FEBRUARY, 1975
Vol. 38, No. 2
Pages 61-124
JMFTA 38(2):61-124 (1975)

Journal of Milk and Food Technology

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ROYAL YORK HOTEL
AUGUST 10-13, 1975
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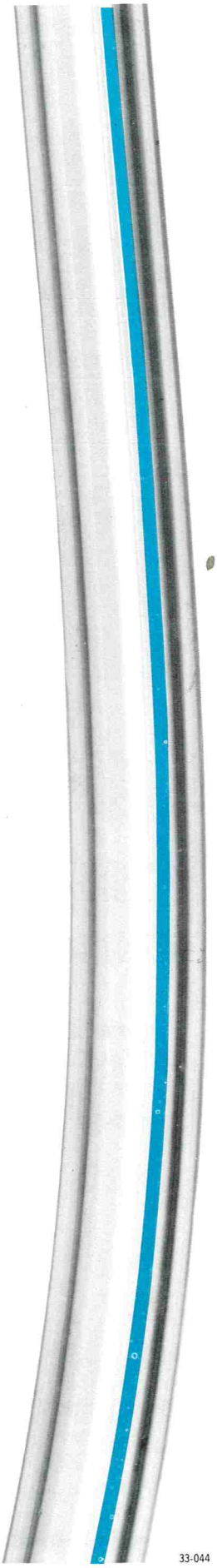

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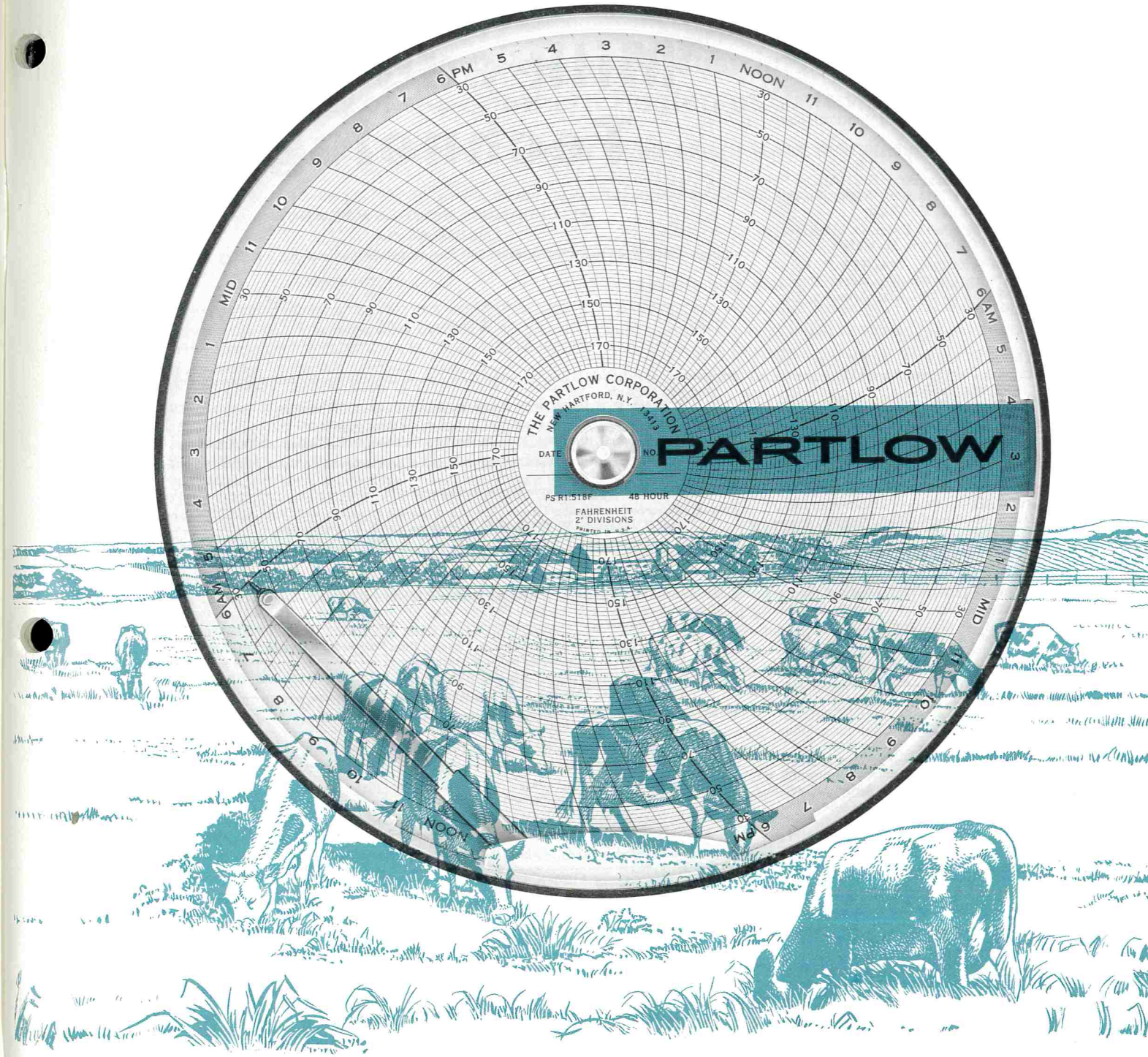

