteria and *Vibrio* spp. together often constituted a major part of the microbial flora of fresh pond shrimp (Table 2). Minor variations in microbial flora existed from species to species of shrimp. In *P. setiferus, Flavobacterium,* and less frequently *Lactobacillus* and *Enterobacteriaceae* were also significant; in *P. vannamei, Bacillus* and *Pseudomonas* spp. In addition, species of *Streptococcus, Moraxella-Acinetobacter, Alcaligenes, Aeromonas,* and molds and yeasts were isolated. Some changes in the microbial flora occurred when *P. setiferus* and *P. vannamei* were stored on ice for 8 days. In both samples, coryneform bacteria and *Pseudomonas* spp. predominated whereas *Vibrio* spp. were absent. *Vibrio* spp. often dominated the microbial flora of pond waters. Coryneform bacteria,

TABLE 1. *Aerobic plate counts of fresh shrimp, stored shrimp, and their pond waters.*

Sample	Aerobic Plate Count per gram or ml during				
	July	Aug.	Sept.	Oct.	Nov.
P. stylirostris-fresh	6.8×10^3	$7{,}8 \times 10^3$	6.0×10^3		
Pond A (water)	2.2×10^4	2.0×10^4	7.1×10^3		
P. setiferus-fresh		1.3×10^4	3.1×10^3	3.5×10^3	
Ice-stored (8 days)				5.1×10^2	
Pond B (water)		6.8×10^3	5.9×10^3	1.2×10^3	
P. vannamei-fresh			6.1×10^3	8.8×10^3	1.5×10^3
Ice-stored (8 days)					9.4×10^2
Pond C (water)			7.2×10^2	9.1×10^2	6.1×10^2

TABLE 2. *Distribution of the microbial flora of fresh shrimp, stored shrimp, and pond waters.*

Sample	Percent distribution of microbial flora												
	Co	Vi	En	St	La	Ps	Mo	Fl	Ba	Al	Ae	Mi	MA
P. styl.													
July 21	17.5	60.0	7.5	5.0	2.5	2.5	5.0						
Aug. 15	47.5	22.5	10.0				7.5	2.5	2.5	2.5			
Sept. 19	12.5	75.0								2.5			
Pond A													
July 21	12.5	10.0				2.5		12.5		55.0			
Aug. 15	27.5	52.5	2.5	2.5					7.5	2.5			
Sept. 19	2.5	42.5											
P. set.													
Aug. 15	35.0	17.5	7.5		2.5	5.0	2.5	20.0	5.0				
Sept. 19	5.0	7.5	30.0		20.0	7.5		27.5			2.5		
Oct. 17	57.5							20.0	10.0			2.5	
Ice-stored	47.5			2.5		22.5		2.5				10.0	10.0
Pond B													
Aug. 15	15.0	67.5	2.5			2.5	5.0	2.5					
Sept. 19		80.0											
Oct. 17	2.5	85.0											
P. van.													
Sept. 19	27.5		2.5			27.5		15.0	17.5				
Oct. 17	37.5	10.0				7.5		2.5	32.5	5.0			
Nov. 18	25.0	12.5	7.5			7.5	2.5	2.5	12.5		5.0	5.0	10.0
Ice-stored	32.5			2.5		25.5		5.0	5.0			27.5	
Pond C													
Sept. 19	10.0	15.0				5.0		17.5					
Oct. 17	15.0	50.0						20.0		2.5			
Nov. 18	27.5	37.5	2.5	2.5		5.0	2.5	10.0		2.5	2.5		

P. sty. = *P. stylirostris, P. set.* = *P. setiferus,* Ice-stored = shrimp stored on ice for 8 days, Co = *Corynebacterium,* Vi = *Vibrio,* En = *Enterobacteriaceae,* St = *Streptococcus,* La = *Lactobacillus,* Ps = *Pseudomonas,* Mo = Yeasts and Molds, FL = *Flavobacterium,* Ba = *Bacillus,* Al = *Alcaligenes,* Ae = *Aeromonas,* Mi = *Micrococcus,* MA = *Moraxella-Acinetobacter, P. van.* = *P. vannamei.* Organisms not identified account for incomplete percentages.

Alcaligenes, and *Flavobacterium* spp. were a significant part of the microflora of one or more of the water samples.

Enterococci, coliform, and fecal coliform counts (MPN/g) are presented in Table 3. Enterococci occurred in all fresh shrimp from July to October and ranged from 3.6 to 23.0 (mean 12.9) per gram. None were detected in the shrimp stored on ice or in the pond waters. Coliform bacteria were present in nearly all shrimp and ranged from 3.6 to 460 (mean 67.0) per gram. Coliforms were present in only two water samples at levels of 3.6 and 9.1 per milliliter. Fecal coliforms in shrimp were present in only one sample at the level of 43.0 per gram. This sample also had the highest coliform count (460 per gram). *Escherichia coli* was not present. No fecal coliforms could be recovered from the pond waters. *Salmonella, V. parahaemolyticus, C. perfringens,* and *B. cereus* were not recovered from either shrimp or pond water.

DISCUSSION

No relationship could be established between changes in numbers and types of microorganisms in pond-reared shrimp or pond waters and changes in pond water characterisitics such as temperature, salinity, dissolved oxygen, and pH.

The bacterial counts of freshly harvested shrimp in this study were somewhat lower than those reported previously (*9,12*) for pond-reared *P. aztecus, P. vannamei, P. setiferus,* and *P. occidentalis* (3×10^4 to 10^6 per gram). The lower counts in the present study could reflect a lower microbial population on shrimp. However, differences in composition of the plating medium may have been in part responsible for this observation. In one of those studies (*9*), the plating medium (TSA) was prepared with 3% NaCl. In the other study, the plating medium, Standard Methods Agar (SMA, BBL) was prepared with seawater diluted with distilled water to approximate the salinity of the pond water.

In a study reported in 1970 (*10*) in which the microbiological methods were similar to those of the present study, aerobic plate counts of *P. aztecus* and *P. setiferus* ranged from 7.2×10^1 to 2.0×10^3 per gram. The plating medium, SMA, was prepared with distilled water. In that study counts on media prepared with seawater or artificial seawater were also higher.

In this study, coryneform bacteria and *Vibrio* species often were the predominant isolates from freshly harvested pond shrimp. In a previous study (*12*) in which the medium (Standard Methods agar) was prepared with diluted seawater, coryneform bacteria, and *Vibrio* species also were dominant. On TSA prepared with 3% NaCl (*9*), the flora of freshly harvested *P. aztecus, P. setiferus, P. vannamei,* and *P. occidentalis* consisted of *Aeromonas, Pseudomonas,* and *Vibrio* species.

Vibrio species were more predominant in the microflora of pond waters in this study than in a previous study (*12*). The salinity of the pond waters in the present study was generally much higher and may have been responsible for this occurrence. In addition to differences in composition of the plating media, differences in pond management practices such as in type of feed, feeding rate, and fertilization of the ponds may have contributed to differences in level and type of microflora reported in the various studies.

The reduction in count after 8 days of storage on ice probably was caused by the washing effect of the melting ice and the inability of some microbial species to survive or grow at low temperatures and at lower salinity on the surface of the shrimp. Changes in the distribution of the microbial flora during refrigerated storage may have been caused by difference in growth rate, symbiotic or antagonistic effects between species, or through reduction of the salinity of shrimp by melting ice. Similar changes were reported by Campbell and Williams (*2*), Carroll et al. (*4*), and Vanderzant et al. (*9*).

Although no nationwide microbiological standards on seafoods have been adopted, certain states and municipalities administer regulations specifying limits on products within their jurisdiction (*6*). Microbiological standards for comparable products are as follows: Enterococci < 1000/g, coliform organisms < 100/g, fecal coliform organism < 2.3/g. The microbiological counts of pond-reared shrimp in this study are well below these limits.

Some investigators suggest that fecal coliform measurements are the most practical and useful methods for determining the degree of disease hazard caused by enteric pathogens in foods. Geldreich and Bordner (*5*) demonstrated a correlation of fecal coliform densitites

TABLE 3 *Enterococci, coliform and fecal coliform levels of fresh shrimp, stored shrimp, and their pond waters.*

	Bacteriological indices of shrimp and pond water during														
	July			August			September			October			November		
Sample	E	Co	FC	E	Co	FC	E	Co	FC	E	Co	FC	E	Co	FC
P. sty.	23.0	43.0	< 3.0	9.1	3.6	< 3.0	23.0	9.1	< 3.0						
Pond A	< 3.0	3.6	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0						
P. set.				3.6	3.6	< 3.0	23.0	460.0	43.0	9.1	3.6	< 3.0			
Iced										< 3.0	< 3.0	< 3.0			
Pond B				< 3.0	9.1	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0			
P. van.							3.6	3.6	< 3.0	9.1	9.1	< 3.0	< 3.0	< 3.0	< 3.0
Iced													< 3.0	< 3.0	< 3.0
Pond C							< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0

P. sty. = *P. stylirostris, P. set.* = *P. setiferus, P. van* = *P. vannamei,* Iced = shrimp stored on ice for 8 days, E = Enterococci, Co = Coliform, FC = Fecal Coliform count (MPN per g or ml).

with occurrence of *Salmonella* in various stream waters. Cann et. (*3*) reported that coliforms, fecal coliforms, and enterococci were present in scampi only in low numbers and that *Salmonella* were absent. In the present study only low levels of enterococci, usually no fecal coliforms, and no *Salmonella* were present in pond-reared shrimp. It has been suggested (*1*) that some correlation may exist between pollution indices and *V. parahaemolyticus*. Vanderzant et al. (*13*) reported that no statistically significant relationship could be established between coliforms, fecal coliforms, and *E. coli* counts, and *V. parahaemolyticus* counts of oysters, water, or sediment samples from Galveston Bay. In the present study, neither *E. coli* nor *V. parahaemolyticus* was recovered from either shrimp or pond waters. The absence of *Salmonella, V. parahaemolyticus, C. perfringens,* and *B. cereus* in pond shrimp indicates that this product can be produced in confined terrestrial environments without the occurrence of common food pathogens.

REFERENCES

1. Baross, J., and J. Liston. 1970. Occurrence of *Vibrio parahemolyticus* and related hemolytic vibrios in marine environments of Washington State. Appl. Microbiol. 20:179-186.
2. Campbell, L. L., Jr., and O. B. Williams. 1952. The bacteriology of Gulf Coast shrimp. IV. Bacteriological, chemical, and organoleptic changes with ice storage. Food Technol. 6:125-126.
3. Cann, D. C., G. Hobbs, B. B. Wilson, and R. W. Horsley. 1971. The bacteriology of scampi (*Nephrops norvegicus*). II. Detailed investigation of the bacterial flora of freshly caught samples. J. Food Technol. 6:153-161.
4. Carroll, B. J., G. B. Reese, and B. O. Ward. 1968. Microbiological study of iced shrimp. Excerpts from the 1965 Iced-Shrimp Symposium, Cir. 284, U. S. Dept. of Interior. Washington, D.C., 17 pp.
5. Geldreich, E. E., and R. H. Bordner. 1971. Fecal contamination of fruits and vegetables during cultivation and processing for market. A review. J. Milk Food Technol. 34:184-195.
6. Liston, J., and J. R. Matches. 1976. Fish, crustaceans and precooked seafoods. pp. 507-521. In Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association. Washington, D.C.
7. Standard Methods for the Examination of Water, Sewage, and Industrial Wastes. 1971. 13th Edition. American Public Health Association, New York, N.Y.
8. U.S. Department of Health Education and Welfare. 1973. Bacteriological analytical manual for foods. Food and Drug Administration. Washington, D.C.
9. Vanderzant, C., B. F. Cobb, C. A. Thompson, Jr., and J. C. Parker. 1973. Microbial flora, chemical characteristics and shelf life of four species of pond-reared shrimp. J. Milk Food Technol. 36:443-446.
10. Vanderzant, C., E. Mroz, and R. Nickelson. 1970. Microbial flora of Gulf of Mexico and pond shrimp. J. Milk Food Technol. 33:346-350.
11. Vanderzant, C., and R. Nickelson. 1969. A microbiological examination of muscle tissue of beef, pork, and lamb carcasses. J. Milk Food Technol. 32:357-361.
12. Vanderzant, C., R. Nickelson, and P. W. Judkins. 1971. Microbial flora of pond-reared brown shrimp (*Penaeus aztecus*). Appl. Microbiol. 21:916-921.
13. Vanderzant, C., C. A. Thompson, Jr., and S. M. Ray. 1973. Microbial flora and level of *Vibrio parahaemolyticus* of oysters (*Crassostrea virginica*), water and sediment from Galveston Bay. J. Milk Food Technol. 36:447-452.

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Use of Alkaline Peeling Effluents from Vegetable and Fruit Processing Operations by *Neurospora sitophila*

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ABSTRACT

Growth of *Neurospora sitophila* on alkaline effluents from rutabaga, potato, and peach processing operations was studied. Submerged fermentation at 30 C reduced COD values from 42 to 68% of initial values for peeling wastes and from 17 to 25% in rinse wastes after 4 days. This procedure for reducing COD would be of interest as a pretreatment technique for use in processing plants discharging into municipal treatment systems. The total amino acid content of potato effluent biomass was nearly quadrupled, whereas the total amino acid content of peach effluent was doubled after 1 day of fermentation.

Alkaline peeling effluents from vegetable and fruit processing operations are typically concentrated sources of suspended solids, oxidizable materials, and alkali. Disposal of these effluents is a universal problem in the food industry. If discharged with other processing wastes, the waste load from peeling can account for 86 to 98% of the total load (*6,19*). Some plants use evaporation ponds; others use spray irrigation and a few are experimenting with cattle feeding after the pH is reduced by anaerobic fermentation.

Church and Nash (*10*) have shown that the waste load of acid effluents from vegetable processing can be reduced by fermentation with species of Fungi Imperfecti. Besides reducing the COD of corn whey by 98%, production of fungal biomass was 50 to 60 g of dry mycelium per 100 g of COD utilized. Feeding trials confirmed this material to be a beneficial source of protein for animals. This work has progressed from laboratory to pilot scale testing (*9*).

The nature of the waste to be used as a substrate for fungal fermentation dictates the organism to be employed. Gregory et al. (*12*) and Reade and Gregory (*14*) studied amylolytic, thermotolerant, filamentous fungi, since such cultures made it unnecessary to hydrolyze starch cassava substrates before fermentation. *Saccharomyces* (*Kluyveromyces*) *fragilis* was selected for studies involving use of coconut water wastes for production of food yeast because of its known ability to assimilate those carbohydrates present in coconut water (*17*). Brewery spent grain liquor has been reported to serve as an excellent substrate for growth of *Aspergillus niger* (*13*) *Candida* spp., *Saccharomyces* spp., and mushrooms (*15,16*).

Previous studies in our laboratory revealed that *Neurospora sitophila* grows readily on a peanut substrate supplemented with tapioca starch and sodium chloride (*4*) and in a potato extract medium (*3*). The organism also was demonstrated to utilize several low molecular weight sugars (*20*). Since effluents from alkaline peeling operations contain substantial levels of carbohydrate and sodium chloride (after pH reduction with hydrochloric acid), *N. sitophila* was the organism chosen for studies to determine the feasibility of simultaneously reducing COD and increasing the protein content of biomass through fermentation.

MATERIALS AND METHODS

Neurospora sitophila NRRL 2884 was grown on potato dextrose agar (pH 5.6) at 30 C for 20 to 25 days. Spores, along with some mycelial fragments, were dispersed in sterile water containing 0.005% Tween 80 by gently rubbing the culture surface with a glass rod. The suspension was filtered through sterile glass wool and the filtrate was used as an inoculum for all experiments. The number of colony-forming units per ml of inoculum ranged from 2×10^3 to 3×10^4.

Grab samples of rutabaga, Irish potato, and peach peeling and rinsing (washing) effluents were collected from a local vegetable and fruit processing plant. These were alkaline effluents from Magnuson peeling and scrubbing equipment which are used commercially for dry caustic peeling. Our objective was not to study high-strength dry caustic peeling wastes, however, but rather to evaluate alkaline wastes which were diluted and adjusted to a suitable fermentation pH. After diluting the peeling effluent 1:4 (vol/vol) with water and adjusting all effluents to pH 5.6 with HCl to facilitate fermentation, 100-ml aliquots of effluents were dispensed into 250-ml Erlenmeyer flasks and autoclaved at 121 C for 15 min. One milliliter of *N. sitophila* spore suspensions was then added to the cooled substrate. Flasks were placed on gyratory shaker (150 rpm, 4-cm stroke) and incubation was at 30 C for 1, 2, 3, and 4 days.

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Ferments were filtered through Whatman #541 paper and the biomass collected was air-dried at 65 C for 16-18 h and weighed. Raw and fermented samples were analyzed for COD by a standard method (*1*). The pH of ferments was monitored during the 4-day incubation period.

Dried biomass was analyzed for amino acid content. Samples (100 mg) were hydrolyzed in 20 ml of 6 N HCl, after flushing with high purity nitrogen, for 2 h, at 145 C. The pH was adjusted to 2.15 ± 0.05 with 12 N NaOH and the sample was diluted to 50 ml. An aliquot was centrifuged using a Beckman Microfuge and amino acids in the supernatant fluid were analyzed by the ion exchange chromatography technique of Spackman et al. (*18*) with a Durrum Model D-500 amino acid analyzer. A 48-cm column (1.75 mm I.D.) packed with a Durrum high resolution cation exchanger (bead diameter 8 ± 2 microns) was used. Running time was 100 min, including regeneration of the column.

RESULTS AND DISCUSSION

Changes in pH of peel and rinse fermentation media are shown in Fig. 1. Although the pH of all effluents was adjusted to 5.6 before autoclaving, some changes in pH were noted as a result of the heat treatment. Growth of *N. sitophila* was accompanied by marked increases in pH, especially early in the fermentation period. This may be due to autolysis and/or the production of ammonia by the organism.

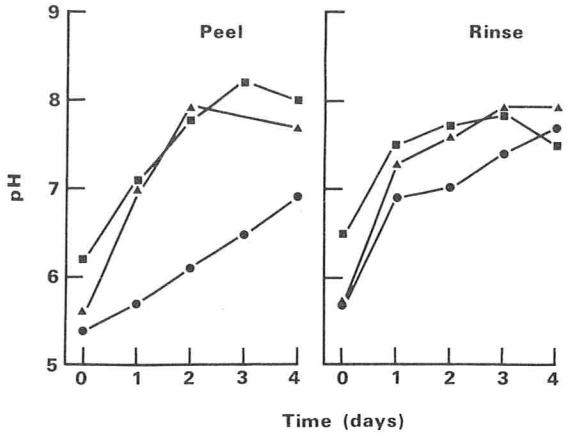

Figure 1. *Changes in pH of alkaline peel and rinse effluents from rutabaga* (■), *potato* (▲), *and peach* (●) *fermented with* N. sitophila.

The composition of peeling and rinse effluents before fermentation is shown in Table 1. The reduction of COD values ranged from 42 to 68% of initial values for the peeling wastes (Fig. 2). The more dilute rinse waters from the Magna Scrubber showed reductions to 17 to 25% of initial values at the end of the 4-day fermentation. Differences in percentage reduction of COD in the two types of waste (peeling versus rinse) may have been affected by the ability of *N sitophila* to grow on substrates having different compositions. Reductions in COD might be of interest as a pretreatment technique for plants discharging to municipal treatment systems. It is becoming increasingly common for municipalities to pass sewerage ordinances requiring industrial wastes to be reduced to the concentration of municipal wastes, which is typically 300 mg/1 for Biochemical Oxygen Demand and suspended solids. Such ordinances are forcing plants

TABLE 1. *Composition of peeling and rinse effluents before fermentation*

Product	Effluent[1]	COD[3] (mg/l)	Filterable materials[4] (mg/l)
Rutabaga	Peel[2]	24,850	838
	Rinse	1,840	36
Potato	Peel[2]	19,600	630
	Rinse	12,500	77
Peach	Peel[2]	62,480	127
	Rinse	6,674	18

[1]The pH of all effluents was adjusted to pH 5.6 before autoclaving.
[2]Peeling effluents after dilution 1:4 (vol/vol) with water.
[3]These COD values are expressed as 100% at zero time in Fig. 2.
[4]Shown as biomass at zero time in Fig. 3.

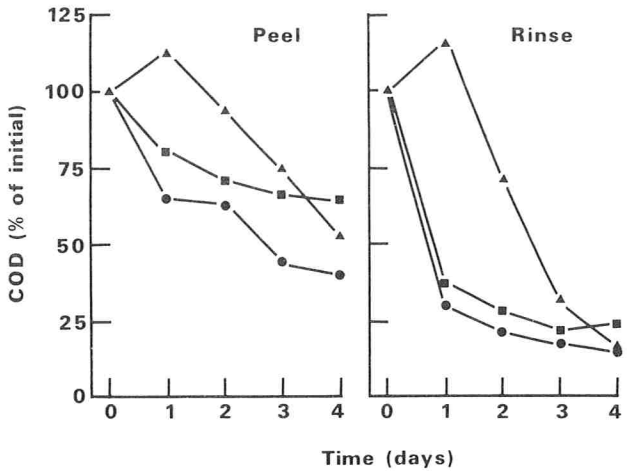

Figure 2. *Changes in COD of filtrates from alkaline peel and rinse effluents from rutabaga* (■), *potato* (▲), *and peach* (●) *fermented with* N. sitophila.

to use in-plant controls, high-rate activated sludge treatment systems, and even physical-chemical treatment of concentrated waste sources. The latter approach has been advocated by Bough in his research on various food processing wastes (*5,8*).

Shown in Fig. 3 are changes in biomass as a result of fermentation of peel and rinse effluents with *N. sitophila*. The biomass product is composed of residual suspended solids and cellular biomass of the organism. Steady increases in biomass accumulation were obtained in potato and peach peel media throughout the 4-day test period whereas a plateau was reached after 2 days in the rutabaga peel substrate. The same trends were noted in peach and rutabaga rinse media. A decrease in biomass content in the potato rinse medium after 1 day may have resulted from sampling or analytical error.

The amino acid contents of nonfermented (control) and fermented rutabaga, potato, and peach alkaline peeling effluents are listed in Table 2. Data are from 1-day ferments and controls are calculated as g amino acid per 100 g dry biomass, i.e. as a percentage of the dry sample weight. Cysteine was not detected in any of the samples; tryptophan was not determined.

Although some changes in levels of individual amino acids occurred during fermentation of the rutabaga effluent, the total amino acid content increased only slightly from 9.48 to 9.66%. The total amino acid content

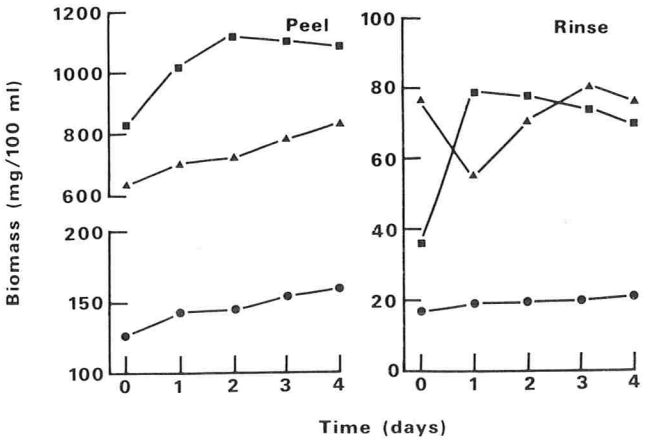

Figure 3. *Changes in biomass in alkaline peel and rinse effluents from rutabaga (■), potato (▲), and peach (●) fermented with* N. sitophila.

TABLE 2. *Amino acid content (g/100 g dry biomass) of nonfermented and fermented (1 day) alkaline peeling effluents*

	Rutabaga		Potato		Peach	
Amino acid	Control	Ferment	Control	Ferment	Control	Ferment
Alanine	.57	.71	.43	3.20	.64	1.15
Arginine	.23	.55	.33	1.59	1.30	.83
Aspartic acid	1.15	.95	1.74	1.56	2.78	1.67
Glutamic acid	1.84	1.68	1.24	4.63	1.20	2.17
Glycine	.45	.56	.40	1.33	.70	1.08
Histidine	.49	.19	.13	1.66	.23	.41
Isoleucine	.42	.51	.40	2.44	.47	.90
Leucine	.77	.86	.75	1.83	.82	1.52
Lysine	.47	.73	.56	.56	.49	1.26
Methionine	.12	.17	.12	1.60	ND[1]	.26
Phenylalanine	.49	.48	.45	2.65	.51	.85
Proline	.71	.44	.42	1.08	.53	.80
Serine	.44	.44	.38	1.56	.63	.86
Threonine	.44	.49	.40	1.16	.46	.80
Tyrosine	.29	.30	.27	2.63	.30	.54
Valine	.60	.60	.52	1.99	.63	1.04
TOTAL	9.48	9.66	8.54	31.47	8.19	16.14

[1]None detected

of dried potato effluent biomass increased by nearly four-fold as a result of fermentation whereas the amino acid content of peach effluent biomass was doubled. Similar trends were noted for rutabaga and potato rinse effluents which increased from 10.32 to 13.19% and from 9.64 to 31.37% total amino acids, respectively. The total amino acid content of the peach rinse effluent actually decreased from 16.78 to 11.73% after 1 day of fermentation with *N. sitophila*.

The total amino acid yield on potato effluent is comparable to that reported by Anderson et al. (*2*). These researchers reported yields of 31.96 to 38.55% protein in dry *N. sitophila* cells grown on molasses and 41.59% when the mold was grown on a starch medium.

The sulfur-containing amino acids are limiting in fermented biomass from various plant sources tested. Diets into which these products might be incorporated would have to be supplemented with these amino acids or with other proteins wherein they are not limiting. Blending fungal mycelium with other protein-containing ingredients has been shown by several researchers to improve the biological value in animal diets. For example, Doctor and Kerur (*11*) reported that a diet containing protein derived from dried *Penicillium chrysogenum* and from peanut meal performed as well as a diet containing a comparable level of protein from casein when fed to rats.

The growth rate of fungi is dependent upon both the nutritional and environmental conditions of the growth medium. In the present study no attempt was made to optimize the carbon:nitrogen ratio, pH, dissolved oxygen content, or temperature for maximum conversion of effluents to protein. It is possible that a more extensive reduction of COD within a shorter period accompanied by higher protein yields could be achieved if these growth parameters were optimized.

It is not so far in the future that food processing plants will be able to offer for sale food waste protein products consisting partly of single-cell protein (SCP). A large U.S. brewery has conducted extensive testing on a SCP product recovered from an activated sludge treatment system. Bough et al. (*7*) have estimated that 322 million pounds per year of dried activated sludge could be produced from food processing and brewery wastes. Containing 28 to 36% protein, activated sludge products are similar in composition to the biomass products produced in this study by fermentation of carbohydrate wastes.

REFERENCES

1. American Public Health Association. 1976. Standard methods for examination of water and wastewater, 14th ed. Washington, D.C. p. 550.
2. Anderson, C., J. Longton, C. Maddix, G. W. Scammell, and G. L. Solomons. 1975. The growth of microfungi on carbohydrates. pp. 314-329. In S. R. Tannenbaum and E. I. C. Wang (eds.) Single cell protein. MIT Press, Cambridge, MASS.
3. Beuchat, L. R., and S. M. M. Basha. 1976. Protease production by the ontjom fungus, *Neurospora sitophila*. Europ. J. Appl. Microbiol. 2:195-203.
4. Beuchat, L. R., and R. E. Worthington. 1974. Changes in the lipid content of fermented peanuts. J. Agric. Food Chem. 22:509-512.
5. Bough, W. A. 1974. Physical chemical treatment of waste effluents from canning of leafy green vegetables. J. Milk Food Technol. 37:205-208.
6. Bough, W. A. 1975. Characterization of waste loads from unit processes of a commercial potato canning operation. J. Milk Food Technol. 38:281-284.
7. Bough, W. A., D. R. Landes, J. Miller, C. T. Young, and T. R. McWhorter. 1975. Utilization of chitosan for recovery of coagulated by-products from food processing wastes and treatment systems. Proc. 6th Natl. Symp. Food Processing Wastes, Madison, Wisc., April 8-11. U.S. Environ. Protection Agency. (in press).
8. Bough, W. A., and A. L. Shewfelt. 1973. Treatment of concentrated pimiento wastes with polymeric flocculating agents J. Milk Food Technol. 12:619-624.
9. Church, B. D., E. E. Erickson, and C. M. Widmer. 1973. Fungal digestion of food processing wastes. Food Technol. 27(2):36-37, 39-40,42.
10. Church, B. D., and H. A. Nash. 1970. Use of Fungi Imperfecti in waste control. Water Pollution Research Series, 12060 EHT 07/70, Federal Water Quality Administration. U.S. Government Printing Office, Washington, D.C.
11. Doctor, V. M., and L. Kerur. 1968. *Penicillium* mycelium waste as protein supplement in animals. Appl. Microbiol. 16:1723-1726.

