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August 18, 19, 20, 21, 1969
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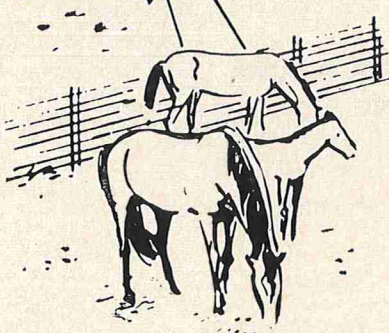
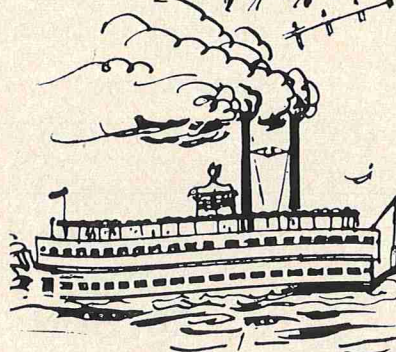
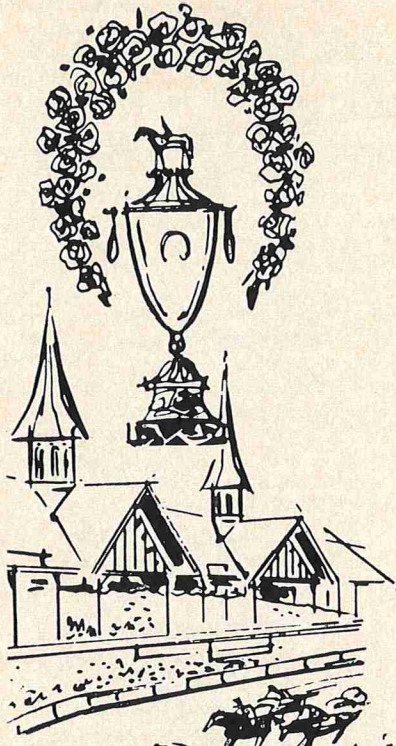
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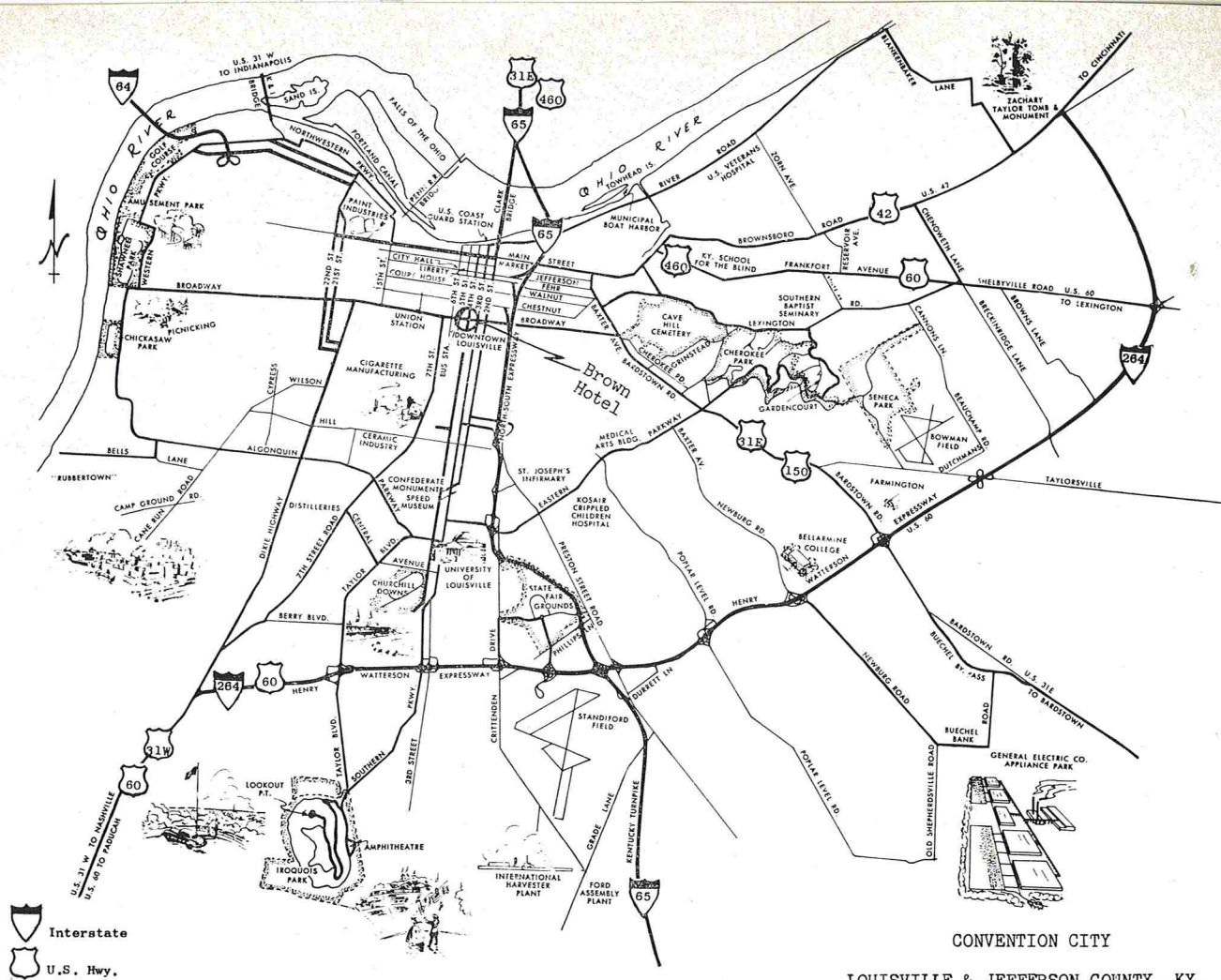
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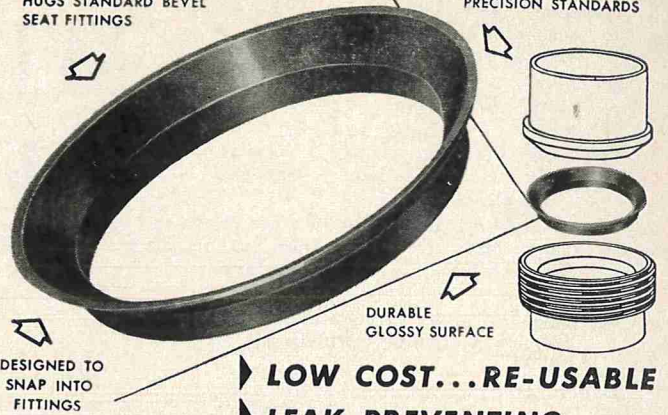
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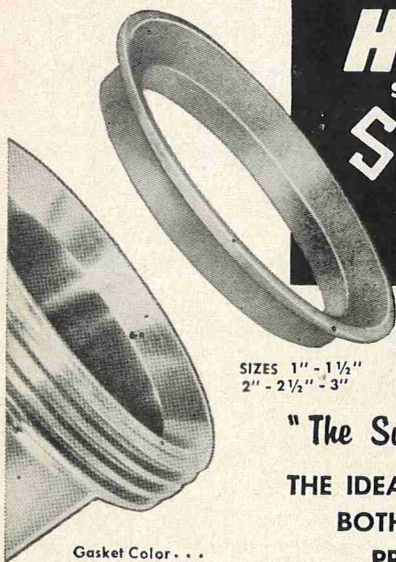
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APPLES FROM ARGENTINA: CHEMICAL AND PHYSICAL CHARACTERISTICS USEFUL FOR TECHNOLOGY

ENRIQUE ROTSTEIN, MELHEM S.S. NAMOR, ANA M. SICA, AND JORGE ARUANND

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(Received for publication March 12, 1968)

ABSTRACT

With a look to apple industrialization in Argentina, apples were analyzed to evaluate them as a raw material. The 9 more important varieties harvested in 1967 were: Red Delicious, Granny Smith, Rome Beauty, Delicious, Black Wine-sap, King David, Yellow Newtown Pippin, Jonathan, and Golden Delicious. They were analyzed for: specific gravity, pH, total acidity (as malic acid), soluble solids, total sugar, reducing sugar, pectin, tannin, ascorbic acid, moisture, starch (qualitative test), dry matter, and texture. Analysis were made on fruit harvested at the picking date, and on samples from the same tree held 1, 2, 4 and 6 weeks (the latter for Delicious and Red Delicious only). All analyses were completed within 24 hr of harvesting time. A review is made of similar data from other countries. Argentinian apples are similar to those grown in other countries.

As part of a general study on industrialization of Argentinian apples, it was felt that those characteristics related to eventual technological utilizations, should be defined. To do so, after consultation with people related to the objective, a series of chemical analyses and physical tests was programed. The aim was to assess the contents of apple components important for eventual processing.

Many studies regarding apple composition have been published the world over. A comprehensive review of pertinent literature was made by Smock and Neubert (33). After this review, Tekeli (38) reported on chemical composition of 4 Turkish varieties, covering items s, n, q, i, and o (See Table 1). Pijanowsky (27) gave data on items f, c, s, q, and a of 10 Polish varieties. Strachan et al. (34) made a comprehensive study of chemical composition of British Columbia tree fruits, reporting on items r, m, c, q, i, s, k, n, b, o, h, a, and ash alkalinity. Ayres et al. (3) gave data on l, s, q, j, g, b, t, a, of English apples. Siberian apples were analyzed for f, o, s, q, and i (5). Michurin and Central Russia varieties were analyzed by Bereznegovskaya (4), reporting f, o, s, q, and i. Lieinbach et al. (20) studied the composition of United States Northwestern Delicious apples (s, k, q, and r) from the standpoint of juice production. Paper chromatography by Aso (2) yielded information on s, n, d, u, g, e, and amino acids. Data on s, q, and a, of Turkmenistan apples was reported by Boltenkov et al. (6). British cider apple juice of 14 varieties was analyzed by Burroughs (7). Tsit-

TABLE 1. KEY FOR CHEMICAL COMPOSITION DATA

Chemical	Key
Ascorbic acid -----	a
Ash -----	b
Dry matter -----	c
Fructose -----	d
Glucose -----	e
Moisture -----	f
Nitrogen -----	g
Pectins -----	h
pH -----	i
P ₂ O ₅ -----	j
Reducing sugars -----	k
Specific gravity -----	l
Soluble solids -----	m
Sucrose -----	n
Tannins -----	o
Texture -----	p
Total acids -----	q
Total solids -----	r
Total sugars -----	s
Viscosity -----	t
Xylose -----	u

sishvili (41) reported s, q, and a of Russian varieties grown in the Gori area. Eliseev (10) took into account different periods of ripening when analyzing Russian varieties for s, n, q, a, and c. He also compared the composition of American varieties grown in Russia with those grown in United States. Lopez (21) studied q, i, m, s, k, n, o, b, p, and h of Virginia apples. Data on s, k, q, o, h, g, a, Fe, Ca, and P of Indian apples was reported by Pruthi (29). Kenworthy et al. (13) gave data on d, e, u, i, m, o, t, and minerals of several varieties, from the standpoint of their relationship to environment and season. The same authors (12) studied the relationship between organic acids in the apple, the variety and source. Wojciechowicz (45) studied pectins of apples.

Several studies have been devoted to sugar contents alone. Minasyan (24) analyzed Michurin varieties. Aso (1) investigated Japanese apples by conventional and chromatographic methods. Williams (44) studied the permanganate method for evaluating tannins in apple juice and cider.

Tanner (36) studied citric, malic, quinic and glycolic acids content of Swiss cider apples. Korabler-

and (17) and Dzamic (9) also worked on this matter. Much work has been done as to the ascorbic acid content of apples. Krauze (18) analyzed Polish varieties. Murneek (26) investigated Missouri Summer, Fall, and Winter varieties, reporting on effect of light intensity, soil, storage, and cooking. Koch (16) studied changes in vitamin C content during ripening. Vodenichaw (42) made a similar study, which also covered the early growing period. Russian apples showed no difference resulting from climate, the content decreasing during maturation and then ris-

ing when apples were ripe (19). Sheherbakova (32) studied the change in vitamin C of apples of the Ryzan region during ripening, making correlative determinations of sugars. Toma (40) also reported ascorbic acid content of apples.

Tables 2 to 9 report representative data of constituents in apples grown in several countries. When the variety was the same as that analyzed here, it was reported individually. Other varieties were reported as a group, unless the nature of the data made it advisable to report individually.

TABLE 2. VITAMIN C CONTENT OF APPLES GROWN IN SEVERAL COUNTRIES

Country	Varieties	References	Ascorbic acid, mg/100		
			Minimum	Maximum	Average
Canada	Average		1.2	20.9	7.8
	Delicious		1.2	17.1	6.9
	Jonathan	(34)	5.5	15.6	9.8
	Golden Delicious		5.1	7.2	6.3
	Rome Beauty		5.1	17.1	11.0
England	Cider	(28)	9.1	34.1	
	table	(3) (14)	1.6	22.5	
France	table	(37)	4.9	58.9	16.3
Germany	table	(25) (30)	2.5	25.8	
India	table	(29)	5.8	9.9	
Japan	table	(35)	4.7	5.6	
Poland	table	(18) (27)	1.8	29.4	
Romania	table	(40)			5.2
West Virginia	Golden Delicious	(11)	10.0	15.0	
	& Jonathan Rome Beauty		7.0	10.0	
New York	Mc Intosh	(13)	3.4	11.1	7.4
Michigan					
U.S.A.	West Virginia	(13)	4.9	12.1	9.6
	Michigan		7.3	18.5	11.6
	Washington				
Washington	Average	(39)	2.5	10.0	
	Jonathan				2.5
	Rome Beauty		3.6	4.2	
	Delicious				3.0
Country-wide	table	(43)			7.0
U.S.S.R.	table	(4) (5) (6) (10) (17)	1.7	29.4	

TABLE 3. TOTAL ACIDITY OF APPLES GROWN IN SEVERAL COUNTRIES

Country	Varieties	References	Total acidity as malic acid, %		
			Minimum	Maximum	Average
Canada	All-average	(34)	0.20	0.86	0.48
	Delicious		0.20	0.58	0.27
	Golden Delicious		0.32	0.51	0.41
	Jonathan		0.48	0.74	0.64
	Rome Beauty				0.55
England	table	(3)	0.23	1.32	0.74
India	table	(29)	0.47	0.73	
Poland	table	(27)			0.45
Turkey	table	(38)	0.08	0.43	
New York Michigan	Mc Intosh	(13)	0.50	0.63	0.56
West Virginia Michigan Washington	Red Delicious	(13)	0.20	0.26	0.23
	Golden Delicious		0.31	0.49	0.34
U.S.A. Massachusetts Minnesota	table	(33)	0.38	1.11	
	table		0.03	1.45	
Virginia	All-average	(8)	0.08	2.50	0.52
	Delicious		0.22	0.31	0.27
	Granny Smith		0.52	0.73	0.66
	King David		0.37	0.65	0.53
	Rome Beauty		0.26	0.39	0.32
U.S.S.R.	table	(4) (5) (6) (10) (17)	0.08	6.74	

EXPERIMENTAL

Sampling

All samples were collected from the Paissanidis orchard, at the Rio Negro Valley, Rio Negro Province, Argentina. This orchard has a sandy soil, typical of the area river coast. Care was taken in selecting trees which were placed in symmetrical positions, not too close to shaded areas or irrigation channels, had regular size and aspect, and were in good condition. The trees were grown according to local practices of good culture and management.

Table 10 indicates all the varieties grown in the area and the percentage of the total crop they represent. The investigation covered the 9 more important: Red Delicious, Granny Smith, Rome Beauty, Delicious, Black Winesap, King David, Yellow Newtown Pippin, Jonathan, and Golden Delicious.

Local practice is to start the harvest at an official picking date, which is set by the Ministry of Agriculture on the basis of a preestablished number of days after full bloom. Samples of each variety were drawn at the picking date, and after 1 and 4 weeks. Delicious and Red Delicious were also sampled after 6 weeks. Samples of each variety were drawn by walking around the tree and random-picking 1 bu. Samples were taken in the early morning and rushed to a nearby laboratory,

where they were immediately analyzed. In each test, 20 apples were taken as the sample for analysis, scrubbed with a brush in running tap water, rinsed in distilled water and dried with cheesecloth. The apples were cored, cut in half at right angles to the core, and quartered by cutting again from stem to blossom end. Diametrically opposite quarters were taken from each apple. Juice was obtained by disintegrating, centrifuging and filtering with a Gigler apparatus. The material for analysis which was not carried on juice, was homogenized with an electric blender. The preliminary steps were similar to those of Kirpatrick (15).