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2nd Class postage paid at Ames, Ia. 50010.

Editorial Offices: Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 52706. Earl O. Wright, P.O. Box 701, Ames, Ia. 50010.

Journal of Milk and Food Technology

INCLUDING MILK AND FOOD SANITATION

Official Publication,

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

Vol. 38 February 1975 No. 2

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

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J. Milk Food Technol. Vol. 38, No. 2, Pages 65-72 (February, 1975)

Organochlorine Insecticides and Industrial Pollutants in the Milk Supply of the Southern Region of Ontario, Canada

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(Received for publication June 27, 1974)

ABSTRACT

A province-wide survey conducted between 1967 and 1969 revealed that milk produced in the Southern region had the highest residues of organochlorine insecticides in Ontario. It was in this region where 90% of the organochlorine insecticides purchased in the province were applied. In 1969-70 restrictions were placed on DDT and the cyclodiene insecticides. Disappearance of these organochlorine insecticides from milk were studied in surveys conducted in 1970-71 and 1973. The mean ΣDDT levels declined from 0.20 ppm in 1967 to 0.05 ppm in 1973, whereas the mean dieldrin residues declined from 0.044 ppm to 0.015 ppm in the same period. These data include counties where there had been only small reductions in the use of DDT and where milk residues remained unchanged until further restrictions were effected in 1971. Residues of lindane and heptachlor epoxide were virtually absent from milk fat. Chlordane, endosulfan, methoxychlor and endrin were absent from all samples in spite of increasing quantities being used of the first two insecticides. Residues of polychlorinated biphenyls appeared slightly higher in the 1973 survey than in the 1970-71 survey. Hexachlorobenzene was present at very low levels in the 1973 survey.

Legislation by the Province of Ontario halted the use of the cyclodiene insecticides aldrin, dieldrin, and heptachlor following the 1969 season and severely restricted use of DDT and TDE in 1970. The legislation on cyclodienes was based partly on the 1967-69 milk survey by Frank, Braun, and McWade (*9*), which revealed that no less than 17 dairy herds in the Province had dieldrin residues that were in violation of the Food and Drug Act and Regulations. At that time 1% of milk tankers in the province had residues at or above 0.1 ppm dieldrin. The western and southern region had, respectively, 1.9% and 1.5% of bulk tankers with residues in violation. Residues of heptachlor epoxide occurred in only the western region giving only 0.1% of the milk supply in the province with residues at or above 0.1 ppm.