12. Gregory, K. F., A. E. Reade, G. L. Khor, J. C. Alexander, J. H. Lumsden, and G. Losos. 1976. Conversion of carbohydrates to protein by high temperature fungi. Food Technol. 30(3):30, 32, 35.
13. Hang, Y. E., D. F. Splittstoesser, and E. E. Woodams. 1975. Utilization of brewery spent grain liquor by *Aspergillus niger*. Appl. Microbiol. 30:879-880.
14. Reade, A. E., and K. J. Gregory. 1975. High-temperature production of protein-enriched feed from cassava by fungi. Appl. Microbiol. 30:897-904.
15. Shannon, L. J., and K. E. Stevenson. 1975. Growth of *Calvatia gigantea* and *Candida steatolytica* in brewery wastes for microbial protein production and BOD reduction. J. Food Sci. 40:830-832.
16. Shannon, L. J., and K. E. Stevenson. 1975. Growth of fungi and BOD reduction in selected brewery wastes. J. Food Sci. 40:826-829.
17. Smith, M. E., and A. T. Bull. 1976. Studies of the utilization of coconut water waste for the production of the food yeast *Saccharomyces fragilis*. J. Appl. Bacteriol. 41:81-95.
18. Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30:1190-1206.
19. Weckel, K. G., R. S. Rambo, H. Veloso, and J. H. von Elbe. 1968. Vegetable canning process wastes. University of Wisconsin; College of Agric. and Life Sciences Res. Rept. 38.
20. Worthington, R. E., and L. R. Beuchat. 1974. α-Galactosidase activity of fungi on gas-forming peanut oligosaccharides. J. Agric. Food Chem. 22:1063-1066.

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Isolation and Identification of Lipolytic Microorganisms Found on Rough Rice from Two Growing Areas

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ABSTRACT

Rice bran and whole brown rice are currently underutilized because free fatty acids are formed from rice oil during storage. Rough rice from two growing areas was tested for presence of lipolytic microorganisms that could release these fatty acids. Approximately 10% of the total bacterial plate count (4×10^6/g for Louisiana and 12×10^6/g for the Arkansas rice samples) were lipolytic. Upon testing, most were classified as nonsaccharolytic, alkali-producing pseudomonads. The average mold plate count for the Louisiana sample was 2×10^3 colony forming units (cfu)/g and 5×10^3 cfu/g for the Arkansas sample. All molds isolated showed various amounts of lipolytic action, as determined by the size of the lipolytic zone. The molds were generally isolated more from the Louisiana than from the Arkansas rice. *Alternaria* and *Helminthosporium* species, the most prevalent molds, were found in all samples.

Rice is one of the most important field crops in the world and is the basic food for over one-half of the world's population (*6*). It is well known that brown rice is more nutritious than polished rice (*8*). Rice bran is an excellent source of B and E vitamins, minerals, and protein, and is higher in protein, fat, fiber, ash, and vitamins than the starchy endosperm (*8*). The bran layer and embryo contain most of the lipids (*8*). Rice bran is underutilized, in view of the quality and quantity of nutrients present (*7*).

Brown rice has a short shelf life because of decomposition of lipids into fatty acids. This decomposition results not only in free acids but also in a bad taste that reduces the marketing potential of brown rice. There are two theories that describe the cause of lipid decomposition. The first holds that the rice grain itself contains lipolytic enzymes that break down the lipids. The other holds that microorganisms are present that decompose the lipids while the product is in storage and on the store shelf. If the major cause of lipolysis could be defined, it should be possible to retard or decrease lipid decomposition, thereby increasing shelf life and market quality of a valuable foodstuff. The object of this work was to show the presence, identification, and numbers of lipolytic bacteria and molds on rough rice that may influence shelf-life of brown rice.

MATERIALS AND METHODS

Rough rice with intact hulls, Starbonnet variety, was obtained from the Louisiana State Experiment Station, Crowley, La., and the USDA Field Experiment Station, Stuttgart, Ark. The moisture content was reduced by mechanical drying to 9-11% after threshing. Samples were kept in sterile plastic bags and stored at 4 C until needed.

Microbiological examination: Bacteria

Five samplings were made of rice from each location. Ten grams of clean, rough rice was weighed aseptically into a sterile Osterizer jar, 90 ml of sterile .02 M phosphate-buffered distilled water, pH 7.2, was added, and the mixture was blended for 1 min. Serial dilutions of 10^{-4}, 10^{-5}, and 10^{-6} were made in sterile, phosphate-buffered distilled water. Triplicate nutrient agar (BBL) pour plates (total plate count) and tributyrin-based agar plates for isolation of lipolytic bacteria (*4*) were inoculated with the appropriate dilutions and incubated at 30 C for 3 days.

Lipolytic bacterial colonies displayed a clear zone, indicating lipase activity on the tributyrin-base medium. These colonies were picked and inoculated on Triple Sugar Iron (BBL) Agar slants and incubated for 24 h at 30 C.

Biochemical media

The following biochemical tests were used to identify the isolated lipolytic bacteria (*3,5*). Gram stains were made from 24-h-old colonies grown on nutrient agar. These colonies were examined for oxidase and catalase production. The carbohydrates tested were in a 0.7% concentration in oxidative-fermentative medium (BBL) as follows: glucose in both open and sealed tubes, and sucrose, xylose, galactose, lactose, maltose, fructose in open tubes only. The tubes containing glucose that were used for fermentation testing were sealed with 3% agar. Although 3% agar does not prevent the egress of oxygen as well as petrolatum does, it was felt that it would retard oxygen diffusion sufficiently during incubation to obtain reliable results. In addition, 3% agar is easier to use. Other tests were citrate, esculin, galactosidase (ONPG), starch, lecithinase, growth on MacConkey agar (Difco), indole, Methyl Red-Voges Proskauer, urea, pigment, motility, flagellar morphology, and reduction of nitrate. After inoculation, media were incubated for 24-48 h at 30 C.

Fungi (molds)

Five samplings were made of rice from each location to survey the mold population. Serial dilutions of rice samples in triplicate (10^{-1} to 10^{-3}) were plated on acidified (pH 3.5 with sterile tartaric acid) potato dextrose agar (PDA; Difco) and incubated at 30 C for 3-5 days. Total mold counts were obtained from the appropriate dilution plates, and representative colonies were isolated on PDA slants.

Each isolate was inoculated on tributyrin-based agar and incubated at 30 C for 5 days. All isolates that exhibited lipolytic activity, as did bacteria, were held for identification.

Lipolytic isolates were cultured by the slide culture method of Riddell (*11*) except for the *Penicillium* and *Aspergillus* species, which were inoculated (tri-point inoculation) on Czapek's solution agar (Difco) and malt extract agar (*10*). However, with zygomycetes, only direct microscopic slides were prepared for positive identification. Amann's solution (*10*) was used to prepare microscopic slides from all cultures.

Microscopic and colonial morphology were employed to determine the taxonomic identity of the fungi (*1, 2, 9, 10*).

RESULTS AND DISCUSSION

Data in Table 1 show that the rough rice used in these studies has a relatively low population of microorganisms. Our study showed that Louisiana and Arkansas rice had similar bacterial populations. In 1970, Goel et al. (*6*) reported plate counts of raw, wild rice which exceeded 10^9 bacteria per gram, whereas our studies on cultivated rice showed only 10^6 per gram. The lipolytic bacterial count in the present study was 10^5 per gram, or approximately 10% of the total bacterial population. Clear zones of lipolysis were developed in 48 h by the lipolytic bacteria, which showed the presence on rough rice of a considerable population of bacteria capable of breaking bran lipids into fatty acids, thereby affecting taste and shortening the shelf life of brown rice. The majority of the lipolytic bacteria were gram negative rods unable to ferment glucose and were classified as alkali-producing pseudomonads. The identity of the lipolytic bacteria in the Louisiana and Arkansas rice was similar, as can be seen in Table 2. Most of these isolates were identified as *Pseudomonas alcaligenes,* followed in number by other nonsaccharolytic alkali-producing pseudomonads and xanthomonads. Also present were some saccharolytic gram-negative bacteria such as *Flavobacterium* species, and a few that were classified as *Chromobacterium typhiflavum.* Two isolates of *Enterobacter agglomerans* were found in Arkansas rice whereas in Louisiana rice a lipolytic isolate identified as *Proteus mirabilis* was found. Few gram-positive lipolytic bacteria were isolated from either sample. All but one belonged to the family *Micrococcaceae,* these being *Micrococcus luteus* in the Arkansas rice and *Staphylococcus saprophyticus* and *Micrococcus varians* in the Louisiana rice. An isolate identified as a *Corynebacterium* sp. was also found in the Louisiana sample. Although individual counts and isolates of the two rice samples were different, the data show that the predominant lipolytic bacteria present on rough rice were nonsaccharolytic, alkali-producing pseudomonads.

TABLE 1. *Microbial quality of clean rough rice*

Type of count	Average (No/g)	Range (No/g)
Louisiana Rice		
Total plate count	3.5×10^6	$2.2\text{-}\ 4.1 \times 10^6$
Lipolytic plate count	6.1×10^5	$3.3\text{-}\ 8.3 \times 10^5$
Molds	2×10^3	$1.8\text{-}\ 2.9 \times 10^3$
Arkansas Rice		
Total plate count	12×10^6	$9.7\text{-}13.5 \times 10^6$
Lipolytic plate count	9.1×10^5	$6.5\text{-}12.0 \times 10^5$
Molds	5×10^3	$1.0\text{-}\ 6.0 \times 10^3$

TABLE 2. *Lipolytic bacteria isolated from Louisiana and Arkansas rice samples at 30 C for 72 hours*

Louisiana		Arkansas	
Isolate	Number isolated	Isolate	Number isolated
Pseudomonas alcaligenes	115	*Pseudomonas alcaligenes*	142
P. diminuta	22	*P. cepacia*	12
P. putrifaciens	2	*P. pseudoalcaligenes*	3
P. cepacia	1	*P. aeruginosa*	1
Xanthomonas sp.	27	*Xanthomonas* sp.	6
Flavobacterium sp.	13	*Enterobacter agglomerans*	2
Chromobacterium typhiflavum	8	*Chromobacterium typhiflavum*	2
Proteus mirabilis	1	*Micrococcus luteus*	1
Micrococcus varians	1		
Staphylococcus saphrophyticus	10		
Corynebacterium sp.	1		

In Table 3 the filamentous fungi isolated for the two locations are identified. Louisiana rice yielded more mold genera than did the samples from Arkansas. *Alternaria* and *Helminthosporium* species were much more prevelant in both locations than any of the other genera, although specific numbers of each genus were not determined. These "field molds" have been associated with rice in other studies (*12, 13*). *Fusarium* sp., on the other hand, were infrequently isolated from Louisiana samples, and none were found in the Arkansas samples. This organism is considered a common member of rice mycoflora.

TABLE 3. *Taxonomic identification of lipolytic molds*[a]

LOUISIANA SAMPLE	ARKANSAS SAMPLE
Alternaria sp.	*Helminthosporium* sp.
Helminthosporium sp.	*Alternaria* sp.
Curvularia sp.	*Penicillium* sp.
Penicillium sp.[b]	*Curvularia* sp.
Aspergillus flavus	Unidentified organism
Unidentified organism	*Rhizopus* sp.
Cladosporium sp.	
Scopulariopsis sp.	
Paecylomyces sp.	
Mucor sp.	
Rhizopus sp.	
Fusarium sp.	

[a]Listed in order of prevalence from the top.

[b]Represents *P. chrysogenum, P. oxalicum* series, and unidentified *Penicillium* species.

Both *Aspergillus* and *Penicillium* species were found in low numbers in samples from Louisiana. *Aspergillus flavus* and *Penicillium chrysogenum* were the principal species found; the former was not isolated from any of the Arkansas samples.

One other lipolytic mold (labeled unidentified organism) was commonly isolated in low numbers. Initially, it produced red pigmentation in the mycelium and in the agar which turned dark with age. Since conidial structures were not observed, its taxonomy was not established.

Although the moisture content of rough rice after drying is between 9-11%, too low for microbial activity,

lipolysis is a problem during prolonged storage of whole brown rice. If brown rice is stored in large containers, such as bags or barrels, heat plus humidity can raise the moisture level to a point where microbial activity could begin. For long periods of storage, whether in a warehouse or on a market shelf, microbial lipases could act on the rice, particularly if the rice is not vacuum packed. This study has shown that both lipolytic fungi and bacteria are present in sufficient numbers to cause rancidity and off-flavor production under appropriate conditions during storage.

REFERENCES

1. Barnett, H. L., and B. B. Hunter. 1972. Illustrated genera of imperfect fungi (3rd ed.). Burgess Publishing Co. Minneapolis, Minn. 241 p.
2. Beneke, E. S., and A. L. Rogers. 1970. Medical mycology manual (3rd ed.). Burgess Publishing Co., Minneapolis, Minn. 226 p.
3. Buchanan, R. E., and N. E. Gibbons (eds.) 1974. Bergey's manual of determinative bacteriology (8th ed.). The Williams and Wilkins Co., Baltimore, Md. 1246 p.
4. Cabes, L. C., Jr., A. R. Colmer, H. T. Barr, and B. A. Turner. 1969. The bacterial population of an indoor poultry lagoon. Poultry Sci. 48:54-63.
5. Elliott, T. B., G. L. Gilardi, R. Hugh, and R. E. Weaver. 1975. Identification of glucose non-fermenting gram-negative rods. Amer. Soc. Microbiol. Washington, 217 p.
6. Goel, M. C., B. L. Gaddi, E. H. Marth, D. A. Stuiber, D. B. Lund, R. C. Lindsay, and E. B. Brickbauer. 1970. Microbiology of raw and processed wild rice. J. Milk Food Technol. 33:571-574.
7. Houston, D. F. 1972. Rice bran and polish. pp. 272-300. *In* D. F. Houston (ed.), Rice chemistry and function. (1st. ed). Amer. Soc. Cereal Chem. St. Paul, Minn.
8. Juliano, B. O. 1972. The rice caryopsis and its composition. pp. 16-74. *In* D. F. Houston (ed), Rice chemistry and function (1st ed.). Amer. Soc. Cereal Chem., St. Paul, Minn.
9. Raper, K. B., and C. Thom. 1949. A manual of the penicillia. The Williams and Wilkins Co., Baltimore, Md. 875 p.
10. Raper, K. B., and D. I. Fennell. 1965. The genus *Aspergillus*. The Williams and Wilkins Co., Baltimore, Md. 686 p.
11. Riddell, R. W. 1950. Permanent stained mycological preparations obtained by slide culture. Mycologia. 42:265.
12. Schroeder, H. W. 1963. The relation between storage molds and damage in high-moisture rice in aerated storage. Phytopathology 43:804-808.
13. Schroeder, H. W., and D. L. Calderwood. 1972. Rough rice storage. pp 166-187. *In* D. F. Houston (ed), Rice chemistry and function (1st ed.). Amer. Soc. Cereal Chem., St. Paul, Minn.

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Precursors of Dimethylnitrosamine in Fried Bacon

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ABSTRACT

Various compounds, all of which are endogenous to bacon systems, were investigated as precursors of dimethylnitrosamine. Low moisture model system studies indicated that dimethylamine and sarcosine were the major contributors to dimethylnitrosamine formation. Under conditions normally encountered in pan-frying of bacon, choline-containing compounds and sarcosine produced measurable quantities of this N-nitrosamine. It was also apparent that sarcosine under conditions of the experiment is a more probable precursor of dimethylnitrosamine. Up to 80% of the N-nitrosamine produced during the frying process was lost in the vapor.

While most of the recent N-nitrosamine research has concentrated on N-nitrosopyrrolidine (N-Pyr) and its precursors in bacon, there have been several reports of dimethylnitrosamine (DMN) being present in both raw (*19*) and cooked bacon (*2,7,19*). As yet, there have been no reports as to the actual precursor of this N-nitrosamine in bacon. Model system studies have implicated several compounds including dimethylamine and trimethylamine (*4,5,18*), quaternary ammonium compounds (*5*), sarcosine (*3,4*), and lecithin (*13,16*).

In view of the extreme carcinogenicity of DMN, the reaction between nitrite and DMN precursors endogenous to cured meat systems was investigated in model systems simulating the pan frying of bacon and the dry surface of cured meat products.

MATERIALS AND METHODS

Since DMN is a potent carcinogen, caution was taken in handling DMN solutions and work was done in efficient fume hoods whenever possible.

Materials

Compounds investigated as potential precursors of DMN included dimethylamine HCl (DMA) and trimethylamine HCl (TMA), choline chloride and sarcosine which were all obtained from Eastman Kodak Chemical Co. Soy lecithin (refined) was obtained from Nutritional Biochemical Co. and phosphoryl choline chloride from Sigma Chemical Co.

N-Nitrososarcosine was prepared using the procedure of Hansen et al. (*11*). Melting point determination and thin layer chromatographic analysis using a solvent system of 95% ethanol-benzene-water (4:1:1) were used for confirmation of purity of the N-nitroso compound.

Analysis of lecithin and related compounds

The method of Pensabene et al. (*16*) was used to determine whether dimethylamine and trimethylamine were present in the soy lecithin and phosphoryl choline chloride samples before reaction with nitrite. Samples were extracted with either; the extracts were concentrated and analyzed by gas-liquid chromatography. A 10 ft × 1/8 inch o.d. stainless steel column containing 4% Carbowax 20M plus 0.8% KOH on Carbopack B (Supelco, Inc.) in a Hewlett-Packard Model 6720A gas chromatograph was used. The carrier gas flow rate was 16 ml/min and the chromatograph was operated isothermally at 65 C.

Production of DMN in a low moisture carboxymethylcellulose (CMC) system

A system containing 1 mM precursor compound, 5 mM $NaNO_2$, 10 g of CMC 7LF (Hercules, Inc.) and 250 ml of biphthalate buffer (pH 3.4 and 6.0) was prepared as previously described (*9*). The mixture was freeze-dried for 24 h in a Virtis RePP Model No. 42 sublimator at a pressure of 5 μ and a platen temperature of 24 C. Residual moisture content was approximately 3%. The dried material was macerated to a fine powder and doubly wrapped in aluminum foil before heating in an oven at the required temperature for 2 h. A temperature range of 100 to 200 C was investigated.

Production of DMN in an oil system

A system, similar to that of Bills et al. (*1*) and Gray and Dugan (*10*) was used to simulate the heating stresses undergone by bacon during pan-frying. The precursors under investigation and sodium nitrite (in the ratio 1:5) except for N-nitrososarcosine where no nitrite was used, were heated in a two-necked round-bottom flask fitted with a reflux condenser and thermometer. Concentrations of the compound used and heating conditions (temperature and time) are given later in the text.

At the completion of the reaction period, the products were distilled under reduced pressure (13 mm Hg, 35 C) after addition of 350 ml of 3 M NaOH. The receiver flask was cooled in a water-alcohol/dry ice mixture at −8 C until 250 ml distillate was collected. This was extracted with 3 × 100-ml aliquots of redistilled dichloromethane after addition of K_2CO_3 (10 g). The extract was dried over anhydrous Na_2SO_4 and concentrated to a volume of 2.5 ml in a Kuderna-Danish apparatus equipped with a Snyder column. Hexane (1 ml) was added and the extract was further concentrated under a stream of nitrogen to a volume of 0.5 ml.

Formation of DMN in nitrite-treated pork samples and quantitation of DMN in the vapor

Two pork bellies, obtained 24 h after slaughter from a commercial processor were skinned, quartered, and cut in thin slices (1/8 in). These

slices were freeze-dried (moisture loss 35%) and placed in sealable plastic bags, flushed with nitrogen and stored at −20 C until required. A 65-g portion of the dried pork samples was rehydrated to its original moisture level using aqueous solutions containing sodium nitrite and sarcosine and allowed to equilibrate at 5 C for 24 h before grinding. Phosphoryl choline choride was added directly to the rehydrated pork sample and the sample ground three times to achieve intimate mixing.

The samples were placed in a two-necked 500 ml round-bottom flask fitted with a distillation head, condenser, and receiving flask. A thermometer was placed in the second neck to record the maximum temperature of the flask's contents reached during cooking. The flask was heated in an oil bath (180 C) for 20 min until the pork sample was crisp and 35 - 40 ml of condensate was collected. A similar cooking period was employed for all samples and the maximum temperature of the flask contents was in the range of 147 - 153 C.

The distillation apparatus was disconnected when cool and the distillation head and condenser were rinsed with distilled water which was then added to the condensate. The combined condensate and washings were extracted with 3 × 50-ml aliquots of dichloromethane after addition of K_2CO_3 (10 g). The extract was dried over anhydrous Na_2SO_4 and concentrated to 0.5 ml as before.

The contents remaining in the heating flask were steam distilled after addition of 300 ml of 3 M NaOH. The distillate (250 ml) was extracted with 3 × 100-ml aliquots of dichloromethane and the combined solvent extracts dried and concentrated as previously described.

Percent recoveries were determined by adding 50 μg of DMN to both the condensate and residues from the pork samples which contained neither sodium nitrite nor added precursor.

Isolation of DMN from the dichloromethane extracts

The formed DMN was isolated from the dichloromethane extracts using the thin layer chromatographic technique described previously (*10*).

Gas chromatographic analysis

A Hewlett-Packard Fand M 402 dual column gas chromatograph equipped with flame ionization detectors and glass columns (6 ft × 1/4 inch o.d.) packed with 6% Carbowax 20M on 80-100 mesh Chromosorb W was used for analysis. The chromatograph was operated isothermally at 140 C. The carrier gas flow rate was 21 ml/min.

Gas chromatography - mass spectrometry

Mass spectra were obtained using a combined GLC - mass spectrometer LKB 900 equipped with a glass column (6 ft × 1/8 inch o.d.) of 3% SP 2100 with an ionizing electron energy of 70 eV.

RESULTS AND DISCUSSION

Model aqueous system studies have implicated a number of compounds which on reaction with sodium nitrite are capable of producing DMN. This study was initiated to establish if DMN could be formed from these compounds in dry systems similar to those encountered in dry foods or on the dry surface of cured meat products and in model oil systems simulating the pan frying of bacon. The compounds investigated all occur naturally in meat systems.

Results of the low moisture CMC system studied indicated that DMA and sarcosine were the principal precursors of DMN (Table 1). This was to be expected based on the results of previous investigations with precursors in aqueous (*5*) and dry systems (*4*). Similar trends were observed for both pH values. TMA and choline chloride produced much smaller concentrations of DMN, which is similar to the findings of Fiddler et al. (*5*) who showed that approximately 10 times more DMN was formed from DMA than from TMA when both were heated with sodium nitrite at 78 C for 4 h in a buffer solution (pH 5.6). Similarly, Scanlan et al. (*18*) showed that in the presence of equimolar concentrations of amines and nitrite in aqueous solution (pH 6.4, 100 C, 2.5 h), more DMN was produced from DMA than from TMA.

TABLE 1. *Effect of pH on formation of dimethylnitrosamine in a low moisture CMC system containing 1 mM precursor compound and 5 mM sodium nitrite, time of heating 2 h at 150 C.*

	Dimethylnitrosamine (mg)[a,b]		%Conversion Precursor → DMN	
Precursor	pH 3.4	pH 6.0	pH 3.4	pH 6.0
Dimethylamine HCl	1.73	0.43	2.38	0.58
Trimethylamine HCl	0.22	0.07	0.30	0.09
Sarcosine	2.36	0.20	3.19	0.27
Choline Chloride	0.23	0.09	0.31	0.12

[a]Uncorrected for losses during extraction and heating procedures.
[b]Average of triplicate determinations.

The effect of temperature on formation of DMN from sarcosine and sodium nitrite in the low moisture system was also investigated (Table 2). Small amounts of DMN were formed at 100 C, while maximum production occurred at 140 C. This low temperature maximum is obviously the result of the formed DMN being expelled from the system. Since the boiling point of DMN is 153 C, it would be expected that some volatilization of the DMN produced would occur around 140 C. Because of the potentially hazardous nature of this compound, the heating oven was enclosed in a well-ventilated fume cupboard, and rubber gloves were worn at all times when working with this substance.

TABLE 2. *Effect of temperature and pH in the formation of dimethylnitrosamine in a low moisture CMC system containing 1 mM sarcosine and 5 mM sodium nitrite, time of heating 2 h.*

	Dimethylnitrosamine (mg)[a,b]		%Conversion Sarcosine → DMN	
Temperature (C)	pH 3.4	pH 6.0	pH 3.4	pH 6.0
100	0.10	0.01	0.14	0.01
120	0.36	0.09	0.49	0.12
140	2.39	0.27	3.23	0.36
160	1.22	1.22	1.65	1.65
180	0.47	0.21	0.64	0.28
200	0.05	0.02	0.07	0.02

[a]Uncorrected for losses during extraction and heating procedures.
[b]Average of triplicate determinations.

Data in Table 3 indicate that this theory of DMN expulsion from the dry system is correct. When sarcosine and sodium nitrite was heated in an oil system equipped with a condensing system, greater yields of DMN, increasing with increasing temperature, were achieved. Similar trends were obtained when N-nitrososarcosine was heated in the oil system, although the amounts of DMN produced were much higher (Table 4). However, the percent conversion of N-nitrososarcosine to DMN was not as high as that obtained by Eisenbrand et al. (*3*) in similar-type experiments. These investigators reported

TABLE 3. *Effect of temperature on formation of dimethylnitrosamine in an oil system containing 1 mM sarcosine and 5 mM sodium nitrite, time of heating 15 min.*

Temperature (C)	Dimethylnitrosamine[a,b] (mg)	% Conversion Sarcosine → DMN
100	0.12	0.16
125	0.88	1.19
150	2.12	2.86
175	2.75	3.71
200	3.75	5.07

[a]Corrected, based on a 80% recovery.
[b]Average of triplicate determinations.

TABLE 4. *Effect of temperature on formation of dimethylnitrosamine from N-nitrososarcosine (1 mM) in an oil system, time of heating 15 min.*

Temperature (C)	Dimethylnitrosamine[a,b] (mg)	% Conversion N-Sarcosine → DMN
100	0.50	0.6
125	2.26	3.05
150	5.75	7.77
175	10.50	14.18
200	16.50	22.29

[a]Corrected, based on 80% recovery.
[b]Average of triplicate determinations.

maximum yields of DMN at 230 C (90% of theory). It is interesting to observe the differences obtained by various researchers in the percent conversion of N-nitrosoamino acids to their respective N-nitrosamines under only slightly different conditions. For example, Eisenbrand et al. (*3*) obtained an 11% conversion of N-nitrosoproline to N-Pyr in their system. Under slightly different experimental conditions, Pensabene et al. (*17*) obtained a much lower yield of N-Pyr.

The role of phoshoryl choline chloride and lecithin as precursors of DMN was investigated in model oil systems. This study was based on the investigation of Fiddler et al. (*5*) who showed that naturally occurring quaternary ammonium compounds reacted with sodium nitrite under mildly acidic conditions to form DMN. One of the quaternary ammonium compounds tested was choline which when heated decomposed to TMA, which then underwent further demethylation to DMA. In a later study, Pensabene et al. (*16*) showed that various lecithins when allowed to react with sodium nitrite in an aqueous system (pH 5.6) at 78 C for 4 h produced DMN in varying concentrations. In the present study, the model system of Bills et al. (*1*) was used to investigate the effect of temperature on formation of DMN from choline-containing compounds and sodium nitrite (Table 5). The molecular weight of the refined soy lecithin was calculated from the given elemental phosphorus analysis. The DMA and TMA contents of the precursors investigated were not as high as those reported by Pensabene et al. (*16*). Soy lecithin had DMA and TMA contents of 0.012 and 0.133 mg/mM, respectively, while phosphoryl choline chloride had values of 0.015 and 0.182 mg/mM, respectively. Results in Table 5 are as expected, higher temperatures producing greater amounts of DMN.

The effect of adding known precursors to pork slices before frying was investigated using the system previously described (*8*). The increasing formation of DMN with increasing levels of amine precursor and nitrite and distribution of DMN between the vapor and the fried product are shown in Table 6. As can be seen under the conditions applied, up to 80% of the DMN formed may be volatilized with the steam. This is essentially in agreement with the results of Eisenbrand et al. (*3*) and Gough et al. (*7*). It is also apparent that sarcosine under the conditions of the experiment is a more probable precursor of DMN. In bacon systems, under normal frying conditions, the situation may not be as clearly defined.

It also can be seen from data in Table 6 that yields of DMN found after frying pork samples containing sarcosine under the conditions applied are much lower than those obtained from heating sarcosine and nitrite in the model oil system (even after taking into account the differences in cooking times). This is understandable since the pork system is only heated to temperatures high enough to result in decarboxylation of the amino acid on the outside of the pork sample whereas the inner part is heated to a lesser extent. The water content of the bacon also contributes to a lowering of the cooking temperature.