Analytical procedures

Total and reducing sugars were analyzed on juice samples, using the Lane and Eynon method. Total acidity was determined by titration with 0.1 N NaOH solution to pH 8.1, using a pH meter. Specific gravity was measured with a densimeter and soluble solids with a refractometer at 20 C. The pH value was determined with a calibrated pH meter. Pectin was determined by the Carré and Haynes method as modified by Mc. Inney. Tannin was measured by titration with 0.1N KMnO₄ solution, using indigo solution as indicator. Moisture was estimated by vacuum drying. Ascorbic acid was titrated with standardized sodium 2:6 dichlorophenolindophenol dye. All the above procedures followed the standards

TABLE 4. DRY MATTER OF APPLES GROWN IN SEVERAL COUNTRIES

Country	Varieties	References	Dry matter, %		
			Minimum	Maximum	Average
Canada	All-average	(34)	12.07	18.39	14.92
	Delicious		13.73	16.35	14.85
	Golden Delicious		14.60	17.05	15.69
	Jonathan		13.22	16.20	14.90
	Rome Beauty				15.23
Illinois	All-average	(22)	12.20	16.65	16.20
	Jonathan		13.40	16.45	14.20
	Golden Delicious Delicious				
New York Michigan	Mc. Intosh	(13)	12.10	16.30	13.70
West Virginia Michigan Washington	Red Delicious Golden Delicious	(13)	12.90 12.57	19.00 19.03	15.73 15.35
U.S.A.					
Michigan	Mc. Intosh	(12)			16.30
	Red Delicious				19.00
	Golden Delicious				19.00
Washington	Golden Delicious	(12)			17.80
Virginia	All-average	(8)	7.63	18.33	12.92
	Delicious				12.46
	Granny Smith		12.64	14.97	13.85
	King David		14.48	15.56	15.02
U.S.S.R.	Early ripening Late ripening	(9)			17.60 15.60

TABLE 5. PECTIN CONTENT OF APPLES GROWN IN SEVERAL COUNTRIES

Country	Varieties	References	Pectins, %		
			Minimum	Maximum	Average
Canada	All-average	(34)	0.36	0.75	0.55
	Delicious		0.36	0.51	0.42
	Golden Delicious		0.61	0.68	0.64
	Jonathan		0.53	0.68	0.59
	Rome Beauty				0.42
India	table	(29)	3.35	3.73	
New York Michigan	Mc. Intosh	(13)	0.669	1.220	0.875
U.S.A.					
West Virginia Michigan Washington	Red Delicious Golden Delicious	(13)	0.863 0.757	1.458 1.441	1.204 1.057

TABLE 6. SOLUBLE SOLIDS CONTENT OF APPLES GROWN IN SEVERAL COUNTRIES

Country	Varieties	References	Soluble solids, %			
			Minimum	Maximum	Average	
Canada	All-average	(34)	11.8	17.2	13.7	
	Delicious		12.3	13.9	13.2	
	Golden Delicious		13.2	15.7	14.3	
	Jonathan		11.9	14.9	13.6	
	Rome Beauty				13.6	
New York Michigan	Mc. Intosh	(13)	10.6	13.4	12.1	
U.S.A.	West Virginia	(13)	13.0	15.9	14.3	
	Michigan					Red Delicious
	Washington					Golden Delicious
Michigan	Mc. Intosh	(12)			11.6	
	Red Delicious				13.8	
	Golden Delicious				15.0	
Washington	Golden Delicious	(12)			14.5	

TABLE 7. REDUCING SUGARS CONTENT OF APPLES GROWN IN SEVERAL COUNTRIES

Country	Varieties	References	Reducing sugars, %			
			Minimum	Maximum	Average	
Canada	All-average	(34)	6.33	10.67	8.37	
	Delicious		8.28	10.13	8.81	
	Golden Delicious		7.75	7.97	7.86	
	Jonathan		7.97	8.91	8.29	
England	table	(3)	4.14	13.06	7.63	
	table	(33)			9.45	
Illinois	Average	(22)	5.26	8.17	6.68	
	Jonathan		7.90			
	Golden Delicious		7.24	8.17		
	Delicious				7.02	
New York Michigan	Mc. Intosh	(13)	6.75	13.03	8.62	
U.S.A.	West Virginia	(13)	7.47	14.97	10.50	
	Michigan					Red Delicious
	Washington					Golden Delicious
Virginia	All-average	(8)	2.94	11.94	7.64	
	Delicious		7.28	9.55	8.10	
	Granny Smith		7.27	10.68	8.27	
	King David		7.79	9.28	8.28	
	Rome Beauty		5.99	6.88	6.43	

APPLES FROM ARGENTINA

TABLE 8. TOTAL SUGAR CONTENT OF VARIETIES GROWN IN SEVERAL COUNTRIES

Country	Varieties	References	Total sugars, %		
			Minimum	Maximum	Average
Canada	All-average	(34)	9.60	14.02	11.64
	Delicious		10.92	12.65	11.79
	Golden Delicious		11.60	13.31	12.39
	Jonathan		10.46	12.33	11.45
	Rome Beauty				11.54
England	table	(3)	5.86	15.36	9.78
	table	(33)			9.45
Poland		(27)	8.40	13.40	
Turkey		(38)	8.39	14.24	
Illinois	Average	(22)	6.69	11.79	9.52
	Jonathan				10.70
	Golden Delicious		9.82	11.79	
	Delicious				10.00
New York Michigan	Mc. Intosh	(13)	11.71	13.05	12.38
U.S.A. West Virginia Michigan Washington	Red Delicious	(13)	8.95	15.80	12.06
	Golden Delicious		10.77	16.44	12.60
	table		(33)	6.6	15.9
Virginia	All-average	(8)	3.22	14.96	10.45
	Delicious		10.10	11.69	10.65
	Granny Smith		10.02	14.18	11.78
	King David		10.70	13.70	12.24
	Rome Beauty		8.22	12.11	9.85
U.S.S.R.		(4) (5) (6) (10)	4.00	14.30	

TABLE 9. TANNINS CONTENT OF APPLES GROWN IN SEVERAL COUNTRIES

Country	Varieties	References	Tannins, %		
			Minimum	Maximum	Average
Canada	All-average	(34)	0.013	0.081	0.034
	Delicious		0.021	0.033	0.026
	Golden Delicious		0.024	0.036	0.028
	Jonathan		0.013	0.033	0.023
	Rome Beauty				0.041
England	table	(3)	0.038	0.290	0.100
U.S.A., Virginia	All-average	(8)	0.038	0.935	0.123
	Delicious		0.076	0.087	0.081
	Granny Smith		0.094	0.115	0.101
	King David		0.692	0.794	0.721
	Rome Beauty		0.072	0.099	0.086
U.S.S.R.	table	(4) (5)		1.400	

TABLE 10. APPLE VARIETIES GROWN IN THE RIO NEGRO VALLEY, ARGENTINA

Variety	1958/59		1959/60		1960/61		1961/62		1962/63		Average	
	thousand boxes	%	thousand boxes	%	thousand boxes	%	thousand boxes	%	thousand boxes	%	thousand boxes	%
Jonathan	290.4	2.15	217.9	1.74	292.0	2.29	199.2	1.44	241.9	1.48	249.0	1.79
Blackjon	26.6	0.10	11.2	0.09	17.9	0.14	10.3	0.07	13.3	0.08	15.8	0.11
King David	891.1	6.53	484.8	3.90	8,957.0	7.04	393.4	2.80	757.8	4.68	685.0	4.95
Red Delicious	6,492.8	47.4	6,601.4	52.70	5,924.0	46.4	7,651.4	54.5	8,647.7	53.00	7,060.0	51.00
Delicious	1,235.6	8.95	1,048.0	8.35	750.6	5.90	1,008.3	7.15	766.9	4.74	978.0	7.05
Golden Delicious	46.5	0.34	37.7	0.30	67.6	0.53	50.9	0.36	82.9	0.51	56.0	0.40
Black Winesap	708.5	5.2	541.8	4.30	792.9	5.20	465.3	3.30	834.1	5.12	669.0	4.87
Winesap	5.9	0.04	21.5	0.17	18.4	0.14	5.1	0.04	4.8	0.03	11.1	0.07
Stayman Winesap	21.0	0.15	8.0	0.06	17.5	0.14	14.4	0.10	16.0	0.10	15.3	0.11
Rome Beauty	1,936.0	14.2	1,529.7	12.20	1,428.4	11.20	1,768.4	12.55	1,406.5	8.63	1,615.0	11.65
Glengyle Red	9.4	0.07	9.6	0.08	7.0	0.05	0.6	0.01	2.1	0.01	5.7	0.04
Yellow Newton												
Pippin	248.6	1.82	402.7	3.21	303.7	2.38	532.2	3.78	451.3	2.77	388.0	2.80
Granny Smith	1,739.0	12.70	1,599.1	12.80	2,173.4	17.00	1,944.8	13.80	3,038.4	18.60	2,100.0	15.30
Red Golden	7.8	0.06	4.4	0.04	35.9	0.28	12.4	0.09	12.5	0.08	14.6	0.10
Various	28.4	0.21	3.3	0.03	5.5	0.04	6.5	0.05	19.5	0.16	12.8	0.09
Total	13,687.5	100.00	12,521.0	100.00	12,667.4	100.00	14,063.2	100.00	16,295.7	100.00	13,875.3	100.00

TABLE 11A. ANALYSES OF THE 1967 CROP OF JONATHAN AND KING DAVID APPLES

Sample	Jonathan			King David		
	I	II	III	I	II	III
Ascorbic acid (mg/100 g)	8.35	7.37	8.95	9.60	11.24	9.25
Acidity, total (%)	0.564	0.519	0.476	0.882	0.815	0.710
Dry matter (%)	12.61	13.39	12.10	13.64	13.97	12.60
Moisture (%)	87.39	86.61	87.90	86.36	86.03	87.40
Pectin (%)	0.632	0.753	0.666	0.726	0.959	0.631
pH	3.35	3.20	3.45	3.25	3.24	3.25
Soluble solids (%) (20 C)	9.69	10.68	10.08	10.30	10.68	10.27
Specific gravity temperature °C reading	23.0	21.0	23.0	23.5	22.0	22.0
Starch	(+)	(+)	(+)	(+)	(+)	(+)
Sugar reducing (%)	7.67	8.65	7.74	7.72	7.60	7.20
Sugar total (%)	8.75	10.72	10.60	11.15	9.48	9.41
Tannin (%)	0.0230	0.0248	0.0200	0.0093	0.0130	0.0108
Texture (lb.)	16.90	18.20	19.11	18.48	19.80	16.33

TABLE 11B. ANALYSES OF THE 1967 CROP OF RED DELICIOUS APPLES

Sample	Red Delicious			
	I	II	III	IV
Ascorbic acid (mg/100 g)	4.88	6.14	8.44	9.06
Acidity, total (%)	0.252	0.213	0.241	0.223
Dry matter (%)	12.23	12.30	12.20	12.75
Moisture (%)	87.77	87.70	87.80	87.25
Pectin (%)	0.699	0.959	0.631	0.515
pH	3.96	3.98	3.77	3.94
Soluble solids (%) 20 C	8.18	8.48	8.62	8.97
Specific gravity temperature, °C reading	24.0	24.0	24.0	23.0
Starch	(+)	(+)	(+)	(+)
Sugar reducing (%)	6.92	7.63	7.41	7.59
Sugar total (%)	7.52	8.00	8.53	8.83
Tannin (%)	0.0108	0.0345	0.0130	0.0212
Texture (lb.)	17.50	14.90	19.70	15.40

TABLE IIc. ANALYSES OF THE 1967 CROP OF ROME BEAUTY AND BLACK WINESAP APPLES

Sample	Rome Beauty			Black Winesap		
	I	II	III	I	II	III
Ascorbic acid (mg/100g)	18.39	10.20	11.36	24.67	13.43	17.75
Acidity, total (%)	0.356	0.304	0.361	0.412	0.397	0.382
Dry matter (%)	13.60	14.20	13.70	13.37	13.87	10.81
Moisture (%)	86.40	85.80	86.30	86.63	86.13	89.19
Pectin (%)	1.010	0.769	0.963	0.971	0.982	1.080
pH	3.52	3.36	3.45	3.45	3.40	3.37
Soluble solids (%) (20 C)	10.00	11.07	9.53	9.93	8.67	9.90
Specific gravity temperature °C	20.00	22.0	20.0	18.0	21.0	18.0
Specific gravity reading	1.046	1.048	1.046	1.041	1.040	1.046
Starch	(+)	(+)	(+)	(+)	(+)	(+)
Sugar reducing (%)	7.75	7.89	7.32	8.14	7.21	8.10
Sugar total (%)	10.40	10.93	11.06	9.59	8.53	9.75
Tannin (%)	0.0157	0.0081	0.0176	0.0146	0.0098	0.0146
Texture (lb.)	21.70	15.38	15.50	23.50	17.99	17.03

TABLE II d. ANALYSES OF THE 1967 CROP OF GRANNY SMITH AND GOLDEN DELICIOUS APPLES

Sample	Granny Smith			Golden Delicious		
	I	II	III	I	II	III
Ascorbic acid (mg/100g)	9.98	8.04	14.04	7.32	8.29	11.95
Acidity, total (%)	0.550	0.503	0.436	0.330	0.331	0.273
Dry matter (%)	12.44	12.30	12.05	13.70	14.20	12.17
Moisture (%)	87.56	87.70	87.95	86.30	85.80	87.83
Pectin (%)	0.622	0.933	0.763	0.658	0.620	0.577
pH	3.26	3.22	3.33	3.70	3.66	3.56
Soluble solids (%) (20 C)	9.74	9.87	9.76	11.16	10.67	10.80
Specific gravity temperature, °C	24.0	21.0	22.0	23.5	23.0	22.0
Specific gravity reading	1.042	1.044	1.044	1.049	1.048	1.044
Starch	(+)	(+)	(-)	(+)	(-)	(-)
Sugar reducing (%)	7.55	7.30	7.63	9.28	9.60	8.45
Sugar total (%)	9.68	9.19	10.11	11.10	11.10	10.53
Tannin (%)	0.0168	0.0129	0.0098	0.0303	0.0216	0.0164
Texture (lb.)	14.50	14.30	13.55	14.70	13.88	12.80

TABLE II e. ANALYSES OF THE 1967 CROP OF DELICIOUS AND YELLOW NEWTON PIPPIN APPLES

Sample	Delicious				Yellow Newton Pippin		
	I	II	III	IV	I	II	III
Ascorbic acid (mg/100 g)	9.79	10.15	11.03	6.44	4.60	9.88	5.40
Acidity, total (%)	0.233	0.238	0.215	0.184	0.388	0.346	0.371
Dry matter (%)	15.60	16.50	15.84	16.10	13.00	12.10	12.97
Moisture (%)	84.40	83.50	84.16	83.90	87.00	87.90	87.03
Pectin (%)	0.964	1.110	0.892	0.737	1.224	1.056	0.931
pH	4.00	3.86	3.84	3.95	3.63	3.58	3.52
Soluble solids (%) (20 C)	10.36	10.56	12.01	12.01	9.40	9.95	9.94
Specific gravity temperature, °C	21.0	24.5	22.0	22.0	20.0	23.0	22.0
Specific gravity reading	1.048	1.046	1.052	1.050	1.042	1.040	1.041
Starch	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Sugar reducing (%)	8.83	9.05	8.75	8.52	7.26	7.23	6.87
Sugar total (%)	10.05	10.77	11.36	11.17	8.88	10.01	9.25
Tannin (%)	0.0144	0.0123	0.0081	0.0113	0.0061	0.0153	0.0066
Texture (lb.)	17.00	15.38	13.95	13.20	17.13	21.00	17.00

TABLE 12. RIO NEGRO VALLEY VARIETIES: MINIMUM, MAXIMUM AND AVERAGE VALUES

Property	Minimum	Maximum	Average
Ascorbic acid (mg/100 g)	4.60	24.67	10.20
Acidity, total (as malic acid) (%)	0.185	0.854	0.400
Dry matter (%)	10.81	16.50	13.32
Moisture (%)	83.50	89.19	83.60
Pectin (%)	0.515	1.224	0.828
pH	3.20	4.00	3.44
Soluble solids (%) (20 C)	8.18	12.01	10.04
Specific gravity	1.033	1.052	1.044
Sugars			
reducing (%)	7.20	9.60	7.88
total (%)	7.52	11.36	9.87
Tannin	0.0061	0.0345	0.0152
Texture (lb.)	12.80	23.50	16.75

set by the Canada Department of Agriculture (31).

Starch was tested qualitatively, following the A.O.A.C. Official Methods. Flesh texture was measured on 3 equidistant sides of each of 20 apples, using a Magness-Taylor pressure tester.

RESULTS AND DISCUSSION

Table 2 reports for each variety the average results of analyses made on the 1967 crop. Minimum, maximum, and average contents, taking all varieties together are reported in Table 12.

In the following discussion, reference is made to the values reported in Tables 2 to 9, as compared to those in Table 11. For the sake of brevity, apples are identified by the State or Country where they were grown, those grown in the Rio Negro Valley being identified as "Argentinian".

The ascorbic acid content range of Argentinian apples is similar to most of the reported data for apples grown elsewhere, particularly for table apples grown in Poland (18, 27), Germany (25, 30), Canada (34), United States (11, 13, 39, 43), Russia (4, 5, 6, 10, 17) and England (3, 14). Some of the cider apples grown in England and table apples grown in France, show outstandingly high values, as reported by Ayres (3) and Tavenier et al. (37), respectively. India (29), Japan (35), and Romania-grown varieties (40), are on the lower side of the range associated with Argentinian grown varieties.

Rome Beauty and Black Winesap have the highest ascorbic acid contents. The values for Rome Beauty are within the range of the Canadian-grown counterpart (34) and they are higher than the Washington (39) and West Virginia-grown (11) Rome Beauty. Argentinian Delicious is above the average Washington-grown Delicious (39) and within the range of the Canadian (34). Red Delicious and Jonathan are within the range of their Canadian similars (34).