In the survey of 1967-69 only three herds were discovered with residues of DDT or TDE above 1.0 ppm. On a Provincial basis, only 0.1% of samples had residues of DDE and DDT over 0.4 ppm and only 0.2% over 0.4 ppm TDE. The residues found in the 1967-69 survey were comparable to residues found previously in U.S. milk supples (*2,3*). While the use of cyclodiene insecticides was brought to a virtual halt, the use of DDT was permitted in the production of tobacco. Applied to rye before plow down or to soil after plow down, the DDT was used to control the dark sided cutworm (*Euxoma messoria*). By 1973 this use was replaced by leptophos and chlorpyriphos.

Three minor uses for DDT were retained in 1970, however, one was cancelled in 1971 (cutworm control on vegetables in muck soils), a second was terminated in 1973 (plant bug control on apples at pre bloom), and a third has been retained (as a tracking powder for the control of bats in structures). Since 90% of the aldrin, dieldrin, DDT, and TDE was used in the Southern region, a resurvey of milk residues in this region could best measure the effects of the legislative restrictions. This was undertaken in 1970-71 and 1973.

MATERIALS AND METHODS

Sampling procedure

All milk from the 11 counties of the southern region of Ontario was monitored in four surveys done in 1967, 1968-69, 1970-71, and 1973, by respectively collecting one liter samples in the field from 284, 374, 337, and 350 bulk tankers. Each bulk tanker represented 2 days milk supply from 5-20 producers. All composite samples were collected by personnel of the Milk Industry Branch, Ontario Ministry of Agriculture and Food, and delivered promptly to the Provincial Pesticide Residue Testing Laboratory of the same ministry.

Analytical procedures

The butterfat was removed immediately upon receipt at the laboratory, according to the procedure described by Moubry, Myrdal, and Jensen (*15*). Approximately 100 ml of milk were transferred to a volumetric flask (200 ml) and, with constant mixing, the flask was filled to the neck with a detergent reagent consisting of 50 g sodium tetraphosphate plus 24 ml of Triton X-100 per liter of water. This mixture was placed in a water bath of 95 C until the clear butterfat layer separated into the neck of the flask. The butterfat was transferred into vials and held under refrigeration for subsequent analysis.

Chlorinated hydrocarbon insecticides were isolated from the butterfat by the Florisil column method described by Langlois et al. (*13*) with some modification. Florisil, 60/100 mesh was heated at 135 C for a minimum of 24 h. Upon cooling to room temperature, the adsorbent was partially deactivated by addition of water at the rate of 5 ml per 100 g Florisil, and tumbled for a 12-h period. Cleanup was carried out in 25 mm × 300 mm Pyrex columns fitted with Teflon stopcocks and 350-ml glass reservoirs.

Butterfat was fluidized by placing in an oven at 100 C and 1.0 g was transferred with a dropping tube to 25 g of conditioned Florisil and mixed thoroughly until a free-flowing powder was obtained. Twenty-five grams of deactivated Florisil were poured into a chromatographic column to form the bottom half of the cleanup system. This was prewashed with 50 ml of a 1:1 mixture of dichloromethane and hexane. The butterfat-Florisil mixture was then introduced to form the top layer. The column was eluted successively with: (*a*) 300 ml of the 1:4 dichloromethane:hexane elution mixture (v/v) for recovery of DDT and its metabolites, lindane, cyclodienes, and PCB's and (*b*) 100 ml of 0.35:50:50-acetonitrile:dichloromethane:hexane for recovery of methoxychlor and endosulfan sulfate; this latter

fraction was further cleaned up using an acetonitrile saturated with hexane to hexane (4:1) for partitioning to remove co-eluted fat (*10*). Eluates from (*a*) and (*b*) were concentrated just to dryness with rotary vacuum at 45 C and re-constituted in 5.0 ml acetone for (*a*) and hexane for (*b*).