Most of the compounds investigated in this study are found in meat systems. Patterson and Mottram (*15*) quantitated the volatile amines in the eye muscle of 10 pork carcasses at various stages of curing. Values of DMA were below 200 ppb before curing while up to 520 ppb of DMA were found in vacuum stored bacon. Singer and Lijinsky (*20*) found 2000 ppb DMA in baked ham and 1000 ppb DMA in frankfurters. Mottram et al. (*14*) investigated formation of DMN in cured pork

TABLE 5. *Effect of temperature on the formation of dimethylnitrosamine in an oil system containing 1 mM precursor compound and 5 mM sodium nitrite; time of heating 30 min.*

	Dimethylnitrosamine (mg)		% Conversion	
			Precursor	DMN
Temperature (C	Soy lecithin	Phosphoryl choline chloride	Soy lecithin	Phosporyl choline chloride
120	ND	0.01	—	0.06
140	0.05	0.07	0.06	0.10
160	0.10	0.28	0.13	0.38
180	0.32	0.74	0.43	0.99

containing added DMA and under laboratory conditions simulating normal bacon curing, the conversion of DMA to DMN was 0.1%.

Choline occurs naturally in tissue either free or combined principally in the form of phospholipid lecithins. Kuchmak and Dugan (*12*) reported a lecithin content of approximately 0.34 g/100 g fresh hog belly. Since most lecithin is found in the adipose tissue, it follows that this content may be very important in view of the fact that high temperatures are reached in the adipose tissue during frying.

Sarcosine is one of the most commonly occurring N-nitrosatable secondary amines (*6*). Sarcosine has been quantitated in the cat ranging from 1.3 mg/100 g of bladder to 4.3 mg/100 g of liver (*21*). There have been no reports on the sarcosine content of pork belly.

CONCLUSIONS

This study has shown that sarcosine and phosphatidyl choline (lecithin) can both contribute to formation of DMN during the frying of bacon. Which is the principal precursor remains to be determined. The relative contribution of each precursor to the formation of DMN should be determined using ^{14}C labelled compounds. The increase in concentration of each precursor during storage of bacon should also be investigated.

TABLE 6. *Distribution of dimethylnitrosamine produced during the heating of pork samples with added sodium nitrite and precursors in a heating flask.*

Sodium nitrite (ppm	Precursor added (ppm)	Dimethylnitrosamine (ppb)[a,b]		Percentage dimethylnitrosamine in condensate
		Condensate	Flask residue	
0	—	—	—	—
150	—	7[c]	ND	—
150	sarcosine (200)	43	21	67.2
150	sarcosine (1,000)	98	41	70.5
150	phosphoryl (200) choline chloride	9[c]	ND	—
150	phosphoryl (1,000) choline chloride	23	10	69.7
1,000	—	20	5[c]	80.0
1.000	sarcosine (200)	117	90	56.5
1.000	sarcosine (1,000)	238	135	63.9
1,000	phosphoryl (200) choline chloride	33	21	61.1
1,000	phosphoryl (1,000) choline chloride	43	29	59.7

[a]Average of duplicate experiments.
[b]Dimethylnitrosamine concentrations based on 100 g pork before heating.
[c]Not confirmed by mass spectrometry.

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REFERENCES

1. Bills, D. D., K. I. Hildrum, R. A. Scanlan, and L. M. Libbey. 1973. Potential precursors of N-nitrosopyrrolidine in bacon and other fried foods. J. Agr. Food Chem. 21:876.
2. Crosby, N. T., J. K. Foreman, J. F. Palframan, and R. Sawyer. 1972. Estimation of steam-volatile N-nitrosamines in foods at the 1 μg/kg level. Nature 238:342.
3. Eisenbrand, G., C. Janzowski, and R. Preussmann. 1976. Analyses, formation and occurrence of volatile and non-volatile N-nitroso compounds: recent results. Paper presented at the Second Symposium on Nitrite in Meat Products, Zeist, The Netherlands.
4. Ender, R., and L. Ceh. 1971. Conditions and chemical reaction mechanisms by which nitrosamines may be formed in biological products with reference to their possible occurrence in food products. Z. Lebensm. Unters.-Forsch. 145:133.
5. Fiddler, W., J. W. Pensabene, R. C. Doerr, and A. E. Wasserman. 1972. Formation of N-nitrosodimethylamine from naturally occurring quaternary ammonium compounds and tertiary amines. Nature 236:307.
6. Friedman, M. A. 1972. Nitrosation of sarcosine: Chemical kinetics and gastric assay. Bull. Envir. Contam. Toxicol. 8:375.
7. Gough, T. A., K. Goodhead, and C. L. Walters. 1976. Distribution of some volatile nitrosamines in cooked bacon. J. Sci. Food Agr. 27:181.
8. Gray, J. I., and M. E. Collins. 1977. A comparison of proline and putrescine as precursors of N-nitrosopyrrolidine in nitrite-treated pork systems. J. Food Sci. 42:1034-1037.
9. Gray, J. I., and L. R. Dugan, Jr. 1974. Formation of N-nitrosamines in low moisture systems. J. Food Sci. 39:474.
10. Gray, J. I., and L. R. Dugan, Jr. 1975. Formation of N-nitrosopyrrolidine from proline and collagen. J. Food Sci. 40:484.
11. Hansen, T., W. T. Iwaoka, and M. C. Archer. 1974. A high-yield synthesis of ^{14}C-labelled nitrosoproline and nitrososarcosine. J. Labelled Compounds 10:689.
12. Kuchmak, M., and L. R. Dugan, Jr. 1963. Phospholipids in pork muscle tissues. J. Am. Oil Chem. Soc. 40:734.
13. Mohler, K. and E. Hallermayer. 1973. Bildung von Nitrosaminen aus Lecithin and Nitrit. Z. Lebensem. Unters.-Forsch. 151:52.
14. Mottram, D. S., R. L. S. Patterson, D. N. Rhodes, and T. A. Gough. 1975. Influence of ascorbic acid and pH on the formation of N-nitrosodimethylamine in cured pork containing added dimethylamine. J. Sci. Food Agr. 26:47.
15. Patterson, R. L. S., and D. S. Mottram. 1974. The occurrence of volatile amines in uncured and cured pork meat and their possible role in nitrosamine formation in bacon. J. Sci. Food Agr. 25:1419.
16. Pensabene, J. W., W. Fiddler, R. C. Doerr, L. Lakritz, and A. E. Wasserman. 1975. Formation of dimethylnitrosamine from commercial lecithin and its components in a model system. J. Agr. Food Chem. 23:979.
17. Pensabene, J. W., W. Fiddler, R. A. Gates, J. C. Fagan, and A. E. Wasserman. 1974. Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. J. Food Sci.

39:314.
18. Scanlan, R. A., S. M. Lohlen, D. D. Bills, and L. M. Libbey. 1974. Formation of dimethylnitrosamine from dimethylamine and trimethylamine at elevated temperatures. J. Agr. Food Chem. 22:149.
19. Sen, N. P., B. Donaldson, J. R. Iyengar. and T. Panalaks. 1973. Nitrosopyrrolidine and dimethylnitrosamine in bacon. Nature 241:473.
20. Singer, G. M., and W. Lijinsky. 1976. Naturally occurring nitrosatable compounds. I. Secondary amines in foodstuffs. J. Agr. Food Chem. 24:550.
21. Tallan, H. H., S. Moore, and W. H. Stein. 1954. Studies in the free amino acids and related compounds in the tissues of the cat. J. Biol. Chem. 211:927.

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Formation of N-Nitrosopyrrolidine in Fried Bacon

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ABSTRACT

Formation of N-nitrosopyrrolidine (N-Pyr) from proline and N-nitrosoproline (N-Pro) was investigated by cooking pork slices to which these precursors had been added. When the cooking system was heated in an oil bath at 200 C for 12 min, 0.33 and 2.18% yields of the N-nitrosamine were obtained from proline and N-Pro, respectively. The N-Pro contents of pork slices to which two levels of nitrite were added were determined after 1 and 8 days of storage at 2 C. Results indicate that the formation of N-Pro occurs at such a rate that 1 day after addition of nitrite there is theoretically sufficient N-Pro formed to account for the quantities of N-Pyr isolated from cooked bacon. However, the rate of decarboxylation of the initial N-Pro in raw bacon is not great enough to account for the N-Pyr isolated from cooked bacon.

Recent research has indicated that N-nitrosopyrrolidine (N-Pyr) occurs in fried bacon but not in other cured meat products (*6,7*). It is also very evident that N-Pyr formation in bacon depends on temperature stresses and the nature of these stresses. This is supported by the fact that no N-Pyr has been isolated from raw bacon (*8*). The presence of N-Pyr in fried bacon and the cooked-out fat has aroused considerable interest as to its mode of formation and consequently various precursors have been suggested, including proline (*2,4,5,12,16*), collagen (*12,16*), spermidine (*15*), and putrescine (*2,16,20*).

Free proline appears to be the most probable precursors of N-Pyr in bacon. However, the mechanism of N-Pyr formation has not yet been clearly defined. Several pathways have been proposed. Sen et al. (*23*) proposed that free proline in bacon is converted to N-nitrosoproline (N-Pro) which when subjected to thermal stresses is decarboxylated to N-Pyr. These findings are consistent with the data of Hwang and Rosen (*17*). Nakamura et al. (*21*) showed that the mechanism involved depends on the cooking temperature. In the temperature range of 100-150 C, amounts of N-Pyr formed from free proline via pyrrolidine were almost similar to those formed via N-Pro. At temperatures above 175 C, the yield of N-Pyr via pyrrolidine was greater than that formed via the N-Pro pathway.

This study was designed to evaluate the contribution of proline and N-Pro to the formation of N-Pyr under actual conditions of frying bacon. The formation of N-Pro in pork belly slices treated with two levels of nitrite (150 and 1000 ppm) was also investigated.

EXPERIMENTAL PROCEDURES

Formation of N-Pro

N-Pro was prepared according to the procedure of Hansen et al. (*14*). Melting point determination and thin layer chromatographic analysis using a solvent system of 95% ethanol-benzene-water (4:1:1) were used for confirmation of purity of the N-nitroso derivative.

Formation of N-Pyr in nitrite-treated pork samples

Pork bellies obtained 24 h after slaughter from a commercial processor were skinned, quartered, and cut in thin slices (1/8 inch). These slices were freeze-dried for 24 h in a Virtis RePP Model No. 42 sublimator at a pressure of 5 μ and a shelf temperature of 24 C. Moisture loss was recorded. The slices were then rehydrated to their original moisture levels using aqueous solutions containing sodium nitrite, proline, or N-Pro, as required. The concentrations of these solutions were calculated so as to give the range of N-Pyr precursor concentrations as indicated in the text.

The amount of N-Pyr formed as a result of cooking the pork slices was determined using the heating system previously described (*10*). A 100-g sample of the rehydrated pork slices containing the added precursor (s) was cut into small pieces and placed in a two-necked, 500-ml round bottom flask fitted with a distillation head, condenser, and receiving flask. A thermometer was placed in the second neck to record the maximum temperature of the flask contents reached during cooking. The flask was heated in an oil bath and the contents of the flask were continuously stirred during the heating period by means of a magnetic stirrer. At the completion of the cooking period, the condenser and distillation head were washed with distilled water and the washings and condensate added back to the original heating flask. The contents of the flask were then steam distilled after the addition of 300 ml of 3 N NaOH.

The distillate (350 ml) was extracted with 3 × 100-ml aliquots of dichloromethane and the combined solvent extracts washed successively with 50-ml aliquots of 6 N HCl and 5 N NaOH. After drying over anhydrous Na_2SO_4, the solvent extract was concentrated to a volume of 2.5 ml in a Kuderna-Danish concentrating apparatus. Hexane (1 ml) was added and the extract further concentrated under a stream of nitrogen to a volume of 1 ml. The solvent concentrate was cleaned up and analyzed for its N-Pyr content using the thin-layer

chromatographic method previously described by Gray and Dugan (*12*).

Isolation of N-Pro from nitrite-treated pork slices

The procedure of Nakamura et al. (*21*) was essentially used. Presence of N-Pro in the nitrite-treated pork slices was established by converting it to its methyl ester and confirming by mass spectrometry. Samples were stored at 2 C and N-Pro analyses were conducted after 1 and 8 days of storage.

Gas chromatographic analysis

A Hewlett-Packard 6720A dual column gas chromatograph equipped with flame ionization detectors and stainless steel columns (10 ft × 1/8 inch o.d.) packed with 6% Carbowax 20M on 80-100 mesh Chromosorb W was used for the analysis of N-Pyr. The chromatograph was temperature programmed from 80 to 180 C at 10 C/min. The carrier gas flow rate was 21 ml/min.

The analysis of N-Pro methyl ester was carried out using columns of 3% SP2100 on 80-100 mesh Chromosorb W. Temperature programming was in the range of 100 to 200 C, at 5 C/min.

Gas chromatography - mass spectrometry

Mass spectra were obtained using a combined GLC-mass spectrometer LKB 900 equipped with a glass column (6 ft × 1/8 inch o.d.) of 3% SP2100 with an ionizing energy of 70 eV. The spectra were reported as bar graphs by means of an on-line data acquisition and processing program (*25*).

Analysis of bacon for nitrite and moisture contents

Residual nitrite was determined by the A.O.A.C. procedure (*1*). Moisture contents were obtained by drying ground samples at 100 C for 24 h.

RESULTS AND DISCUSSION

Four commercial samples of bacon were purchased in a local retail store and analyzed for their moisture and nitrite contents (Table 1). Average nitrite levels for the lean and adipose tissues were 43 and 25 ppm, respectively, while the average moisture contents were 67.5 and 11.9%, respectively. Assuming that all the nitrite was dissolved in the water, concentrations of residual nitrite in the aqueous phases of the lean and adipose would be 63.7 and 210 ppm, respectively. A previous study (*9*) has shown that the average free proline contents in five green pork bellies stored at 2 C for 8 days were 24.1 μM/100 g of lean tissue and 10.5 μM/100 g of adipose tissue. From the moisture contents obtained in this study, and assuming that most of the free proline is dissolved in the water, the aqueous phases of lean and adipose tissues would contain 35.7 and 88.2 μM proline/100 g of tissue, respectively. Thus it would appear that conditions are favorable in both the lean and adipose tissues for N-Pro formation, although the latter seems the more probable location because of the higher concentration of nitrite. This study was designed to quantitate the presence of N-Pro in pork slices treated with nitrite.

Two levels of nitrite were used; 150 ppm which is the legal amount permitted in the curing of side bacon in Canada and 1000 ppm which was used to enhance formation of N-Pro (Table 2). Results indicate that formation of N-Pro occurs at such a rate that 1 day after addition of nitrite, there is sufficient N-Pro formed to account for the quantities of N-Pyr isolated from cooked bacon provided the percent conversion of N-Pro to N-Pyr during frying is sufficiently great. The N-Pro content increased slightly during storage at 2 C for 8 days, although sample 4 doubled its N-Pro content during this storage period. The values reported were based on a 50% recovery and were much lower than those reported by Kushnir et al. (*19*). These authors reported values ranging from 0.38 to 1.18 ppm for three bacon samples and these values were not corrected for losses incurred during the extraction procedure. Nakamura et al. (*21*) analyzed five bacon samples, both raw and fried, and failed to detect any N-Pro. Ivey (*18*) reported that bacon cured in brines containing 1600 ppm of nitrite and having a residual nitrite content of 100 ppm contained greater than 100 ppb of N-Pro. Frying of the bacon reduced the N-Pro concentration by 86-100%. As expected, the higher level of nitrite used in the present study produced greater amounts of N-Pro.

The precursor role of proline and N-Pro generally has been studied in model systems simulating the pan-frying of bacon. Many of these studies have been conducted in the dry state (*5,12,16*) and in oil systems (*2,12,23*). In this study, the contribution of proline and N-Pro to formation

TABLE 2. *Effect of nitrite level on formation of N-nitrosoproline in pork slices.*

Carcass number	Nitrite added (ppm)	N-Nitrosoproline (ppb)[a,b]	
		Day 1[c]	Day 8[c]
1	150	101	115
2	150	78	92
3	150	43	61
4	150	210	416
1	1,000	340	389
2	1,000	243	430
3	1,000	782	913
4	1,000	833	1,083

[a]Calculations based on 100-g pork sample.

[b]Limit of detection, 20 ppb.

[c]Day 1 and Day 8 refers to the number of days of storage at 2 C after the addition of nitrite.

TABLE 1. *Analysis of four commercial bacon samples for moisture and nitrite contents.*

Sample number	Percent composition of sample		Percent moisture in			Nitrite content (ppm)	
	Lean	Adipose	Whole	Lean	Adipose	Lean	Adipose
1	58.1	41.9	41.2	63.8	13.8	48	28
2	45.1	54.9	37.0	69.2	11.3	34	31
3	42.3	57.7	36.3	65.9	12.8	61	30
4	50.6	49.4	41.9	71.2	9.8	28	12
Average	49.0	51.0	39.1	67.5	11.9	43	25

of N-Pyr was evaluated by frying pork slices containing the added precursors. The cooking was done in an all glass distillation system so that the volatilized N-Pyr could be collected to quantitate the total N-Pyr produced during the cooking of the pork slices. Tables 3 and 4 show that when the cooling system was heated in an oil bath at 200 C for 12 min, 0.33 and 2.18% yields of N-Pyr were

TABLE 3. *Effect of temperature on formation of N-nitrosopyrrolidine in fried pork slices containing 1 mM N-nitrosoproline, time of nitrite, time of heating, 12 min.*

Temperature of oil bath	Temperature (max) of cooking system (C)	N-Nitrosopyrrolidine[a,b,c] (mg)	Percent conversion Pro → N-Pyr
100	92	ND	—
120	104	0.02	0.02
140	125	0.08	0.07
160	149	0.16	0.13
180	172	0.24	0.21
200	189	0.38	0.33

[a]Average of duplicate determinations.
[b]Uncorrected for contribution from naturally occurring free proline in the pork slices.
[c]Confirmed by mass spectrometry.

TABLE 4. *Effect of temperature on formation of N-nitrosopyrrolidine in fried pork slices containing 1 mM N-nitrosoproline, time of heating, 12 min.*

Temperature of oil bath (C)	Temperature (max) of cooking system (C)	N-Nitrosopyrrolidine[a,b] (mg)	Percent yield N-Pro → N-Pyr
100	94	ND	ND
120	108	0.03	0.03
140	123	0.18	0.18
160	147	0.52	0.52
180	168	1.48	1.48
200	187	2.18	2.18

[a]Average of duplicate determinations.
[b]Confirmed by mass spectrometry.

obtained from proline and N-Pro, respectively. Based on the N-Pro contents reported in Table 2 for the 150 ppm level of nitrite, this rate of conversion of N-Pro to N-Pyr would not be great enough to account for the levels of N-Pyr found in fried bacon (*3,6,11,23*). However, Nakamura et al. (*21*) reported that N-Pro is formed from proline and nitrite during the frying of bacon. Maximum N-Pro formation occurred at 125 C, above which temperature there was a gradual decrease in N-Pro content up to 175 C. At 200 C, there was a rapid decrease in N-Pro formation.

Gray et al. (*11*) reported an average value of 23.8 μM proline/100 g of uncooked bacon. This is equivalent to 27.4 ppm. From data in Table 3 it can be seen that a 0.33% conversion of proline to N-Pyr was obtained when the sample was heated in an oil bath at 200 C. Based on this percentage conversion, the free proline in bacon should theoretically produce approximately 90 ppb of N-Pyr during frying. When the conditions of 200 C (oil bath temperature) and 12 min were used in this study, the pork slices were overcooked. At an oil bath temperature of 180 C (maximum temperature of cooking system, 172 C), the pork slices were crisp but not burnt. Under these conditions, using the proline to N-Pyr factor of 0.21, bacon should theoretically produce 57 ppb of N-Pyr. This value is similar to those reported for fried bacon, assuming that during conventional frying procedures, approximately 35-40% of the N-Pyr is lost in the vapor (*10*).

Yields of N-Pyr obtained from N-Pro and proline in this study are in general agreement with the results of some of the previous studies. However, the absolute values obtained depend on the system used. Eisenbrand et al. (*4*) heated N-Pro in a sealed tube containing 2 ml of silicone oil and reported an 11% yield of N-Pyr at 230 C for 10 min. When ham containing added N-Pro was fried in a pan containing 3 ml of vegetable oil, yields of 0.24 and 0.42% N-Pyr were obtained when the samples were cooked at 180-190 C and 210-220 C for 8 min, respectively. These investigators explained these lower yields by reporting loss of N-Pyr in the vapor. The ham sample also was only heated to a temperature sufficiently high to effect decarboxylation on the outside whereas the inner part was obviously heated to a lesser extent. The water content of ham also contributes to lowering the effective cooking temperature.

In the present study, the temperature of the contents in the heating flask did not attain the same temperature of oil bath. The temperatures reported in Tables 3 and 4 were the maximum temperatures reached in the system after 12 min of heating. This temperature was only reached because some of the water in the pork slices was evaporated during the cooking process. For example, when the pork slices were heated in an oil bath at 180 C for 12 min, 25 ml of distillate was collected. When the system was cooked under reflux conditions at 180 C for 12 min, a maximum temperature of only 110 C was achieved. Since bacon is normally cooked in a frying pan with no lid attached, it is expected that higher temperatures are reached towards the end of the cooking process when the moisture content is decreased through evaporation.

Hwang and Rosen (*17*) heated a 250 mg slice (2-mm thick) of bacon to which N-Pro had been added at 185 C for 5 min and reported a 1.43% yield of N-Pyr. Under similar conditions, a yield of 0.33% N-Pyr was obtained from proline and nitrite. These bacon samples were cooked under reflux but because of the size of the bacon samples used, it would be expected that the moisture present did not greatly reduce the sample temperature below that of the oil bath.

This present study has shown that free proline in bacon can be N-nitrosated and decarboxylated to account for the presence of N-Pyr in fried bacon. This is consistent with the findings of Sen et al. (*24*) which support the pathway of free proline converted to N-Pro with the latter being decarboxylated during the frying process. However, as recently reported by Nakamura et al. (*21*), an alternative mechanism, decarboxylation of proline followed by reaction with nitrite is also a

possiblity. From these studies it was concluded that in the temperature range 100 to 150 C with a heating time of 10 min, the amounts of N-Pyr formed from free proline via pyrrolidine were almost the same as those formed via N-Pro. At temperatures of 175 C and above, the yield of N-Pyr via pyrrolidine formation was greater than that formed via N-Pro.

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REFERENCES

1. A.O.A.C. 1970. Official methods of analysis, 11th ed. Association of Official Analytical Chemists, Washington, D.C.
2. Bills, D. D., K. I. Hildrum, R. A. Scanlan, and L. M. Libbey. 1973. Potential precursors of N-nitrosopyrrolidine in bacon and other fried foods. J. Agr. Food Chem. 21:876.
3. Crosby, N. T., J. K. Foreman, J. F. Palframan, and R. Sawyer. 1972. Estimation of steam-volatile N-nitrosamines in foods at the 1 μ g/kg level. Nature 238:342.
4. Eisenbrand, G., C. Janzowski, and R. Preussmann. 1976. Analyses, formation and occurrence of volatile and non-volatile N-nitroso compounds:recent results. Paper presented at the Second Symposium on Nitrite in Meat Products, Zeist, The Netherlands.
5. Ender, R., and L. Ceh. 1971. Conditions and chemical reaction mechanisms by which nitrosamines may be formed in biological products with reference to their possible occurrence in food products. Z. Lebensm. Unters-Forsch. 145:133.
6. Fazio, T., R. H. White, L. R. Dusold, and J. W. Howard. 1973. Nitrosopyrrolidine in cooked bacon. J. Assoc. Offic. Anal. Chem. 56:919.
7. Fiddler, W., J. W. Pensabene, J. C. Fagan, E. J. Thorne, E. G. Piotrowski, and A. E. Wasserman. 1974. The role of lean and adipose tissue on the formation of nitrosopyrrolidine in fried bacon. J. Food Sci. 39:1070.
8. Gray, J. I. 1976. N-Nitrosamines and their precursors in bacon: a review. J. Milk Food Technol. 39:686.
9. Gray, J. I., and M. E. Collins. 1977. The development of free proline during the storage of green pork bellies. Can. Inst. Food Sci. Technol. J. 10:97.
10. Gray, J. I., and M. W. Collins. 1977. A comparison of proline and putrescine as precursors on N-nitrosopyrrolidine in nitrite-treated pork systems. J. Food Sci. 42:1034-1037.
11. Gray, J. I., M. E. Collins, and L. F. Russell. 1977. Formation of N-nitrosohydroxypyrrolidine in model and cured meat systems. Can. Inst. Food Sci. Technol. J. 10:36.
12. Gray, J. I., and L. R. Dugan, Jr. 1975. Formation of N-nitrosopyrrolidine from proline and collagen. J. Food Sci. 40:484.
13. Gough, T. A., K. Goodhead, and C. L. Walters. 1976. Distribution of some volatile nitrosamines in cooked bacon. J. Sci. Food Agr. 27:181.
14. Hansen, T., W. T. Iwaoka, and M. C. Archer. 1974. A high-yield synthesis of ^{14}C-labelled nitrosoproline and nitrososarcosine. J. Labelled Compounds 10:689.
15. Hildrum, K. I., R. A. Scanlan, and L. M. Libbey. 1975. Identification of γ-butenyl-(β-propenyl) nitrosamine, the principal nitrosamine formed in the nitrosation of spermidine or spermine. J. Agr. Food Chem. 23:34.
16. Huxel, E. T., R. A. Scanlan, and L. M. Libbey. 1974. Formation of N-nitrosopyrrolidine from pyrrolidine ring containing compounds at elevated temperatures J. Agr. Food Chem. 22:698.
17. Hwang, L. S., and J. D. Rosen. 1976. Nitrosopyrrolidine formation in fried bacon. J. Agr. Food Chem. 24:1152.
18. Ivey, F. J. 1975. The determination of N-nitrosoproline in cured meats. Dissertation Abstracts International, b35 (2):879.
19. Kushnir, E., J. I. Feinberg, J. W. Pensabene, E. G. Piotrowski, W. Fiddler, and A. E. Wasserman. 1975. Isolation and identification of nitrosoproline in uncooked bacon. J. Food Sci. 40:427.
20. Lijinsky, W., and S. S. Epstein. 1970. Nitrosamines as enviromental carcinogens. Nature 225:21.
21. Nakamura, M., N. Baba, T. Nakoka, Y. Wada, T. Ishibashi, and T. Kawabata. 1976. Pathways of formation of N-nitrosopyrrolidine in fried bacon. J. Food Sci. 41:874.
22. Patterson, R. L. S., A. A. Taylor, D. S. Mottram, and T. A. Gough. 1976. Localized occurrence of N-nitrosopyrrolidine in fried bacon. J. Sci. Food Agr. 27:257.
23. Pensabene, J. W., W. Fiddler, R. A. Gates, J. C. Fagan, and A. E. Wasserman. 1974. Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. J. Food Sci. 39:314.
24. Sen, N. P., B. Donaldson, J. R. Iyengar, and T. Panalaks. 1973. Nitrosopyrrolidine and dimethylnitrosamine in bacon. Nature 241:473.
25. Sweeley, C. C., B. D. Roy, W. I. Wood, J. F. Holland, and M. Kritchevsky. 1970. On-line digital computer system for high-speed single focusing mass spectrometry. Anal. Chem. 42:1505.

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Microbiological Evaluation of Shrimp (*Pandalus borealis*) Processing

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ABSTRACT

Line samples from three different shrimp processing plants (brine-cooked shell-on, hand-peeled raw, and machine-peeled cooked) in Maine were examined for microbiological quality. Aerobic plate count (APC) of freshly caught shrimp (*Pandalus borealis*) was found to be about 530/g (Plate Count Agar at 35 C) while salt-requiring (SR) organisms were at significantly higher concentration (1.11×10^5/g; Salt Water Medium at 21 C). Some increase in psychrotrophic-mesophilic flora of shrimp delivered to the plant was observed. Cooking in-plant or on board the boat drastically reduced the SR flora, which was subsequently observed to increase after culling and inspection in the brine-cooked shell-on process. No such significant fluctuation due to processing was detected in APC. Shrimp sampled from steel barrels before a hand-peeled raw operation exhibited relatively high APC (7.2×10^4/g) and SR microflora (2.78×10^6/g). Heading and hand-peeling reduced the APC and SR bacterial loads by 71 and 95%, respectively. Subsequent processing and holding at room temperature resulted in a product with an APC and SR load of about 4×10^4/g. Similarly, high APC (1.66×10^5/g) and SR bacterial loads (1.84×10^5/g) were detected in samples obtained from a storage hopper of the machine-peeled cooking process. Although significant reduction in bacterial load was detected on line samples of this process (fluming, preheating, and cooking), the total bacterial load reached about 4×10^4/g before the canning step. Low levels of contamination with coliform and/or coagulase-positive staphylococci were detected in the three processes studied.