The latter has a higher ascorbic acid content than that reported by Todhunter for Washington grown Jonathan (39).

Total acidity of the average Argentinian apple is quite similar in range and average value to Canadian apples (34). It is within the range of British apples, as reported by Ayres (3) although none of the analyzed samples reached the higher values of said range. Its average is also close to Polish varieties (27). Data for United States show a wider scattering. Turkish varieties range in the lower values of Argentinian apples (38), whereas Indian apples are higher (29). Jonathan is within the range of the data reported by Strachan (34). King David is well above the data given in the same report, even at the late picking date. Delicious and Rome Beauty are within the range of their American counterparts (8, 13), but the Canadian grown Rome Beauty has a higher average acidity (34). The acid content of Granny Smith is similar to that of the Virginia-grown (8) only at the early picking date. At the subsequent picking dates it falls down below the reported range. Golden Delicious and Delicious are on the low side when compared with Canada and United States-grown varieties (34, 8, 12, 13, 22). The limited data available for Russian apples, put them on the higher side (9). Where individual varieties could be compared, Argentinian apples fall below those grown in other countries. This is true of Canadian-(34) and Illinois-grown Jonathan (22), Virginia-grown King David (8), West Virginia-, Michigan-, and Washington-grown Red Delicious (12, 13), Canada-grown Rome Beauty (34), and Virginia-grown Granny Smith (8). The Argentinian Golden Delicious is closer to the lower range of some of the American Golden Delicious (13, 22) but it is below the values reported for Canada (34), Michigan, and Washington (13). Delicious is the only exception where data for the Argentina-grown apple corresponds to the higher values for the Canadian Delicious (34) and it is higher than the Virginia- (8) and Illinois-grown (22).

Pectin content of the average Argentinian variety is, in general, above that of Canadian (34) and Indian (39) apples. American varieties have a wide range of coincidence with the Argentinian varieties, but there is much data showing higher values (13). Comparing those varieties for which there is available data, it is seen that Argentina-grown Jonathan, Rome Beauty, and Delicious, are above the same varieties grown in Canada (34). Golden Delicious grown in Argentina are within the range of those grown in Canada (34), but quite below those grown in West Virginia, Michigan and Washington (13). Also, Red Delicious grown in United States have a

higher pectin content than those grown in Argentina (13).

Soluble solids are, in general, lower for the Argentinian apples than for the Canadian (34) and American (12, 13) varieties for which data was available. The reducing sugar content for the average Argentinian apple is within the range reported for apples grown in Canada (34), England (3), and United States (22). The range of values is increasingly wider for those countries, in the order they are stated. Reducing sugars for all Argentinian varieties average 79.9% of the total sugars, slightly above the 74.0% reported for Canadian apples (34). Argentine-, Canada- (34), and Illinois-grown (22) Jonathan are similar in reducing sugar content. Red Delicious is within the lower side of the same variety grown in West Virginia, Michigan, and Washington (13). The same can be stated for Granny Smith as compared with its Virginia-grown counterpart (8). King David is well below and Rome Beauty above the data available for Virginia (8). Golden Delicious rates higher than Canadian (34) and Illinois (22) Golden Delicious apples but within the range of those grown in West Virginia, Michigan and Washington (13). Delicious rates higher in reducing sugars than the same variety grown in Illinois (22), but it is within the range of the Canada- (34) and Virginia-grown Delicious (8).

Red Delicious is the variety with lower total sugar content: 7.52%. Delicious picked 4 weeks after the official picking date had the highest content: 11.36%. Taking all varieties together, they are within the range of English (3, 33) and American (8, 13, 22, 33) apples. They agree with the lower values of Canadian (34), Turkish (38), and Polish (27) apples. Jonathan shows values below those reported for Canada (34). Its higher values are equal to the average reported for Illinois (22). The higher values of King David are equal to the lower data reported for Virginia-grown King David (8). Red Delicious is well below the data reported for the same variety grown in West Virginia, Michigan and Washington. The range of total sugar content of Rome Beauty equals the higher content of the Virginia-grown (8) and is below the Canada-grown Rome Beauty (34). Granny Smith is below its Virginian counterpart (8). Golden Delicious is within the range of Illinois data (22), it agrees with the lower data for West Virginia, Michigan, and Washington (13), and it is below Canadian Golden Delicious. Virginia (8) and Argentina-grown Delicious have a very similar total sugar content. They are above the average reported for Illinois-grown Delicious (22). Their higher values are equal to the lower Canadian data (34). The tannin content of Argentinian varieties as a whole, is on the lower side of the range re-

ported for Canada (34), England (3), United States (8), and Russia (4, 5). For the 3 countries, the range of data includes considerably higher values. Actually, some Russian varieties grown in Tomsk range between 0-0.725%, whereas Siberia-grown varieties reportedly rate as high at 1.400% (5). Comparing individual varieties, the difference is not so great. Jonathan and Golden Delicious are similar in range to the same varieties grown in Canada (34). Delicious is close but below Virginia-grown Delicious (8). The same can be said of Rome Beauty grown in Canada (34) and Virginia (8) and Granny Smith grown in Virginia (8). On the other hand, Virginia-grown King David is higher than King David grown in Argentina.

The 1967 harvest was not a very good one. In general, fruit failed to develop good color and size, even at very late picking dates. The maturity pattern is not well reproduced by the successively picked samples. As pointed out by Martin (23), differences in the characteristics of fruit of different branches become significant even when differences caused by variation in fruit size between branches are eliminated. As stated, the objective of the work was not to follow the maturity pattern, but to pick samples at different dates in the same ways associated with the product that reaches the market. It is well known that environment, climate, season, culture, and management, are very influential on the content of each apple constituent (12, 13, 33). Therefore the data reported should not be considered as an average representation of all Argentinian apples.

The data gathered indicates that the analyzed varieties are similar to those grown in other countries. The more significant differences are some peak values of ascorbic acid content and low total sugar content in many instances. As far as the discussed properties, the data should be regarded as an indication that it is acceptable to extrapolate to local varieties those technological findings developed for varieties grown in other countries, when they are related to said properties.

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EFFECTS OF MEDIUM AND INCUBATION TEMPERATURE ON RECOVERY OF MICROORGANISMS FROM MANUFACTURING-GRADE, GRADE-A AND PASTEURIZED MILK¹

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ABSTRACT

Recovery of microorganisms by Standard Methods Agar and Eugonagar was compared at 7, 21, 28, and 32 C incubation temperatures for 10, 5, 4, and 2 days, respectively, from manufacturing-grade, grade-A, and pasteurized milk. The incubation temperature made a statistically significant difference in the mean logarithm of the count for all three grades of milk. The highest mean logarithm of the count with manufacturing-grade milk was obtained at 21 C for both agars. With grade-A raw milk, the greatest mean logarithm of the count was obtained at 21 C for Standard Methods Agar and at 28 C for Eugonagar. The highest mean logarithm of the count for pasteurized milk was obtained at 28 C with Standard Methods Agar and at 21 C with Eugonagar. There was a significant difference between means on Standard Methods Agar and Eugonagar on grade-A milk samples only; recovery was highest with Eugonagar.

Because of the presence of psychrophilic microorganisms, an incubation temperature lower than 32 C is needed for maximum recovery. Incubation at 28 C for 4 days was the optimum temperature-time combination in this study.

Since there is no recognized group of microorganisms that can be used as an index of production conditions on grade-A dairy farms, we must rely on recovering the maximum number of microorganisms from the milk as a partial measure of farm sanitation. The purpose of this work was to compare the use of different media and incubation temperatures to be sure the maximum number of microorganisms is recovered. The scope of the experiment was broadened to also include recovery from two other grades of milk. Two agars were incubated at each of four different temperatures with samples of manufacturing-grade, grade-A, and pasteurized milk.

METHODS

Fifty-six manufacturing-grade, and 103 grade-A samples were aseptically collected from properly agitated bulk-tank milks and were placed in 6-oz. Whirl-Pak plastic bags for refrigerated transportation to the laboratory. The samples were collected in Iowa and Southern Minnesota. Most were collected by accompanying bulk-tank drivers on their routes; the remainder by driving to the farms. Samples were an-

alyzed the evening of the collection day or on the following day. The grade-A producers' milk was collected every other day. Milk from 37 of the 50 manufacturing-grade farms was collected every third day or less frequently. Fifty pasteurized-milk samples were obtained from local supermarkets, the Iowa State University Dairy, and from products sent to the Iowa State University Food Products Analysis Laboratory for routine examination. Because pasteurized milk of different ages was plated, some counts were high. Several pasteurized samples were eliminated from the study because the Standard Plate Count (SPC) and the count on Eugonagar (EA) (4) at 32 C were both >100,000/ml.

Analysis of samples

Procedures listed in the 11th edition of *Standard Methods for the Examination of Dairy Products* (2) were followed in analyzing the samples unless another procedure is specified. Duplicate plates with Standard Methods Agar (SMA) (2) and EA (4) were incubated at each of the following temperature-time combinations: 32 C for 2 days, 28 C for 4 days, 21 C for 5 days, and 7 C for 10 days. The incubation temperatures were maintained within 1 C of the specified temperatures. Plates were counted within 3 hr of the specified time. Cover layers of the same plating medium were poured on plates incubated at 21, 28, and 32 C to minimize the problem with spreaders.

Counts of <1 were recorded as 0, and the data were transformed by taking the logarithm₁₀ (count + 1). A least-squares analysis was performed.

RESULTS AND DISCUSSION

Mean logarithms of counts for each incubation temperature-time combination for the three grades of milk are presented in Table 1. The F tests of sample source, incubation temperature, and medium are presented in Table 2. The source of sample and the incubation temperature made a significant difference in the count of all three grades of milk. Both these sources of variation were expected to be statistically significant.

With grade-A milk samples, there was a significant difference in geometric means on the two agars (Table 2). Higher geometric means were obtained with EA, with the greatest increase occurring at 7 C (Table 1). On the other hand, there was no significant difference between the means obtained with the media on the other two grades of milk. Pelczar and Vera (13), and Pessin and Black (14) obtained

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TABLE 1. MEANS OF TRANSFORMED^a COUNTS OF MILK SAMPLES INCUBATED^b AT FOUR TEMPERATURES WITH TWO AGARS

Agar	Incubation Temperature			
	7 C	21 C	28 C	32 C
[Means of Log ₁₀ (Count + 1)]				
56 Manufacturing-grade milks				
SMA ^c	5.849	6.233	6.196	6.072
EA ^d	5.823	6.213	6.178	6.056
103 Grade-A milks				
SMA	3.359	4.174	4.155	4.074
EA	3.546	4.189	4.210	4.133
50 Pasteurized milks				
SMA	1.885	3.180	3.203	3.075
EA	1.914	3.106	3.091	2.967

^aLogarithm₁₀ (count + 1).

^bWith incubation temperatures of 7, 21, 28, and 32 C, the respective incubation times were 10 days, 5 days, 4 days, and 2 days.

^cStandard Methods Agar.

^dEugonagar.

essentially the same results when comparing a medium with approximately the same composition as EA with tryptone-glucose-beef extract-milk agar (TGEM). The highest recovery of microorganisms from grade-A milk was obtained with EA at 28 C incubation. With SMA, the highest geometric mean was obtained at 21 C. With both agars, geometric means at 21 and 28 C were higher than those at 32 C. Further, there was a significant interaction between medium and incubation temperature because of the greater recovery on EA at 7 C. With improved cooling facilities and longer storage periods, psychrophiles are becoming more significant in raw milk. Because some psychrophiles do not grow at 32 C, lower incubation temperatures result in better recovery.

Research workers have recognized that incubation temperatures too high for many of the bacteria in milk have often been used. Bacteriological determinations on milk were first made at 37 C because that was the temperature laboratories were using for water analysis. Also, earlier investigators wanted to incubate at a temperature favorable for growth of pathogens. Pederson and Yale (11), 1934, observed that 37 C was not the optimum temperature for bacterial counts of milk and that, at temperatures slightly above 37 C, the count was much lower. They said the optimum, which varied somewhat depending on the sample, was close to 32 C. In later studies, using an improved agar (21), they also found

32 C better than 37 C. Other workers reported higher counts at 32 than at 37 C (1, 5, 6, 8, 15, 16, 20). Pederson and Breed (12), in 1940, recommended 32 instead of 37 C for determining milk counts. Thomas and Jenkins (17) obtained significantly higher counts at 30 C for 3 days, than at 37 C. Babel et al. (3), 1955, reported that the logarithmic mean count at 32 C was generally higher than at 35 C. Mean counts at 26 and 32 C were essentially the same after 2 days, but after longer incubation, plates at 26 C gave the higher counts. They suggested that an intermediate temperature between 26 and 32 C possibly would give a higher count. In this experiment, 21 and 28 C gave higher geometric means than 32 C incubation. Although *Standard Methods for the Examination of Dairy Products* (2) recommends incubation at 32 C, this is not the optimum for many bacteria in milk.

With manufacturing-grade milk, there was no statistically significant difference in recovery with the two agars (Table 2); however, the higher geometric mean at all temperatures was with SMA (Table 1). No significant interaction indicates that relative recovery on the two agars was independent of the incubation temperature. The counts at 7 C were high. From samples collected in the same geographical area, LaGrange and Nelson (9), 1958, reported that the psychrophilic count (PBC) on manufacturing-

TABLE 2. ANALYSIS OF VARIANCE OF BACTERIAL COUNTS^a OF MILK SAMPLES

	Degrees of freedom	Mean squares	F values
56 Manufacturing-grade raw bulk-tank milk samples			
Farms	55	8.3491	95.31**
Temperatures	3	3.4174	39.01**
Agars	1	0.0427	0.49
Temperature X agar	3	0.0005	0.006
Error	385	0.0876	
103 Grade-A raw bulk-tank milk samples			
Farms	102	1.8323	41.95**
Temperatures	3	25.7618	589.78**
Agars	1	1.2897	29.53**
Temperature X agar	3½	0.2858	6.54**
Error	714	0.0437	
50 Pasteurized milk samples			
Samples	49	7.6217	29.14**
Temperatures	3	36.5944	139.91**
Agars	1	0.4332	1.66
Temperature X agar	3	0.1075	0.41
Error	343	0.2616	

^aCounts were transformed by taking logarithm₁₀ (count + 1).

**P < 0.01.

grade milk was nearly as high as the SPC and sometimes exceeded it greatly.

The smallest difference in count between agars and incubation temperatures was obtained with manufacturing-grade milk. This was probably because of the complex flora present, which included many psychrophiles. The highest geometric mean occurred at 21 C with both agars. The means at 28 C incubation were both higher than those obtained at 32 C. Although better recovery was obtained at temperatures lower than 32 C, this is of little or no significance when we consider the high bacterial population present.

With pasteurized milk, the incubation temperature had a significant effect on the count, but there was no statistically significant effect of agars. There was, however, more experimental variation with pasteurized milk (Table 2). With pasteurized milk, Pessin and Black (14) reported that counts were significantly lower on a medium with approximately the same composition as EA than on TGEM. With a 7 C incubation temperature, the higher geometric mean was obtained with EA. At the other three incubation temperatures, the highest mean count was obtained with SMA. The different recovery at 7 C was not large enough to cause significant interaction. The highest recovery was obtained with SMA at 28 C. With EA, the highest geometric mean was obtained at 21 C. The means for both agars were higher at 21 and 28 C than at 32 C.

Many workers have obtained greater increases in the count of pasteurized milk than in raw milk by lowering the incubation temperature from 37 to 32 C, which shows that the flora of pasteurized milk is more sensitive to the higher incubation temperature (5, 7, 8, 11, 15, 16). Nelson and Baker (10) observed that incubation at 25 C for 3 days detected large numbers of bacteria in many market-milk products that did not develop colonies at 35 C. Babel et al. (3) reported higher counts from pasteurized milk samples by incubating at 26 C for 3 days than at 32 C for 2 days. Comparing 21, 28, 32, and 35 C incubation of plates of laboratory pasteurized milk, Thomas, Reinbold, and Nelson (18) found that 28 C incubation for 4 days was the temperature-time combination that produced the highest count. Studies with pure cultures indicated that thermophilic bacteria generally were more exacting in growth temperature requirements after they had been subjected to laboratory pasteurization than before. Also, they were more exacting in nutrient requirements (19). Commercial market-milk samples, like those used in this experiment, will have a flora much different from laboratory pasteurized samples, however. The counts were quite high in the pasteurized milk used in this experiment.

Although *Standard Methods for the Examination of Dairy Products* (2) specifies a 32 C incubation temperature, results of this study show that higher counts were obtained at the lower temperatures. Because psychrophiles constitute an important part of the milk flora and since some psychrophiles may not grow at 32 C, a lower incubation temperature should be used for grade-A milk evaluation. Because incubation at 28 C requires 1 less day than incubation at 21 C, this would be the preferred incubation among those studied.