The charcoal column procedure as described by Berg et al. (*1*) with modifications was used to separate chlorinated hydrocarbon pesticides from PCB's and HCB. Charcoal (50-200 mesh, Fisher No. 5-690) was washed with acetone, filtered with suction, and the absorbent was dried and stored at 135 C. Columns used were 9 × 250 mm Pyrex fitted with Teflon stopcocks and solvent reservoirs. The columns were prepared as follows: glass wool plug, 3 inches of washed charcoal, sandwiched between ½-inch layers of Ottawa sand. The column was pre-wetted with 1:3 diethyl ether:acetone (v/v). Four milliliters of the samples in acetone solution were introduced to the column. The samples were successively eluted with 180 ml 1:3 diethyl ether:acetone, 80 ml benzene, and 100 ml of toluene at percolation rates of approximately 5 ml/min. The eluates were concentrated just to dryness with rotary vacuum evaporation at 45 C and residues were re-dissolved in 4 ml of hexane. The diethyl ether-acetone fraction contained the organochlorine insecticides, the benzene fraction contained the PCB's and the toluene fraction contained hexachlorobenzene as described by Holdrinet (*12*).

Analyses were done with a Varian Model 204 gas chromatograph equipped with a 250 mc tritium electron capture detector. Operating conditions were as follows: (*a*) column: 150 cm × 3 mm o.d. Pyrex packed with 4% SE-30 plus 6% QF-1 on Chromosorb W, pre-conditioned 72 h at 225 C (*13*); (*b*) temperature: column - 175 C, detector - 200 C, injection block - 225 C; and (*c*) carrier gas: nitrogen at 60 ml/min.

Injection volumes of 5 μl were used for both sample solutions and comparison standards. When necessary, sample solutions were diluted until peak heights were within the linear range of the detector. Because all analyses were done at isothermal and isobaric conditions, peak heights alone were used for quantitation.

Where possible, pesticides, PCB, and HCB levels were confirmed by thin layer chromatography. This was done using glass plates with a 250μm silica gel layer developed with 1% chloroform in *n*-heptane. The chromatograms were outlined with alcoholic $AgNO_3$ spray. Alternatively *p,p'*-TDE and *p,p'*-DDT were confirmed by treatment with 5% methanolic KOH to produce the corresponding dehydrochlorinated derivatives. These were re-identified by gas chromatography. HCB was confirmed by hydrolysis and methylation to the methyl ether of pentachlorophenol as described by Holdrinet (*12*).

Recoveries of pesticides, PCB (Arochlor 1254) and HCB were checked periodically by fortification directly into butterfat. Averaged recoveries were as follows: *p,p'*-DDE, 98%, *p,p'*-TDE, 95%; *p,p'*-DDT, 90%; dieldrin, 89%; methoxychlor, 84%; endosulfan sulfate, 90%; endrin, 83%; Aroclor 1254, 85-90% and HCB, 79-81%. Separation of PCB's from HCB and DDT and dieldrin was 90% effective. The data do not include corrections for percent recovery. PCB's were measured quantitatively by comparing the sum of the peak heights of peaks VII, VIII, and X as by the Reynolds numbering system (*16*), with the sum of the corresponding peaks of an Aroclor 1254 mixture.

RESULTS

Agricultural production

The southern region of Ontario comprises the most intensively farmed region of the Province. Here, high value cash crops are produced, along with considerable quantities of beef and milk. Between 1967 and 1973 the annual milk production of this region represented 15 to 20% of the total annual production in the Province. In 1967, when the first pesticide residue survey was undertaken, 385 million kg of fluid milk were produced. This rose to 661 million kg by the time of the 1973 residue survey (Table 1). Oxford being the leading dairy county of the region produced between 18-20% of the regional supply. Middlesex (13-16%), Niagara (8-14%), and Haldimand (7-11%) are other important dairy counties (Fig. 1).