Bacteriological quality of freshly caught shrimp has been investigated in Louisiana by Green (*4*) through processing and distribution. Harrison and Lee (*5*) reported qualitative changes in the microbial flora of Pacific shrimp, *Pandalus jordani*, during processing by hand-peeling and cooking. The Food and Drug Administration (*18*) investigated the bacteriological quality of cold water shrimp, but restricted its study to raw peeled operations. The present study has been conducted to determine the bacterial load of shrimp (*Pandalus borealis*) processed in Maine, taking line samples of three different processes (brine-cooked shell-on, hand-peeled raw, and machine-peeled cooked).

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MATERIALS AND METHODS

In the brine-cooked shell-on process (Plant A), shrimp were cooked within 4 h of catch either on board the boat or after landing. After cooking by boiling for 5 min in 10-12% brine, shrimp were air-cooled and refrigerated overnight. The next day they were inspected, culled, and packed in insulated containers for shipment.

The hand-peeled, raw process in Plant B used shrimp which were delivered iced in steel barrels. The shrimp were first brined for 2 h in 10-12% NaCl solution, headed, hand-peeled, washed in a rod reel washer, rinsed in a chlorine solution (200 ppm), inspected, and culled. After weighing they were packed in trays and frozen. Personnel handling the shrimp wore rubber gloves and hair nets, and had access to a 200-ppm chlorine solution in which they frequently rinsed their hands. All tables had stainless steel tops, which were washed and rinsed with chlorinated water (200 ppm) every hour.

The machine-peeled cooked operation in Plant C received shrimp in open trucks or trailors at ambient temperature. The shrimp were moved by conveyor belt to a storage tank (hopper), where they were stored up to 24 h at ambient temperature (-5 to 0 C) before processing. The hopper was then filled with seawater and shrimp were conveyed by wooden flume and belts to preheater tanks where they were heated at about 93 C for 2 to 3 min, and subsequently machine-peeled and headed. They were then washed in seawater, drained, rinsed with fresh water, and passed through a grader onto an inspection belt. Next, the shrimp were boiled for 3 min in 10-12% brine solution, drained, inspected, and culled. Finally, the shrimp were mechanically size graded into trays and held at ambient room temperature until packed in a solution of 2.5% citric acid in 2.5% sodium chloride, sealed, and retorted.

Composite samples (> 100 g) were collected aseptically, placed in sterile containers and transported in ice to the laboratory for examination. Total viable counts were obtained by the pour-plate method using Plate Count Agar (PCA) (Difco) as well as Salt Water Medium (SWM) (*11*). Samples of 50 g were homogenized with 450 ml of Butterfield's Buffer (*2*) or artificial seawater, and appropriate serial decimal dilutions using buffer or artificial seawater were prepared to inoculate PCA and SWM, respectively. The SWM plates were incubated at 5, 21, and 35 C for 2.5 and 10 days, respectively, while the PCA plates were incubated at 35 C (APC) for 2 days. The SWM was included to detect salt-requiring (SR) flora. Most probable number (MPN) of coliforms (confirmed test) and coagulase-positive staphylococci were determined according to FDA recommendations (*1*).

RESULTS AND DISCUSSION

The shrimp season off the New England coast usually lasts from January through April, at which time shrimp are caught in relatively shallow waters of various bays and estuaries. During the 1968-1969 season, when shrimp were more plentiful and 10,693 metric tons were landed, significant quantities were caught from August through April (*3*). Experiments in this investigation were conducted during February and March, when average ambient temperatures were about –5 and 0 C, respectively.

Plant A

Total viable counts obtained for line samples of the brine-cooked shell-on process are shown in Table 1. The total bacterial load of shrimp freshly landed on board the boat, as determined by using SWM at 21 C, was 1.11×10^5/g. This value is within limits of the bacterial load reported for skin (10^2 to 10^7/cm^2) and gill samples (10^3 to 10^6/g) of fish caught in the North Atlantic (*12*). Although Green (*4*) detected an average viable count of 4.2×10^4/g for shrimp caught in the Gulf of Mexico, using nutrient agar, it must be pointed out that Liston (*7*) obtained significantly higher viable counts on freshly caught fish using salt water medium as compared to fresh water medium.

It is interesting to note that freshly caught shrimp (Table 1) had a 50% higher count on salt water medium incubated at 5 C rather than 21 C. Similar results were reported by Liston (*7*), who studied the bacterial flora of flatfish, and noted that during the winter months the 20-C count was similar or slightly lower than the 0-C count in 25% of the cases.

APC (530/g) obtained in this study is comparable to results reported by Zapata and Bartolomeo (*18*), who detected counts between < 100 and 1.0×10^3/g on freshly caught cold-water shrimp.

Raw shrimp obtained in the plant revealed a 6 to 7 fold increase in count as determined on SWM at 21 C and 5 C, respectively (Table 1). This increase accompanying a delay in cooking indicates a buildup of psychrotrophic-mesophilic flora, which is not surprising in view of the ambient temperatures during that time of the year. The cooking process in the plant as well as on board the boat drastically reduced viable counts of SR bacteria while very little reduction in APC was observed.

After the shrimp were cooked, they were cooled and stored in a refrigerator overnight before culling, inspecting, and packaging for shipment. Samples taken after inspection showed 45- and 130-fold increase in the 21- and 5-C counts, respectively. Although this increase in viable count may be attributed to recontamination during handling (*8*), part of this increase may be due to recovery of bacteria sustaining sublethal damage during the cooking operation. This view is partially supported by the fact that only a slight increase in APC was detected. Recovery under suboptimal growth conditions is well known with UV and ionizing radiation (*10,14,15*) and has been observed more recently in heat inactivated cells (*16*). It is noteworthy that the population surviving the cooking step was about the same regardless of plating media used.

There were no coliforms or coagulase-positive staphylococci detected in either raw or cooked samples obtained from the boat. The raw shrimp samples in the plant contained coliforms (23/g), perhaps indicating inadequate sanitation. No coagulase-positive staphylococci were detected and there was no appreciable increase in APC.

No coliforms were detected in the product after cooking or after inspection. However, coagulase-positive staphylococci (4/g) were detected after inspection. Detection of staphylococci is not surprising since 40% of the human population harbor this organism in the nasopharynx (*9,17*).

Plant B

The bacterial load of shrimp delivered in steel barrels to the processing plant was about 7.2×10^4/g, as determined by the APC (Table 2). This count is within the limits (0.14 to 75.0×10^4/g) reported by Zapatka and Bartolomeo (*18*) for whole stored shrimp obtained before processing from plants considered to have poor sanitary conditions. In fact, the observed change in viable count, from 530/g in freshly caught shrimp to 7.2×10^4/g of stored shrimp, represents about a 130-fold increase. This increase is assumed to be primarily due to contamination, since the psychrotrophic-mesophilic SR flora (measured at 21 C) increased only 25-fold. Furthermore, Green (*4*) observed only a 10-fold increase in viable count of whole and headed shrimp stored for 24 h in ice.

Harrison and Lee (*5*) reported the psychrotrophic-mesophilic count on shrimp before processing as being

TABLE 1. *Viable bacterial counts of line samples for brine-cooked shell-on shrimp process (Plant A).*

Sample	Aerobic plate count/g				Coliforms/g	Coag-positive Staphylococci/g
	PCA	SWM				
	35 C	35 C	21 C	5 C		
On board boat (raw)	5.30×10^2	4.00×10^4	1.11×10^5	1.69×10^5	< 3	< 3
On board boat (cooked)	3.90×10^2	1.00×10^2	8.00×10^1	4.00×10^1	< 3	< 3
In plant (raw)	8.90×10^2	2.41×10^5	7.10×10^5	1.21×10^6	23	< 3
In plant (cooked)	1.80×10^2	2.50×10^1	4.00×10^1	10	< 3	< 3
Inspection table	2.00×10^2	7.90×10^2	1.80×10^3	1.30×10^3	< 3	4

1.3 and 3.0×10^6/g, respectively. These counts cannot be directly compared to the APC in the present study, since the former counts were obtained by incubating nutrient plates at 27 C. In fact, the count on Pacific shrimp might have been reduced anywhere from 1.5 to 40-fold had the plates been incubated at 35 C. Such difference in total viable count using PCA medium incubated at 22 and 35 C has been observed in a storage study of blue mussel shell stock (*13*).

With such a heavy bacterial load (contamination) on the raw material, it is not surprising to find the presence of coliforms (4/g), although no coagulase-positive staphylococci were detected.

A 2-h brining step, that preceded the hand peeling operation, appeared to reduce the viable count on PCA as well as SWM plates, although an increase in coliforms (15/g) was detected. The washing effect of the brining step is evident from the bacterial load of the brine as observed using SWM and PCA media (see Table 2).

Following the brining operation, shrimp were dipped out with a net, placed in a colander, and taken to picking tables. Shrimp then were headed and peeled manually. Composite samples of peeled shrimp revealed a 71% reduction of the APC and a 95% reduction of SR flora (Table 2). The reduction in the APC compared very favorably with data reported by Green (*4*) where 75% reduction by heading shrimp under aseptic conditions was observed. These counts indicate no buildup of bacterial load in Plant B at the peeling table, implying good sanitary practices at this processing step. This contention is further supported by absence of an increase in coliforms.

Washing peeled shrimp and dipping in chlorinated water resulted in an appreciable reduction in SR bacteria, while the APC increased slightly. There appeared to be a subsequent increase in bacterial load following inspection, resulting in a finished product with an APC of 4×10^4/g. This increase in bacterial count may have been due to recontamination in the holding trays as well as bacterial growth, since the weighing step required excessive handling as well as prolonged holding at room temperature. The extensive handling of peeled shrimp was also reflected by an increase in the coliform count (23/g) and by the appearance of coagulase-positive staphylococci (4/g). In a similar process, Zapatka and Bartolomeo (*18*) reported an average of 5.2 and 7.6 coliforms per gram on shrimp samples obtained from plants considered to have marginal and poor sanitary conditions, respectively.

Plant C

Raw shrimp delivered in open trucks and trailers had a relatively high APC (6.6×10^3/g), as compared to that of freshly caught shrimp. This increase can be attributed to contamination since a relatively smaller increase in SR bacteria was observed. However, samples obtained from the storage hopper showed an excessively high APC, although SR organisms exhibited a significant decrease (see Table 3). Similarly, a high APC on raw samples

TABLE 2. *Viable bacterial counts of line samples for hand-peeled raw shrimp process (Plant B).*

Sample	Aerobic plate count/g				Coliform/g	Coag-positive Staphylococci/g
	PCA	SWM				
	35 C	35 C	21 C	5 C		
Barrel	7.20×10^4	1.54×10^6	2.78×10^6	1.66×10^6	4	< 3
Brine tank (whole shrimp)	1.47×10^4	2.51×10^5	1.20×10^6	7.70×10^5	15	< 3
Brine tank (brine)	4.10×10^4	5.20×10^5	1.19×10^6	9.10×10^5	—	—
Peeling table (headed & peeled)	4.20×10^3	1.36×10^4	6.50×10^4	3.90×10^4	9	< 3
Inspection table	8.80×10^3	7.50×10^3	1.72×10^4	1.00×10^4	4	< 3
Packaged product	4.00×10^4	2.42×10^4	3.70×10^4	3.00×10^4	23	4

TABLE 3. *Viable bacterial counts of line samples for machine-peeled cooked shrimp process (Plant C).*

Sample	Aerobic plate count/g			
	PCA	SWM		
	35 C	35 C	21 C	5 C
Trailer	6.60×10^3	2.01×10^5	8.10×10^5	8.10×10^5
Storage hopper	1.66×10^5	2.37×10^4	1.84×10^5	1.90×10^5
Preheater tank (before)	2.56×10^3	1.64×10^4	1.13×10^5	6.80×10^4
Preheater tank (after)	3.80×10^2	1.26×10^3	4.30×10^3	2.12×10^3
Peelers	1.08×10^3	1.71×10^3	7.90×10^3	4.20×10^3
Seawater wash	2.10×10^2	2.53×10^3	1.01×10^3	1.09×10^4
Fresh water rinse	2.60×10^2	1.63×10^3	9.70×10^2	5.50×10^2
Inspection belt (1)	2.70×10^2	3.50×10^3	8.00×10^2	9.50×10^2
Cooker	8.00×10^1	7.00×10^1	< 10	4.00×10^1
Inspection belt (2)	1.39×10^3	6.80×10^3	5.10×10^4	1.40×10^1
Size grader	1.60×10^4	6.50×10^4	9.00×10^4	2.55×10^3
Before retorting	2.25×10^4	2.45×10^4	4.00×10^4	7.30×10^2

obtained from holding bins has been reported by others (*18*) who investigated plants categorized as having poor sanitary conditions.

Despite the fact that the above plant employed wooden flumes to convey raw shrimp to preheater tanks, fluming with seawater apparently resulted in about a 60-fold reduction of bacterial load on shrimp in the hopper as determined by APC (see Table 3). A similar reduction due to this step has been reported by Zapatka and Bartolomeo (*18*). The SR bacteria were not reduced as dramatically (only 1.6-fold as determined at 21 C).

A brief heating step, to facilitate the peeling operation, significantly reduced the APC (7-fold), which was more pronounced with SR bacteria (26-fold as determined at 21 C). It is noteworthy that machine heading and peeling, instead of reducing bacterial load as in the hand operation, resulted in a significant increase in APC with subsequent reduction of this flora after the salt-water wash. No such significant change in counts of SR organisms was observed (see Table 3). No further buildup of APC was observed in subsequent steps (fresh water rinse and inspection), yet a significant reduction of SR psychrotrophic-mesophilic flora occurred.

The 3-min cooking process which followed significantly reduced the viable count; this was most pronounced with SR bacteria. The sudden increase in viable count at the second inspection belt appears to have been due to contamination, but contribution by bacteria recovering from sublethal damage cannot be ruled out.

Trays collecting graded shrimp were emptied infrequently and their reuse would allow bacterial growth and recontamination. Samples analysed at this stage of processing had an APC of 1.6×10^4/g. Significantly higher concentrations of SR bacteria were detected at this sampling point except for the 5 C count. This was also the only sampling where coagulase-positive staphylococci (43/g) were detected. Brief holding of shrimp in cans in a solution of 2.5% citric acid in 2.5% NaCl before sealing and retoring reduced the viable count of SR bacteria, but the APC showed an increase. It is encouraging to note that no coliforms were detected in any of the samples taken throughout this plant.

In conclusion, it may be pointed out that the brine-cooked shell-on process was operated under good sanitary conditions and had the advantage of processing shrimp which had minimal increase in bacterial count after catch. Both the hand-peeled raw and the machine-peeled cooked processes had the disadvantage of receiving raw material which revealed significant increase in bacterial load after catch as well as after in-plant storage, especially in the latter process. Reduction in viable bacterial count occurred in both processes and was more pronounced during cooking, but because of delays in handling and questionable sanitary practices, especially in the machine-peeled cooked process, rapid bacterial build-up took place. All shrimp samples examined at the final stage of production appeared to be acceptable from a microbiological standpoint (*6*), although some loss in quality may be suspected due to the initial high bacterial load of the hand-peeled raw and the machine-peeled cooked process as revealed using SWM.

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REFERENCES

1. AOAC. 1975. Official methods of analysis. W. Horowitz (ed). Association of Official Analytical Chemists, Washington, D.C.
2. Butterfield, C. T. 1932. The selection of a dilution water for bacteriological examinations. J. Bacteriol. 23:355-368.
3. Dow, R. 1977. Natural abundance fluctuations in the Maine shrimp fishery. National Fisherman. 57(11):4B-5B.
4. Green, M. 1947. Bacteriology of shrimp. II. Qualitative studies on freshly caught and iced shrimp. Food Res. 14:372-383.
5. Harrison, J. M., and J. S. Lee. 1969. Microbiological evaluation of Pacific shrimp processing. Appl. Microbiol. 18:188-192.
6. International Commission on Microbiological Specifications for Foods. 1974. Microorganisms in foods. 2. Sampling for microbiological analysis: Principles and specific applications. University of Toronto Press, Toronto, Canada.
7. Liston, J. 1956. Qualitative variations in the bacterial flora of flatfish. J. Gen. Microbiol. 15:305-314.
8. Olson, J. C., Jr., and L. R. Shelton. 1973. Microbiology of prepared and precooked fishery products. *In* C.O. Chichester and H. D. Graham (eds.), Microbial safety of fishery products. Academic Press Inc., New York.
9. Ortel, S. 1958. Lysotypie von *Staphylococcus aureus* zur Klärung epidemiologischer Zusammenhänge bei durch Fischkonserven hervorgerufenen Staphylococcen — Nahrungsmittelvergiftungen. Z. Hyg. Infektionskrankh. 144:407-424.
10. Patrick, M. H., and R. H. Haynes. 1964. Dark recovery phenomena in yeast. II. Conditions that modify the recovery process. Rad. Res. 23:564-579.
11. Scholes, R. B., and J. M. Shewan. 1964. The present status of some aspects of marine microbiology. Adv. Marine Biol. 2:133-169.
12. Shewan, J. M. 1961. The microbiology of sea-water fish. *In* G. Borgstrom (ed.) Fish as food. Academic Press Inc., New York.
13. Slabyj, B. M., and C. Hinkle. 1976. Handling and storage of blue mussels in shell. University of Maine, Res. Life Sci. Agric. 23(4);1-13.
14. Stapleton, G. E., D. Billen, and A. Hollaender. 1953. Recovery of x-irradiated bacteria at suboptimal incubation temperatures. J. Cell Comp. Physiol. 41:345-377.
15. Stapleton, G. E., A. J. Sbarra, and A. Hollaender. 1955. Some nutritional aspects of bacterial recovery from ionizing radiations. J. Bacteriol. 70:7-14.
16. Stevenson, K. E., and L. R. Richards. 1976. Thermal injury and recovery of *Saccharomyces cerevisiae*. J. Food Sci. 41:136-137.
17. Wilson, G. S. 1949. The bacteriology of food poisoning. Practitioner 162:445-451.
18. Zapatka, F. A., and B. Bartolomeo. 1973. Microbiological evaluation of cold-water shrimp (*Pandalus borealis*). Appl. Microbiol. 25:858-861.

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Functional Properties of Plasmids as Related to Enumeration of Bacteria Important to the Food Microbiologist

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ABSTRACT

Plasmids are extrachromosomal elements that behave like auxiliary chromosomes and contain the basic structure of the replicating unit. They were initially recognized by the unusual phenotypic characteristics they confer upon a cell, such as ability to promote genetic transfer by conjugation; resistance to antibiotics and metal ions; production of bacteriocins, toxins, antigens; and other factors. Much of the work on plasmids and plasmid-mediated characteristics has been conducted with various genera and species of the family *Enterobacteriaceae*. Present data indicate that although plasmid transfer occurs with variable frequency, intergeneric transfer within the family is invariably successful. Although a great deal of investigation has been focused on plasmid-mediated resistance to antibiotics, many workers have determined that a number of plasmids control other phenotypic characters. For example such plasmids appear to mediate production of bacterial products and/or enzymes which may confer a selective ecological advantage to bacteria in their inter-relationships with their hosts and other microorganisms. The isolation of these phenotypically altered microorganisms by food, medical, and public health microbiologists may signify an important ubiquitous phenomenon. There is increasing evidence that selective forces operate in both the laboratory and in nature which should alert the microbiologist that any of the present-day identification schema must be utilized with some caution. To this end technical advances in identification of microorganisms by use of a large battery of biochemical tests has increased the accuracy of identification and measurably reduced the misidentification of atypical bacteria. Availability of such data may assist the microbiologist to establish baseline information essential for development of prospective studies for defining the reservoirs of phenotypically altered microorganisms and their pathogenic potential for the general population. The full importance of plasmid-mediated characteristics in bacterial populations in food products and the role they will play in the future is largely unknown. The origin and selective advantage that strains harboring plasmids in the natural ecology is also unknown. Detailed knowledge of microorganisms maintaining plasmids may be important economically, since the emergence of a number of antibiotic-resistant microorganisms may have contributed a significant role in development of disease among domestic animals because of the manner in which animal husbandry is practiced in many countries. Extrapolated, one could speculate about an ever-increasing reservoir of similar bacterial species, potentially transmissible to man. Because the factors which control mobilization of plasmids and the important ecological selective advantage conferred upon bacterial populations by plasmids, it is essential that we as microbiologists become cognizant of their existence and to fully understand the mechanisms of perpetuation of plasmid-mediated characteristics.

In many species of bacteria portions of the genetic complement of the cell are carried on extrachromosomal elements, which behave like small auxiliary chromosomes and are referred to as plasmids.

Plasmids can be defined as extrachromosomal elements capable of stable replication in a cell and contain the basic structure of the replicating unit. Generally, plasmids are initially recognized by the unusual phenotypic properties they confer on a cell; for example, the ability to promote genetic transfer by conjugation, resistance to various antibiotics and metal ions, production of bacteriocins, toxins, and other factors. A plasmid is generally named for the particular property that it characteristically specifies. Bacterial plasmids can be divided into two general categories: those that promote conjugal transfer of DNA are referred to as conjugative, transmissible, or sex-factor plasmids; and those that are incapable of promoting conjugative, non-transmissible, or non-sex-factor plasmids (*18*).

All plasmids, whether transmissible or not, share certain basic characters. They are autonomous replicating genetic elements which direct their own replication and segregation during cellular division. Their replication is generally controlled and synchronized with the chromosome, and there may be multiple copies of a plasmid. The genes carried by plasmids are not essential for cell survival, but may confer enhanced survival, under particular environmental circumstances. Plasmids can be lost from a cell and are transferred at varying frequencies. Isolated plasmids appear to be covalently-closed circles of doublestranded DNA. Finally, presence of a plasmid will often confer a kind of immunity to superinfection and replication of a second closely related plasmid (*21*).

ANTIMICROBIAL RESISTANCE

Much of the work on plasmids and plasmid-mediated characteristics has been conducted with various genera and species of the family *Enterobacteriaceae*. Medical

and public health microbiologists were first introduced to the importance of plasmid-mediated properties in bacteria, following an epidemic of shigellosis in Japan. In 1956 a group of Japanese investigators reported careful epidemiological observations on the changing pattern of antibiotic susceptibility of *Shigella* strains (*7,11*). It was repeatedly observed that some patients excreted *Shigella* strains that were completely susceptible to antimicrobial agents; whereas, other patients excreted multiple-drug-resistant strains of the same serological type. Moreover, it was found that multiple-drug resistance was not restricted to *Shigella.* In one epidemic, *Escherichia coli* strains were isolated that had the same pattern of resistance to chloramphenicol, streptomycin, tetracycline, and sulfonamides as the implicated *Shigella* serotype. Later, it was demonstrated that this property of drug resistance was transmissible. Since then, R-factors (resistance-transfer-factors) have been demonstrated in virtually all serotypes of *E. coli*, all four species of *Shigella* and the Alkescens-Dispar group, many of the *Salmonella*, *Arizona, Citrobacter,* all four species of *Proteus,* and members of the genera *Providencia, Klebsiella, Enterobacter, Serratia, Aeromonas, Pseudomonas, Yersinia,* and others.

INTERGENERIC PLASMID TRANSFER

Intergeneric plasmid-transfer has been documented for many years (*14*). Present data indicate that although plasmid transfer occurs with variable frequency between all members of the family *Enterobacteriaceae* and also members of other families, it is often dependent as much on the properties of the specific plasmid as on the donor and recipient tested.

Studies on the intergeneric transfer within bacteria, including the *Enterobacteriaceae,* demonstrate invariably successful intercrosses between the following genera: *Escherichia, Shigella, Salmonella, Citrobacter, Klebsiella, Serratia,* and *Proteus.* Transfers that have been less frequently successful have been observed with the genera *Yersinia, Arizona, Enterobacter,* and *Providencia*

Different groups of plasmids appear to have specific host ranges. F-like R-factors are transmissible from *E. coli* to *Proteus,* but not to the *Pseudomonodaceae.* The apparent frequency of plasmid transfer in interspecific crosses is usually much lower that in intraspecific crosses, but this may be due to factors having limited value in determining relatedness.

OTHER PHENOTYPIC FACTORS

Although a great deal of investigation has been focused on plasmid-mediated drug resistance in many different genera of bacteria, a number of workers have determined that plasmids may control other phenotypic characters. For example, plasmids have been described from intestinal bacteria which appear to mediate the synthesis of a wide variety of bacterial products, many of which are significant to survival of the bacteria and in their inter-relationships with their hosts and other microorganisms. Moreover, plasmids also appear to be associated with the virulence of some intestinal bacteria in that they may control elaboration of enterotoxins, specific antigens, hemolysins, and a variety of other factors.

Lautrop and co-workers (*12*) reported the isolation of 26 strains of hydrogen sulfide-producing *E. coli* recovered over a fourteen month period in Denmark. The isolates belonged to 13 different serotypes and represented 11 distinguishable biotypes. One of the strains transmitted its hydrogen sulfide-producing capacity to a non hydrogen sulfide-producing strain of *E. coli*, indicating plasmid transfer. Sixteen of the 26 isolates were recovered from patients with urinary tract infections.

Maker and Washington (*13*) later reported the isolation of seventeen strains of hydrogen sulfide-producing *E. coli*, 10 from fecal specimens, five from urinary tract infections, and two from post-mortem studies. These strains were unstable in their production of hydrogen sulfide and demonstrated biochemical reactions and antimicrobial susceptibility profiles of typical *E. coli* strains. In a later study, these same authors (*20*) reported on the isolation of three strains of gram-negative bacilli that closely resembled *E. coli* except their citrate reactions were definitely positive. Darland and Davis (*3*) reported on over 200 strains of hydrogen sulfide-producing *E. coli*, identified between 1966 and 1972, which represented 46 different serogroups. Fifty-four percent of these isolates were resistant to three or more antibiotics and 20% demonstrated typical antimicrobial susceptibility profiles. Sixty-five percent of the total isolates were recovered from fecal or urine specimens. No doubt some of the characteristics of these isolates represent an example of multiple plasmid transfer.

Johnson et al. (*10*) reported a strain of *Salmonella tennessee* from a clinical specimen, which transferred its ability to ferment both lactose and sucrose to various strains of *E. coli* by conjugation. The ability to utilize both lactose and sucrose appeared to be transferred as a single entity.

In another study by Wohlhieter and colleagues (*22*) two of seven sucrose-fermenting strains of *Salmonella* were capable of transmitting this property to *E. coli* K12. Earlier, Easterling et al. (*4*) demonstrated six of seven lactose fermenting *Salmonella* strains that transferred this property to *Salmonella typhi*, which in turn transferred it to a strain of *Salmonella typhimurium,* at high frequencies. Their strain of *S. typhi* also could transfer this lactose-fermentation property to *Proteus mirabilis*. The beta galactosidase produced by these *lac+* elements was quantitatively and qualitatively distinguishable from that produced by typical strains of *E. coli.*

Rowe and co-workers (*16*) described a new *Salmonella*

species, *Salmonella crossness*, isolated from activated sludge. This organism fermented sucrose rapidly in Triple Sugar Iron Agar, but failed to produce hydrogen sulfide in this medium. Hydrogen sulfide production was subsequently demonstrated with the use of lead acetate medium. Reactions with *Salmonella* 0 antisera, 1-66, were uniformly negative. Preparation of homologous antiserum to the new strain demonstrated presence of a new somatic antigen, designated 67. The ability to utilize sucrose was shown to be transmissible to strains of *E. coli* and *S. typhimurium.*

Smith and Parsell (*19*) reported on the ability of three strains of *E. coli* to transfer separately their sucrose and raffinose characteristics to strains of *S. typhi, Shigella flexneri,* and *Shigella sonnei.* The transmissible raffinose utilizing capacity was found to be a feature of porcine enteropathogenic *E. coli,* possessing the K88 fimbrial antigen. Moreover the determinants controlling raffinose utilization and production of fimbrial antigen were commonly transmitted together; this was also true of the determinants controlling enterotoxin production and tetracycline resistance, but to a lesser extent.

Quantitatively different phenotypic characters of lactose fermentation, on MacConkey Agar, have been demonstrated with a variety of *Klebsiella* strains (*15*). Some strains demonstrate a strong (ML+) lactose fermentation capacity, while others utilize lactose to a much lesser extent (ML−/+). ML+ strains of *Klebsiella* possess approximately 10 times the beta-galactosidase activity of ML −/+ strains. Thirteen of 14 ML + strains tested carry a *lac* operon which is transmissible to *E. coli.* Loss of the *lac* operon results in strains similar to ML−/+ strains.

Cornelis et al. (*1*) described a strain of *Yersinia enterocolitica* which was capable of transmitting its lactose fermentation capability to various strains of *E. coli* (at a frequency less than 10^{-6}) and back to the original *Yersinia* which could transfer it to other strains of *Y. entercolitica* at greater frequency (5×10^{-3}).

These few examples of plasmid-mediated alterations of typical phenotypic characteristics of a variety of bacterial genera should be a signal to food, medical, and public health microbiologists of an important ubiquitous phenomenon. There is increasing evidence that selective forces operate in both the laboratory and in nature which should alert us that identification schema must be used carefully and intelligently. Moreover, these schema always should be viewed as continuously evolving and not "cast in stone."