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OCEANS VAST RESERVOIRS OF FOOD

Oceans represent a vast potential of almost virgin territory as a reservoirs of food for people, according to Dr. Ernest R. Pariser of the Massachusetts Institute of Technology, Cambridge. Dr. Pariser told an audience of food processor executives, planners and researchers that in order to utilize this reservoir—whether plant, squid or fish—powerful prejudices and taboos against such products “must be overcome by processing, marketing and educational skills.” This task, he emphasized, is the most difficult to accomplish. The scientist was addressing the Third Biennial Food Forum, a feature of the Food & Dairy Industries Expo, Oct. 13-17, at Chicago’s International Amphitheatre. Both are sponsored by the Dairy and Food Industries Supply Association, a national trade group headquartered in Washington, D. C.

Dr. Pariser noted that while the total quantity of ocean plant biomass is “vastly superior to that of land plants,” most marine plants are microscopic and difficult to spot and harvest. On the other hand, the larger marine plants being harvested and consumed are “entirely leafy vegetables having no roots, tubers, fruits, nuts or other food concentrating and storage members.” Thus, these are only of “limited food value.”

Although currently not used as human food, invertebrates account for more than 80% of the weight of marine animals, and, they represent an important protein reservoir that must be slowly tapped as other, more conventional supplies become insufficient to meet the world demand. Squids are being harvested in large quantities in some areas, but are used mainly for bait. These and their relatives could be used more

extensively as human food, since they contain a high protein concentration, are perfectly safe and edible, widely distributed over the world’s oceans, and easily harvested.

Of the vertebrates, fish represent the best known and widely used. However, only a handful of species of a total of 20 or 25,000 known species are consumed by man, and the annual world harvest is only 54 million metric tons compared to a potential annual harvest estimate of as many as 2 billion metric tons.

Considering the urgent need for food in general and for protein in particular, Dr. Pariser explained why more marine foods are not reaching hungry peoples of the world. “It’s a complex question,” he said, requiring “changes at different levels and directions—technological, economic, socio-psychological.” First, the art of fishing—locating and surrounding a catch—is still almost prehistoric. New and more sophisticated methods must be developed. Second, marine organisms spoil more easily than most other foods, necessitating processing and preservation. Although freezing, freeze-drying, radiation preservation and canning are excellent procedures, he noted, they are expensive and for a long time will remain out of the reach of the poor. Less expensive methods are being developed, and new foods incorporating such preserved products will have to be formulated. Last, marine foods—especially fish—have been consumed and marketed in their recognizable forms for many years. Slowly and against much resistance, it’s being established that marine proteins from one source or another can and should be used in a new form in which the original raw material loses its identity.

DETECTION OF LEAKS IN THE REGENERATION SECTION OF EGG PASTEURIZERS

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ABSTRACT

A method is proposed for the detection of leaks in the heat transfer surface of the regeneration section of an egg pasteurizer. When the unit is not in use, the raw side of this section is pressurized with a fluorocarbon refrigerant gas and air on the pasteurized side is then checked with a halogen leak detector for the presence of fluorocarbon. Laboratory tests indicate that presence of a hole too small for any flow of egg white under normal conditions can be detected. Some information is given on the flow of egg whites, air, and fluorocarbon gas through small holes. The problem of assuring that holes will not be plugged when tests are made was extensively investigated. Conditions required to use the method, based on laboratory tests, are indicated. The test method appears to offer a positive and practical means to determine presence of leaks in the regeneration section that might permit flow of raw to pasteurized product.

In the usual egg pasteurization unit the heat exchanger contains 3 parts: a heating section, a cooling section, and a regeneration section. In the latter, part of the heat in the pasteurized egg product coming from the holding tube is transferred to the incoming cold raw material. A positive displacement pump moves the product consecutively through one side of the regeneration section, the heating section, the holding tube, the other side of the regeneration section, and then the cooling section. On the first side of heat transfer plates in the regeneration section there is non-pasteurized material that may contain pathogenic bacteria; on the other side is a pasteurized product. With flow following the pattern described, pressure on the non-pasteurized side is higher than on the pasteurized side. If a leak occurs between these two sides, there will be flow from the non-pasteurized into the pasteurized product and the latter will be contaminated. The frequency of such leaks is apparently extremely low, but the possibility of such leaks and the resulting contaminated products has been of serious concern to people in the industry and to health and regulatory agencies.

The milk pasteurization industry has met the same problem since the same type of heat exchange equipment is used for milk. Their solution has been to use different pumping arrangements and to regulate the pressures on the two sides of the regeneration section so that the pasteurized product is always at a higher pressure than the raw material. If there is a leak in the heat transfer surface of the regeneration sec-

tion of a milk pasteurizer, flow will be from pasteurized into raw product and the pasteurized product will not become contaminated. Two pumping arrangements are commonly used to accomplish this pressure relationship. If a single pump is used, it is located after the raw product side of the regeneration section so that the raw product is drawn through that side and is below atmospheric pressure. The rest of the system is under positive pressure. If a 2-pump system is used, the first pump precedes the raw product side of the regeneration section and a second pump follows. The first is a centrifugal, developing a limited pressure; the second is a positive displacement unit capable of greater pressure. Pressure regulating equipment is added to assure desired relative pressures. Certain other refinements of the system are necessary for control when starting operations and in the event of power failure.

The method used by the dairy industry has not been used by the egg industry. Egg products are more difficult to pump because of the wide range of viscosities and the presence of dissolved and entrained gases. High viscosities of some cold products result in large pressure drops across the raw side of the regeneration section. For these reasons suction is an impractical way to move egg products. A single pump placed after the raw side of the regeneration section, as used by the dairy industry, has not been feasible. The 2-pump system with pressure regulating equipment may be feasible, but to our knowledge, has not been tested on the various egg products. There may be a serious problem with pressure regulation because of pressure fluctuations and the range of pressures to be covered. Sudden pressure changes have been observed in egg pumping systems, possibly caused by gases that have separated from the liquid product and that go through the pumps as bubbles or foam. We now have a unit in a commercial egg pasteurization plant that is being altered so that problems of using a 2-pump system can be studied.

Until more is known about the feasibility of using pressure control in meeting the problem of possible leaks in the regeneration section of egg pasteurizers, other methods are needed. As described in the *Egg Pasteurization Manual* (3) current practice in plants operating under continuous United States Depart-

TABLE 1. DATA ON HOLES TESTED

Hole Number	Maximum air flow rate — (ml/min)	Equivalent diameter — (Inches)	Pressure at start of flow — (psi)	Flow of egg white before plugging ^a	
				Average (ml)	Range (ml)
A	630	.0065	.5	18	.2 to 39
B	540	.0060	.5	7.3	.5 to 29
C	500	.0058	.4	32	0 to 89
D	408	.0053	.5	21.	.06 to 44
E	346	.0048	.9	1.	.1 to 2.0
F	260	.0042	1.0	.5	.03 to 1.3
G	252	.0041	.5	27.	.5 to 73
H	164	.0033	1.1	.75	0 to 2.3
I	146	.0031	.9	.5	.2 to 1.2
J	104	.0027	.9	1.	.4 to 1.8
K	78	.0023	1.2	.1	0 to .4
L	67	.0021	1.3	.06	0 to .2
M	52	.0019	2.2	.02	0 to .03
N	44	.0017	1.2	.01	0 to .03
O	22	.0012	5.8	.01	0 to .01
P	21	.0012	2.6	.01	.01
Q	.50	.00018	19.	0	0
R	.25	.00013	47.	0	0

^aFor 12 trials on holes A through N, for 4 trials on holes O through R.

ment of Agriculture inspection is to depend on visual observations to prevent occurrence of leaks. The inspector checks all regenerative heat exchanger plates before each run not only for cleanliness but also for evidence of etching, corrosion, or cracking that might lead to leakage. Any suspected plates should be removed. The adequacy of this method of finding leaks has been questioned. The method proposed in this paper is a supplement to visual inspection and offers a sensitive and positive method of finding actual leaks of any type.

The heat exchanger is assembled and connections made so that the raw product side of the regeneration section can be pressurized with a fluorocarbon gas. R-12 (CCl_2F_2) and R-22 (CHClF_2), which are the most commonly used refrigerants, work very well. A slow moving air stream at atmospheric pressure is swept through the pasteurized side of the regeneration section and checked for the presence of the fluorocarbon to see if a leak exists. A sensitive halogen leak detector of a type commonly found in the refrigeration industry is used. Two problems appear with this test when applied to a commercial unit: (a) sensitivity of the test and (b) keeping holes in the transfer system from being temporarily plugged with egg or other material when the test is made.

LABORATORY EQUIPMENT AND PROCEDURES

To conduct the necessary laboratory tests of the proposed detection method various size holes that corresponded to what might be found in commercial pasteurizers were needed. Attempts to make these holes in 20-gage, 304 stainless steel by

corrosion were abandoned because of the slow corrosion rates. Holes could be made by this method in aluminum but the resulting sizes were unpredictable. In addition aluminum would be further corroded by the cleaning compounds used in some of the subsequent testing.

The following method of making holes was finally adopted. Discs of 1-1/8 inch diameter were punched from 20-gage, 304 stainless steel. A hole 3/32 inch diameter was drilled in the center of each disk until an appreciable dimple showed on the opposite side but not so far as to break through. A disk with the dimple facing out was then connected on the end of a 1 inch sanitary elbow leading from a flexible air line. Air pressure was applied and the disk placed under water in a pan. The dimple was gently ground down with a fine motor-driven emery wheel, such as is found in hobby kits. Air bubbles showed when the hole broke through. With practice the size of the hole could be roughly judged by the rate of air flow which could then be measured by displacing water in an inverted graduated cylinder.

After some exploratory tests the set of 18 disks listed in Table 1 was assembled. Larger holes were eliminated as they did not offer any problems not encountered with the small sizes. The last 4 disks were added part way through the study to give experience with very small holes.

The primary purpose of testing so many holes was to experiment with various treatments to unplug them. Non-pasteurized egg white that had passed through the filter screen following the egg breakers in a commercial plant was run through the holes at 20 psi and room temperature until the holes were completely plugged. Table 2 lists 21 consecutive treatments which the first 14 holes of Table 1 were given in studying the problem of plugging. After each of the indicated treatments the rates at which air (at 20 psi and room temperature) flowed through the holes were determined. The holes were kept wet between the time of the treatment and the determination of air flow rates unless drying was indicated. When the holes were dry, air flow was started before they were submerged to determine the air flow rates.

TABLE 2. TREATMENT OF HOLES PRIOR TO DETERMINATION OF AIR FLOW RATES

	Per cent open	
	Average	Range
1. Holes ground through under water.	83	51 to 100
2. Washed with direct jets in test tube washer at 180 F for 3 minutes with TSP, (trisodium phosphate).	78	38 to 97
3. Holes plugged with egg white at 20 psi at room temperature, washed as above	48	0 to 85
4. Same as 3.	61	1 to 95
5. Same as 3.	76	25 to 100
6. Same as 3.	83	44 to 95
7. Holes plugged with egg white, washed under water tap without direct impact on holes.	10	0 to 45
8. Dried 18 hours at room temperature.	10	1 to 69
9. Washed at 160 F for 10 minutes with 0.5 ounce/gallon TSP flowing 1.75 feet/second.	42	0 to 88
10. Same as 9.	54	4 to 95
11. Same as 2.	86	28 to 100
12. Holes plugged with egg white, washed at 160 F for 30 minutes with 1 ounce/gallon TSP flowing 1.75 feet/second.	71	20 to 97
13. Same as 12.	61	16 to 100
14. Same as 12.	56	13 to 95
15. Washed at 160 F for 30 minutes with phosphoric acid wash 1 quart/25 gallons flowing 1.75 feet/second.	50	12 to 81
16. Washed at 160 F for 60 minutes with 2% caustic flowing 2 feet/second	94	73 to 100
17. Holes plugged with egg white, washed same as 16.	97	83 to 100
18. Same as 17.	95	90 to 100
19. Dried for 48 hours at room temperature.	93	81 to 100
20. Same as 17.	94	83 to 100
21. Same as 17.	95	79 to 100

In order to check the sensitivity of the proposed leak detection method, a plate pasteurizer was set up to make the necessary tests. The unit contained 21 plates with the flow arranged to correspond to a regeneration section in an egg pasteurizer. To provide leaks in the unit 2 plates near the center were modified as shown in Fig. 1. By use of the various disks listed in Table 1 the desired size of leak could be obtained for the heat exchanger. A tank of R-22 was connected with valves and pressure gages to one side of the plates. This side was provided with a vent valve at the opposite end from the R-22 inlet so that at the start of a test the air in that side could be displaced with the fluorocarbon gas. The other side of the heat exchanger was closed with rubber stoppers until a test for the presence of fluorocarbon was made. At that time the stoppers were removed and a plastic bag containing about 5 ft³ of air was temporarily attached to one end. By gently squeezing the bag, air could be swept through the air side of the heat exchanger at the desired rate. The air stream flowing from the unit was checked with a halogen detector for the presence of fluorocarbon to see if a leak occurred.

The detector (2) used in this work contained a probe

through which air was drawn by a small vacuum pump in the unit. This air passed between two electrodes. If a halogen compound such as a fluorocarbon refrigerant was present in the air, the current between the electrodes was increased. This current was amplified causing a small neon light in the probe to flash. For testing refrigeration systems the unit was rated as capable of picking up a leak of 0.1 ounce/year. Use of the unit was improved by putting a pointer and chart on the balance control adjustment.

In making a test, a disk with the desired size leak was placed in the heat exchanger. Stopcock grease was used on the "O" ring to keep it and the disk in place while the heat exchanger was being closed. The valve on the fluorocarbon tank was opened until the pressure in the unit was 15 psi. This valve was then closed and the vent valve opened briefly so that air was displaced from the fluorocarbon side of the heat exchanger. The pressure was then raised, usually to 5 psi and maintained at this level for the test. After elapse of the desired length of time, air was swept through the air side of the heat exchanger and the exit stream checked for fluorocarbon.

Fluorocarbon pressure of 5 psi was used because first, it was roughly the amount needed to induce gas flow through any wet hole that permits significant leakage of egg white (Table 1), and second, low fluorocarbon pressure in the heat exchanger was desirable to reduce leakage by the gaskets to the air surrounding the unit. The unit was tightened manually as much as possible but specific gasket areas gave leak signals even at 5 psi. When the air around the unit became contaminated with fluorocarbon, use of the leak detector became more difficult.

A 12-inch fan was operated continuously to keep fresh air in the work area. Care was needed to keep out fluorocarbon from the air used to fill the plastic bag. With these precautions leakage to the outside air gave no problems in testing for internal leaks. Tightness of the "O" ring seal on the perforated plate was checked by tests of 1 hr duration using a disk without a hole.

As later discussion indicates, it was found best to conduct the tests with the disks dry so that no water was in the holes. For laboratory tests, drying could readily be done by allowing the disks to remain exposed to normal room conditions overnight. For commercial pasteurizers, a similar procedure may at times be inadequate because of low temperatures and high humidities that may occur. Various other methods of drying may be feasible; namely, forced circulation of room air over open plates by an oscillating fan, circulation of heated air over open plates with an electric air heat-

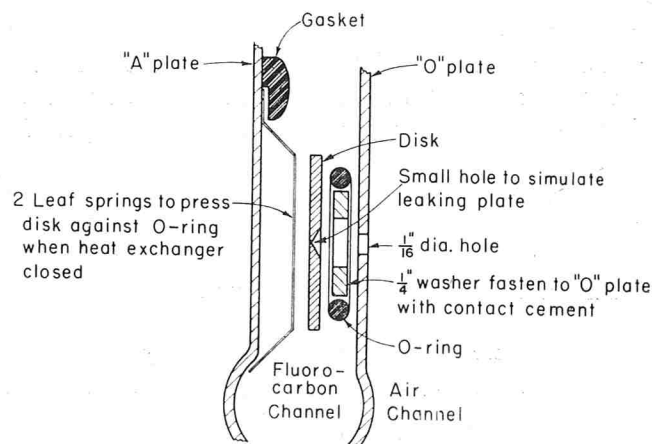


Figure 1. Modification of plates in test heat exchanger.

er which contains a fan, and blowing heated air through the closed heat exchanger. The latter seems preferable, based on some exploratory testing in the laboratory. It provides more uniform drying conditions for all heat transfer surfaces and overcomes the problem of slow drying, if low temperatures and high humidity occur in the pasteurizing rooms.

An expedient method in the laboratory for forcing heated air through the heat exchanger was to use a household canister type vacuum cleaner as a blower. This particular unit was rated at 1.25 horsepower, and after leaks were sealed it delivered 15 ft³/min of air at 1 psi with a temperature rise of about 30 F or 75 ft³/min at 0.1 psi with a temperature rise of 25 F.

RESULTS AND DISCUSSION

As an indication of the size of each hole, an "equivalent diameter" was calculated. The following orifice formula for critical flow of air (4) was used for the calculation assuming the coefficient of discharge was 1, even though the holes were not shaped like orifices nor were they similar to each other in shape. Microscopic examination showed the holes to be quite irregular in shape. The maximum air flow rate (Table 1) at any time was assumed to be the rate for the hole when completely open and it was used as the basis for the calculations.

$$w = \frac{.533 C A p}{\sqrt{T}}$$

where w = flow rate, pound/second
 C = coefficient of discharge
 A = area of throat, square foot
 p = upstream pressure, pound/square foot
 T = upstream temperature, 460 + F

The above formula can be converted to the following for the conditions of our tests.

$$d = 2.6\sqrt{V} \times 10^{-4}$$

where d = equivalent diameter, inches
 V = volume of flow, ml/min measured at atmospheric pressure and saturated at 70 F

Table 1 shows the equivalent diameters calculated by this formula range from .00013 inch to .0065 inch. Some preliminary testing included holes slightly larger than .01 inch. Plugging of such holes with egg white took a long time and the larger holes provided the same information as the smaller holes. Comparative trials with the fluorocarbon gas, R-22 gave volumetric flow rates about 40% of those for air.