TABLE 1. *Production of milk by county in the southern region during the survey years (1967-1973)*

County	Mean annual production of fluid milk (× 1000 kg)			
	1967[1]	1968-69[2]	1970-71[2]	1973
Brant	27,710	27,807	33,624	34,027
Elgin	20,590	25,698	37,915	40,890
Essex	35,110	32,350	26,157	21,795
Haldimand	42,270	42,349	49,360	49,024
Kent	8,210	7,790	7,282	7,072
Lambton	17,010	20,372	28,821	32,449
Middlesex	49,990	53,583	85,147	92,771
Niagara	54,930	56,388	52,531	50,689
Norfolk	15,700	16,453	19,213	18,537
Oxford	69,940	78,772	155,810	165,250
Wentworth	43,730	41,157	39,797	36,305
Southern Region	385,190	402,519	535,657	660,559

[1]1967 production of milk by county was estimated from Agricultural Statistics for Ontario 1967, Publication 20. Ministry of Agriculture & Food.

[2]1968-73 production of milk by county was obtained from Record of the Ontario Milk Marketing Board, 50 Maitland Street, Toronto 5, Ontario.

Corn production predominated in Elgin, Kent, Lambton, Middlesex, and Oxford and it was in these counties where the use of aldrin was greatest. Essex and Kent are prime areas for processing vegetables and Niagara and Wentworth are main fruit growing areas and both aldrin and DDT were widely used. Norfolk County was the main tobacco county and here the largest volume of DDT was used.

Pesticide use

In 1968-69, 88% of the DDT and 89% of the aldrin and dieldrin sold in the Province of Ontario were used in the Southern region. In 1968 and 1969, before any restrictions had been applied, just over 310,000 kg of DDT were used in this region. The major portion of this DDT was employed in tobacco production centered in Norfolk County, with smaller areas in the surrounding counties of Brant, Elgin, and Oxford (Table 2). From 1961 onwards, cutworms attacking tobacco exhibited an increasing resistance to the cyclodiene insecticides, and DDT was increasingly used for this purpose. During the mid and late sixties DDT became the major insecticide for the control of the darksided cutworm (*Euxoma messoria*) in tobacco (*11*).

In 1970 restrictions were placed on use of DDT and permits were granted for one major and three minor uses. The major use was for the control of cutworm in tobacco at the reduced rate of around 1.5 kg per hectare. The minor uses included the control of cutworms in muck soils (mainly Essex and Kent), the control of the tarnished plant bug (*Lugus lineolaris*) on apples (mainly Niagara and Essex) and the control of bats in urban centers. In 1970-71 almost 140,000 kg of DDT were used annually for the above mentioned permit uses. During this period leptophos and chlorpyriphos were developed

Figure 1. *Map of Southern Ontario showing the eleven south regional counties.*

TABLE 2. *Reported amounts of DDT and dieldrin sold in counties of southern region of Ontario.*

County	Total sales of DDT (Kg)			Sales of aldrin & dieldrin (Kg)[1]
	1968 & '69	1970 & '71	1972 & '73	1968-69
Brant	27,000	16,376	—	13,576
Elgin	46,260	26,860	112	153,140
Essex	6,150	1,948	135	263,540
Haldimand	200	—	—	133
Kent	1,613	1,427	15	266,640
Lambton	938	697	25	175,840
Middlesex	9,302	8,121	—	252,040
Niagara-N.&S.	13,211	601	85	14,494
Norfolk	171,510	68,764	41	3,192
Oxford	31,880	13,622	185	184,760
Wentworth	2,939	1,416	29	6,521
Southern region	311,003	139,832	627	1,333,876
(Percent of Provincial use)	(88.0%)	(89.8%)	(40.1%)	(89.3%)

[1]Sales of dieldrin in 1968 & 1969 represented only 0.01% of the total sales.

for cutworm control of cutworm in tobacco and in 1972 they were recommended as replacements for DDT and no further permits were issued for DDT.