IDENTIFICATION OF MICROORGANISMS

Technical advances in the accurate identification of gram-negative bacilli and particularly the glucose-fermenting strains have substantially diminished the misidentification of atypical bacteria. In this regard routine use of a large battery of biochemicals increases the accuracy of identification, especially at the species and subspecies (or biotype) level. Biochemical reactions form the primary basis for classification of these bacteria and to achieve reproducibility of results, the importance of accepting a standardized procedure becomes self-evident.

Several authors (*2,5,6,8*) have compiled extensive biochemical data on large numbers of gram-negative bacilli. These and other authorities feel that use of a larger battery of biochemical tests coupled with the increasing occurrence of aberrant biochemical behavior enhances the need for development of new interpretative schemas. Until a few years ago only the larger microbiology laboratories had the capability to do larger batteries of biochemical tests for identification of their gram-negative bacilli. However, with the advent of several commercial test systems employing a preset battery of biochemicals, i.e. API, Enterotube, Inolex, etc. even the smallest of laboratories can now enjoy the pleasures of reliable definitive identification. Moreover, many of these systems include some sort of numerical identification key to assist in the identification process.

Computer-assisted programs for identification of gram-negative bacilli have been in use for several years particularly at the NIH and the Central Public Health Laboratory in England. For example, the NIH computer identification system maintains both the sophistication of calculation and the availability of readily interpretable and accessible data for the laboratory. Many of the aforementioned test systems now provide similar computer identification systems based on thousands of biochemical results. Availability of such data banks should definitively assist the microbiologist in the identification process regardless of the aberrant nature of the organism isolated due to plasmids or whatever other factors that may be present.

Space does not permit us to discuss all of the identification systems that are available or being developed but one of the more important advances which is currently taking place in several laboratories is the use of gas-liquid chromatography (GLC) as an aid in the rapid identification of bacteria. Offering a very high degree of sensitivity and resolution, GLC is relatively easy to do employing relatively inexpensive instrumentation. Basically, there are three identification techniques employed by this procedure, and these are: (*a*) detection of metabolic end products as a result of growth in media, (*b*) detection of components of the organism such as with pyrolysis, and (*c*) detection of microorganisms or their products direct from body fluids, i.e. urine, abscesses, CSF, etc.

It seems to us that on the basis of what has been accomplished in the diagnostic laboratory, GLC would be equally suited in the laboratory of the food microbiologist.

OVERVIEW

The full importance of plasmid-mediated characteristics in bacterial populations in food products and the role they will play in the future is largely unknown. The

origin and selective advantage that such strains have in the natural ecology is also unknown. However, it is clear that as we become more aware of the existence of these phenotypically aberrant strains, baseline and prospective studies will be required to try to define the reservoirs of these organisms and their potential for pathogenicity to the general population.

It is conceivable that detailed knowledge of microorganisms carrying plasmids may be important economically. For example, the emergence of resistant bacterial organisms within animal populations has been increasing. Animals provide a significant amount of protein for the world's population. The conditions under which animal husbandry is practiced certainly provide the impetus for outbreaks of various diseases caused by enteric and other bacteria. To effectively treat severe diarrheal disease in cattle, swine, and poultry populations, extensive use of antibiotics, which are essentially identical to those for man, have been employed. This and the wide spread use of antibiotics in animal feed has caused a definite increase in the number of isolates of drug-resistant microorganisms. Extrapolated, one could easily speculate about an ever-increasing reservoir of bacterial species, harboring and transmitting a variety of plasmids, potentially transmissible to man. Since the factors which control the mobilization of plasmids are largely unknown, and probably uncontrollable at this point, the need to understand the ecologically important selective factors involved in the emergence of these strains is essential.

Because it will be largely the task of the food, medical, and public health microbiologists to recognize and characterize the large number of newly emerging phenotypically atypical bacteria, it becomes imperative for them to understand the existence and mechanism(s) of plasmid-mediated characteristics. Equally important will be their awareness of the rapid changes taking place in the technology of the identification process.

REFERENCES

1. Cornelis, G., P. M. Bennett, and J. Grinsted. 1976. Properties of PGGl, a *lac* plasmid orginating in *Yersinia enterocolitica.* J. Bacteriol. 127:1058-1062.
2. Cowan, S. T., and K. J. Steel. 1974. Manual for the identification of medical bacteria. 2nd ed. Cambridge Univ. Press, Cambridge, U. K.
3. Darland, G., and B. Davis. 1974. Biochemical and serological characterization of hydrogen sulfide positive variants of *Escherichia coli.* Appl. Microbiol. 27:54-58.
4. Easterling, S. B., E. M. Johnson, J. A. Wohlhieter, and L. S. Baron. 1969. Nature of lactose-fermenting *Salmonella* strains obtained from clinical sources. J. Bacteriol. 100:35-41.
5. Edwards, P. R., and W. H. Ewing. 1972. Identification of *Enterobacteriaceae,* 3rd ed. Burgess Publ. Co., Minneapolis, MN.
6. Ewing, W. H., and W. J. Martin. 1974. *Enterobacteriaceae.* Manual of clinical microbiology. E. H. Lennett, E. H. Spaulding, and J. P. Truant (ed). 2nd ed. American Society for Microbiology, Washington, D. C.
7. Falkow, S. 1975. Infectious multiple drug resistance. Pion Limited, Brondesbury Park, London.
8. Gilardi, G. L. 1973. Nonfermentative gram-negative bacteria encountered in clinical specimens. Antonie van Leenwenhoek 39:229-242.
9. Gyles, C. L. 1972. Plasmids in intestinal bacteria. Amer. J. Clin. Nutrit. 25:1455-1458.
10. Johnson, E. M., J. A. Wohlhieter, B. P. Placek, R. B. Sleet, and L. S. Baron. 1976. Plasmid-determined ability of a *Salmonella tennessee* strain to ferment lactose and sucrose. J. Bacteriol. 125:385-386.
11. Kitamoto, O., N. Kasai, A. Fukaya, and A. Kawashima. 1956. Drug sensitivity of the *Shigella* strains isolated in 1955. J. Japan. Assoc. Infect. Dis. 30:403.
12. Lautrop, H., I. Orskov, and K. Gaarslev. 1971. Hydrogen sulfide producing variants of *Escherichia coli* Acta Path. Scand., Sec. B. 79:641-650.
13. Maker, M. D., and J. A. Washington II. 1974. Hydrogen sulfide producing variants of *Escherchia coli.* Appl. Microbiol. 28: 303-305.
14. Marmur. J., S. Falkow, and M. Mandel. 1963. Ann. Rev. Microbiol. 17:329-372.
15. Reeve, E. C., and J. A. Braithwaite. 1973. Lac+ plasmids are responsible for the strong lactose-positive phenotype in many strains of *Klebsiella* species. Genet. Res. 22:329-333.
16. Rowe, B., M. L. M. Hall, and J. H. Mc Coy. 1976. *Salmonella crossness*, a new serotype containing a new somatic antigen. J. Hyg. 77:355-357.
17. Sanderson, K. E. 1976. Genetic relatedness in the family *Enterobacteriaceae.* Ann. Rev. Microbiol. 30:329-349.
18. Sherratt, D. J. 1974. Bacterial plasmids. Cell 3:189-195.
19. Smith, H. W., and Z. Parsell. 1975. Transmissible substrate-utilizing ability in Enterobacteria. J. Gen. Microbiol. 87:129-140.
20. Washington, II, J. A., and M. D. Maker. 1976. Unclassified citrate-positive member of the family *Enterobacteriaceae* resembling *Escherchia coli.* J. Clin. Mcrobiol. 4:165-167.
21. Wilson, G. S., and A. Miles. 1975. Topley and Wilson's Principles of Bacteriology, Virology, and Immunology. 6th ed., Vol. 1. Edward Arnold, London.
22. Wohlhieter, J. A., J. R. Lazere, N. J. Snellings, E. M. Johnson, R. M. Synenki, and L. S. Baron. 1975. Characterization of transmissible genetic elements from sucrose-fermenting *Salmonella* strains. J. Bacteriol. 122:401-406.

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Nondestructive Quality Evaluation of Agricultural Products: Introduction

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Precise objective quality grades for agricultural products have long been the goal of many people who are concerned with marketing and processing of our agricultural production. The role that 20th Century technology is playing in developing improved equipment for quality evaluation was the subject of a recent Food Science Symposium titled "Nondestructive Quality Evaluation of Agricultural Products." The symposium was held at the 1977 meeting of the Southern Association of Agricultural Scientists in Atlanta, GA. The technology used in nondestructive quality evaluation has evolved through the efforts of several disciplines, so the program was co-sponsored by the Food Science, Horticultural, and Agricultural Engineering Sections of the Association.

The primary objective of this group of four papers is to bring into focus, for people in the food industry, the wide array of quality evaluation problems that can be resolved with nondestructive measurements. Development of a nondestructive test depends entirely upon establishing a relationship between the quality factor of interest and one or more physical properties of the sample, such as density or the interaction between the product and some form of energy. The authors have addressed themselves to describing procedures and equipment that are based upon proven relationships. Furthermore, the primary concern is with active devices; that is, equipment that produces an electrical signal proportional to the quality of the product. This signal may be read out directly as in a quality control laboratory (Fig. 1) entered into a computation via a computer (Fig. 2) or used to control an automatic sorting apparatus (Fig. 3).

In recent years we have seen very rapid advances in the electronics industry. This can be illustrated with the inexpensive pocket calculators that are on the market which can do the same computations that required very large expensive computers 25 years ago. With this improved technology we are at a new threshold in nondestructive quality evaluation.

Figure 1. *Read-out of electrical signal proportional to product quality.*

The capacity of computers to handle large quantities of data very fast provides many new possible applications of nondestructive quality evaluation equipment. For example, at packinghouses for fresh produce where automatic sorting is used, statistical information about the quantity and quality of the produce being packed could be obtained simultaneously with the sorting operation. At processing plants, process control could be based, in part, upon the quality of the raw material going into the plant. Thus process control changes could be

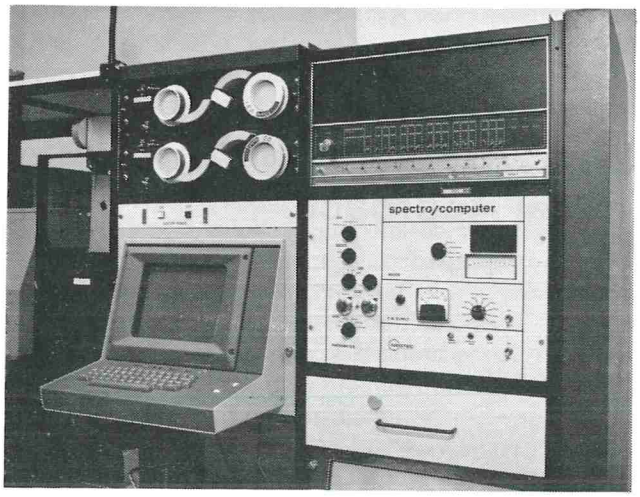

Figure 2. *Computer for calculation of product quality.*

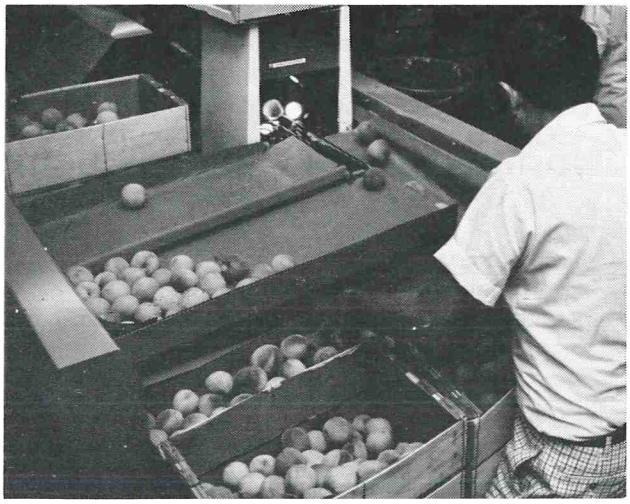

Figure 3. *Computer-controlled automatic sorting device.*

anticipated. With the increased sophistication of systems engineering, there is an unlimited number of future applications for nondestructive quality evaluation equipment in the handling and processing of our agricultural products.

As an engineer who has been involved in the development of nondestructive quality evaluation techniques for several years, I look forward to the increased uses of the technology with considerable enthusiasm.

Along with the authors of the symposium papers Drs. Dull, Marion, Finney, and Ballinger, I want to express our appreciation to the editor of the *Journal of Food Protection* for offering to publish the papers presented at the 1977 SAAS Food Science Symposium.

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Nondestructive Quality Evaluation of Agricultural Products: A Definition and Practical Approach

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ABSTRACT

A definition of terms in this research area is presented. A general approach for applying nondestructive quality evaluation (NDQE) to horticultural crops, for both fresh and processing purposes is discussed. The approach includes consideration of the importance of biochemical, morphological and physical parameters of quality. The objective and subjective determination of single and multiple parameter judgements of quality at different stages of physiological development is a critical part of this approach. The concept of "Optimum Quality Range" is presented in relation to the development of nondestructive quality evaluation techniques and applications.

In approaching the subject of nondestructive quality evaluation (NDQE) of agricultural products, one should appreciate that man has always made this type of judgment. Historically, the color and texture of fruits and vegetables have been evaluated by eye and by feel without destroying the integrity of the product. A formal research approach to NDQE includes basic research to define quality, to develop and compare methods of measuring quality, to develop highly specialized instruments, and to study the practical applications of this analytical approach in the total food delivery system. This approach has received increased interest and support in the last two decades. In my opinion, it deserves recognition as a research area with a great deal of potential for valuable practical applications. The potential value of this work was the basis for planning this symposium.

THE TERMS

In setting the stage for this symposium, there is need to define the terms "nondestructive," "quality," and "evaluation." The term "non-destructive" indicates that the sample, (the physical form is not critical), can be analyzed and meaningful data collected by some means so that the physical and chemical attributes of that sample are not altered. The sample can then be used for other purposes, such as eating. The word "quality" has several meanings, but I believe the one we are looking for is "The degree of excellence which a thing possesses; hence, excellence or superiority." Another more basic definition of quality is "that which makes something what it is." "Evaluation" means "to determine the worth of; to appraise." Putting these three words together, nondestructive quality evaluation could be simply stated as "the gaining of meaningful information that can be used in making judgments, both positive and negative, about the degree of excellence of a food without altering the physical and chemical properties of that food."

The methods of obtaining these objective analyses by nondestructive means include weighing, sizing, spectrophotometric analysis by reflectance or transmittance measurements, X-ray analysis, the use of sonics to determine some degree of tissue integrity, and densiometric measurements using light, liquid, or possibly radiofrequencies.

QUALITY FACTORS

One of the purposes of this presentation is to give a general approach for the application of NDQE to agricultural products with emphasis on horticultural crops. In keeping with this approach the first step should be selection of the positive quality factors that are critical for a specific comodity.

For example, man considers most apples to be red, peaches to be yellow-orange, some avocados to be green colored when ripe, and tomatoes to be red, in general. The fact that color varies from commodity to commodity has not deterred man from using that parameter as an index of quality.

A second quality factor is flavor. Flavor is a subjective judgment but has a number of objective components which include sugar content, acidity, the amount and type of volatile compounds, and the presence or absence of materials that contribute to astringency or bitterness.

A third quality factor is texture. It is generally accepted that celery should be crunchy, peaches and cantaloupes should be firm to soft but not mushy,

avocados should be soft, and snap beans should be free from an excessive number of fibers. These textural properties can be evaluated by means of a shear press, but that is destructive. Density or optical measurements may prove to be important in this area. Some evidence indicates that light-scattering measurements might be useful in assessing texture.

These three attributes (color, flavor, and texture) are generally positive. Attention must be given to negative quality factors—defects. Characteristics in this category include bruises, cuts, insect bites, diseased or rotted areas, brown or black spots, and flower parts. Any factor that detracts from the final product quality grade can be considered a negative quality factor.

MEASURING CHANGES IN QUALITY FACTORS

After considering the above quality factors and selecting the specific quality parameters for a commodity, the next step is to determine changes in these parameters between different stages in the development of the fruit or vegetable. The changes usually are measured by classic chemical analyses which are, for the most part, destructive. This approach can be illustrated by the changes in physiochemical properties of pineapple fruit during the period from flowering to senescence (Fig. 1).

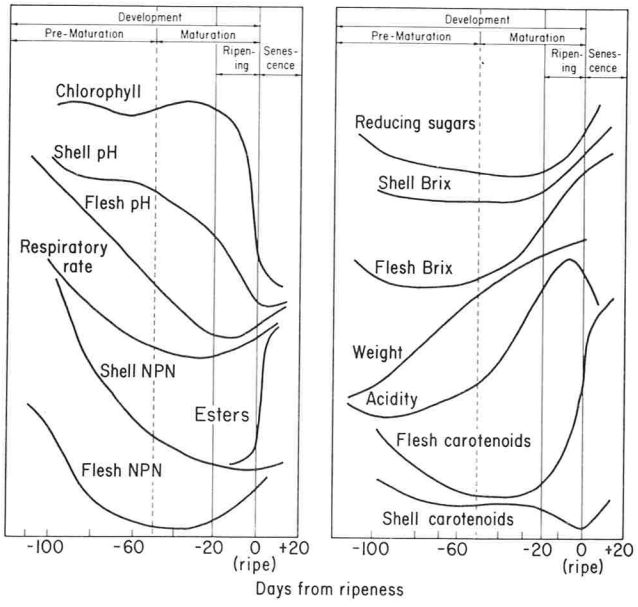

Figure 1. *Changes in physiochemical properties of pineapple fruit during period from flowering to senescence.* [*From Gortner et al. (2)*].

As defined by Gortner et al. (*2*), development is the period from the end of blossoming to and including the ripening of the fruit. A subdivision of the development time is the prematuration period, which is the developmental period before onset of maturation processes and generally includes at least half of the interval between blossoming and harvest. Maturation covers the stage of development during which the fruit emerges from the incomplete stage to attain a fullness of growth and a *maximum edible quality.* Ripening is the terminal period of maturation during which the fruit attains its full development and its maximum aesthetic and edible quality. Senescence is the period following fruit development during which growth has ceased and the biochemical processes of aging replace the perfective changes of ripening.

The critical factor in this approach is to recognize that each time a fruit is produced, it goes through a predictable series of compositional changes. It reaches a predictable endpoint, a ripe fruit. It is these changes during development which are of concern in the application of nondestructive quality evaluations.

Using pineapple as an illustration, note (Fig. 1) that during prematuration and the early part of maturation, chlorophyll is high, meaning that the fruit is very green in appearance. During the latter stages of maturation or ripening, there is a rapid degreening of the fruit. This trend is characteristic. During the ripening stage of pineapple the flesh Brix (a measure of soluble solids which are primarily sugars) increases markedly. This increase is interpreted by man as an increase in sweetness. The acidity has an interesting change since it reaches a maximum just before the fruit becomes ripe, and then it decreases. This is a moderating effect and the fruit is less tart. The fruit color changes in two ways. It degreens through a loss of chlorophyll, and the yellow becomes more intense due to the increase in the amount of flesh carotenoids during ripening. This increase continues into senescence. The esters show a marked increase in the last few days of ripening from basically zero to a very high level (over 100 ppm). These are examples of selected quality parameters which are dynamic in their changes. These sophisticated measurements are really of little value unless they are related to human judgments or choices of quality.

The concept of optimum quality range (OQR), introduced by Dull and Hulme (*1*) offers a way to understand the interrelations of objective and subjective measurements of quality. The concept of OQR is presented graphically in Fig. 2. Although Fig. 2 is based on data developed with pineapple and a panel of 50

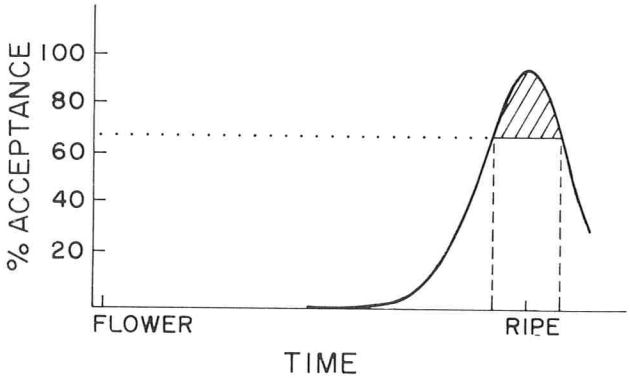

Figure 2. *A generalized presentation of changes in percent acceptance as ripe for fruit during the period of flowering to senescence. The crosshatched area represents the Optimum Quality Range.*

people, the concept is considered applicable to fruits in general. Time from flowering until ripeness is represented on the X axis, and percent acceptance on the Y axis. In developing the panel, two questions were asked. First, do you know what a pineapple is? Second, do you like pineapple fruit? If both questions were answered affirmatively, the person was qualified to serve on the panel. This approach is concerned with acceptance rather than preference.

After the panel was constituted, a sampling schedule was established and samples were collected, prepared, and presented to the panel. The panelists were asked, "Do you accept this sample as characteristic of a ripe fruit?" The response had to be either yes or no.

At the flowering stage, no one said the fruit was acceptable as ripe. Proceeding along the time axis at selected sampling dates, responses continued to be of zero percent acceptance as ripe until one point, an individual said "Yes, this fruit is ripe." At the next sampling more panel members said "Yes, this fruit is acceptable as ripe." At one point on the time axis all of the panel said "Yes, I accept this fruit as ripe."

Continuing on the time axis, a point was reached at which some panel members did not accept the sample as ripe. The connotation was that the fruit had entered senescence and so was considered over-ripe and therefore not acceptable.

To delineate the 70% acceptance range, a line was drawn at the 70% acceptance level to intercept the curve on both the positive and negative slopes. The time between these two intercepts was designated as the 70% acceptance range or the optimum quality range (OQR). The 70% value is arbitrary and would be subject to the judgment of individual researchers. Within the OQR, fruit have specific ranges in composition. In illustration of this point, for pineapple expected ranges would be 8 to 15% for brix, 0.6 to 1.1% for acids, 10 to 100 ppm for esters, and 0.4 to 1.2 ppm for yellow pigments.

Physiological development is an ongoing process from flowering to senescence during which any point can be called a stage of development. In the idealized situation all fruit at the same stage of development would have the same chemical and physical properties. The OQR can thus be viewed as a continuum of stages of physiological development, all of which are acceptable as ripe for the fruit population being evaluated. Saying it in another way, in a given population (fruit from the same field) all fruit at the same stage of physiological development could be expected to have similar compositions.

If fruit were selected from different populations, e.g., a different field or a different year, the OQR could still be described, but the absolute values for the ranges might differ. For example, a ripe fruit in Field 1 may have a sugar level of 12% and an acid content of 1.0%. In Field 2, a ripe fruit may have 14% sugar and 0.6% acid. Based upon the judgment of panelists, both fruit could fall within the OQR, but they would taste very different.

A more familiar example is the wine grape. Based upon accepted quality parameters, there are good and bad grape years. All wine grapes are picked at the peak of their physiological development each year, and that peak composition is the best that can be obtained from that field that year. The peak composition can be expected to vary from year to year.

Moving towards the application of NDQE to horticultural crops, another point needs to be raised; more than one quality parameter may be needed to make the final maturity or quality index judgment. In pineapple, the amount of light (at 468 nm) transmitted through a sample has been measured. When transmittance was correlated with chemically measured pigments in the same tissues, the coefficient of determination (r^2) was relatively low, in the range of 40% for some samples. It became obvious that the samples, for which the correlation was low, had an opaque appearance indicating there was air in the interstitial spaces. This opacity was measured by illuminating the same sample with white light from a tungsten bulb and using a selenium photovoltaic cell to detect radiation which passed through the sample. By simple calculation of the square of absorbance at 468 nm, times the instrument reading of the photovoltaic cell output, a pigment index was derived. This index, when correlated with the pigment measurement, gave an r^2 value of 74. Thus, a two-parameter measurement was necessary to estimate pineapple pipgments nondestructively. An example of a three-parameter measurement for maturity index would be the simultaneous determination of color, density, and size in peaches.

INSTRUMENTAL QUALITY MEASUREMENT

Although extensive research has already been conducted on how to determine quality factors of agricultural products nondestructively, more effort in this area is necessary. The papers following will address this subject more specifically. The NDQE sensing of specific quality parameters of horticultural products in the context of automatic high speed sorting is an accomplished fact. The number of specific applications continues to grow. After developing an instrument system for sensing quality for a given commodity, it seems logical to move on to practical applications—the prototype sorting machine. This is not to be confused with a commercial unit which handles commercial quantities of product, but is really a pilot plant model in which the sensing operation can be combined with several handling operations, such as unloading, washing, singulation, and disposition. Each of these steps could be expected to influence the validity of the specific NDQE measurements. For example, after a "best" means of sample presentation has been ascertained, considerable work can still be required to actually effect that sample presentation in a pilot plant sorting unit.

In considering a prototype sorting unit we should recognize that the speed of the sensing operation is not likely to be the limiting factor. The combined sensing,

calculation, and decision-making operation can be accomplished in milliseconds. However, the operations of singulation (the transformation of a stationary group of fruit into a moving line of separated fruit) and disposition (the movement of a fruit from a moving line of separated fruit to an area established for fruit of similar quality) pose interesting challenges. They must be accomplished with minimum damage to the product and maximum accuracy of quality evaluation. In terms of accomplishing the high speed separation of fruit based on NDQE, the operations of singulation and disposition are as important as the sensing operations.

In addition to the concern with problems of high velocity handling of fruit, there is also a need to be concerned with the problems of evaluating an entire fruit, as opposed to a small portion of the surface. The filling of this need is difficult because of fruit size, irregularities in shape and surface, and inherent variations in the composition of different parts of a single fruit. Allowances for this consideration must be designed into the prototype sorting unit as required.

The complexity of the sorting unit could increase even more if there were a need to examine small areas on the surface of a fruit. This need could hinge on the relative importance of specific defects such as cuts, bruises, insect bites, and brown or black spots.

Our present day knowledge of electronics technology, chemical analysis, physiochemical properties of foods, computers, and produce handling technology is highly sophisticated. We do have the capability of making rapid (millisecond) judgments on the quality of soft flesh products (peaches, blueberries, apples) as well as the capability of handling large quantities of products at rates that are commercially feasible and acceptable.

We have before us an open door. As we look behind us, we see a considerably greater amount of application of nondestructive quality evaluation today than we had a decade ago. Through the door we see a much greater appreciation for the application of NDQE in the future. I look forward to moving through that door with you and to our success in bringing NDQE to a high level of acceptance and application.

REFERENCES

1. Dull, G. G., and A. C. Hulme. 1971. Quality. Chapter 22 *IN* A. C. Hulme, (ed.) The biochemistry of fruits and their products. Vol. 2 Academic Press, New York.
2. Gortner, W. A., G. G. Dull, and B. H. Krauss. 1967. Fruit development, maturation, ripening, and senescence: A biochemical basis for horticultural terminology. Hort Science 2:141.

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Nondestructive Quality Evaluation of Agricultural Products — Industrial Application

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ABSTRACT

Use of nondestructive quality evaluation techniques for agricultural products is steadily increasing. Reasons for increased application and refinements of techniques in processing of agricultural products are discussed, especially with regard to more automation and less labor in processing, increased processing rates, and demand for more quality and uniformity of product. Information is presented on a number of nondestructive techniques presently being used, and the need for additional development is covered. Special emphasis is given to the efficiency, practicality, and economic feasibility of various techniques. Agricultural products covered include peanuts, pecans, poultry, eggs, pork, and seeds. Nondestructive techniques discussed that are used for processing these commodities into uniform, high-quality products include visual examination, weighing, screening, gravity sorting, air classification, electronic sorting, and estimation of chemical composition. A more detailed discussion of electronic color sorting of peanuts, pecans and almonds is presented, particularly in relation to aflatoxin sampling and reduction, preparing for further processing, and evaluation of commercial sorters for speed and accuracy.

Humans have used their five senses to assess quality of food long before instruments or measuring devices were invented. Former Secretary of Agriculture Butz mentioned in a speech at the Institute of Food Technologists' meeting in June, 1976 that he could remember well the practice of sorting fruits and vegetables on the farm, and sorting out for immediate consumption those of poorer quality because the better quality products would store better for later consumption. A number of quality control techniques in use today are still based on sight, smell, taste, texture, and with apples, the crisp sound of the "bite".