While the air flow rates through the holes were being measured, it was observed that a significant pressure was required to initiate air flow through the holes when they were wet. The required pressures varied from test to test and were quite high for the smaller holes (Table 1). Tests with fluorocarbon gas, R-22, required similar pressures. Table 1 also

gives some information on the amount of egg white required to plug the holes. Quantities under 0.1 ml are estimated. A drop of egg white is about 0.06 ml. When a fraction of a drop came through, the quantity was visually estimated down to 0.01 ml. If the quantity was insufficient to be observed on the metal disk but showed as a small spot on a fingertip after touching the hole, it was called 0.01 ml. For the small flows, plugging commonly occurred within 1 min. For the large flows, the times were as long as 1 hr or more. As anticipated, the results were quite variable, probably because of the variability in the occurrence and amount of suspended material in the egg white. Some smaller holes never showed any indication that egg white had flowed through even when the pressure was raised to 40 psi.

We can compare the degree of contamination caused by a small leak in a commercial pasteurizer with that remaining after pasteurization. Assuming that a leak with a total flow of 0.01 ml is diluted with 5 min of production in a plant running 3,000 pounds/hr, the degree of contamination of the pasteurized product is 1×10^{-7} of that of the raw product. Pasteurization requirements for killing *Salmonella* give a similar order of reduction (holding time = 3.5 min; decimal reduction time = 0.4 min; holding tube efficiency, 74%). From a comparison of equivalent diameters and flow of egg white in Table 1 and the above discussion, it appears that a method capable of detecting leaks with an equivalent diameter of 0.001 inch or smaller is needed. The average pressure required to initiate flow of gas through this size hole when it is wet is about 5 psi.

Table 2 indicates the size (per cent open) of the first 14 holes of Table 1 after each treatment. These figures show some completely plugged and many partially plugged until the cleaning method listed in treatment 16 was used. This method is similar to a recommended washing procedure for the cleaning-in-place of egg pasteurizers (1). In this laboratory, disks were placed in a rack that could be inserted in a 1.5 inch sanitary pipe for washing. The face of the disks was parallel to the line of flow. A 2% caustic solution containing some additional cleaning agents was pumped through the pipe with the indicated conditions. It may be assumed from the data of preceding trials that there were some thoroughly cooked-on egg solids in the holes at the start of this cleaning operation. Opening of holes in any commercial unit should not be any more difficult. In subsequent tests the same methods showed equally good results in opening holes. It is believed that if any holes exist in the plates of an egg pasteurizer, they will be open after proper cleaning.

Tests conducted with hole R indicate the sensitivity of using the fluorocarbon testing method for leaks.

The equivalent diameter of .00013 inch for this hole (Table 1) is based on the maximum air flow at any time which occurred immediately after it was ground and probably before the hole became wet. This diameter is of an order of magnitude of one-tenth that which permits flow of egg white in any significant amount. After this hole was ground and the initial air flow determined it never again, while wet, permitted any gas flow at 20 psi. It never permitted any detectable flow of egg white. After treatments 16 through 21, the air flow through this hole while wet was .075 ml/min at 60 psi. The hole was then dried overnight at room temperature and tested for leakage in the heat exchanger. On consecutive tests at 5 psi R-22 pressure and with the air side closed for the indicated length of time the following results were obtained: 2 min-weak signal; 5 min-strong signal; 2 min-no signal; and 5 min-strong signal.

Holes with equivalent diameters of .002 inch to .003 inch when dry always gave strong signals at 5 psi in 1 to 2 min. Some tests as low as 1 psi gave equivalent results. Hole R, when wet, never gave a positive signal with pressures up to 20 psi. The larger holes, when wet, gave inconsistent results for various pressures. While the figures cited in Table 1 for the pressures required to start flow of gases varied appreciably, the tests conducted in the heat exchanger varied more. Because of the inconsistency of tests on wet holes, but primarily because of the much greater sensitivity of those on dry holes, we

believe it is better to use dry heat transfer surfaces than to try to overcome the effect of wetness.

For drying a commercial plate heat exchanger we propose the following procedure. (a) After the surfaces are thoroughly cleaned, the open plates should be rinsed with hot water and promptly dried with a sponge or towel so that no visible drops of water remain. (b) The exchanger is then closed and warm air is forced through each side of the section to be tested for 30 min. If either side is more open to flow, that side should be dried first to produce more uniform temperatures through the exchanger. Air leaving the unit at the end of the drying period should be at least 15 F warmer than the room air.

ACKNOWLEDGMENT

The advice and encouragement of Mr. G. W. Putnam in this work has been deeply appreciated.

Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

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2. General Electric Company. 1966. Operation and Service, Type H-10 Leak Detector, West Lynn, Massachusetts.
3. Lineweaver, H., H. H. Palmer, G. W. Putnam, J. A. Garibaldi, and V. F. Kaufman. 1968. Egg Pasteurization Manual. U. S. Dept. Agr. ARS 74-48 series. (*In press*).
4. Perry, J. H. 1963. Chemical Engineers' Handbook. McGraw-Hill Book Co., New York. Section 5, pp. 9.

**HOLDERS OF 3-A SYMBOL COUNCIL
AUTHORIZATIONS ON FEBRUARY 20, 1969**

"Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to C. A. Abe'e, Secretary-Treasurer, 2617 Hartzell St., Evanston, Ill. 60201."

**0101 Storage Tanks for Milk and Milk Products,
as Amended**

97	Beseler Steel Products, Inc.	(3/24/58)
	417 East 29th, Marshfield, Wisconsin 54449	
116	Jacob Brenner Company, Inc.	(10/ 8/59)
	450 Arlington, Fond du Lac, Wisconsin 54935	
28	Cherry-Burrell Corporation	(10/ 3/56)
	575 E. Mill St., Little Falls, N. Y. 13365	
102	Chester-Jensen Company, Inc.	(6/ 6/58)
	5th & Tilgham Streets, Chester, Pennsylvania 19013	
1	Chicago Stainless Equipment Corp.	(5/ 1/56)
	5001 No. Elston Avenue, Chicago, Illinois 60630	
2	CP Division, St. Regis	(5 / 1/56)
	1243 W. Washington Blvd., Chicago, Illinois 60607	
117	Dairy Craft, Inc.	(10/28/59)
	Holdingsford, Minnesota 56340	
76	Damrow Brothers Company	(10/31/57)
	196 Western Avenue, Fond du Lac, Wisconsin 54935	
115	DeLaval Company, Ltd.	(9/28/59)
	113 Park Street, So., Peterborough, Ont., Canada	
109	Girton Manufacturing Company	(9/30/58)
	Millville, Pennsylvania 17846	
21	The J. A. Gosselin Co., Ltd.	(9/20/56)
	P. O. Box 280, Drummondville, Quebec, Canada	
44	The Heil Company	(10/26/56)
	3000 W. Montana Street, Milwaukee, Wisconsin 53235	
114	C. E. Howard Corporation	(9/21/59)
	9001 Rayo Avenue, South Gate, California 90280	
127	Paul Mueller Company	(6/29/60)
	1616 W. Phelps Street, Springfield, Missouri 65801	
197	Mueller/Richardson Ltd.	(9/ 9/67)
	84 Wellington St., South, St. Marys, Ont.	
143	Portersville Stainless Equipment Div.,	(5/16/63)
	Gibson Industries, Inc.	
	Portersville (Butler County), Pennsylvania 16051	
31	Walker Stainless Equipment Co.	(10/ 4/56)
	Elroy, Wisconsin 53929	

**0201 Pumps for Milk and Milk Products
Revised, as Amended**

29R	Cherry-Burrell Corporation	(10/ 3/56)
	105 W. Adams St., Chicago, Ill. 60603	
63R	CP Division, St. Regis	(4/29/57)
	1243 W. Washington Blvd., Chicago, Illinois 60607	
180R	The DeLaval Separator Co.	(5/ 5/66)
	Poughkeepsie, N. Y. 12602	
65R	G & H Products Corporation	(5/22/57)
	5718 52nd Street, Kenosha, Wisconsin 53140	
145R	ITT Jabsco, Incorporated	(11/20/63)
	1485 Dale Way, Costa Mesa, Calif. 92626	
26R	Ladish Co., Tri-Clover Division	(9/29/56)
	2809 60th Street, Kenosha, Wisconsin 53140	
148R	Robbins & Myers, Inc.	(4/22/64)
	Moyno Pump Division	
	1895 Jefferson Street, Springfield, Ohio 45501	
163R	Sta-Rite Products, Inc.	(5/ 5/65)
	234 South 8th Street, Delavan, Wisconsin 53115	

72R	L. C. Thomsen & Sons, Inc.	(8/15/57)
	1303 53rd Street, Kenosha, Wisconsin 53140	
175R	Universal Milking Machine Div.,	(10/26/65)
	National Cooperatives, Inc.	
	First Avenue at College, Albert Lea, Minn. 56007	
52R	Viking Pump Div.—	
	Houdaille Industries, Inc.	(12/31/56)
	406 State Street, Cedar Falls, Iowa 50613	
5R	Waukesha Foundry Company	(7/ 6/56)
	Waukesha, Wisconsin 53186	

**0400 Homogenizers and High Pressure Pumps of the
Plunger Type, As Amended**

87	Cherry-Burrell Corporation	(12/20/57)
	2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404	
37	CP Division, St. Regis	(10/19/56)
	1243 W. Washington Blvd., Chicago, Illinois 60607	
75	Manton-Gaulin Mfg. Co., Inc.	(9/26/57)
	44 Garden Street, Everett, Massachusetts 02149	

**0501 Stainless Steel Automotive Milk Transportation
Tanks for Bulk Delivery and/or Farm Pick-up Service,
As Amended**

131	Almont Welding Works, Inc.	(9/ 3/60)
	4091 Van Dyke Road, Almont, Michigan 48003	
98	Beseler Steel Products, Inc.	(3/24/58)
	417 East 29th, Marshfield, Wisconsin 54449	
70	Jacob Brenner Company	(8/ 5/57)
	450 Arlington, Fond du Lac, Wisconsin 54935	
40	Butler Manufacturing Co.	(10/20/56)
	1000 Berry Avenue, St. Paul, Minnesota 55114	
118	Dairy Craft, Inc.	(10/28/59)
	Holdingsford, Minnesota 56340	
66	Dairy Equipment Company	(5/29/57)
	1919 So. Stoughton Road, Madison, Wisconsin 53716	
123	DeLaval Company, Ltd.	(12/31/59)
	113 Park Street, South, Peterborough, Ont., Canada	
190	Eastern Industries, Limited	(11/18/66)
	830 Blvd., Lemire, Drummondville, Quebec, Canada	
121	The J. A. Gosselin Co., Ltd.	(12/ 9/59)
	P. O. Box 280, Drummondville, Quebec, Canada	
45	The Heil Company	(10/26/56)
	3000 W. Montana Street, Milwaukee, Wisconsin 53235	
201	Paul Krohnert Mfg., Ltd.	(4/ 1/68)
	West Hill, Ontario, Canada	
80	Mueller/Richardson, Ltd.	(11/24/57)
	84 Wellington Street, So., St. Marys, Ont., Canada	
93	Pennsylvania Furnace & Iron Co.	(2/ 6/58)
	316 Pine Street, Warren, Pennsylvania 16365	
85	Polar Manufacturing Company	(12/20/57)
	Holdingsford, Minnesota 56340	
144	Portersville Stainless Equipment Div.,	(5/16/63)
	Gibson Industries, Inc.	
	Portersville (Butler County), Pennsylvania 16051	
71	Progress Industries, Inc.	(8/ 8/57)
	400 E. Progress Street, Arthur, Illinois 61911	
47	Trailmobile Div. of Pullman, Inc.	(11/ 2/56)
	16th & Howell Streets, North Kansas City, Mo. 64116	
189	A. & L. Tougas, Ltée	(10/ 3/66)
	1 Tougas St., Iberville, Quebec, Canada	

- 25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

**0800 Fittings Used on Milk and Milk Products
Equipment, and Used on Sanitary Lines Conducting
Milk and Milk Products and Supplements 2, 3, 4, 5,
and 6 As Amended**

- 79 Alloy Products Corporation (11/23/57)
1045 Perkins Avenue, Waukesha, Wisconsin 53186
- 138 A.P.V. (Canada) Equipment, Ltd. (12/17/62)
103 Rivalda Rd., Weston, Ont., Canada
- 82 Cherry-Burrell Corporation (12/11/57)
105 W. Adams St., Chicago, Ill. 60603
- 124 DeLaval Company, Ltd. (2/18/60)
113 Park Street, South, Peterborough, Ont., Canada
- 184 The DeLaval Separator Co. (8/ 9/66)
Poughkeepsie, New York 12602
- 67 G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140
- 199 Gray Company, Inc. (12/ 8/67)
60 Eleventh Ave., N.E., Minneapolis, Minn. 55413
- 203 Grinnell Company (11/27/68)
260 W. Exchange St., Providence, R. I. 02901
- 204 Hills McCanna Company (2/10/69)
400 Maple Ave., Carpentersville, Ill. 60110
- 34 Ladish Co., Tri-Clover Division (10/15/56)
2809 60th St., Kenosha, Wisconsin 53140
- 200 Paul Mueller Co. (3/ 5/68)
1616 Phelps St., Springfield, Mo. 65601
- 149 Q Controls (5/18/64)
Occidental, California 95465
- 89 Sta-Rite Industries, Inc. (12/23/68)
P. O. Box 622, Delavan, Wis. 53114
- 73 L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street, Kenosha, Wisconsin 53140
- 191 Tri-Canada Fittings & Equipment Ltd. (11/23/66)
21 Newbridge Road, Toronto 18, Ontario
- 151 Tubular Components, Inc. (11/18/64)
Butternut Drive, East Syracuse, New York 13057
- 86 Waukesha Specialty Company (12/20/57)
Walworth, Wisconsin 53184

**0900 Thermometer Fittings and Connections Used
on Milk and Milk Products Equipment and
Supplement 1, As Amended**

- 32 Taylor Instrument Companies (10/ 4/56)
95 Ames Street, Rochester, New York 14611

**1000 Milk and Milk Products Filters Using Disposable
Filter Media, As Amended**

- 35 Ladish Co., Tri-Clover Division (10/15/56)
2809 60th Street, Kenosha, Wisconsin 53140

**1100 Plate-Type Heat Exchangers for Milk and Milk
Products, As Amended**

- 20 A.P.V. Company, Inc. (9/ 4/56)
137 Arthur Street, Buffalo, New York 14207
- 30 Cherry-Burrell Corporation (10/ 1/56)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404
- 14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets, Chester, Pennsylvania 19013
- 38 CP Division, St. Regis (10/19/56)
1243 W. Washington Blvd., Chicago, Illinois 60607

- 120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street, South, Peterborough, Ont., Can.
- 17 The DeLaval Separator Company (8/30/56)
Poughkeepsie, New York 12602
- 15 Kusel Dairy Equipment Company (8/15/56)
100 W. Milwaukee Street, Watertown, Wisconsin 53094

**1200 Internal Return Tubular Heat Exchangers,
for Milk and Milk Products, As Amended**

- 103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street, Chester, Pennsylvania 19013
- 96 C. E. Rogers Company (3/31/64)
8731 Witt Street, Detroit, Michigan 48209
- 152 Sanitary Processing Equipment Corp. (11/18/64)
Butternut Drive, East Syracuse, New York 13057

**1301 Farm Milk Cooling and Holding Tanks —
Revised, As Amended**

- 11R CP Division, St. Regis (7/25/56)
1243 W. Washington Street, Chicago, Illinois 60607
- 119R Dairy Craft, Inc. (10/28/59)
Holdingford, Minnesota 56340
- 4R Dairy Equipment Company (6/15/56)
1919 S. Stoughton Road, Madison, Wisconsin 53716
- 92R DeLaval Company, Ltd. (12/27/57)
113 Park Street, South
Peterborough, Ontario, Canada
- 49R The DeLaval Separator Company (12/ 5/56)
Poughkeepsie, New York 12602
- 10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846
- 95R Globe Fabricators, Inc. (3/14/58)
7744 Madison Street, Paramont, California 90723
- 179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
635 Laurel St., Preston, Ont., Canada
- 12R Paul Mueller Company (7/31/56)
1616 W. Phelps Street, Springfield, Missouri 65801
- 58R Schweitzer's Metal Fabricators, Inc. (2/25/57)
806 No. Todd Avenue, Azusa, California 91702
- 50R Emil Steinhurst & Sons, Inc. (12/20/56)
612-616 South Street, Utica, New York 13503
- 134R Universal Milking Machine Division (5/19/61)
National Co-operatives, Inc.
First Avenue at College, Albert Lea, Minn. 56007
- 182R Vacooler Co. (5/20/66)
700 Gaylord Ave., Elyria, Ohio 44035
- 42R VanVetter, Inc. (10/22/56)
2130 Harbor Avenue S.W., Seattle, Washington 98126
- 18R Whirlpool Corporation, St. Paul Division (9/20/56)
850 Arcade Street, St. Paul, Minnesota 55106
- 55R John Wood Company (1/23/57)
Superior Metalware Division
509 Front Avenue, St. Paul, Minnesota 55117
- 170R The W. C. Wood Co., Ltd. (8/ 9/65)
5 Arthur Street, South, Guelph, Ont., Canada
- 16R Zero Manufacturing Company (8/27/56)
Washington, Missouri 63090