In 1972 and 1973 permits were issued for the two minor uses remaining, namely plant bug control in apples and bat control in urban structures, and only 627 kg were used annually. During this period dimethoate was developed to replace DDT for the control of plant bugs in the 1974 season.

Large quantities of aldrin were used in the corn producing counties of Elgin, Kent, Lambton, Middlesex, and Oxford and in the vegetable producing county of Essex until 1969. Aldrin, Dieldrin, and heptachlor were severely restricted following the 1969 growing season and only one minor use was retained into the 1970 season, this being the control of termites in urban structures. Chlordane and a number of organophosphorus insecticides were increasingly used from 1970 onwards to control the northern corn rootworm (*Diabrotica longicornis*) and soil borne insects (Table 3). Chlordane

TABLE 3. *Sale of chlordane, endrin, and lindane in the southern region.*

Insecticide	1968-69	1970-71	1972-73
Chlordane	1,507	25,400	37,300
(Percent of Provincial sales)	(22%)	(41%)	(39%)
Endrin	1,477	1,385	25
(Percent of Provincial sales)	(74%)	(48%)	(15%)
Lindane	776	859	1,300
(Percent of Provincial sales)	(40%)	(38%)	(46%)

use rose from 1500 kg annually in 1969 to 37,000 kg in 1972 and 1973.

Many label uses for endrin were cancelled between 1971 and 1972 especially for tobacco and potato production. By 1972-73 only 25 kg were used in the

TABLE 4. *Residues of DDT and its metabolites in butterfat or milk for the southern region*

	Weighted mean in butterfat (ppm) & Percent metabolites DDE/TDE							
	1967		1968-69		1970-71		1973	
	ΣDDT	DDE & TDE	ΣDDT	DDE & TDE	ΣDDT	DDE & TDE	ΣDDT	DDE & TDE
Brant	0.29	51	0.29	84	0.20	87	0.09	95
Elgin	0.19	86	0.21	82	0.11	76	0.05	100
Essex	0.26	60	0.26	74	0.09	77	0.04	94
Haldimand	0.18	63	0.17	86	0.15	81	0.04	95
Kent	0.23	56	0.23	60	0.05	81	0.02	95
Lambton	0.11	72	0.10	72	0.06	65	0.01	100
Middlesex	0.12	78	0.13	82	0.08	70	0.03	100
Niagara	0.25	60	0.19	80	0.15	83	0.06	96
Norfolk	0.36	64	0.29	51	0.28	87	0.09	100
Oxford	0.16	78	0.16	78	0.10	78	0.06	98
Wentworth	0.19	72	0.18	82	0.16	82	0.07	99
Southern region	0.20	67	0.19	80	0.12	80	0.05	98

region (Table 3). Use of lindane doubled between 1968 and 1973 while its use around milk and milk rooms were cancelled. Use of endosulfan rose markedly following restrictions on DDT and dieldrin, but comparative sales figures were not available.

Insecticide residue in milk

Dieldrin, and DDT and its metabolites appeared in all milk supplies of the region, heptachlor epoxide and lindane occurred occasionally while residues of *a* and *γ* chlordane, endrin, endosulfan and its sulfate, and methoxychlor were not detected.

DDT residues

Before the 1970 restrictions the mean residues of ΣDDT in butterfat for the southern region were 0.20 ppm in 1967 and 0.19 ppm in 1968-69. Following the restrictions residues of ΣDDT in milk fat declines to 0.12 ppm in 1970-71 and 0.05 ppm in 1973 (Table 4). A rough correlation was noted between the sales of DDT per county and the ΣDDT residue in the butterfat produced in these counties. The largest quantities of DDT were applied in Norfolk County and here the highest ΣDDT residues were observed (Tables 2 and 4). Conversely, the county using one of the lowest quantities of DDT, namely Lambton, had milk fat that had the lowest residues of ΣDDT. Following the restrictions of 1970, residues in milk fat, showed a marked decline in the 1970-71 survey. Only milk from Norfolk County exhibited little or no