The field of quality control has been recognized in the twentieth century, not as a science per se but as a profession in which one can practice after having been educated in one of many different scientific areas. Undoubtedly the recognized leader in the discipline of quality control is Dr. J. M. Juran. In a course taught by Dr. Juran, he lists one of the earlist recorded quality control practices as that of measuring dimensions of stone behind the stonecutter in early Egyptian culture. The picture portraying this might bring on comments about some of our quality control practices, maybe some regulatory practices also. There is one man working and one man measuring. With increased automation in processing plants, line inspectors and quality control personnel may actually outnumber production workers on the line. After taking an associate through a poultry plant recently, he commented that automation has reduced all manpower requirements except U.S.D.A. inspectors, and company managers.

NONDESTRUCTIVE TECHNIQUES

Eggs

Only a few nondestructive quality techniques have been developed for eggs, but those used have been highly successful. The practice of candling eggs has been used for years to detect defects such as small cracks and checks, presence of blood and meat spots, stability of the yolk in the center of the egg, and in the case of hatching eggs, to eliminate infertiles. This method was first used with single-orifice candlers, but has been adapted for use with eggs in trays or on conveyors. Eggs are sized automatically by weight, and rough and thin shells are downgraded by candling or sight.

A nondestructive method of determining egg shell quality is by flotation in graded concentrations of salt water. However, this is not normally used in processing and is primarily a research tool.

In egg breaking plants, eggs are candled before breaking, and then are observed after breaking for blood and meat spots, smelled for off-odors or spoilage, and then measured for color. Visual comparison may be made with colored standards (*15*), chemical determination (*2*), or by instrumental methods (*6,7*). An illustration of Hunter *a* and *b* values for eggs is shown by Philip et al. (*11*).

One of the greatest potentials for salvaging eggs for human foods would be a technique for detection of egg fertility during early incubation. Presently infertile eggs are discarded at 18 to 20 days of incubation when eggs

are transferred from incubators to hatchers. At this time, the infertiles are processed with hatchery waste into a feed ingredient, or discarded to a land fill. We have heard that a technique is being studied for early fertility detection but were unuable to find a lead on the method.

Seeds

Processing methods used for cleaning and purifying seeds are about as numerous as the varieties of seeds we handle in the diversified agri-business of southeastern U.S. In seed processing, quality control techniques and processing methods are frequently one and the same. Some of the methods most frequently used are screening for size, separation by density in air flows, separation by shape of seeds (round or flat in corn), color sorting for uniform size, and hand picking defects. The seed industry is unique in that blending of different quality lots can be done legally so long as the resultant blend meets State and Federal requirements for purity, germination, and levels of noxious seeds and inert material. Electronic color sorting is successfully used for a number of different seeds but particularly for peanuts seeds. In sorting peanuts for seeds, parameters are reversed so that light colored seeds (or those lacking skins) are discarded. Automation may bring problems to production workers since jobs are eliminated, but can result in considerable savings. At the Gold Kist Seed Research Farm, breeder and foundation peanut seeds are now being graded by a two-channel electronic sorter that previously required six workers hand picking from a moving belt.

Except for germination, the final assessment of seed quality is essentially nondestructive in that representative samples are subdivided as sound seeds and other material (inert, noxious, etc.). Germination potential of some seeds can now be determined with a degree of accuracy by tetrazolium staining technique, and by X-ray (*8*).

Pork

The type of hog grown for pork products has changed very dramatically over the past 50 years. As vegetable oils replaced lard as shortening for cooking, lard-type hogs have been replaced with bacon-type. In adopting new breeds, and in selecting for lean hogs, they were evaluated for production efficiency and carcass characteristics. Loin eye area and back-fat thickness were usual carcass measures. Unfortunately these were of little value in measuring genetic potential of breeding stock since animals must be slaughtered before these measures could be made. Later a back-fat probe method was developed that could be used with live animals. More recently ultrasonic techniques have been used with limited success to measure loin eye area and back fat thickness of live animals (*12*), thus allowing breeding of animals after these measurements were made. Most recently Domermuth et al. (*4*) used "Electronic Meat Measuring Equipment," based on the difference in electrical conductivity of fat and lean tissues, to predict ratios of these two tissues in live hogs. They found that this method, or ^{40}K counts with liquid scintillation, correlated well with carcass protein and lean cuts.

In cured meat and in emulsion products, salt, moisture, fat and protein levels are determined by recognized A.O.A.C. methods. These tests help assure uniform quality, least cost products, and conformance to U.S.D.A. specifications. The Babcock (Paley) method of fat analysis and toluene distillation for moisture analysis have gained acceptance for quicker, in-plant use. More recently, X-ray (Anyl-Ray), specific gravity (Honeywell), and solvent extraction-dielectric (Steinlite) methods for fat analysis have continued to gain acceptance [see Kramlich et al. (*9*) for a discussion of these methods]. Of these methods, the X-ray and specific gravity methods are nondestructive in that samples are analyzed and returned to the processing line.

The greatest need in the pork industry is to supply uniform hog carcasses for processing at the plant, and then to measure rapidly and accurately the composition of different ingredients going into processed pork products. In-line analysis of emulsion product mix for fat and moisture would be of tremendous benefit to the pork industry.

Poultry

Quality control in poultry is different from many agricultural products because factors concerning production such as feed additives, antibiotics, etc., are under the control of FDA, the product during processing is controlled by USDA and is then again under FDA while in marketing channels if interstate shipment is made. Most in-plant tests such as weights, carcass fat level, carcass grades, skin color, etc. are nondestructive and are designed to segregate product into uniform groups. Sizing methods by weights are used routinely but some method of segregating product by body and parts conformation is needed to provide uniform lots of birds for specialized markets. Also machine measurement of skin pigment would be helpful in supplying market outlets for "pale" and "yellow" birds.

Peanuts

In the processing of peanuts, grading, screening, air separation, etc. have been used for years to separate stems, hulls, stones and trash from edible nuts. These methods have been refined and successfully combined with electronic sorting to remove undesirable peanuts and foreign materials to produce uniform lots of whole edible peanuts, splits, and peanuts with skins intact for seeds.

When it was definitely established that aflatoxin contamination was a problem in peanuts, a study was financed by the peanut industry and conducted by the A. D. Little organization which showed clearly that reject peanuts from the electronic sorter stream (off-color, dark, shriveled) were higher in aflatoxin than normal raw and roasted peanuts in the accept stream. With this finding, color sorting became doubly important as a

nondestructive technique for quality control. Electronic sorters are generally designed so the peanuts feed between the scanning system in single file. The product is illuminated and viewed by "electric eyes." The difference in light reflectance of the product and the background determines if the product is accepted or rejected. The control system activates an air valve that fires an ejector to remove off-color product.

The efficiency of electronic color sorters versus hand picking peanuts has been studied by Dickens and Whitaker (*3*). Using aflatoxin-contaminated lots, they found that careful hand-picking for discoloration was far more selective for aflatoxin-contaminated kernels than was electronic color sorting.

Unpublished data from our organization (*14*) showed that three different commercial electric sorters varied greatly in their ability to separate foreign material and rejects from a uniform product stream. Machines were evaluated for four basic components: feed system, scanning system, control system, and reject system. Feed rates were studied, and a sustained sorting rate of 500 lb. per hour of peanut kernels is considered optimum for these sorters.

In removing splits from whole peanuts, the three machines tested varied greatly in sorting efficiency. On a scale of 0 to 25 (poor to best), the machines rated 0, 17, and 22. The two best machines had different operating characteristics that tended to equalize them as test parameters were changed (i.e. loss in value of accept with reject material, cost of sorting rejects, or in complete removal of rejects from accept product stream). One machine rated lowest in all sorting tests (splits and wholes, foreign material and rejects from edible peanuts, rejects from seed peanuts, etc.) while the other two were consistently superior in sorting ability.

Electric sorters employed in the peanut industry on raw and roasted peanuts perform a dual function of eliminating foreign material and rejects, and simultaneously eliminating much of the material most likely to be contaminated by aflatoxin. This same principle seems to apply in almonds and pecans, both of which are sorted by electric sorters and have a potential for aflatoxin contamination.

Almonds and pecans

A study was conducted by Shade et al. (*13*) in which aflatoxin was found in 14% of 74 samples of unsorted, in-shell almonds as received by processors in 1972. Aflatoxin levels were low (below 20 ppb in 90% of the contaminated samples). Commercial sorting procedures were effective in reducing the incidence of aflatoxin since none of the processed whole nut meats contained detectable aflatoxin levels.

In pecans, the potential for aflatoxin contamination has been demonstrated (*5,10*). To date, no extensive study has been published to indicate actual severity of the aflatoxin problem in pecans. However, methods for preventing and controlling mold growth and aflatoxin development are being studied, along with methods for economical sampling for aflatoxin analysis (*1*). If aflatoxin in pecans should prove to be a problem, studies should be instituted to improve color sorting of pecan halves and chopped pieces to reduce the problem.

REFERENCES

1. Ayres, J. L. 1977. Aflatoxins in pecans: problems and solutions. J. Am. Oil Chem. Soc. (Accepted for publication)
2. Dalby, G. 1948. The determination of color in eggs. Cereal Chem. 25:413-417.
3. Dickens, J. W. and T. B. Whitaker. 1975. Efficacy of electronic color sorting and hand picking to remove aflatoxin contaminated kernels from commercial lots of shelled peanuts. Peanut Sci. 2:45.
4. Domermuth, T. L., M. A. Alexander, H. B. Hedrich, J. Clark and D. Eklund. 1976. Prediction of lean body composition of live market weight swine by indirect methods. J. Anim. Sci. 43:966.
5. Doupnik, B., and D. K. Bell. 1971. Toxicity to chicks of *Aspergillus* and *Penicillium* species isolated from moldy pecans. Appl. Microbiol. 21:1104-1106.
6. Francis, F. J., and F. M. Clydesdale. 1972. Color measurement of foods. XXXV. Egg Yolks. Food Prod. Dev. 6(8):47.
7. Hinton, C. F., E. M. Ahmud, J. L. Fry, and R. A. Haims. 1970. Reflectance colorimetric evaluation of egg yolk pigmentation. Poultry Sci. 49:1397.
8. Kamra, S. K. 1964. Determination of seed quality by X-rays. Advan. Frontiers Plant Sci. 9:119.
9. Kramlich, W. E., A. M. Pearson, and F. W. Tauber. 1973. Processed meats. Avi Publ. Co., Westport, Conn. pp.280-311.
10. Lillard, H. S., R. T. Hanlin, and D. A. Lillard. 1970. Aflatoxigenic isolates of *Aspergillus flavus* from pecans. Appl. Microbiol. 19:128-130.
11. Philip, T., C. W. Weber, and J. W. Berry. 1976. Rapid measurement of egg yolk color. Food Technol. 30(11):58-59.
12. Price, J. F., A. M. Pearson, and J. A. Emerson. 1960. Measurement of the cross-sectional area of the loin eye muscle in live swine by ultrasonic reflections. J. Anim. Sci. 19:786-789.
13. Schade, J. E., K. McGreevy, A. D. King, Jr., B. Mackey, and G. Fuller. 1975. Incidence of aflatoxin in California almonds. Appl. Microbiol. 29:48-53.
14. Steele, B., and J. L. Ayres. 1977. Gold Kist Inc., Atlanta, Ga. Personal communication.
15. Vuillenmier, J. P. 1969. The "Roche Yolk Color Fan" — an instrument for measuring color. Poultry Sci. 48:767-779.

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Engineering Techniques for Nondestructive Quality Evaluation of Agricultural Products[1]

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ABSTRACT

A number of nondestructive methods have been developed to detect defects and evaluate physical characteristics associated with quality of agricultural products. The methods involve the application of a well-characterized source of energy (such as x-rays, light, infrared radiation, or sonic energy) to the test object. Because of the composition and condition of the material, the input energy is modified in some unique manner. The difference between the input energy and energy response can be measured and recorded, and may provide a basis for development of empirical relations and correlations for assessing quality-related factors. This paper reviews some of the nondestructive techniques which have been developed for testing agricultural products.

Quality is often thought of as the degree of excellence of a product. Kramer and Twigg (*48*), however, defined quality as "...the composite of those characteristics that differentiate individual units of a product, and have significance in determining the degree of acceptability of that unit to the buyer." Thus, quality may be specified in terms of a number of factors or characteristics, such as size, shape, color, tenderness, hardness, sweetness, each of which may be defined and evaluated independently. Grade standards of the United States Department of Agriculture (USDA) are based largely upon such quality factors (*36,81*). Quality evaluation instruments have been developed in an attempt to improve the objectivity of methods used to assess factors of quality. This paper highlights some of the nondestructive engineering techniques used to evaluate physical characterisitics associated with quality of agricultural products.

THE NONDESTRUCTIVE TESTING CONCEPT

A simplified concept of the principles involved in nondestructive testing is illustrated in Fig. 1. Nondestructive tests (NDT) have been used for many years to evaluate engineering materials (*6,99*), and many of these tests can be adapted to measure agricultural products. Nondestructive implies that the sample is not damaged or destroyed during measurement.

Nondestructive testing commonly involves application

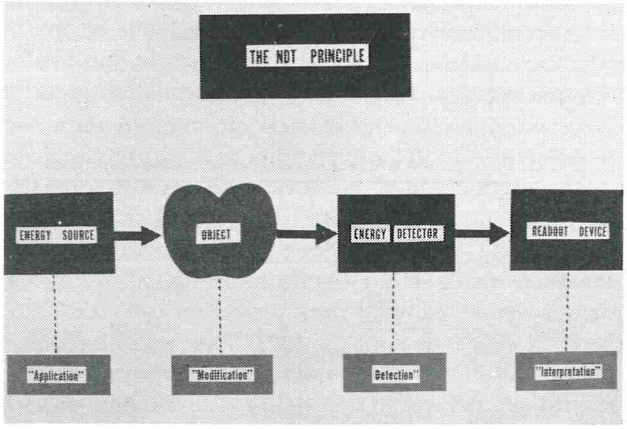

Figure 1. *Block diagram of the elements of a nondestructive test (NDT) system.*

of a well-characterized source of energy to the test object or sample (Fig. 1). This energy may be in the form of x-rays, ultraviolet, visible (light), or infrared radiation, microwaves, sonic or ultrasonic energy, etc. The type of energy to use as a source depends upon a number of factors, such as the material to be tested and the physical characteristics or anomaly which is of primary interest (*2,6,99*). The energy which reaches the test object (Fig. 1) interacts with it and is modified in some unique manner because of the chemical and/or physical properties of the object. Generally, the input energy is reflected, transmitted, and/or absorbed by the object. The difference between the input energy and the energy response is measured and recorded, and provides a basis for development of empirical relations and correlations to predict product quality.

An element which is crucial to performance of the entire test system is the receiving transducer or the energy detector (Fig. 1), and its interface with the test object. The transducer converts the energy response into an electrical signal. Zurbrick (*99*) emphasizes that the receiving transducer is responsible for efficient, low-noise, high-compliance reception; also, it should have

long-range stability, precision, and high sensitivity. A major consideration in the design of any NDT instrument, therefore, must focus upon matching detector performance to the energy response characteristics of the test object. Norris (*59,60*) and others (*62,74,88*) have discussed factors to consider in the selection of detectors for light-transmission and reflectance measurements; and Finney (*25*) has described the effect which detectors may have upon the measured sonic vibration response of fruits.

The final component of the NDT system is the readout device (Fig. 1). This unit processes the electrical signal from the detector and enables the investigator to interpret the measurement. A wide variety of electronic signal processing and display equipment is available, from simple digital or analog meters to sophisticated computer-controlled systems. The readout device or system should be designed to match the test situation. For basic studies that require energy response data over a range of frequencies, the readout device should be able to record and display spectra or differences in spectra for different samples. Such systems have been developed for spectrophotometric studies (*55,72*). They include a computer to record, store, and calculate correlations and regression relationships between measured variables. In other instances, when the basis for a measurement has been previously established, the readout system may simply be the pointer on a meter or a digital indicator. Birth and Norris (*14*), for example, used a special recording spectrophotometer to obtain information that was then used in the design of an abridged instrument to indicate directly the chlorophyll concentration in fruit.

Many NDT methods have been developed and used to evaluate agricultural products. Some of the applications are listed in Table 1, according to the type of energy used to interrogate the product. The discussion that follows further illustrates some of the principles involved in nondestructive quality evaluation.

NONDESTRUCTIVE TEST METHODS

Light techniques

External appearance and internal color are important factors to consider in the quality evaluation of agricultural products; and they can be objectively

TABLE 1. *Nondestructive techniques for evaluating some of the factors of quality in agriculutural products.*[a]

Commodities	Defects or quality factors	X-rays	Ultraviolet	Light	Infrared	Microwaves	Nuclear magnetic resonance	Ultrasonic	Sonic	References
Apples	Color			X						*16,51,52,93*
	Maturity			X						*14,16,52,71,95*
	Texture								X	*1,23,25,28*
	Watercore			X						*14,15,68*
Butter	Moisture content					X				*17, 80*
Cherries	Color			X						*14,97*
Corn	Moisture content				X	X	X			*29,39,80,85*
	Mold damage		X							*90*
Eggs	Bloodspots			X						*18,64*
	Green rot			X						*58*
	Shell strength	X								47,73
Forages	Protein, fiber content				X					*65*
Lettuce	Maturity, firmness	X								*33,50*
Meat	Fat content	X			X		X	X		10,22,34,53,54,80, *84,89*
	Moisture content				X	X	X			*10,78,80*
Milk	Fat content			X	X			X		*31,35,40,75,98*
Oranges	Color			X						*32,43,45,46*
	Internal granulation	X								*3,27*
Peaches	Maturity, color			X						*14,16,19,76*
	Firmness								X	*19,24,25,26*
Peanuts	Maturity			X						*7.49*
	Moisture content				X					*63,80*
Pecans	Aflatoxin		X							*87,90*
Potatoes	Black spot			X						*12,14*
	Hollow heart	X		X						*12,14,30,67*
Raspberries	Firmness								X	*66*
Rice	Milling quality			X						*8,83*
Seeds	Viability, purity	X								*9,82*
Soybeans	Moisture content				X	X		X		*11,39,42,63,80*
	Oil content				X		X			*41,44,61,70,78,80*
	Protein content				X					*41,44,61,92*
Tomatoes	Maturity, color			X						*14,94,96*
	Firmness, ripeness								X	*5*
Wheat	Moisture content				X	X	X			*57,63,78,80,92*
	Protein content				X					*4,41,92*

[a]The methods indicated are applicable; but selection is influenced by such considerations as geometry, structure, and composition of the commodity, defect orientation, and equipment cost.

measured by use of visible radiation (light). A number of techniques are available for isolating narrow beams of light of a specific wavelength and measuring that which is transmitted, reflected, or absorbed by the sample (*13,59,62,88*). Instruments have been designed to specifically measure the color of agricultural products; that is, the spectral reflectance of specified wavelengths in the visible region (*20,43,69,96*). For some quality evaluations, however, internal characteristics may be of equal or even greater importance than external appearance. Chlorophyll content in the flesh of apples and peaches, for example, is correlated with maturity and ripeness (*13,14,76,93,95*). Quality defects such as hollow heart in potatoes (*12,67*) and watercore in apples (*15,68*) exist internally without any external evidence of abnormality. These internal quality factors can affect the USDA grade, and light-transmittance instrumentation can be used for their detection (*14*). Chlorophyll in flesh is associated with a specific type of pigmentation; hollow heart, with discoloration; and water core, with light scatter within the product. Thus, the energy transmitted by the product in selected portions of the visible region of the spectrum can provide an empirical basis for separating desirable products from those which are not. The principles and methods involved in applying these NDT techniques to evaluate interior quality of agricultural products have been described in a number of publications (*13,14,56,59,71,94,95*).

Infrared techniques

Radiation in the infrared and near-infrared region of the spectrum (wavelengths longer than 780 nm) can provide information related to the chemical composition of agricultural products. Water, for example, is an important component of most agricultural commodities, and has strong absorption bands at 0.76, 0.97, 1.19, 1.45, and 1.94 μm (*21*). These bands can be used to measure the moisture content of seed, grain, and meat preparations (*10,11,39*), and of whole, intact products (*29,63*).

Fat content of meat and meat products can be estimated rapidly from infrared reflectance and transmittance measurements. Ben-Gera and Norris (*11*) identified two prominent absorption bands for fat at 1.725 and 1.76 μm. Measurements made at these bands provided a correlation coefficient of 0.974 with percent fat. A simple table model instrument has been developed to measure the intensities of the moisture and fat absorption bands in ground beef and to convert the measurements to fat content, which is displayed directly on a panel meter (*89*).

During recent years, infrared reflectance techniques have been extended to evaluate other quality factors important in agriculture. Absorption bands within the wavelength region from 1.0 to 2.4 μm have been correlated with the oil and protein content of soybeans (*41,44,61,70*), protein in cereal grains (*44,92*), and with the nutritional quality of forages (*65*). By use of special optical components and digital processing equipment, table model instruments have been developed to provide a direct quantitative readout of such quality factors as oil, protein, and moisture in less than 2 min (*92*). Such instrumentation for rapid compositional analyses could have a marked influence upon the system for marketing cereal and feed grains (*4*). In each of these applications, the basic design of the test instrumentation is based upon an empirical relation between the energy response of the sample to a well-defined source of near-infrared radiation and the quality factor of interest.

X-ray techniques

The energy generated by x-ray tubes and radioactive sources, such as cobalt-60, iridium-192, cesium-137, is highly penetrating to biological materials. The material-energy response tends to be especially sensitive to structural discontinuities (such as voids and cracks), density differences or variations, and shape or geometry (*2*). In agricultural products, those quality factors which are associated with mass-density variations may often be evaluated nondestructively by x-rays. Hollow heart of potatoes, for example, is not visible externally, but can be detected clearly in an x-ray picture (*27,67*). Radiographic techniques have been used for a number of years to detect this defect nondestructively, and recently a scanning technique has been suggested for automatic sorting of hollow heart potatoes (*30*). X-rays have been used also to sort stones from potatoes on the basis of density differences (*77,79*).

Significant correlations have been established between density and maturity of lettuce (*33,50*). Lettuce becomes firm and dense as it matures; and the density change provides a basis for nondestructive evaluation of lettuce maturity by use of x-rays, and hence for selective mechanical harvesting (*33*).

The density and x-ray absorption properties of the fat components differ significantly from those of lean meat. The specific gravity of meat, for example, is significantly correlated with the percentage of fat (*91*), and the absorption of x-radiation by ground meat has been linearly related to the percentage of fat (*54*). These empirical relationships provide a basis for evaluating the fat content of meat nondestructively (*34*).

Nondestructive x-ray techniques have been developed to sort frost-damaged, granulated oranges from good quality fruit (*3,27*). Frost damage or internal granulation in oranges is known to cause changes in the specific gravity of the fruit (*86*). Cells within the fruit tend to become dry, and the air-filled voids cause mottling of the x-ray image. A normal fruit, on the other hand, shows fairly uniform absorption characteristics across the projected area of the fruit.

These examples indicate the applicability of x-ray energy to evaluate quality nondestructively. In these applications, response of the product to the input energy tends to reflect mass-density characteristics associated with internal voids or structural variations.

Sonic techniques

The response of materials to sonic energy is influenced by such factors as elasticity, mechanical resistance, mass, and geometry of the material (*2*). Finney and Abbott (*28*) have reviewed a number of techniques for applying sonic energy to agricultural products and measuring their responses. Response is usually measured in terms of vibration amplitude, frequency, and damping characteristics. Results of sonic vibration studies indicate that the natural resonant frequency of fruits decreases as they soften (*1,19,25*) and that firm hard fruit transmit high frequency energy (above 2,000 Hz) better than soft fruit (*24,26,66*). A number of studies have been conducted to correlate sonic response to other indices of fruit quality, such as pressure tests and sensory panel ratings (*19,23,24,26*). The studies show a significant correlation between the nondestructive sonic measurements and fruit texture. Instruments have been developed to sort tomatoes, blueberries, and grapes for ripeness on the basis of their responses to sonic vibrations (*5,37,38*).

SUMMARY

In this paper, some of the principles involved in development of nondestructive tests for agricultural products have been reviewed. I have emphasized that such tests must be based upon some type of unique interaction between the energy source and the product under test. Four test methods were discussed and are based upon the use of sonic energy, x-rays, light, and infrared radiation to characterize the product. With each method, the energy response is sensitive to certain characteristics of the product, such as mechanical resistance, mass density, pigmentation and color, or composition. Other techniques and energy sources such as microwaves (*17,80*), beta backscatter (*47*), ultrasonics (*31,42,84*), ultraviolet (*87,90*), and nuclear magnetic resonance (*78,80,85*) may also be applied to test agricultural products. The NDT principles described in this paper apply equally to these latter approaches. That is, the effectiveness and reliability of the measurement must be based upon established empirical relationships or correlations between a measured product-energy response and the quality factor of interest. Because of the wide differences in structure and composition, and the inherent natural variability of agricultural products, nondestructive quality evaluation instruments often must be customized for the particular commodity and quality factor of interest. Many approaches, however, are available for the engineer to consider in the design of such quality evaluation instrumentation.