**1400 Inlet and Outlet Leak Protector Plug Valves
for Batch Pasteurizers**

- 122 Cherry-Burrell Corporation (12/11/59)
105 W. Adams St., Chicago, Ill. 60603
- 69 G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140

- 27 Ladish Co. - Tri-Clover Division (9/29/56)
2809 60th Street, Kenosha, Wisconsin 53140
- 78 L. C. Thomson & Sons, Inc. (11/20/57)
1303 43rd Street, Kenosha, Wisconsin 53140

- 155 Paul Mueller Co. (2/10/65)
1616 W. Phelps Street, Springfield, Missouri 65801
- 195 Mueller/Richardson, Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada
- 165 Walker Stainless Equipment Co. (4/26/65)
New Lisbon, Wisconsin 53950

1600 Evaporators and Vacuum Pans for Milk and Milk Products, As Amended

- 132 A.P.V. Company, Inc. (10/26/60)
137 Arthur Street, Buffalo, New York 14207
- 111 Blaw-Knox Company, (2/12/59)
Dairy Equipment Division
750 E. Perry, Buffalo, N. Y. 14210
- 110 Arthur Harris & Company (11/10/58)
210-218 North Aberdeen Street, Chicago, Illinois 60607
- 128 Mojonier Bros. Co. (7/ 6/60)
4601 W. Ohio Street, Chicago, Illinois 60644
- 164 Mora Industries, Inc. (4/25/65)
112 South Park Street, Mora, Minnesota 55051
- 107 C. E. Rogers Company (8/ 1/58)
8731 Witt Street, Detroit, Michigan 48209
- 186 Marriott Walker Corporation (9/ 6/66)
925 East Maple Road, Birmingham, Mich. 48008

1700 Fillers and Sealers of Single Service Containers, For Milk and Milk Products, As Amended

- 192 Cherry-Burrell Corporation (1/ 3/67)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52404
- 139 Exact Weight Sale Company (4/15/68)
538 East Town Street, Columbus, Ohio 43215
- 137 Ex-Cell-O Corporation (10/17/62)
P. O. Box 386, Detroit, Michigan 48232
- 140 General Films, Inc. (4/23/63)
Covington, Ohio 55318
- 142 Polygal Company (4/15/63)
Div. of Inland Container Corp.
P. O. Box 68074, Indianapolis, Indiana 46268

1900 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

- 141 CP Division, St. Regis (4/15/63)
1243 W. Washington Blvd., Chicago, Illinois 60607
- 146 Cherry-Burrell Corporation (12/10/63)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404

2200 Silo-Type Storage Tanks for Milk and Milk Products

- 168 Cherry-Burrell Corporation (6/16/65)
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1243 W. Washington Blvd., Chicago, Illinois 60607
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- 198 Mueller/Richardson, Ltd. (9/ 9/67)
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PHYSIOLOGICAL CHARACTERISTICS OF ENTEROPATHOGENIC AND NON-PATHOGENIC COLIFORM BACTERIA ISOLATED FROM CANADIAN PASTEURIZED DAIRY PRODUCTS¹

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ABSTRACT

Three enteropathogenic *Escherichia coli* serotypes (PE) were compared with three non-pathogenic *E. coli* strains (E) and three *Enterobacter aerogenes* strains (A) on the basis of their biochemical, thermal death time, and low temperature growth characteristics. All nine strains were isolated from Canadian pasteurized dairy products. It was not possible to differentiate consistently between the PE and E strains from the results of any biochemical tests performed. Differentiation was achieved, however, on the basis of differences in colonial morphology of the strains when grown on Tergitol-7 agar containing 0.004% 2, 3, 5-triphenyltetrazolium chloride; the PE strains produced rough or intermediate rough colonies on this medium, whereas the E and A strains produced smooth or mucoid colonies. Thermal death time studies showed that one or more of the E strains might survive a commercial high temperature process if the number of cells present in the raw milk were unusually large; the PE and A strains were relatively heat sensitive and would be unlikely to survive pasteurization. z -Values found for the strains varied from 9.6 to 16.5, but the magnitude of these values was not directly correlated with the identity of particular groups of strains. Viable counts of the E strains suspended in skim milk at 7 C decreased with time; counts of the A strains and of one of the PE strains declined during the first 2 to 4 days of storage but thereafter increased rapidly. One PE strain grew only weakly under these conditions but the third showed a remarkable capacity for rapid initiation of fast growth. When cells were suspended in skim milk at -28 C, viable counts of all nine strains decreased during storage. It was concluded that particular significance can be attached to the capacity of the PE strains to multiply in skim milk held at 7 C if the serological evidence obtained is a valid criterion of their potential enteropathogenicity. The results emphasize the need for a simple and rapid test to detect enteropathogenic *E. coli* contamination of dairy products, and it is suggested that a cultural test, using Tergitol-7 agar containing 2, 3, 5-triphenyltetrazolium chloride as a differential medium, might be developed for this purpose.

Contamination of dairy products by coliform bacteria is of three-fold importance. First, the very wide distribution of coliforms in nature makes them use-

ful indicators of the extent of recontamination after pasteurization and therefore also of the sanitary quality of pasteurized products (1). Second, coliforms themselves cause rapid spoilage of products held under conditions conducive to microbial growth. Third, some strains of *Escherichia coli* are potential enteric pathogens for which dairy products may act as vehicles of dissemination (5).

No simple and reliable non-serological test for the differentiation of pathogenic from non-pathogenic *E. coli* serotypes has yet been devised. This is because no absolute correlation has been found in this biotype group between pathogenicity and any single physiological characteristic. In comparative experiments, however, correlations of various degree have been found to exist between pathogenicity and, respectively, sucrose fermentation (15, 27), inhibition of growth by serine (22, 23) and colicin (19), mucinase production (21) and hemolysis (14, 29). The most reliable single method for determining the potential pathogenicity of an isolate is still serological analysis involving agglutination testing (8) and most workers employ a combination of biochemical and serological tests in the characterization and typing of *Escherichia* isolates (15, 18).

Numerous studies have been reported concerning the sensitivity to heat treatment of coliform bacteria when suspended in milk (7, 9, 13, 17, 20). These have clearly shown that the thermal death time for a particular strain is dependent upon several factors, including initial cell concentration of the heated suspension (7, 17), growth temperature of the cells (17), and their physiological age (16). Olson et al. (17) found that within a group of 139 coliform strains isolated from a variety of sources, including several dairy products, there were considerable differences in heat sensitivity from one strain to another. *E. coli* biotypes were generally the most heat resistant, intermediate biotypes were the most heat sensitive, and *Aerobacter aerogenes* biotypes occupied a position between these extremes. When thermal death time curves for one of the most heat resistant *E. coli* strains were plotted using data obtained from experiments in which the initial population of cells grown in skim

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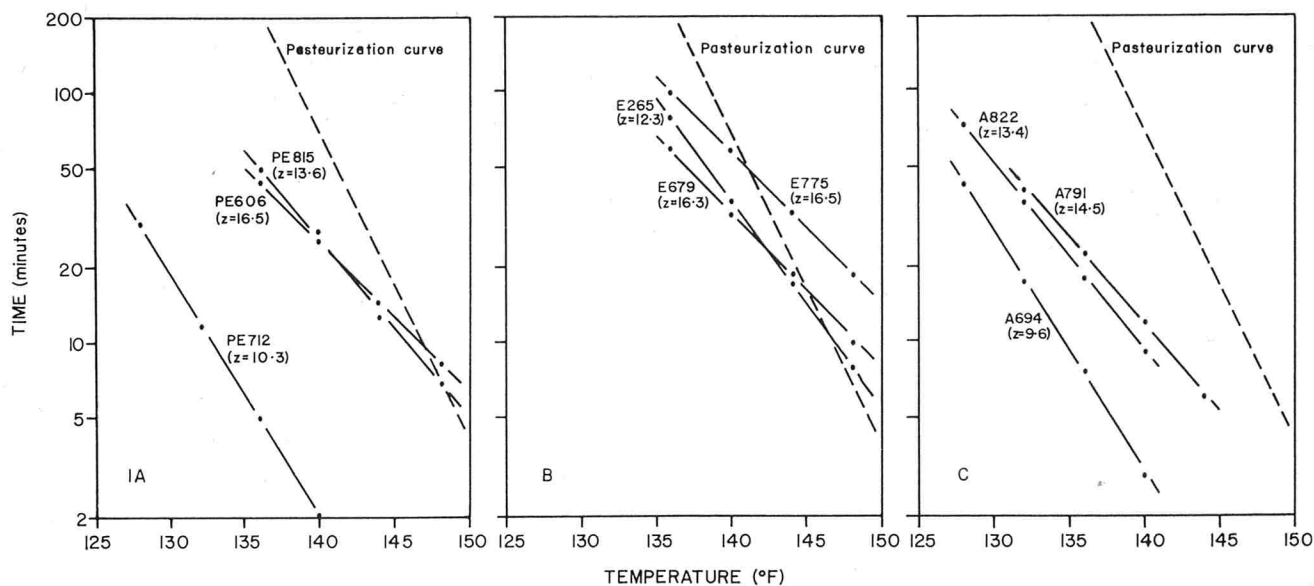


Figure 1. Thermal death time curves for coliform bacteria suspended in skim milk. (A) enteropathogenic *E. coli* serotypes, (B) non-pathogenic *E. coli* strains, (C) *E. aerogenes* strains.

milk for 16 hr at 37 C was approximately 50,000 per ml, z -values (2) of 10.5 and 10.8, respectively, were obtained in two replicate trials. The thermal death time at 143 F was approximately 19 min. Read et al. (20), using 1×10^8 cells per ml of raw milk, obtained a z -value of 10.2 for *E. coli* ATCC 9637.

The normal temperature range for growth of coliforms is 10 to 45 C, but milk frequently contains psychrotrophic strains capable of growth below 10 C (32). The mean generation time of strains grown in pure culture at refrigeration temperatures is usually quite long. Skelton and Harmon (26) found mean generation times for *E. coli* and *A. aerogenes* grown in skim milk at 10 C of 15.7 and 19.9 hr, respectively. At 4 C the populations of both organisms progressively declined, the rate of apparent death of *A. aerogenes* being faster than that of *E. coli*. Populations in skim milk held at 0 C decreased during the first 21 days of storage but thereafter remained stationary until the 76th day.

During a survey recently carried out in this laboratory (12) three enteropathogenic *E. coli* serotypes were recovered from Canadian pasteurized dairy products. The purpose of the present study was to compare the biochemical, thermal death time, and low temperature growth characteristics of these strains with those of representative non-pathogenic *E. coli* serotypes and *Enterobacter* (= *Aerobacter*) *aerogenes* strains isolated in the same survey.

MATERIALS AND METHODS

Organisms

The nine strains of coliform bacteria used in this investigation comprised three pathogenic *E. coli* serotypes (strain

PE616, serotype O128:B12; PE712, O26:B6; and PE815, O119:B14), three non-pathogenic *E. coli* serotypes (strains E265, E679, and E775) and three *E. aerogenes* strains (A694, A791, and A822). All the strains had been isolated from Canadian pasteurized dairy products and identified in a previous study (11, 12). The six non-pathogenic strains (*E* and *A* groups) were selected at random from groups of strains of similar identity for comparison with the three pathogenic strains (*PE* group). Stock cultures of all strains were maintained on stock culture agar (Difco) at 4 C and transferred at 2-week intervals. Inocula for cultural tests were prepared by growing the strains in brain heart infusion broth (Difco) at 37 C for 24 hr. A loopful of cells was suspended in 2 ml of sterile distilled water and one loopful of the suspension was used to inoculate each test medium.

Colonial morphology

Comparative observations of colonial morphology were made on violet red bile agar, blood agar, and Tergitol-7 agar (Difco) containing 0.004% 2, 3, 5-triphenyltetrazolium chloride (TTC). The latter medium was prepared as described by Scherer (24). Colonies were examined under a 39-power binocular microscope after incubation of the plates at 37 C for 24 hr followed by a subsequent 24 hr period of incubation at room temperature. Colonies on Tergitol-7 medium were described using the terminology of Scherer (24).

Biochemical tests

Tests for motility, capsule formation, indole production, acetoin production (Voges-Proskauer test), acid production in dextrose broth (methyl red test), citrate utilization and carbohydrate fermentation capacity were performed on each strain using methods recommended in *Manual of Microbiological Methods* (28). Capacity to hydrolyze casein, to liquefy gelatin, and to produce oxidase were tested by the methods of Collins (6). Tests for hydrogen sulfide production, urease production, and nitrate reduction (8); for methylene blue reduction (25); and for starch hydrolysis and catalase production (10) were also performed. In all these tests each strain was treated in duplicate and control tests on uninocu-

lated media were conducted in parallel with each set of tests performed. Agglutination tests were done as previously described (11). The capacity of the strains to decarboxylate 18 amino acids was tested using the method of Edwards and Ewing (8). L-forms of substrate amino acids were used at 0.5% and DL-forms at 1% concentration. Duplicate control tests in the absence of substrate and inoculum respectively, were conducted in each instance. Unless otherwise indicated, all inoculated media were incubated at 37 C and cultures were observed after 48 hr.

Thermal death times

A thermal death time curve for each test organism was constructed using a procedure based on that of Olson et al. (17). Cells were grown in nutrient broth at 37 C for 24 hr, harvested aseptically by centrifugation, and washed twice with sterile 0.0003 M phosphate buffer (pH 6.8). They were finally suspended in sterile distilled water. The suspension was standardized optically at 600 $m\mu$ to contain an appropriate cell density, using a standard curve for each strain which related the optical density of cell suspensions to their counts on violet red bile agar. One milliliter aliquots of the suspension were then added to 99 ml volumes of sterile, antibiotic-free skim milk at 4 C and these were shaken vigorously to distribute the cells. One milliliter aliquots of inoculated milk, containing approximately 50,000 cells per ml as determined by SPC (1), were sealed into 6 x 100 mm glass tubes in such a way that heating of the milk was avoided. After cooling, the tubes were immersed in a water bath maintained at the desired temperature. Preliminary experiments using thermocouples sealed into similar tubes of skim milk were carried out to establish the average warm-up time, and this time interval was considered in computing the effective exposure time at each bath temperature employed. After removal from the bath the tubes were immediately cooled in ice-water and checked for the presence of viable cells as previously described (11). Bath temperatures between 128 F and 152 F were used and effective exposure times varied from

1 to 120 min. Strains were tested in duplicate under each set of temperature and time conditions employed and the thermal death time at any temperature was considered to be the shortest exposure time which consistently resulted in complete destruction of cells.

Effect of low temperature storage on viability and growth

Suspensions of washed cells in sterile reconstituted 10% skim milk (pH 6.9) were prepared as described above. Five milliliter aliquots of the milk, containing approximately 50,000 cells per ml, were distributed in sterile rubber-stoppered test tubes. Sets of tubes were stored at 7 C and at -28 C. Initially and at appropriate time intervals thereafter tubes were removed and thawed if necessary in a water bath held at 30 C, and the viable cell density of their contents was determined by SPC. The pH of the contents was measured at room temperature. All results reported are the average of triplicate determinations.