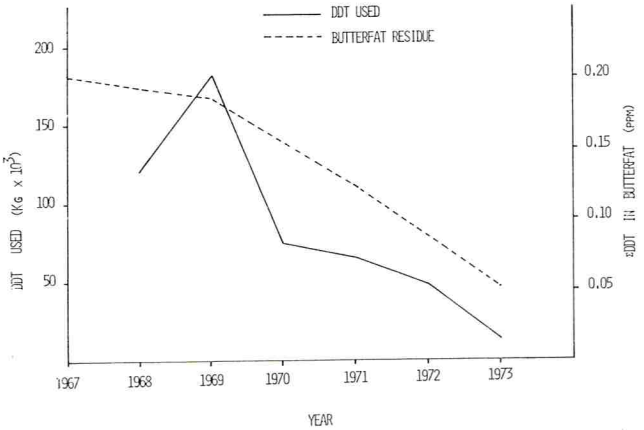

Figure 2. *Correlation between DDT used in the southern region and the average concentration of ΣDDT in butterfat.*

change and here the quantity of DDT applied was still considerable. With the termination of DDT use in tobacco after the 1971 crop season residues in milk from that county declined markedly as observed in the 1973 survey. By 1973 the residues of ΣDDT had declined to almost one quarter of that level before the restrictions were imposed. A correlation was observed between the quantity of DDT used in the region and the mean residues found in the milk fat (Fig. 2).

In 1967 the two metabolites DDE and TDE represented between 51 to 86% of the ΣDDT with a mean of 67% (Table 4). The percentage of ΣDDT present as metabolites slowly increased to 94 to 100% by 1973 with a mean of 98%. The actual quanities of DDT present in the milk supply of the region declined from about 3 kg in 1967 to 1.1 kg in 1973 while the total milk supply rose by 71% (Fig. 3).

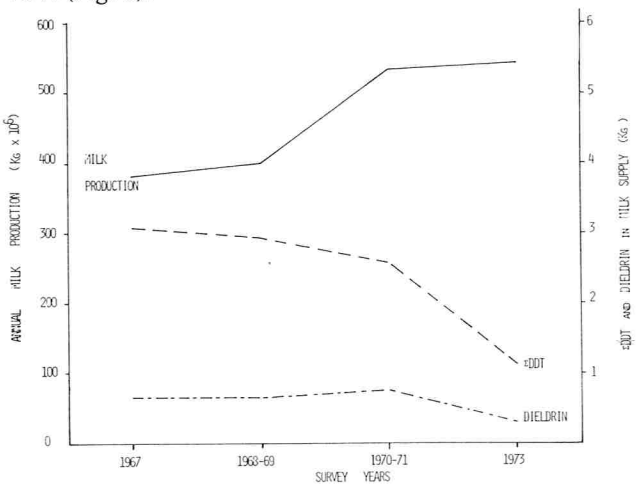

Figure 3. *The decline in the actual quantity of ΣDDT and dieldrin present in milk following the legislative restrictions in 1969-70 inspite of an increase in actual milk volume.*

Before the restrictions only 1.3% of bulk tankers had ΣDDT residues below 0.101 ppm (Table 4). At that time no bulk tanker from Norfolk had residues below this level. By 1973 94% of the bulk tankers in the region had residues below 0.101 while in Norfolk 71% were below this residue level. During the 1967 to 1969 surveys, 2% of the bulk tankers of the region had ΣDDT residues of over 0.5 ppm in the butterfat. These were found in Brant,

Essex, and Norfolk where respectively 10%, 4%, and 11% of bulk tankers contained these concentrations. By the 1970-71 survey there were no bulk tankers with residues above these concentration levels (Fig. 4).