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REFERENCES

1. Abbott, J. A., G. S. Bachman, N. F. Childers, J. V. Fitzgerald, and F. J. Matuski. 1968. Sonic techniques for measuring texture of fruits and vegetables. Food Technol. 22(5):101-112.
2. Anonymous. 1968. Recommended practice for selecting nondestructive testing methods for reinforced thermosetting plastics. Materials Res. Stands. 8(7):37-40.
3. Anonymous. 1970. A system for the seventies. California Citrograph 55(4):129-130.
4. Anonymous. 1972. Grain quality analyzer wins an award. Industrial Res. 14(10):42.
5. Anonymous. 1976. Pilot model is 95% accurate. Amer. Veg. Grower 24(6):38.
6. Ballard, D. W. 1965. Nondestructive testing. Industrial Res. 7(11):68-71.
7. Beasley, E. O., and J. W. Dickens. 1968. Light transmittance of peanut oil as an objective measurement related to quality of raw peanuts. Trans. ASAE 11:680-682.
8. Beerwinkle, K. R., and R. A. Stermer. 1976. A device to facilitate optical sorting of milled rice based on translucence differences. p. 219-224. In: Quality detection in foods. Amer. Soc. Agricul. Engrs. St. Joseph, Mich.
9. Belcher, E. W. 1968. Use of soft x-ray in tree seed testing and research. Proc. Southeastern Forest Radiography Workshop, p. 74-96.
10. Ben-Gera, I., and K. H. Norris. 1968. Direct spectrophotometric determination of fat and moisture in meat products. J. Food Sci. 33:64-67.
11. Ben-Gera, I., and K. H. Norris. 1968. Determination of moisture content in soybeans by direct spectrophotometry. Israel J. Agric. Res. 18:125-132.
12. Birth, G. S. 1960. A nondestructive technique for detecting internal discolorations in potatoes. Amer. Potato J. 37(2):53-60.
13. Birth, G. S. and K. H. Norris. 1958. An instrument using light transmittance for nondestructive measurement of fruit maturity. Food Technol. 12:592-595.
14. Birth, G. S., and K. H. Norris. 1965. The difference meter for measuring interior quality of foods and pigments in biological tissues. U.S. Dept. Agric. Tech. Bull. No. 1341, 20 p.
15. Birth, G. S., and K. L. Olsen. 1964. Nondestructive detection of water core in Delicious apples. Proc. Amer. Soc. Hort. Sci. 85:74-84.
16. Bittner, D. R., and K. H. Norris. 1968. Optical properties of selected fruits vs. maturity. Trans. ASAE 11:534-536.
17. Bleackley, W. J. 1969. A microwave instrument for measuring the moisture content of butter. Bull Radio Elect. Eng. Div., Nat'l. Res. Council of Canada 19(1):1-4.
18. Brant, A. W., K. H. Norris, and G. Chin. 1953. A spectrophotometric method for detecting blood in white-shell eggs. Poultry Sci. 32:357-363.
19. Clark. R. L., and P. S. Shackelford. 1973. Resonance and optical properties of peaches as related to flesh firmness. Trans. ASAE 16:1140-1142.
20. Clydesdale, F. M. 1972. Measuring the color of foods. Food Technol. 26(7):45-51.
21. Curcio, J. A., and C. C. Petty. 1951. The near infrared absorption spectrum of liquid water. J. Optical Soc. Amer. 41:302-304.
22. Davis, J. K., R. S. Temple, and W. C. McCormick. 1966. A comparison of ultrasonic estimates of rib-eye area and fat thickness in cattle. J. Animal Sci. 25:1087-1090.
23. Finney, E. E. 1971. Dynamic elastic properties and sensory quality of apple fruit. J. Texture Studies 2:62-74.
24. Finney, E. E. 1971. Random vibration techniques for nondestructive evaluation of peach firmness. J. Agric. Eng. Res. 16:81-87.
25. Finney, E. E. 1972. Vibration techniques for testing fruit firmness. J. Texture Studies 3:263-283.
26. Finney, E. E., and J. A. Abbott. 1972. Sensory and objective measurements of peach firmness. J. Texture Studies 3:372-378.
27. Finney, E. E. 1973. Meausrment techniques for quality control

of agricultural products. Publ. by Amer. Soc. Agric. Eng., St. Joseph, Mich. 53 p.
28. Finney, E. E., and J. A. Abbot. 1977. Methods for testing the dynamic mechanical response of solid foods. Proc. First International Congress on Engineering and Food. Boston, Mass. (In press)
29. Finney, E. E., and K. H. Norris. 1977. Determination of moisture in corn kernels by near-infrared transmittance measurements. Paper 77-3021. Amer. Soc. Agric. Eng., St. Joseph, Mich.
30. Finney, E. E., and K. H. Norris. 1977. X-ray scans for detecting hollow heart in potatoes (In preparation for the Amer. Potato J.)
31. Fitzgerald, J. W., G. R. Ringo, and W. C. Winder. 1961. An ultrasonic method for measurement of solids-not-fat and butterfat in fluid milk. Publ. M-58. Presented before the 56th Annual Meeting of the Amer. Dairy Sci. Assoc.
32. Gaffney, J. J. 1973. Reflectance properties of citrus fruits. Trans. ASAE 16:310-314.
33. Garrett, R. E., and W. K. Talley. 1970. Use of gamma ray transmission in selecting lettuce for harvest. Trans. ASAE 13:820-823.
34. Gordon, A. 1973. Anyl-ray determines lean/fat ratio. Food Processing Industry 42:49.
35. Goulden, J. D. S. 1964. Analysis of milk by infrared absorption. Dairy Res. 31:273-284.
36. Grange, G. R. 1966. Standards — Their meaning and use. Agricultural Marketing (October), U.S. Dept. Agric., Washington, D.C., p. 4-5.
37. Hamann, D. D., and D. E. Carroll. 1971. Ripeness sorting of Muscadine grapes by use of low-frequency vibrational energy. J. Food Sci. 36:1049-1051.
38. Hamann, D. D., L. J. Kushman, and W. E. Ballinger. 1973. Sorting blueberries for quality by vibration. J. Amer. Soc. Hort. Sci. 98: 572-576.
39. Hart, J. R., K. H. Norris, and C. Golumbic. 1962. Determination of the moisture content of seeds by near-infrared spectrophotometry of their methanol extracts. Cereal Chem. 39:94-99.
40. Haugard, G. 1966. Photometric determination of fat in milk. J. Dairy Sci. 49:1185-1189.
41. Hilliard, J. H., and T. B. Daynard. 1976. Measurment of protein and oil in grains and soybeans with reflected near infrared light. J. Canadian Inst. Food Sci. Technol. 9(1):11-14.
42. Hoki, M., and K. Tomita. 1976. Mechanical properties of soybeans by means of ultrasonic measurements. Paper No. 76-3044, Amer. Soc. Agric. Eng., St. Joseph, Mich.
43. Hunter, R. S. 1967. Development of the citrus colorimeter. Food Technol. 21:906-911.
44. Hymowitz, T., J. W. Dudley, F. I. Collins, and C. M. Brown. 1974. Estimation of protein and oil concentration in corn, soybean, and oat seed by near-infrared light reflectance. Crop Sci. 14: 713-715.
45. Jahn, O. L. 1970. Effects of maturity changes on nondestructive measurements of citrus fruit quality. Tech. Bull. No. 1410, Agric. Res. Ser., U.S. Dept. Agric., Washington, D.C., 50 p.
46. Jahn, O. L. 1972. Photoelectric color sorting of citrus fruits. U.S. Dept. Agric. Tech. Bull. No. 1448, 56 p.
47. James, P. E., and H. J. Retzer. 1967. Measuring egg shell strength by beta backscatter technique. Poultry Sc. 46:1200-1203.
48. Kramer, A., and B. A. Twigg. 1966. Fundamentals of quality control for the food industry. AVI Publ. Co., Inc., Westport, Conn. 541 p.
49. Kramer, H. A., J. E. Gates, K. D. Demaree, and A. P. Sidwell. 1963. Spectrophotometric investigations on peanuts with particular reference to estimation of maturity. Food Technol. 17(7):90-92.
50. Lenker, D. H., nd P. A. Adrian. 1971. Use of x-rays for selecting mature lettuce heads. Trans. ASAE 14:894-898.
51. Lott, R. V. 1963. Variability in color and associated quality constituents in Golden Delicious apple packs. Proc. Amer. Soc. Hort. Sci. 83:139-146.
52. Lott, R. V. 1966. Changes in skin colour during the ripening of 'Golden Delicious' apples, in relation to harvesting at different stages of maturation. Hort. Res. 6:113-125.
53. Lynch, G. 1967. Ultrasonic and direct measurement of backfat thickness in bacon pigs. Irish J. Agric. Res. 6:41-47.
54. Madigan, J. 1961. Method and apparatus for determining the quantitative relationship of components in products by measurement of gamma ray penetration thereof. U.S. Patent No. 2,992,332.
55. Massie, D. R., and K. H. Norris. 1975. A high-intensity spectrophotometer interfaced with a computer for food quality measurement. Trans. ASAE 18:173-176.
56. McClure, W. F. R. P. Rohrbach, L. J. Kushman, and W. E. Ballinger. 1976. Design of a high-speed fiber optic blueberry sorter. p. 189-192. In: Quality detection in foods. Amer. Soc. Agric. Eng., St. Joseph, Mich.
57. Miller, B. S., and H. D. Kaslow. 1963. Determination of moisture by nuclear magnetic resonance and oven methods in wheat, flour, doughs, and dried fruits. Food Technol. 17:650-653.
58. Norris, K. H. 1955. Photoelectric inspector detects green rot in eggs. Electronics 28(7):140-142.
59. Norris, K. H. 1958. Measuring light transmittance properties of agricultural commodities. Agric. Eng. 39:640-643, 651.
60. Norris, K. H. 1962. Instrumentation of infrared radiation. Trans. ASAE 5:17-20.
61. Norris, K. H. 1970. Characterization of the near-infrared absorption and reflectance properties of ground soybean. Abstracts of Papers, Part 3, 3rd Int. Congress of Food Sci. Technol. Washington, D.C.
62. Norris, K. H., and W. L. Butler. 1961. Techniques for obtaining absorption spectra on intact biological samples. IRE Trans. Bio-Medical Electronics 8(3):153-157.
63. Norris, K. H., and J. R. Hart. 1965. Direct spectrophotometric determination of moisture content of grain and seeds. Proc. 1963 Int. Symposium on Humidity and Moisture, Reinhold Publ. Corp., New York. Principles and methods of measuring moisture in liquids and solids, Vol. 4, p. 19-25.
64. Norris, K. H., and J. D. Rowan. 1962. Automatic detection of blood in eggs. Agric. Eng. 43:154-159.
65. Norris, K. H., R. F. Barnes, J. E. Moore, and J. S. Shenk. 1976. Predicting forage quality by infrared reflectance spectroscopy. Animal Sci. 43:889-897.
66. Nybom, N. 1962. A new principle for measuring firmness of fruits. Hort. Res. 2:1-8.
67. Nyland, R. E., and J. M. Lutz. 1950. Separation of hollow heart potato tubers by means of size grading, specific gravity, and x-ray examination. Amer. Potato J. 27:214-222.
68. Olsen, K. L., H. A. Schomer, and G. S. Birth. 1962. Detection and evaluation of water core in apples by light transmittance. Washington State Hort. Assoc. Proc. 58:195-197.
69. Powers, J. B., J. T. Gunn, and F. C. Jacob. 1953. Electronic color sorting of fruits and vegetables. Agric. Eng. 34:149-154, 158.
70. Rinne, R. W., S. Gibbons, J. Bradley, and C. A. Brim. 1975. Soybean protein and oil percentages determined by infrared analysis. U.S. Dept. Agric. Publ. ARS-NC-26, 4 p.
71. Rosenthal, R. D., and D. R. Webster. 1973. On-line system sorts fruit on basis of internal quality. Food Technol. 27(7):52-60.
72. Rosenthal, R. D., R. Hayden, and S. Engler. 1976. A highly flexible 9.0 OD light transmittance reflectance research spectrophotometer. p. 16-19, 23. In: Quality detection in foods, Amer. Soc. Agr. Eng., St. Joseph, Mich.
73. Schollard, A. V., and A. E. Comstock. 1969. Egg handling and grading machine. West German Patent Application 1,507,103.
74. Shapiro, P. 1969. Infrared detector outlines materials and characteristics. Electronics 42(2):91.
75. Shipe, W. F. 1969. Collaborative study of the Babcock and Foss Milko-Tester Methods for measuring milk fat in raw milk. J. Assoc. Offic. Anal. Chem. 52:131-138.
76. Sidwell, A. P., G. S. Birth, J. V. Ernest, and C. Golumbic. 1961. The use of light transmittance techniques to estimate the chlorophyll content and stage of maturation of Elberta peaches. Food Technol. 15:75-78.
77. Siepman, A. H. J. 1967. Aardappelen scheiden van stenen en

kluiten met rontgenstralen (Separating potatoes from rocks and clods by x-rays). Landbouw Mechanisatie 18:243-245.
78. Simpson, R. J. 1968. A survey of applications of low-resolution NMR. Abs. and Reviews of the British Scientific Instrument and Res. Assoc. 23(3):75-79.
79. Slight, D. L. 1966. Some x-ray absorption and scatter properties of potatoes and stones. J. Agric. Eng. Res. 11:148-151.
80. Slight, H. A. 1970. Continuous measuring techniques for process control. Food manufacture 45(11):61-64.
81. Southerland, F. L. 1968. Forty years of processed fruit and vegetable standards. Agricultural Marketing (December), p. 14, U.S. Dept. Agric., Washington, D.C.
82. Stermer, R. A. 1960. An x-ray device for rapid evalution of purity of grass seed. Paper No. 60-328, Amer. Soc. Agric. Eng., St. Joseph, Mich. 10 p.
83. Stermer, R. A., H. W. Schroeder, A. W. Hartstack, and C. H. Kingsolver. 1962. A rice photometer for measuring the degree of milling of rice. Rice J. (May) p. 22-29.
84. Stouffer, J. R., M. V. Wallentine, G. H. Wellington, and A. Diekmann. 1961. Development and application of ultrasonic methods for measuring fat thickness and rib-eye area in cattle and hogs. J. Animal Sci. 20:759-767.
85. Sudhakar, S., M. P. Steinberg, and A. I. Nelson. 1970. Bound water defined and determined at constant temperature by wide-line NMR. J. Food Sci. 35:612-615.
86. Ting, S. V., and J. G. Blair. 1965. The relation of specific gravity of whole fruit to the internal quality of oranges. Proc. Florida State Hort. Soc. 78:251-260.
87. Tyson, T. W., and R. L. Clark. 1974. An investigation of the fluorescent properties of aflatoxin infected pecans. Trans. ASAE 17:942-948.
88. Ulrich, W. F. 1969. How to select and evaluate a spectrophotometer. Laboratory Management 7(2):26-32.
89. U.S. Dept. Agriculture. 1974. Instant fat tester goes to market. Agricultural Res. 23(1):8-9.
90. Velasco, J. 1975. Fluorometric measurement of aflatoxin absorbed on florisil in minicolumns. J. Assoc. Offic. Anal. Chem. 58:757-763.
91. Whitehead, R. C. 1970. Fat analysis of boned meat by the specific gravity method. Food Technol. 24(4):165-169.
92. Williams, P. C. 1975. Application of near infrared reflectance spectroscopy to analysis of cereal grains and oil seeds. Cereal Chem. 52:561-576.
93. Workman, M. 1963. Color and pigment changes in Golden Delicious and Grimes Golden apples. Proc. Amer. Soc. Hort. Sci. 83:149-161.
94. Worthington, J. T., D. R. Massie, and K. H. Norris. 1976. Light transmission technique for predicting ripening time for intact green tomatoes. p. 46-49. In: Quality detection in foods. Amer. Soc. Agric. Eng., St. Joseph, Mich.
95. Yeatman, J. N., and K. H. Norris. 1965. Evaluating internal quality of apples with new automatic fruit sorter. Food Technol. 19(3):123-125.
96. Yeatman, J. N., A. P. Sidwell, and K. H. Norris. 1960. Derivation of a new formula for computing raw tomato juice color from objective color measurement. Food Technol. 14(1):16-20.
97. Yeatman, J. N., G. S. Birth, J. V. Ernest, R. W. Bender, and A. P. Sidwell. 1961. Spectrophotometric evaluation of anthocyanin pigment development and scald damage in intact red tart cherries. Food Technol. 15:521-525.
98. Zacharias, E. M., and R. A. Parnell, Jr. 1972. Measuring the solids content of foods by sound velocimetry. Food Technol. 26(4):160-166.
99. Zurbrick, J. R. 1968. The mystery of reinforced plastics variability: Nondestructive testing holds the key. Materials Res. Standards 8(7):25-36.

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Nondestructive Quality Evaluation From a Horticulturist's Point of View

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ABSTRACT

Application of nondestructive sorting of fruits can be direct or indirect. Direct applications involve mainly objective means of establishing grades and quality of fruits and vegetables, as well as use of light-sorting and other nondestructive means for determining when a crop should be harvested or whether it should be marketed fresh or processed immediately. Indirect applications might be termed "research" usage of nondestructive sorting. Plant breeders would find nondestructive techniques useful for rapidly evaluating quality characteristics during the development of high quality cultivars. Physiologists could utilize it to rapidly determine the effects of treatments upon the quality of the commodity. Examples of development of techniques of light-sorting of blueberries and grapes for ripeness are discussed.

From a horticultural standpoint, the potential of nondestructive sorting of horticultural crops for quality is truly exciting. Applications can be seen as both direct and indirect. *Direct* refers to its commercial use in determining grades and standards. Since the inception of our present inspection system in the 1920's, most of the quality characteristics of fruits and vegetables have been estimated subjectively by the inspector. This is done amazingly accurately by eye, touch, etc. However, development of accurate, rapid, and objective means of measuring quality would be of great value to the inspection system. After proper estimators (non-destructive) of maturity and quality for each product (often for the particular cultivar) have been determined by researchers, these objective estimators can be used as grade standards. For example, internal and external color can be described in terms of which color-sorting machine settings are used to sort a crop in the packinghouse. Also, the uniform ripeness of a given lot of fruit coming into one of many hoppers from a light-sorter machine can, in many instances, be rapidly and easily categorized by determining the soluble solids to acid ratio (SS/Ac) of one or two small random subsamples of that lot of fruit. Soluble solids to acid ratio is generally recognized today as one of the more reliable destructive indices of ripeness of fruits. Light-sorters can also potentially be used to sort out internal defects of fruits that the eye cannot see, such as water core or cork spot inside apples, internal rots, and insect damage.

We foresee that growers could better determine when to harvest and thus establish a basis for meeting these new standards and grades by collecting random samples from the field or orchard, bringing these to the packinghouse, and running them through nondestructive sorters. The results could indicate whether or not the crop was of optimum quality for fresh or processing markets. Inspectors could use portable light-sorters to establish the grade into which a load falls. Wholesalers and retailers could also utilize portable light-sorters to check on quality and grades of produce coming into their facilities. For example, the fat content of meat analyzer developed recently by the USDA (*4*) is widely used today by inspectors and marketing personnel. Food processors, knowing more about the physico-chemical properties of raw products, could materially reduce costs by being able to make rational judgements concerning processing time involving high energy inputs. They would also know how much sugar and other additives were needed to attain desired organoleptic properties.

Indirect applications might also be termed "research" uses of nondestructive sorting. Plant breeders would find the use of nondestructive techniques invaluable for evaluating several quality characteristics of plants and plant parts during development and selection of high-quality cultivators and in determining the degree of uniformity of ripening of fruits of a seedling or selection.

Research physiologists could use nondestructive techniques to rapidly determine the effects of treatments upon the quality of a plant part, or, if the treatment influences rates of ripening, to eliminate ripeness effects and thus unmask the inherent influence of the treatment upon quality of a plant or plant part.

According to recent research findings by the USDA,

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quality characteristics such as sugars, proteins, starch, fiber, and lignin can perhaps be determined nondestructively.[4] Research on dried forage and dried tobacco so far looks promising.

OUR EXPERIENCE

We, as post-harvest physiologist, plant breeder, agricultural engineer, or enologist at North Carolina State University have worked in one way or another with quality of highbush blueberries and muscadine grapes for a number of years. Thus, we have for some time been keenly aware of the need for developing objective means of nondestructive sorting fruits of quality. Some 10 years ago, L. J. Kushman, formerly of USDA-ARS, working with researchers Karl Norris and Gerald Birth, brought light-sorting to the small fruit industry in North Carolina. Our first work was undertaken with an instrument called the Light Transmittance Difference Meter (LTDM), developed by Birth and Norris (*5*). That was the beginning of our rewarding adventures in nondestructive sorting. Our first work was to investigate the implications and relationship of light-sorting to highbush blueberries.

Blueberries

Until recently, blueberries were picked by hand. A person looked at the blueberries on a bush and made a decision as to which berries should be picked. Blue fruit were picked directly into pint boxes for shipment to market. Green, red, and purple fruit were left on the bush for subsequent harvest. Little work in the packinghouse was necessary. However, the recent expense and scarcity of labor has resulted in development of mechanical harvestors which are non-selective in removal of berries from the bush. These machines harvest bulk quantities of fruits representing the full range of ripeness from small-green to overripe-blue. This results in a tremendous problem of sorting for ripeness in the packinghouse. There the human eye can easily and quickly detect green fruits going by on a conveyer belt of a sorting/packing line but cannot easily differentiate between an all blue fruit is "just ripe" and one that is blue and "overripe." Thus, small green fruits can be removed by sizing, air blast, or by hand, but overripe fruit cannot. Inclusion of these overripe fruits in fresh market packs presents a problem. Overripe blueberries (SS/Ac greater than 30) decay very quickly and therefore should be processed immediately while quality is high (*1*). Just ripe blue fruit have a longer shelf/storage life and are more ideally suited for fresh marketing. Green fruit are not acceptable in the fresh market trade but perhaps could be processed along with overripe fruits into products such as pop-tarts. Thus, we were interested in developing a means of rapidly, effectively, and nondestructively sorting blueberries for ripeness before they are packaged for shipment to fresh markets or are sent to processing plants.

Development of a light-sorting technique was begun by selecting unripe and ripe blueberries of the North Carolina cultivars Wolcott, Croatan, Murphy, and Morrow, using subjective criteria of skin color, degree of oblateness, and distention of stem scar. These berries were carried to Beltsville, MD to Mr. Karl Norris in the USDA's Instrumentation Laboratory. There, the spectral curves (600-850 nm) of these berries were determined on the computerized biological spectrophotometer (*3,7, 8,10*). From these curves, we selected two pairs of wavelengths (710-800 and 740-800 nm) such that the Δ OD for a given pair of wavelengths changed progressively as the berries changed in ripeness. Filters for these wavelengths were purchased and installed in the LTDM borrowed from Dr. Watada, UDSA-ARS, to test-sort several lots of blueberries into categories of "Δ OD". Destructive estimations of pH, titratable acidity (Ac) (% as citric), % soluble solids (SS), SS/Ac, and anthocyanin (Acy) content of those sorted berries were determined analytically. The wavelengths 740-800 nm were more effective in sorting according to ripeness than were the wavelengths 710-800 nm. Correlations of Acy contents and Δ OD ($r = 0.94$) and of Ac and Δ OD ($r = -0.82$) of these fruits indicated that the LTDM (740-800 nm) did indeed sort blueberries according to ripeness. These results supported those reported earlier by Dekazos and Birth (*6*).

However, sorting with the LTDM was slow. Six people relieving each other could sort only 2,000 berries during a 10-h day. Each berry had to be placed on the machine before lowering the photomultiplier-tube assembly; the voltmeter had to be read, and the berry then had to be placed manually in one of several boxes, each marked with a given range of Δ OD (category of ripeness). This was tedious. A high speed sorter was essential if we were to undertake experiments requiring large numbers of optical determinations.

Therefore, over 5 years ago, working in cooperation with agricultural engineers, we established design criteria for development of a semi-automatic sorter which has come to be known as the "Berrymatic" (*9*). This machine sorts small fruits (blueberries, grapes, etc.) into five categories of ripeness at a maximum rate of 64 berries per minute. Berries are placed on 16 cups on a rotating horizontal disc. An aperture in the bottom of each cup permits light from a stationary incandescent lamp to strike the bottom of a given berry as the berry and cup pass over the lamp (reading station). Each fruit, being an excellent diffuser, then glows like a low wattage lamp. Sufficient light is scattered from the berry in all directions to permit fibre optics to be offset from the vertical. This prevents saturation of the detection system on the other end of the bifurcating fibre optic bundle when a fruit is not in the cup.

[4] *Report by Mr. K. H. Norris presented at an in-depth seminar entitled "Optical measurements for non-destructive quality evaluation of horticultural products." Sponsored by the Horticultural Crops Laboratory of the Richard B. Russell Agricultural Research Center, Athens, GA, March 7, 8, and 9, 1977.*

Each fruit is thus "read" as it traverses the light/reading station. A portion of the light transmitted through the berry is transmitted by the fibre optics to each of two narrow-band interference filters (740-800 nm for blueberries) covering each of the bifurcations of the fibre optics. Two red-sensitive photomultiplier tubes detect the energy transmitted through the filters. The "reading" from each fruit is stored in an electronic shift register until the fruit moves to one of five sorting stations, as established by the reading. One of five solenoid valves is activated to release a compressed air blast that removes the berry from the sample wheel and sends it into one of five sample boxes at the rear of the machine. In the Berrymatic, the larger the reading, the riper is the fruit. Thus, the machine reads, stores the reading, and sorts the berry at the appropriate time into one of five categories of ripeness.

Using this machine, over 10,000 berries can be easily sorted on a given day. For example, over 20,000 berries were sorted to set up a blueberry storage test involving 216 one-hundred-berry samples, five stages of ripeness, three storage temperatures, and six sampling dates. The test's objective was to determine the effect of stage of ripeness on the storage life of blueberries at 1.1, 10, and 22.2 C. Results of the test indicated that blueberries held at 22.2 C decay quickly regardless of stage of ripeness. The real economic benefit of sorting blueberries for ripeness was realized when the fruits were stored at 1.1 C. Just-ripe blueberries (SS/Ac 9) required 35 days to develop 20% decay as compared to only 16 days for overripe blueberries (SS/Ac 32). These findings hopefully should introduce the fresh market trade to the extreme importance of sending only just-ripe blueberries to transoceanic markets where up to 10 days are required for transport. The high cost of transportation dictates that only the highest quality fruit be shipped.

Grapes

Our work with nondestructive sorting of muscadine grapes (*Vitis rotundifolia* Michx.) was prompted by our grape breeder, Dr. W. B. Nesbitt. He desperately needed a rapid, non-destructive means of eliminating the ripeness variable from his evaluation of quality characteristics of the fruit in his grape breeding program. He tried specific gravity (citric acid, sucrose, or sodium chloride solutions), firmness (Instron Universal Testing Machine), and similar techniques. The specific gravity and firmness methods worked, but were considered too slow and "messy."

A cooperative effort was then put forth to evaluate the potential for light-sorting his grapes. We hoped that we could utilize the Berrymatic that was developed for the blueberries. Again, a series of grapes ranging from green to overripe was chosen subjectively according to firmness by touch, degree of visual surface gloss, and external visual coloration. Since muscadine grapes include cultivars that produce berries that are either bronze or black in color when ripe, a series of ripenesses was selected for each of these types and taken to Mr. Karl Norris' laboratory in Beltsville, MD. There, spectral curves from the sets of fruit were developed. These fruits were then returned to Raleigh, NC where pH, Ac, SS, and SS/Ac were determined destructively in the analytical laboratory. Correlations of Δ OD and SS/Ac ($r = +0.92$), Δ OD and SS ($r = +0.90$), and ΔOD and Ac ($r = -0.89$) indicated that the pair of wavelengths (540 and 610 nm) had potential for use in light-sorting of bronze muscadine grapes. For black grapes, the 740-800 nm wavelengths, as used for sorting blueberries, were selected. We subsequently sorted large quantities of both types of grapes into several stages of ripeness using the Berrymatic (*2*). Subsequent destructive testing of these grapes for ripeness confirmed our selection of wavelengths.

A further confirmation of the effectiveness of light-sorting into categories of ripeness was obtained by a recent study of the effect of ripeness of grapes upon the quality of wines made from the grapes.

FUTURE OUTLOOK

Thus far, we have developed techniques for nondestructive sorting blueberries and grapes for ripeness. We have developed a semi-automatic Berrymatic TM sorter for use in our research programs. Currently, we are developing an in-line light-sorter to mass sort blueberries rapidly. A second-generation, breadboard model was evaluated last summer. It sorted 400 berries per minute using fibre optics and electronics similar to those developed for the Berrymatic. It has five sorting channels, each of which can sort into three categories: green, ripe, and overripe. Continued success in its development can hopefully provide a commercially available in-line sorter within a few years.

We are greatly encouraged by our success in light-sorting thus far and hope to develop similar techniques for other crops. Thus, an inter-disciplinary research project has been initiated at North Carolina State University. A computerized biological spectrophotometer of our own is being established. It consists of a Cary 17 Monochrometer, a Nova 2/10 Computer with 325 words of memory, a 3-Drive Cassette Tape, a Model 611 Tektronic Scope, Teletype, an X-Y Plotter for Hardcopy of Spectra, and a Model 306 Centronic Printer. It has a scan range of 0.4 to 3.0 mm. A silicon detector can be used to scan from 0.4 to 1.0 μm; a lead sulfide detector can scan from 1.0 to 3.0 μm. It is blazed at 1.6 μm for high efficiency in near infrared. Resolution is 0.01 nm. We are truly excited about the potentials of nondestructive sorting.

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REFERENCES

1. Ballinger, W. E., and L. J. Kushman. 1970. Relationship of stage of ripeness to composition and keeping quality of highbush blueberries. J. Amer. Soc. Hort. Sci. 95:239-242.
2. Ballinger, W. E., W. F. McClure, and W. B. Nesbitt. 1974. Progress in maturity measurement of muscadine grapes using a light transmittance technique. HortScience 9:283 (Abstr.)
3. Ballinger, W. E., J. N. Yeatman, and L. J. Kushman. 1968. Progress in maturity measurements of blueberries using light transmittance technique. HortScience 3:99. (Abstr.)
4. Ben-Gera, I., and K. H. Norris. 1968. Direct spectrophotometric determination of fat and moisture in meat products. J. Food Sci. 33:64-67.
5. Birth, G. S., and K. H. Norris. 1965. The difference meter for measuring interior quality of foods and pigments in biological tissues. USDA Tech. Bul. 1341.
6. Dekazos, E. D., and G. S. Birth. 1970. A maturity index for blueberries using light transmittance. J. Amer. Soc. Hort. Sci. 95:610-614.
7. Kushman, L. J., and W. E. Ballinger. 1975. Effect of season, location, cultivar, and fruit size upon quality of light-sorted blueberries. J. Amer. Soc. Hort. Sci. 100:564-569.
8. Kushman, L. J., and W. E. Ballinger. 1975. Relation of quality indices of individual blueberries to photoelectric measurement of anthocyanin content. J. Amer. Soc. Hort. Sci. 100:561-564.
9. McClure, W. F., R. P. Rohrbach, L. J. Kushman, and W. E. Ballinger. 1975. Design of a high-speed fibre optic blueberry sorter. Trans. ASAE. 18:487-490.
10. Norris, K. H., and W. L. Butler. 1961. Techniques for obtaining absorption spectra on intact biological samples. I.R.E. Trans. Bio-Med Electronics BME. 8(3):153-157.

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Errata

HACCP Models for Quality Control of Entree Production in Foodservice Systems

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This paper appeared on pages 632 to 638 of Volume 40 (September, 1977) of the *Journal of Food Protection.* Several errors appeared in Table 4. The corrected table appears below.

TABLE 4. *Time-temperature critical control points at control points during entree production in a cook/chill foodservice system*

Control point	Critical control point	
	Time	Temperature[1]
Preparation	Minimal	7-60 C
Heating	—[2]	≥60 C[3]
Chilling	≤ 4 h[4]	≤ 7 C
Chilled storage	≤20 h[4]	≤ 7 C
Portioning and assembly	Minimal	≤ 7 C
Cold holding and distribution	—[2]	≤ 7 C
Heating	—[2]	74-77 C
Service	Minimal	≥60 C

[1]Internal temperature of entree at completion of control point activity.
[2]Time will vary with entree, equipment, and/or system.
[3]Minimum temperature; will vary with entree.
[4]Combined time of chilling and chilled storage should be ≤24 h.

Microbial Growth in Bulk Whole Corn Kernels Subjected to High Pressure Compaction

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This paper appeared on pages 686 to 688 of Volume 40 (October, 1977) of the *Journal of Food Protection.* K. Ostovar should be added as an author, the Department of Microbiology should be eliminated as a sponsoring organization, and the Department of Food Science should be added as a sponsoring organization.