RESULTS

Colonial morphology

Comparisons of the colonial morphology of the nine coliform strains under investigation failed to reveal strain differences when violet red bile agar and blood agar were the plating media. Differences were observed, however, when the strains were plated on Tergitol-7 medium (Table 1); the PE strains were differentiated from the E and A strains by the roughness of their colonies. PE712 produced two distinct colony types, intermediate rough and TTC-reducing (24). Colonies representing the two types were isolated and the cultures were compared on the basis of several tests. They produced identical indole, Voges-Proskauer, methyl red, and citrate utilization reactions and agglutinated with the same specific

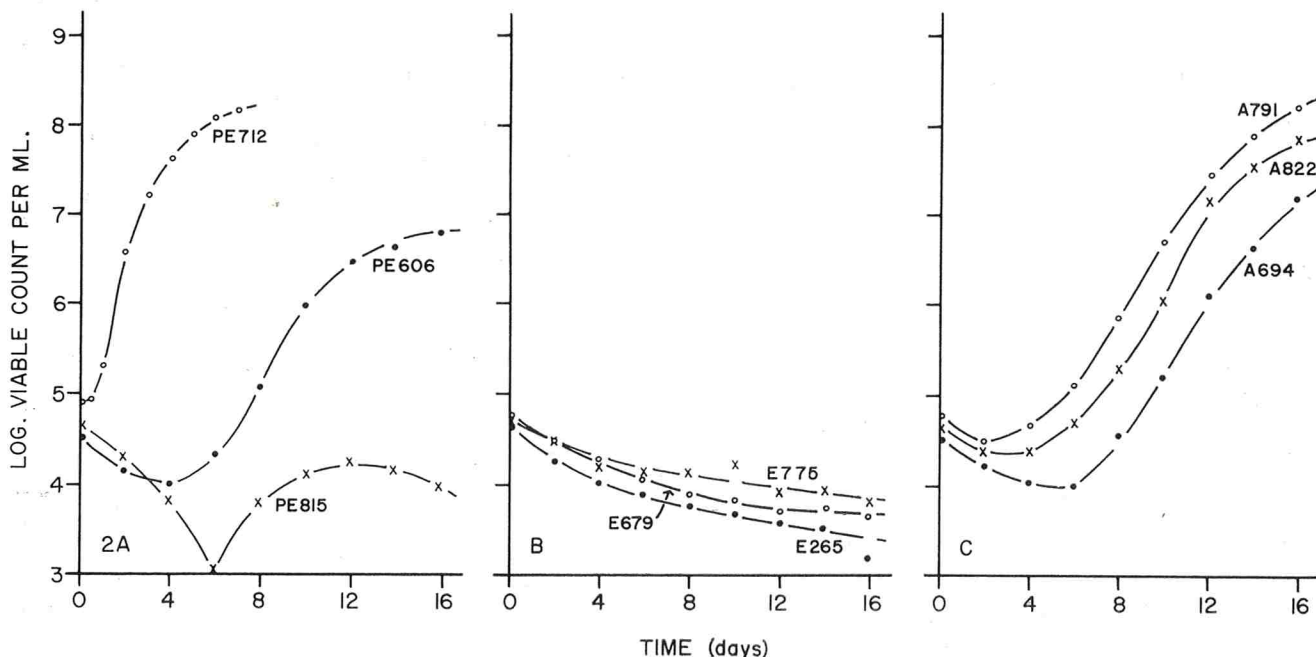


Figure 2. Changes with time in viable counts of coliform bacteria suspended in skim milk stored at 7 C. (A) enteropathic *E. coli* serotypes, (B) non-pathogenic *E. coli* strains, (C) *E. aerogenes* strains.

TABLE 1. COLONIAL MORPHOLOGY OF NINE COLIFORM STRAINS ON TERGITOL-7 AGAR CONTAINING 0.004% TTC

Strain	Colonial type					
	Rough	Intermediate rough	Intermediate smooth	Mucoid type A	Mucoid type B	TTC-reducing
PE606		+				
PE712		+				+
PE815	+					
E265			+			
E679			+			
E775			+			
A694				+		
A791				+		
A822					+	

TABLE 2. FERMENTATION OF LACTOSE AND CELLOBIOSE BY TWO COLONIAL TYPES OF COLIFORM STRAIN PE712

Colonial type	Lactose			Cellobiose		
	7 C	37 C	44 C	7 C	37 C	44 C
Intermediate rough	AG ¹ (2) ²	AG(1)	O ¹	AG(2)	A(1)	O
TTC-reducing	AG(30)	AG(1)	O	AG(2)	A(1)	O

¹A: acid produced; G: gas produced; O: no fermentation.

²Days required for visible reaction.

antiserum (O26:B6). Their respective capacities to ferment lactose and cellobiose at different temperatures were generally similar (Table 2), but they were differentiated by the rate at which acid and gas were produced from lactose at 7 C. The TTC-reducing colony type required 30 days to produce a visible reaction, whereas the intermediate rough type required only 2 days to do so. Apparently PE712 comprised two physiological types of cells which were differentiable on the basis of certain fermentation reactions and under conditions of growth on Tergitol-7 medium. In subsequent tests the two colonial types of this strain were not separated and the results probably relate to cultures containing both types.

Biochemical tests

Many biochemical tests performed on the strains failed to distinguish one from another. All the strains were encapsulated, produced catalase, and were facultatively anaerobic; all of them produced acid and gas from arabinose, galactose, glucose, lactose, levulose, maltose, mannitol, mannose, trehalose, and xylose. None of the strains hydrolyzed starch or casein, liquefied gelatin, produced hydrogen sulfide or oxidase, and none was hemolytic on blood agar; no strain decarboxylated alanine, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, or valine; and no strain fermented erythri-

tol, inulin, or melizitose within 15 days at 37 C. Results of biochemical tests in which the response of the strains varied are shown in Table 3; in no instance, however, do these results permit a clearcut distinction to be drawn between the PE and E groups of strains.

Thermal death times

Using cell suspensions containing between 43,000 and 70,000 cells per ml, thermal death times of the various strains were determined over a range of temperatures; the resulting curves are plotted in Fig. 1. z -Values for the respective strains, which are also shown in these figures, were calculated from these curves. Considerable variation occurred in the position and slope of the curves for the different strains. The PE and E strains were generally more heat resistant than the A strains, in agreement with the findings of Olson et al. (17) for strains of similar identity. PE712, however, was the most heat sensitive strain tested. All strains had previously been shown to be incapable of surviving laboratory pasteurization at 145 F for 30 min (11). The most heat sensitive strains, PE712 and A694, had the lowest z -values, whereas the most heat resistant strain, E775, had the highest. Ranking the strains in decreasing order of sensitivity to heat reveals the existence among them of two series with respect to z -value. Thus PE712 and the three A strains form one series in which heat resistance and z -value were correlated,

TABLE 3. BIOCHEMICAL CHARACTERS AND CARBOHYDRATE FERMENTATION REACTIONS OF NINE COLIFORM STRAINS

Test or substrate	Strain								
	PE606	PE712	PE815	E265	E679	E775	A694	A791	A822
Motility	+	-	+	+	+	-	-	+	+
Indole produced	+	-	+	+	+	+	-	-	-
Dextrose broth pH 4.5	+	+	+	+	+	+	-	-	-
Acetoin produced	-	-	-	-	-	-	+	+	+
Citrate utilized	-	-	-	-	-	-	+	+	+
Nitrate reduced	+	+	+	+	+	+	+	+	-
Methylene blue reduced	+	-	+	+	+	+	+	-	+
Urease produced	-	-	-	-	-	-	+	+	+
Aspartic acid decarboxylated	+	-	+	+	+	+	-	-	-
Lysine decarboxylated	+	-	-	+	-	-	-	-	-
Arginine decarboxylated	-	-	-	-	-	-	-	+	-
Ornithine decarboxylated	-	-	-	-	-	+	+	+	+
Adonitol	O ¹	AG ¹	O	O	AG	O	O	O	O
Cellobiose	O	A	O	O	O	O	AG	AG	AG
Dulcitol	AG	O	AG	AG	O	AG	O	O	O
Esculin	AG	A	O	AG	AG	O	AG	AG	AG
Inositol	O	O	O	O	O	O	A	A	A
Melibiose	AG	A	AG	AG	AG	AG	AG	AG	AG
Raffinose	AG	O	AG	O	AG	O	AG	AG	AG
Rhamnose	A	A	A	AG	AG	A	AG	AG	AG
Salicin	AG	AG	O	AG	AG	O	AG	AG	AG
Sorbitol	AG	O	AG	AG	AG	AG	AG	AG	AG
Sucrose	A	O	O	O	AG	O	AG	AG	AG

¹A: acid produced; C: gas produced; O: no fermentation.

and the remaining PE and E strains form another.

Effect of low temperature storage on viability and growth

The effect of storage in skim milk at 7 C on the capacity of the strains to grow is shown in Fig. 2. None of the E strains was capable of multiplying at this temperature, and the viable counts of these strains gradually decreased during the storage period. Viable counts of the A strains declined during the first 2 to 4 days of storage but increased almost 10,000-fold during the following 12 days. Despite the capacity of these strains to multiply under the experimental conditions used, however, the pH of the cultures dropped by less than 0.5 of a unit during the entire period of growth. The individual PE strains varied considerably in their response to storage in milk at 7 C. PE606 behaved similarly to the A strains, whereas PE815 showed only a weak capacity to grow after an initial steep and extensive decline in cell numbers. PE712 showed a remarkable capacity to grow at 7 C. There was apparently no initial decline in cell numbers but rather a short stationary phase followed by a phase of rapid multiplication which resulted in a 1000-fold increase in population within 6 days.

When similar experiments were carried out using a storage temperature of -28 C instead of 7 C, results shown in Fig. 3 were obtained. All strains tested showed an almost linear decrease in numbers

of viable cells with time during the first 14 to 28 days of storage. Thereafter populations were maintained at a relatively constant level which varied from strain to strain. The E strains died initially more slowly than the PE or A strains and their subsequent stable populations were generally larger. PE712 and A694 were particularly sensitive to the test conditions used and less than 5% of the respective initial viable cell populations remained after 14 days; thereafter the viable populations continued to decrease through the 98th day.

DISCUSSION

The object of this investigation was to compare selected physiological and biochemical properties of three enteropathogenic *E. coli* serotypes with those of three non-pathogenic *E. coli* strains and three *E. aerogenes* strains. All nine strains had been isolated in a previous study (12) from Canadian pasteurized dairy products. It was hoped at the outset that such a comparative approach might reveal consistent differences between the groups of strains which could form the basis for subsequent development of a simple and rapid test to differentiate between pathogenic and non-pathogenic coliform bacteria suitable for routine use in product control. In addition, information was sought concerning the behavior of the enteropathogenic strains in milk during heat treatment and storage, because such information could

have important implications for the epidemiological significance of the presence of pathogenic coliforms in dairy products.

Although the results of a number of biochemical tests clearly differentiated the A strains from the PE and E strains, responses of the latter two groups of strains were generally so similar that it was impossible, on the basis of any test or combination of tests, to differentiate between them. In particular, results with the present strains did not confirm the relationship between potential pathogenicity and sucrose fermentation capacity reported to exist in other strains (15, 27).

Differentiation of the PE strains from the remaining six strains was, however, achieved on the basis of differences in their colonial morphology on Tergitol-7 agar containing 0.004% TTC. Of the nine organisms tested only the PE strains produced colonies on this medium which were rough or intermediate rough in appearance. The E and A strains produced colonies which, in appearance, were either smooth or mucoid. It is recognized that these results were obtained with a relatively small number of strains; nevertheless, they support the suggestion of Scherer (24), who found that mucoid colonies could not be typed serologically, that Tergitol-7 medium might be valuable for screening purposes in epizootiological studies of *E. coli* infections. We suggest, therefore, that these observations could form the starting point for work designed specifically to develop a cultural test for enteropathogenic *E. coli* contamination of dairy products. Tergitol-7 agar is inherently, although probably not absolutely, selective for coliform bacteria (3), and the presence in it of a low concentration of TTC permits differentiation between

strains on the basis of colony appearance (3, 4, 24, 31). A test based on this medium would be simple to perform with the equipment found in routine control laboratories and results could be obtained rapidly (4). Experiments on the behavior of the PE strains in skim milk under various conditions have clearly demonstrated the need for such a test.

Results of simple laboratory pasteurization tests performed previously on the nine coliform strains under investigation had shown them all to be post-pasteurization contaminants of the products from which they were isolated (12). Results of the present study, in which thermal death times over a range of temperatures were determined, have confirmed this conclusion. The possibility exists, however, that cells of one or more of the E strains, especially E775, might survive a commercial high temperature process if the number of cells present in the milk prior to treatment were unusually large (17). The generally higher heat resistance of *E. coli* biotypes compared to *E. aerogenes* biotypes, which is illustrated by these results, is well known (17), but z -values calculated from the thermal death time curves constructed for the various strains were, in some instances, higher than those reported by other workers (17, 20) for different strains of coliform bacteria.

Striking differences were observed in the relative capacities of the PE and E strains to multiply in skim milk stored at 7 C. Whereas populations of the E strains consistently lost viability under these conditions, all three PE strains were capable of growth, generally following an initial reduction in cell numbers. This reduction in viable count and consequent pre-multiplication lag can be attributed to the time taken for selection of a population of cells adapted to

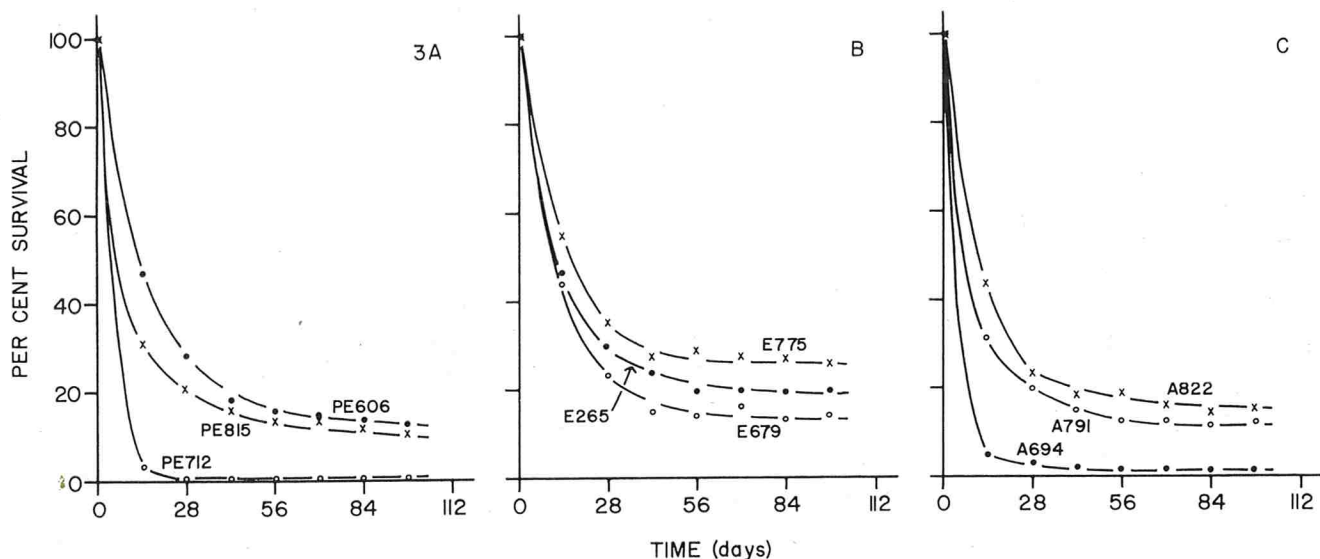


Figure 3. Rates of death of coliform bacteria suspended in skim milk stored at -28°C . (A) enteropathogenic *E. coli* serotypes, (B) non-pathogenic *E. coli* strains, (C) *E. aerogenes* strains.

grow at the low temperature. PE712 showed a remarkable capacity for rapid initiation of fast growth at 7 C. Cultures of this organism, however, were shown to consist of two physiological types of cells, but whether the increase in viable count under the experimental conditions was attributable to growth of only one type or to growth of both types was not determined. The two types differed in the rate at which they were capable of fermenting lactose at 7 C but not in the rate at which cellobiose was fermented at the same temperature; nevertheless, they were indistinguishable on the basis of the serological tests performed. If the PE strains are indeed capable of initiating enteric disease in the human, the fact that they have been shown to multiply in skim milk held at 7 C may be of considerable epidemiological significance because pasteurized milk and dairy products are frequently stored for periods of several days at temperatures close to 7 C. This conclusion assumes that the behavior of the organisms when suspended in dairy products other than skim milk would be similar to their behavior in skim milk; although identical behavior in different menstrua could not be expected (30), it seems both prudent and reasonable to assume that radical differences in behavior would not result. When cells were suspended in skim milk stored at -28 C, viable counts of all the strains tested decreased during storage; this is in general agreement with previous findings (26) where storage temperatures of 0 C and below have been used.

Strain PE712 had been previously identified as an *E. coli* II biotype and found to agglutinate in a test tube with specific O26:B6 antiserum at an antiserum dilution of 1:2560 (11, 12). The present results, however, raise a question as to the taxonomic status of this strain, for some of its characteristics differed from those of the remaining *Escherichia* strains tested. It failed to reduce methylene blue and to decarboxylate aspartic acid, and its heat sensitivity when suspended in skim milk more nearly resembled that of the A strains than that of the *Escherichia* strains; of the nine strains tested it was in fact the most heat sensitive and, in addition, it had the lowest z -value. Moreover, cultures of PE712 were shown to comprise two physiological types of cells. Crucially, however, agglutination by both types of cells with specific *E. coli* OB antiserum was confirmed in the present study, and it would therefore be necessary to provide an explanation for this very strong specific agglutination reaction if the identification of this organism as an *E. coli* biotype were postulated to be incorrect. On the other hand, whether or not PE712, and for that matter PE606 and PE815, are indeed potential enteric pathogens could only be determined by carefully controlled animal experiments (29), and such experiments have not been performed.

The significance to be attached to the presence of these organisms in pasteurized dairy products, and to their behavior in products stored at low temperature, therefore depends upon the validity of the serological evidence as a criterion of their potential enteropathogenicity.

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ASSOCIATION AFFAIRS

PENNSYLVANIA DAIRY FIELDMEN'S CONFERENCE

The 1969 Dairy Fieldmen's Conference will be held on Tuesday and Wednesday, June 10 and 11 at the J. O. Keller Building at The Pennsylvania State University. This new Conference Center is located adjacent to the Nittany Lion Inn in State College.

The program will include talks on dairy management; financing and labor; instrumental testing of DHIA samples; vitamin and mineral feeding; CIP cleaning of farm dairy equipment; bulk tank calibration; 3A standards for milking equipment and IMS inspection. A half-day program on sampling, handling, and interpretation of raw milk sample quality tests will be a major feature.

The annual meeting of the Pennsylvania Dairy Sanitarians Association will be held on Tuesday, June 11.