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Instructions for Authors

Journal of Food Protection

SCOPE OF THE JOURNAL

The *Journal of Food Protection*, an international monthly journal in the English language, is intended for publication of research and review papers on all topics in food science and on the food aspects of the animal (dairy, poultry, meat, seafood) and plant (cereals, fruits, vegetables) sciences. Major emphases of the journal will be on: (*a*) cause and control of all forms (chemical, microbial, natural toxicants) of foodborne illness, (*b*) contamination (chemical, microbial, insects, rodents, etc.) and its control in raw foods and in foods during processing, distribution, and preparing and serving to consumers, (*c*) causes of food spoilage and its control through processing (low temperatures, high temperatures, preservatives, drying, fermentation, etc.), (*d*) food quality and chemical, microbiological, and physical methods to measure the various attributes of food quality, (*e*) the foodservice industry, and (*f*) wastes from the food industry and means to utilize or treat the wastes. Prospective authors with questions about the suitability of their material for publication are invited to request an opinion from the Editor.

SUBMITTING MANUSCRIPTS AND OTHER ITEMS

All manuscripts, including "Letter to the Editor," should be submitted *in duplicate*, in flat form, and by first class mail to the Editor, Dr. E. H. Marth, Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706. Revised manuscripts also should be submitted *in duplicate* to the Editor.

All materials intended for the "Association Affairs" and "News and Events" sections of the *Journal* should be submitted in flat form by first class mail to the Managing Editor, Mr. E. O. Wright, Box 701, Ames, Iowa 50010. Subjects suitable for inclusion in the "News and Events" section include: announcements of meetings, short courses, or other events of interest to the readership; notices of position changes and promotions; announcements of new products of interest to the readership; and notices of death and obituaries of members. Any questions on suitability of material can be answered by the Managing Editor.

Correspondence dealing with membership in the International Association of Milk, Food, and Environmental Sanitarians, Inc., subscriptions, advertising (including classified advertising), etc. should be sent to the Managing Editor at the address given above.

PUBLICATION OF MANUSCRIPTS

Manuscripts are accepted for publication, subject to editorial review. Most papers are reviewed by two members of the Editorial Board or by other specialists who may be called on by the Editor when in his opinion the subject of a paper is outside of the specialties represented by Editorial Board members. After review, a manuscript generally is returned to the author for revision in accord with suggestions made by reviewers. Authors can hasten publication of their papers by revising and returning them promptly. With cooperation by authors, research papers nearly always are published within 6 months after they are received and often they appear sooner.

The author is notified when a manuscript is received and also when it is sent to the printer for preparation of proofs. An author must return proofs promptly or publication of his paper may be delayed.

Membership in the International Association of Milk, Food, and Environmental Sanitarians, Inc. is *not* a prerequisite for acceptance of a manuscript for publication. Nonmember scientist from the U.S. and from other countries are invited to submit papers for consideration for publication.

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Submission of a paper implies that all authors and their institution(s) have agreed to its publication. It also implies that the paper is not being considered for publication in another domestic or foreign journal.

MANUSCRIPT SERVICE CHARGE

Upon recommendation of the Journal Management Committee, it was voted by the Executive Board of IAMFES to institute a manuscript service charge of $25.00 per printed page for publication of all *research* papers received after January 1, 1969. This charge is necessitated by increases in costs and also will permit expansion of the *Journal* so that more research papers can be published promptly.

Most institutions accept the manuscript service charge as a necessary cost of conducting research and communicating the results. Nevertheless, it is realized that some authors may not have funds available for this purpose and hence exceptions can be made when necessary. *Inability to pay the service charge shall not prohibit publication of an acceptable manuscript*. An author will be informed of the manuscript service charge when proofs are sent. The manuscript service charge does not apply to review papers or other general interest papers that are not research papers.

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Reprints of a paper may be ordered by the author when he returns the proofs. An appropriate form for this purpose is attached to proofs. Reprints may be ordered with or without covers in multiples of 100. The cost varies according to the number of printed pages in the paper. No free reprints are provided.

Reprints also may be ordered after a paper has been published. The IAMFES office can supply reprints of any papers published in the *Journal of Food Protection, Journal of Milk and Food Technology,* or *Journal of Milk Technology*. Arrangements to obtain such reprints should be made with the Managing Editor.

TYPES OF PAPERS

The *Journal* enjoys a wide readership in the United States and in other countries. Readers include persons at various levels in industrial, regulatory, and academic organizations. As a consequence, the *Journal* attempts to publish a variety of papers so that it is of maximum benefit to its readers. The following types of papers are acceptable for publication in the *Journal.*

Research paper

The research paper reports results of original research which has not been published elsewhere. It usually consists of 8 to 12 double-spaced typewritten pages plus appropriate tables and figures. A research paper deals in some depth with its subject.

Research note

A research note is a short paper which describes observations made in a rather limited area of investigation. Negative results are sometimes best reported in the form of a research note. The research note should

not be used as a vehicle for reporting inferior research. A research note generally consists of less than 5 double-spaced typewritten pages of text together with appropriate figures or tables. Organization of a research note is the same as that of a research paper. The designation, "A Research Note" will appear above the titles of these papers since the *Journal* does not devote a separate section to research notes.

The author should specify that his manuscript is research note so that it can be properly evaluated during the reviewing process.

Review papers

Well written, thorough, well documented review papers on subjects of concern to the readers of the *Journal* are encouraged and will be published promptly. If a review paper substantially exceeds approximately 35 double-spaced typewritten pages, it may be necessary to subdivide the manuscript so that it will appear in several issues of the *Journal.* Authors are invited to discuss their plans for review papers with the Editor.

General interest-nontechnical

The *Journal* regularly publishes some nontechnical papers as a service to those readers who are not involved with the technical aspects of dairy and food science. These "grassroots" papers might deal with such topics as working with people, organization of a food control program, organization of a regulatory agency, organization of an educational program, use of visual aids, and similar subjects. *Papers of this type should be well written and properly organized with appropriate subheadings.* Often talks given at meetings can be modified sufficiently to make them appropriate for publication. Authors planning to prepare general interest nontechnical papers are invited to correspond with the Editor if they have questions about the suitability of their material.

Letter to the Editor

Readers are invited to submit letters to the editor to express their opinion on papers published in the *Journal* or on other matters which may be of concern to the entire readership. The letter to the editor also may be used to report limited observations made in the field or in the laboratory which cannot be published as a research note. This mechanism should be particularly valuable for the exchange of information by persons who are unable to attend annual meetings of IAMFES or by laboratory workers whose duties preclude publication of full-fledge research papers. A letter to the editor must be signed by its author(s).

Book reviews

Authors and publishers of books in the fields covered by this *Journal* (see earlier discussion of scope) are invited to submit their books to the Editor. Books will then be reviewed by a specialist in the field covered by the book and a review will be published in an early issue of the *Journal.*

PREPARATION OF MANUSCRIPTS

A. All manuscripts should be typed double-spaced on 8.5 by 11-inch bond paper. *Lines on each page should be numbered to facilitate review of papers.* Use of paper with prenumbered lines is satisfactory. Side margins should be one inch wide and pages should not be stapled together.

B. The Editor assumes that the senior author has received proper clearance from his/her organization for publication of the paper. An author should be aware of procedures for approval within his/her own organization.

C. A manuscript should be read critically by someone other than the author before it is submitted. This will help to eliminate errors and to clarify statements.

D. The current edition of CBE *Style Manual* (published by the American Institute of Biological Sciences, 3900 Wisconsin Ave. N.W., Washington, D.C. 20016) has been adopted by the *Journal* and should be consulted by authors for technical details of manuscript preparation. Abbreviations for botanical, chemical, physical, mathematical, and statiscal terms should conform to the *Style Manual.*

E. *Organization of research papers and research notes*

1. The *title* should appear at the top of the first page. It should be as brief as possible, contain no abbreviations, and be truly indicative of the subject matter discussed in the paper. Care should be exersised by the author in preparing the title since it is often used in information retrieval systems. *Good information can be lost through a poor title!*
2. *Name(s) of author(s) and affiliation(s)* should follow under the title. If an author has changed location since the work was completed, his new address should be given in a footnote.
3. The *Abstract* appears at the beginning of the paper. It should be brief, factual, and not exceed 200 words. The abstract should be intelligible without reading the remainder of the paper. Generally, an abstract should not contain abbreviations. Abstracts of papers are reprinted by abstracting journals and so will be disseminated beyond the readership of the *Journal* to people who often do not have access to the entire paper. Hence abstracts should be prepared with great care.
4. The *text* should contain: (*a*) introductory statements, objectives or reasons for research, and related literature, (*b*) materials and methods (*c*) results, (*d*) discussion (may be combined with results), (*e*) conclusions (only if needed; should not repeat the abstract) (*f*) acknowledgments, and (*g*) references.
5. *Citation of references* must follow the style of the *CBE Style Manual.* Several examples of proper citations are given below.

   A. *Paper in a journal*

   Alderman, G. G., and E. H. Marth. 1974. Experimental production of aflatoxin in citrus juice and peel. J. Milk Food Technol. 37:308-313.

   b. *Paper in a book*

   Marth, E. H. 1974. Fermentations. pp. 771-882. In B. H. Webb, A. H. Johnson, and J. A. Alford (eds.) Fundamentals of dairy chemistry (2nd ed.), Avi Publishing Co., Westport, Conn.

   c. *Book*

   Fennema, O. R., W. D. Powrie, and E. H. Marth. 1973. Low-temperature preservation of foods and living matter. Marcel Dekker, Inc., New York. 598 p.

   d. *Patent*

   Hussong, R. V., E. H. Marth, and D. G. Vakaleris. 1964. Manufacture of cottage cheese. U. S. Pat. 3, 117, 870. Jan. 14.

   For citation of bulletins, annual reports, publications of federal agencies, etc., *see CBE Style Manual. References must be listed in alphabetical order and numbered. Numbers in parentheses, independently or in conjunction with last names of authors, must be used in the text for designating references.*

F. *Organization of review and general interest papers*

These papers must have a title followed by names(s) of author(s) and affiliation(s), and the text must begin with an abstract. See items 1, 2, and 3 under E. The remainder of the text should begin with an introductory statement and then should be subdivided into appropriate sections each with a subheading which is decriptive of the subject matter in the section. Review papers by their nature, utilize numerous references. Citation of references in the text and listing of references at the end of the paper should be done as mentioned in Section E-5 above.

G. *Preparation of figures*

Figures consisting of drawings, diagrams, charts, and similar material should be prepared in India ink on 8.5 by 11-inch tracing paper, white drawing paper, or blue linen. Do not use paper with green, red, or yellow lines since they cannot be removed and will appear in the final copy. A lettering guide must be used to prepare all letters which appear on figures. *Titles for all figures must be on separate sheets and not on the figures.* Use Arabic numbers for numbering of figures. Glossy prints of figures are suitable for use. They should be at least 4 by 5 inches in size. If photographs of equipment, etc. are submitted, the images should be sharp, there should be good contrast, and a minimum of distracting items

should appear in the picture.

H. *Preparation of tables*

Each table should be typed on a separate sheet of 8.5 by 11-inch bond paper. *Tables should not be included in the text of the paper.* Use Arabic numbers for numbering of tables. *Titles should be as brief as possible but fully descriptive.* Headings and subheadings should be concise with columns or rows of data carefully centered below them. Use only horizontal lines to separate sections of tables. *Data in tables should not be repeated in figures.* When possible use figures instead of tables since the latter are more costly to prepare for publication.

Book Review

Normal and Therapeutic Nutrition, Fifteenth Edition. By Corinne H. Robinson, and Marilyn R. Lawler. Macmillan Publishing Co., Inc., New York. 1977. $12.95.

As the title indicates this text is divided into two parts, normal nutrition (Chapters 1-24) and therapeutic nutrition (Chapters 25-46). In addition, there are sections containing (1) tabular materials (nutritive value of foods, weight-height for age-sex categories, and normal constituents of human blood, etc.), (2) list of audiovisual materials, (3) common abbreviations, and (4) a glossery of nutrition terms.

The authors have successfully accomplished their objective of updating this text by revision and inclusion of new materials related to normal and therapeutic nutrition. It is rather comprehensive, although superficial, well-written and illustrated. A major strength of this text is the review questions and the list of references at the end of each chapter.

Part one, normal nutrition, basically takes a classical approach to the presentation of nutrition. The first portion of part one is devoted to the chemistry and the physiological role of proteins, carbohydrates, lipids, energy, minerals, fluids and electrolyte balance, and the fat- and water-soluble vitamins. For example, emphasis upon zinc nutrition and the mechanism of vitamin D activity are valuable materials in this part. To complete part one, topics concerning meal planning, food habits, patterns and selection, and nutrition during different stages of growth and development are presented.

Part two, therapeutic nutrition, concerns the type of diet required in diseases, disturbances and disorders. Some examples of the materials presented on health conditions requiring therapeutic nutrition of current interest, and diets for these conditions, are obesity, diabetes mellitus, hyperlipidemia and atherosclerosis, diverticulosis, anemias, and allergies. Each of these conditions are adequately described, and then sample menus are presented as well as in a number of conditions, dietary counseling is prescribed.

This textbook very adequately meets its intended use for students in nursing and dietetics and it is also useful for others interested in normal and therapeutic nutrition.

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Coming Events

February 15-16, 1978. DAIRY INDUSTRY CONFERENCE. Center for Tomorrow, Ohio State University, Columbus, OH. Contact: J. Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Rd., Ohio State University, Columbus, OH 43210.

March 7-8, 1978. SYMPOSIUM, "ALFALFA—ENERGY, PROTEIN AND NITROGEN" Radisson South Hotel, Bloomington, MN. Contact: Richard Meronuck, Office of Special Programs, University of Minnesota, St. Paul, MN 55108.

March 15, 1978. NEW YORK STATE CHEESE MANUFACTURERS ASSN. ANNUAL CONFERENCE Hotel Syracuse, Syracuse, NY. Contact: David K. Bandler, Dept. of Food Science, Cornell University, 11 Stocking Hall, Ithaca, NY 14853.

February 26-March 3, 1978. 8th CONFERENCE ON ENVIRONMENTAL ENGINEERING IN THE FOOD PROCESSING INDUSTRY. Asilomar Conference Grounds, Pacific Grove, California. Contact: Sandford Cole, Engineering Foundation, United Engineering Center, 345 East 47 St., New York, NY 10017.

February 26-March 1, 1978. 20th ANNUAL MEAT SCIENCE INSTITUTE. University of Georgia, Athens, GA. Co-sponsored by the University of Georgia and the National Independent Meat Packers Association. Contact: Dr. John Carpenter, Food Science Dept., College of Agriculture, University of Georgia, Athens, GA 30602.

March 20-24, 1978. MIDWEST WORKSHOP IN MILK AND FOOD SANITATION. Center for Tomorrow, Ohio State University, Columbus, OH. Contact: J. Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Rd., Ohio State University, Columbus, OH 43210.

March 20-30, 1978. UNIVERSITY OF MARYLAND 38th ANNUAL ICE CREAM SHORT COURSE. Dept. of Dairy Science, Animal Sciences Center, College Park, MD. Contact: J. Mattick, Dept. of Dairy Science, University of Maryland, College Park, MD 20742.

March 27-29, 1978. AMERICAN CULTURED DAIRY PRODUCTS INSTITUTE ANNUAL TRAINING SCHOOL AND JUDGING CONTEST. Stouffer's Inn, Indianapolis, Indiana. Contact: C. Bronson Lane, ACDPI, P.O. Box 7813, Orlando, FL 32804.

March 28-30, 1978. WESTERN FOOD INDUSTRY CONFERENCE, Freeborn Hall, University of California at Davis. Contact: John C. Bruhn, Dept. of Food Science and Technology, University of California, Davis, CA 95616, Phone: 916-752-2192.

April 17-19, 1978. DAIRY AND FOOD INDUSTRIES SUPPLY ASSN., 59th ANNUAL MEETING, Canyon Hotel Racquet & Golf Resort, Palm Springs, CA. Contact: DFISA, 5530 Wisconsin Ave., Suite 1050, Washington, DC 20015

June 4-7, 1978. 1978 ANNUAL MEETING OF THE INSTITUTE OF FOOD TECHNOLOGISTS, Dallas, Texas. Contact: Dan Weber, Director of Convention Services, IFT, 221 N. LaSalle St., Chicago, IL 60601.

June 25-28, 1978. CANADIAN INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY 21st ANNUAL CONFERENCE, Edmonton, Alberta. Contact: P. Jelen, Dept. of Food Science, University of Alberta, Edmonton, Alta. T6G 2N2.

Larry J. Gordon Promoted

Larry J. Gordon, currently serving as New Mexico Administrator for Health and Environmental Programs in which role he adminsters the New Mexico Environmental Agency, New Mexico Health Agency, New Mexico Scientific Laboratory System, and New Mexico Health Planning and Development Agency, has been promoted to Deputy Secretary of New Mexico's newly-created Department of Health and Environment. The new Department will include the previously-listed agencies as well as New Mexico programs for Mental Health, Drug Abuse, Alcoholism, and Developmental Disabilities.

A recipient of the IAMFES Sanitarians Award, Gordon is a Past-President of the Rocky Mountain Association of Milk and Food Sanitarians, and is a Past-Chairman of the Conference of Local Environmental Health Administrators. While serving as Director of the Albuquerque Health Department, his Department twice received the Samuel J. Crumbine Award. He is a Founder of the American Academy of Sanitarians, and currently serves on the Executive Board of the American Public Health Association.

Illinois Team Tops Collegiate Dairy Products Evaluation Contest Honor

Balance in scoring and geographic location marked the individual and team judging competition in the 56th Collegiate Dairy Products Evaluation Contest at Denver, Oct. 24, 1977.

Thirteen of the nineteen participating college teams were winners of the top three awards in each of the seven team events or had students who won the first three places in the individual product competition.

The University of Illinois' predominantly female team outdistanced the field as the best All-Products team and also won first place in the ice cream competition. Illinois' last All-Products victory was in 1972.

Winner of the All-Products individual award was Mary Nilsson of the University of Illinois, who recorded the highest average score of the 72 participating students in the taste testing of milk, ice cream, cottage cheese, butter, cheddar cheese and yogurt. She received the $100 Robert Rosenbaum award, presented by the Dairy Remembrance Fund, for her accomplishment.

South Dakota State University captured the cottage cheese trophy and won its fifth successive butter cup. It was the 13th time since 1917 that the SDSU team walked off with the butter honors.

Other team winners were the University of Georgia, winner of the milk trophy, its first in contest history; University of Minnesota, which won its fifth cheddar cheese cup since 1937; and Michigan State University, victor in the first yogurt competition ever staged. Yogurt was added to the product lineup this year, the first new category since 1962.

Prof. Joseph Tobias, Illinois coach, was presented the Coach-of-the-Year award by Joseph Rubis of the U.S. Department of Agriculture, who served his first year as contest superintendent.

Top three winners in each product are:

All-Products teams: Illinois, Minnesota, Missouri; Individuals: Mary Nilsson, Illinois; Bill Berkbigler, Missouri; Carol Martin, Illinois.

Milk teams: Georgia, Mississippi State, Oregon State; individuals: Phil Underwood, Georgia; Holly Hoffman, Cornell; Tom Williams, Cal Polytechnic State University.

Cottage Cheese teams: SDSU, Minnesota, Cal Poly; individuals: Paulette Pierson, Minnesota; Randy Child, Wyoming; Carol Hamilton, West Virginia.

Butter teams: SDSU, Michigan State, Missouri; individuals: Brent Weckworth, SDSU; Carol Martin, Illinois; Doug Freier, SDSU.

Cheddar Cheese teams: Minnesota, Illinois, Oregon; individuals: Wendy Thompson, Minnesota; Paulette Pierson, Minnesota; Andrea Hummel, Oregon State.

Ice Cream teams: Illinois, Cal Poly, Tennessee; individuals: Antonette Andruzzi, West Virginia; Bill Berkbigler, Missouri; Vonnie Romano, Wyoming.

Yogurt teams: Michigan State, Missouri, Cal Poly; individuals: Garret White, Michigan State; Antonette Andruzzi, West Virginia; Susan Fierke, Michigan.

Other college teams participating were Brigham Young University, Iowa State, Moraine Park Technological Institute (a first-time entrant), Ohio State, Oklahoma State and University of Wisconsin.

The contest was conducted during the annual convention of the International Association of Ice Cream Manufacturers (IAICM) and Milk Industry Foundation (MIF). Students whose decisions most nearly agree with the professional judges' receive appropriate honors.

Public Health Professionals

Strong endorsement of the "preventive health value" of single-use cups, plates and other paper and plastic food service and packaging products has been voiced by the American Public Health Association through action by its Governing Council at the Association's recent annual meeting in Washington, D.C.

At the same time the Association urged caution on the part of the federal government in any efforts at curtailment of single service articles for resource conservation or other environmental purposes.

The American Public Health Association is a national professional organization representing 28,000 public health workers, health and environmental agency officials, and academic specialists. State and local health associations affiliated with APHA add approximately 22,000 physicians, nurses, social workers, epidemiologists, environmental health scientists, administrators of hospitals, clinics and health departments, and university professors involved in public health.

In an APHA policy statement approved on November 2 by the 170-member Governing Council, the public health professionals stated:

"The American Public Health Association affirms the preventive health value of single service articles for selected food service operations and cautions that curtailment of single service articles may introduce environmental hazards that are far more significant in terms of overall impact upon human health and safety than the possible reductions that resource conservation may achieve."

"With the APHA statement, the sanitary and safety benefits of single service products have now been endorsed by all three major professional public health societies in the United States," commented Robert W. Foster, Executive Vice President of the Single Service Institute. "Our industry is gratified now just as it was when similar positions were taken by the National Environmental Health Association and the International Association of Milk, Food and Environmental Sanitarians." The Institute is the national trade association of manufacturers of paper and plastic single-use food service and packaging products.

In its policy statement, the APHA stresses that "resource conservation can have serious consequences to public health by removing from the market place disposable devices which have come to be part and parcel of modern preventive helath practice." This is a reference to the "source reduction" approach to preventing waste generation defined by the U.S. Environmental Protection Agency as involving the substitution of reusable products for single-use articles. In commenting on this approach, the APHA statement said "it is the opinion of the American Public Health Association that substitution of reusable food service items for disposable products in many cases will be a regressive step, contrary to established public health practice."

For these reasons, the policy statement concludes, "the APHA urges the Environmental Protection Agency, the Council on Environmental Quality, and the Resource Conservation Committee to make a thorough analysis of the public health and safety impact of any disposable devices before recommending their curtailment for the sake of resource conservation."

Upturn to Continue in 1978 DFISA Survey Reveals

Food and dairy processors are expected to increase expenditures for capital and noncapital goods by 5 to 10 per cent in 1978, based on a survey conducted by Dairy and Food Industries Supply Association of its member companies.

The increase is a continuation of the upturn, reflected in the 1976 survey, in capital investment for new plant, equipment and tooling, and noncapital purchases of ingredients, packaging, materials, supplies and services.

While projecting the modest increase in expenditures by processors, equipment manufacturers, supply and service firms reported cost control at all levels as by far the No. 1 concern of their customers. The burden of government regulations was singled out as an equally serious problem for processors by ingredient, packaging, and transportation suppliers. Other factors identified as customer problems or requirements were growing dependence on pollution and energy-conserving equipment, greater expectations from advanced processing methods, such as aseptic and membrane technology, and intensified interest in automated systems.

The DFISA member companies estimated their own business volume in 1978 would go up domestically by 14.6 per cent and export business by 19 per cent.

A majority of all companies responding expect to maintain capital investment in plant and equipment at about the same level in 1978 as in 1977. A third of the processing equipment companies indicated increased capital spending averaging 24 per cent. Half of the container suppliers projected a 30 per cent rise. Transportation and delivery suppliers forecast a 15 per cent hike.

Equipment and supply sales in 1978 to food processors are expected to be 13 per cent higher than 1977 and to dairy processors 11 per cent above the current year.

Factors expected to cause DFISA companies the greatest problems in 1978 are, in order of importance listed, controlling costs, rising cost of raw materials, complying with government regulations, maintaining productivity, and pricing to maintain competitiveness.

All companies are reported being handicapped with higher energy costs, with 8 companies reported having to convert to new energy sources.

Association Affairs

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Jointure Being Studied by IAMFES and NEHA Committees

The Executive Boards of both NEHA and IAMFES have appointed special committees to study and plan for a jointure of the two organizations.

The jointure being studied is somewhat different than the merger that was attempted a few years ago. The jointure as being studied by the committees now is the joining together of these organizations without loosing the identities of either organization. Many of the same things can be accomplished by a Jointure without merging. There are many legal problems that might be avoided by using a jointure procedure. When such organizations change names many legal problems arise such as Federal registration changes for tax exemptions, patents, etc.

The joint committees on sight selection have met and have chosen Milwaukee, Wisconsin for the sight of a joint annual meeting in 1980. The dates for this meeting will be July 26-31. This should prove to be a most rewarding meeting for both organizations.

The committees appointed are as follows:

IAMFES
E. O. Wright
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H. E. Hutchings
C. K. Luchterhand
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Harry Steigman
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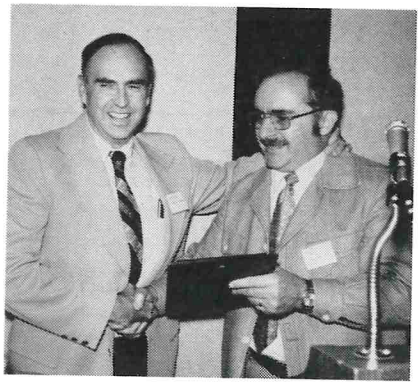
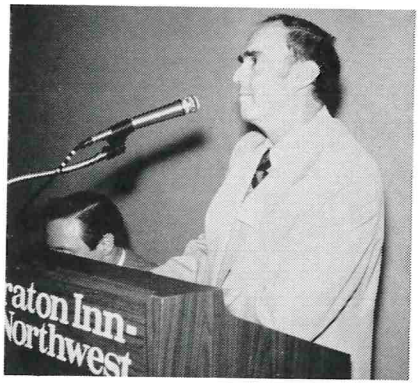
Index to Advertisers

1977 Shogren Award Winners Meet

1977-78 MINNESOTA SANITARIANS ASSOCIATION OFFICERS (l. to r.) President, Peter Patrick, St. Paul; Secretary-Treasurer, Roy E. Ginn, St. Paul; and Past President, Arnold O. Ellingson, Fergus Falls.





Dr. Vernal S. Packard, University of Minnesota's Department of Food Science and Nutrition, received the annual Minnesota Certificate of Achievement Award, presented by the Minnesota Sanitarians Association. Making the presentation, Dr. Edmund Zottola (r.) also from the Department of Food Science & Nutrition, U. of M.

The Minnesota Sanitarians Association held its Annual Fall Sanitarians Conference September 15-16, 1977 at the Sheraton Inn Northwest in Minneapolis.

MSA achieved recognition as outstanding affiliate and thus received the C.B. Shogren Award at the IAMFES Annual Meeting in Sioux City, Iowa last August.

In 1966 MSA hosted the IAMFES Annual Meeting. Its current membership of some 250 includes two former IAMFES presidents and a former editor of the *Journal of Milk and Food Technology.*

This year, MSA's Annual Sanitarians Conference, with sessions on food and environment plus dairy sanitation, covered a wide range of current topics. Examples are U.S.D.A.'s Dairy Farm Program highlights, animal waste handling, implementation of the Federal Drinking Water Act, and standards in food sanitation education.

The meeting highlight was the Annual Awards Banquet. Honorary Life Membership was given to Ing Lein, recently retired from Land O' Lakes. In addition, the Certificate of Achievement was given to Dr. Vernal S. Parkard in recognition of exceptional services to MSA.

ROY E. GINN, (l.) Secretary-Treasurer of the Minnesota Sanitarians Association, accepting the C. B. Shogren Award for the Minnesota Sanitarians Association from Orlowe M. Osten, St. Paul, Past President of the Association and Past President of the International Association of Milk Food and Environmental Sanitarians.

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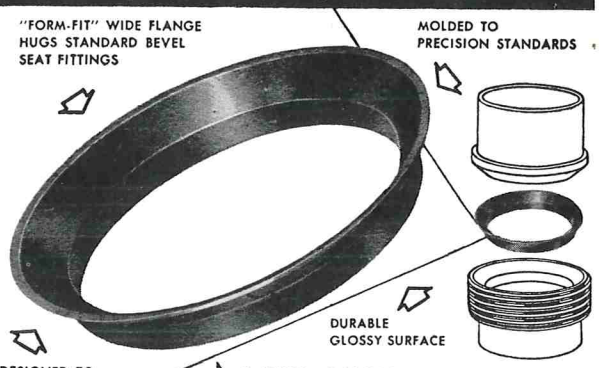

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Stimulation is more than cleaning the udder before milking. Properly done, stimulation substitutes completely for the natural signal provided by the calf to tell the cow she is hungry. Oxytocin, a hormone released into the blood stream after stimulation,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

Complementary Milk: Profits Left in the Udder

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

Thirty Seconds of Profitable Time

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

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