Pre-registration forms and housing information can be obtained by writing to the Agricultural Conference Coordinator, Room 410, J. O. Keller Building, The Pennsylvania State University, University Park, Pennsylvania 16802.

LETTER TO THE EXECUTIVE SECRETARY

Mr. H. L. Thomasson, Executive Secretary
International Association of Milk, Food,
and Environmental Sanitarians, Incorporated
Box 437
Shelbyville, Indiana 46176
Dear Red:

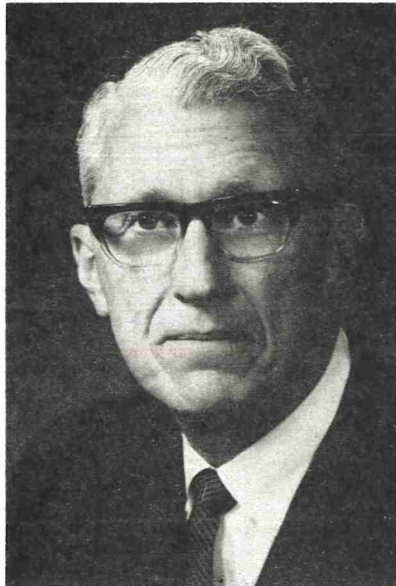
We are down to the final count in preparations for our annual meeting March 6 and 7. As you can see by the enclosed brochure, our Program Committee has once again come up with a timely and interesting group of speakers. We are making a concerted effort this year to attract new membership from those people in our state involved in laboratory activities, and this is reflected in the makeup of the program. I want to extend to you an invitation to be with us at our annual meeting. Your presence is always stimulating.

One of the accomplishments during the year of the IAMFES Section of the Virginia Association has been the organization of the Dairy Farm Methods Committee. I am enclosing a copy of the minutes from the organizational meeting. As you can see we have started very cautiously in this area with the idea of trying to cover one area well rather than a large number of areas poorly. As chairman of the Education and Publicity Subcommittee, I would appreciate your providing me with any information concerning the organization, objectives, and accomplishments of the International Dairy Farm Methods Committee. Any help which you can give in this area will be greatly appreciated.

Yours truly,
A. C. Holliday, President
Virginia Association of Sanitarians
and Dairy Fieldmen

NOMINATIONS FOR OFFICES OF IAMFES, INC.—1969-1970

FOR SECOND VICE-PRESIDENT AND SECRETARY-TREASURER



ELMER E. KILSTRUM

A native Minnesotan, Elmer E. Kihlstrum has been employed by Johnson & Johnson or one of their family of companies for over 30 years. These assignments have included Filter Products Division, Vetco and, at present, as sales manager, Dairy Department, Chicopee Mills, Inc., Non-Woven Division.

He has been a member of the International Association of Milk, Food & Environmental Sanitarians for 25 years. He has served on the Farm Methods Committee for the past 11 years and at present is chairman of subcommittee "Sediment in Fluid Milk."

Elmer is a member of Executive Committee, National Mastitis Council and served as 1966 program chairman. He is presently Chairman, N.M.C. State Council Coordination Committee.

He has served on several committees in Dairy & Food Industries Supply Association, including Milking Systems Task Committee. He is, also, a member of the American Dairy Science Association.

He and his wife, Jane, live in Western Springs, Illinois and have one son, John, a research biologist, who is married and has one son.

Parnell "Specs" Skulborstad, as he is known to his many friends, is Vice President of Sales and Marketing of Babson Bros., Co. "Specs" was born July 5, 1926 in Madison, Minnesota and joined the Navy upon graduation from high school in 1944. He



PARNELL SKULBORSTAD

served two years as a Pharmacist Mate 2nd Class. Upon being discharged from the Navy he entered the University of Minnesota, School of Business graduating in 1950 with a B.S. degree in accounting and economics.

"Specs" serves on the Board of Directors of the National Dairy Council and the National Mastitis Council he is a longtime member of IAMFES, and the American Management Association. He and his wife Elaine have three sons and live at 12 Hamill Lane, Clarendon Hills, Illinois.

SECRETARY-TREASURER

Roy B. Fairbanks is Milk Sanitation Survey Officer for the Division of Milk Control, Illinois Department of Public Health. Mr. Fairbanks attended public schools in Illinois and after one year in the University of Illinois, he began his first task with the dairy industry as a tester for the Dairy Herd Improvement Association.

He spent a number of years in the fluid milk and cheese industry in Illinois and Missouri. He has been in public health work on local and state level for the past 20 years. In 1948, he became Chief Milk Sanitarian for the City of Aurora, Illinois, until accepting a position as Milk Sanitarian for the Illinois Depart-



ROY B. FAIRBANKS

ment of Public Health in 1956. Since 1963, he has devoted full time to milk sanitation survey activities.

Mr. Fairbanks has been a member of the International since 1950 and is a past president of the Illinois affiliate, member of the Illinois Public Health Association and is a Registered Sanitarian with the Illinois Department of Registration and Education.

Mr. and Mrs. Fairbanks live at 21 Salmon Court, Springfield, Illinois. They have a married daughter, Carol, at Aurora, Illinois, and a son, Ronald, in his first year of graduate work at Berkley, California.

ELEVENTH ANNUAL MEETING ONTARIO MILK SANITARIANS, JANUARY 29, 1969 HOLIDAY INN, TORONTO, ONTARIO



J. L. Baker, Ontario Dept. of Agriculture and Food making presentation of Sanitarian of the Year Award to Les Forquharson. Les is a fieldman with Ontario Department of Agriculture and Food working out of Alliston. Dairy Princess is Beth Stansell.



Past Presidents Ontario Milk Sanitarians: Glen White, Herm Carruthers, Bill McCorquadale, Bill Lawrence, George Hazelwoll and Bob Sinclair.



Ontario Milk Sanitarians'—Holiday Inn. J. L. Baker, Dept. of Agriculture and Food; Tom Dickison, Borden Co.; J. C. Palmer, Dept. of Agriculture and Food; E. Sing, Mosley Laboratories, Beth Stansell, Dairy Princess, Dr. Arnott, Dept. of Food Science, Guelph; and Ray Bowles, President Ontario Milk Sanitarians.

SEVENTY-THIRD ANNUAL CONFERENCE OF THE ASSOCIATION OF FOOD AND DRUG OFFICIALS OF THE UNITED STATES

The 73rd Annual Conference of the Association of Food and Drug Officials of the United States (AFDOUS) will be held June 15-19, 1969, at the Eden Roc Hotel, 4525 Collins Avenue, Miami Beach, Florida.

The Conference will have under consideration uniform rules and standards affecting foods, drugs, cosmetics, devices and hazardous substances which will promote consumer protection.

For detailed information contact Mr. Eaton E. Smith, President, 153A State Office Building, 165 Capitol Avenue, Hartford, Connecticut 06115; Mr. Evan Wright, Secretary-Treasurer, State Health Department, Box 1494, State Office Building, Topeka, Kansas 66603; or Mr. Vincent Giglio, Local Arrangements and Program Chairman, Room 235, Mayo Building, Tallahassee, Florida 32304.

NEWS AND EVENTS

NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS

Plans for the 1969 meeting of the NCIMS to be held at the New Albany Hotel, Denver, Colorado, May 26-29 have been finalized. Earle Wright, Chairman of the Program Committee, will have the programs in the mail early in March. Registration fee for the Conference has been set at \$15.00 by the Executive Board.

Hotel reservations

The management of the New Albany Hotel has guaranteed a rate of \$10 for a single room and \$16 (for 2) for a twin-bedded room. The hotel management has asked me to emphasize that this flat rate includes all single rooms which normally sell from \$10 to \$18 and all twin-bedded rooms which normally sell from \$14 to \$22. In other words, some will be assigned to much more elaborate rooms than others, based entirely on the "luck of the draw". The hotel room reservation card will be included with each program when it is mailed. In order for individuals to obtain the special rates quoted above, reservations must be received by the hotel no later than May 9, 1969.

Ladies Activities

Harold Barnum and his Local Arrangements Committee have provided several activities which will occupy the ladies while the men are participating in program deliberations. On Monday, May 26, two tours have been scheduled, one to the famous Red Rocks Theatre and the other to the Museum of Natural History. The Red Rocks Theatre represents one of nature's most magnificent natural theatre settings while the Museum of Natural History has one of the most outstanding displays of animals, birds in realistic habitats. A special ladies luncheon will be held at the famous Laffitte Restaurant on Tuesday, May 27. This will be followed by a tour through famous Larimer Square which represents a private renewal program of an area that was once the most famous street in the frontier West.

Wednesday afternoon, May 28 will be devoted to relaxation and entertainment for both men and women. It will involve a tour of the Air Force Academy at Colorado Springs and a Chuckwagon Dinner at the Garden of the Gods. Bus fare for the complete trip is \$2.75 per person and a genuine Chuckwagon Dinner will cost \$5.00 for an adult and \$2.50 for children under 9 years of age. Persons may participate in the Chuckwagon Dinner without taking the

Air Force Academy tour. The Chuckwagon Dinner will be followed by genuine old-fashioned western entertainment.

The ladies can see from the above outline that the Local Committee has pulled out all stops in an attempt to make your stay in Denver most memorable.

NATIONAL RESTAURANT ASSOCIATION TRAINING PROGRAM FOR FOOD SERVICE EMPLOYEES

A new training program for food service employees has just been completed by the National Restaurant Association. The program called "Protecting The Public," uses sound-film-strips to explain the "why" and "how" of sanitation in food service. The program is designed for use by restaurants, public health agencies, and schools. It was developed by the NRA's Education and Public Health and Safety Committees with the assistance of the U. S. Public Health Service, state and local health officials, educators and food service operators.

"Safe and sanitary food service requires the efforts of individual employees as well as management," said NRA President Page Yaw. "Through this training program," he said, "the high motivation towards sanitation should be reinforced, and the latest safe techniques for food handling can be communicated to the widest possible audience. The entire food service industry should make use of this program."

The training program includes three 10-minute sound-filmstrips, which provide an entertaining and convenient-to-use treatment of food service sanitation, aimed at employees such as waiters and kitchen workers as well as supervisory and management personnel. The basic purpose of the program is to impress on each individual connected with a food service operation the fact that he holds the key to protecting the public. The program was produced for the NRA by Norm Pierce and Associates, a leading audio-visual training firm. The sound-filmstrips in the "Protecting The Public" series include:

Part one—*The Personal Side*: This filmstrip emphasizes the individual employee's role in safeguarding food through good personal hygiene. It emphasizes the sanitary practices every employee should follow during work and before coming to work, and it illustrates the way contamination can be spread by one person.

Part two—*Food Protection*: Rules for safe food

handling through sanitary cooking, re-heating, serving, and storage are explained in this filmstrip. The way time and temperature affect germ growth is explained simply but thoroughly, as are the rules employees should follow.

Part three—*Establishment and Equipment Sanitation*: The sanitary care of tableware, utensils, and other food service equipment is illustrated with special emphasis being given to the protection of food contact surfaces. The difference between cleaning and sanitizing is explained.

The set of three full-color sound-filmstrips is available from the Educational Materials Center of the NRA, 1530 North Lake Shore Drive, Chicago, Illinois 60610. Cost of each set is \$27.50.

SHORT COURSE IN MANUFACTURING PRACTICES AND SANITATION

September 15-18, 1969—Short Course in Manufacturing Practices and Sanitation, University of Florida, Gainesville, Florida 32601. Sponsored by Florida Section IFT and Florida Agricultural Extension Service. Fee—\$30.00. For further information, write Dr. R. F. Matthews, Department of Food Science, University of Florida, Gainesville, Florida 32601.

TRAINING PROGRAM IN PRINCIPLES AND TECHNIQUES OF OBTAINING PUBLIC SUPPORT

The creation of lasting improvement in any environmental health condition requires support from the people. State and local health administrators, heads of programs, project managers and environmental health professionals generally, as well as city, county and state officials, need public support if they are to provide health services needed by the people they serve.

The Cincinnati Training Program of the Environmental Control Administration, U. S. Public Health Service, will present the principles and techniques of obtaining public support in the course *Generating Community Action for Environmental Health* during the week April 21-25, 1969, in Cincinnati, Ohio.

The course will present an understanding of the use of public education to generate support from the people. The course will also present ways to guide public groups seeking solutions to health problems. Topics to be discussed during the course will include proper attitudes toward the public, making the health problem visible to the people, presenting the health message to public groups, and utilizing newspapers, radio and television to reach the public.

Application for the course may be made by writ-

ing Chief, Cincinnati Training Program, Environmental Control Administration, 222 E. Central Parkway, Cincinnati, Ohio 45202.

The telephone number for registrations is (513) 871-1820, extension 298. There is no tuition or registration fee. Trainees are expected to provide for their own housing and transportation while attending the course.

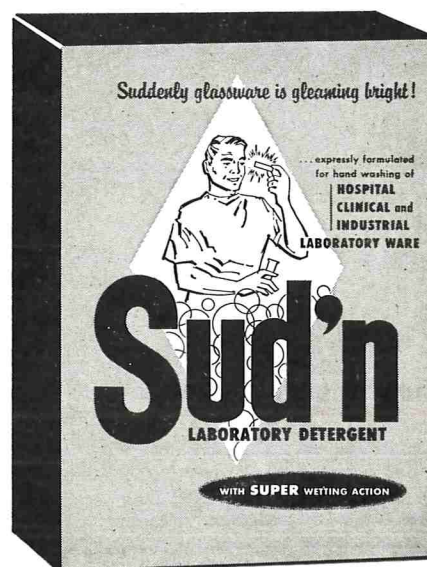
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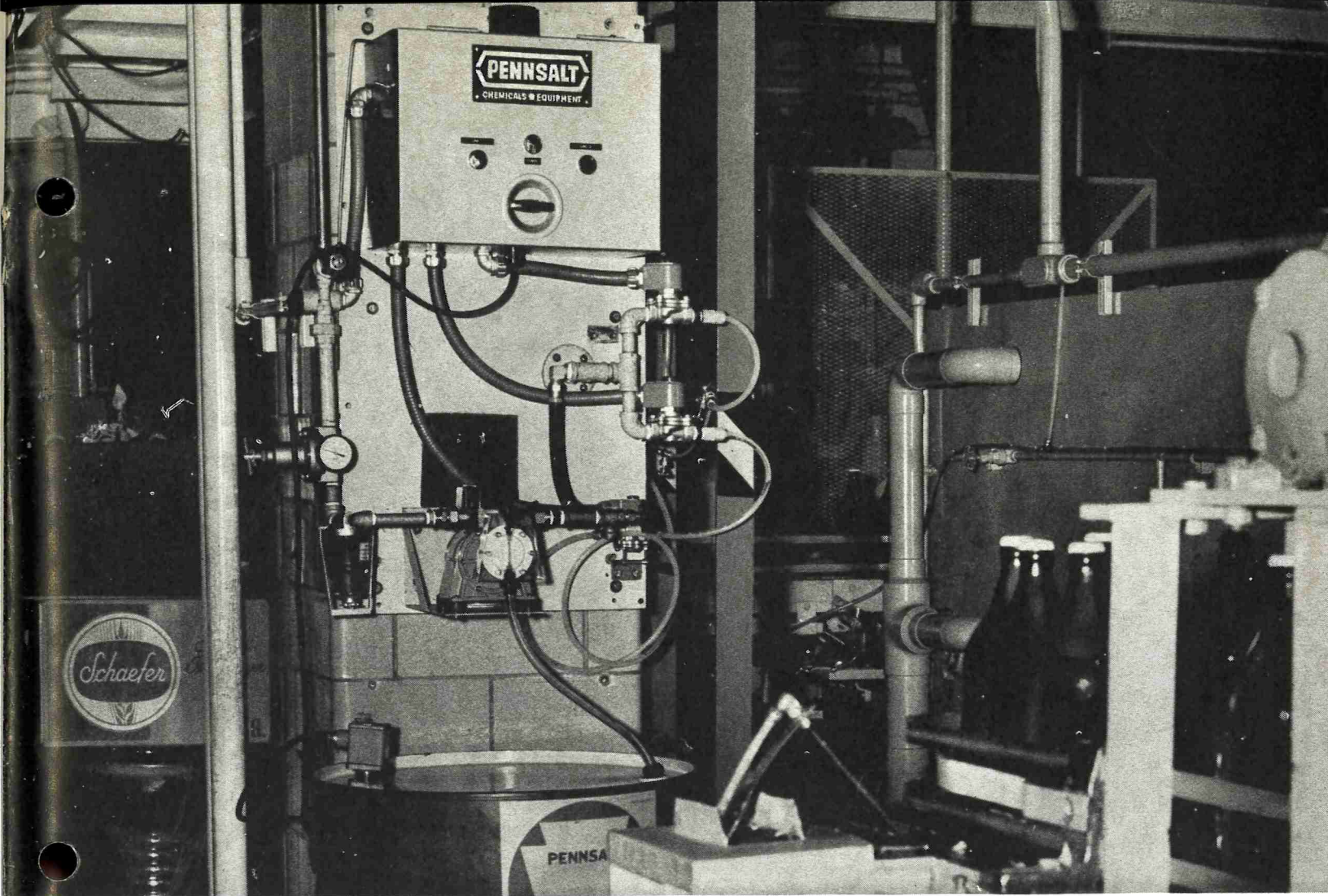
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