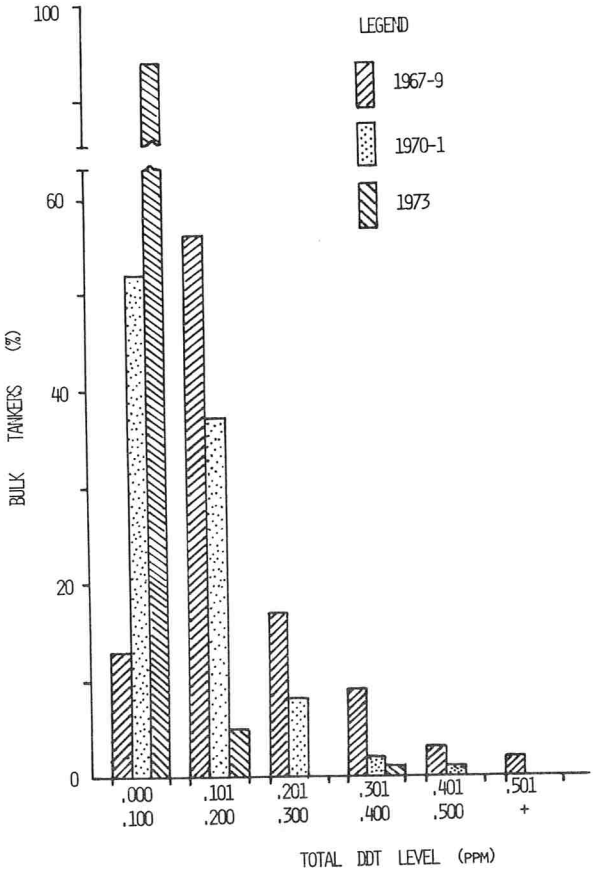

Figure 4. *Distribution of ΣDDT residues in butterfat by bulk tankers in the surveys before and after legislative restrictions in 1970.*

Dieldrin residues

Residues of dieldrin in milk fat from the southern region of Ontario averaged 0.44 and 0.41 ppm during the 1967 and 1968-69 surveys respectively (Table 5). Following the restrictions of late 1969, residues declined to 0.035 ppm in the 1970-71 survey and 0.015 ppm in the 1973 survey.

TABLE 5. *Residues of dieldrin in butterfat of milk in the southern region of Ontario.*

County	Weighted mean content in butterfat (ppm)			
	1967	1968-1969	1970-71	1973
Brant	0.053	0.070	0.041	0.019
Elgin	0.062	0.058	0.033	0.018
Essex	0.051	0.037	0.048	0.019
Haldimand	0.047	0.038	0.035	0.017
Kent	0.062	0.051	0.040	0.013
Lambton	0.032	0.033	0.034	0.009
Middlesex	0.044	0.040	0.029	0.012
Niagara	0.030	0.029	0.038	0.012
Norfolk	0.071	0.051	0.058	0.024
Oxford	0.043	0.041	0.030	0.016
Wentworth	0.031	0.031	0.036	0.013
Southern region	0.044	0.041	0.035	0.015

In 1967, the milk fat from five counties (Brant, Elgin, Essex, Kent and Norfolk) had mean residues above 0.05 ppm. The number dropped to four counties in the 1968-69 survey and then to only one in the 1970-71 survey. By the 1973 survey the highest mean residue in any county was 0.024 ppm. Among the five counties involved Elgin, Essex and Kent were areas of high use of aldrin during 1968 and 1969 (Table 2).

The quantity of aldrin and dieldrin used in Brant and Norfolk during the 1968-69 period was among the lowest of the region, however, in the early and mid sixties aldrin was used in large quantity for the control of cutworm. Lambton, Middlesex, and Oxford were counties with large corn acreages and numerous dairy farms using considerable amounts of aldrin, however, residues of dieldrin in the butterfat were among the lowest for the region.

In the two surveys done before the restriction on aldrin and dieldrin it was found that 37% and 31% of bulk tankers contained milk fat with residue levels of dieldrin above 0.05 ppm. Following the restriction, the number of tankers in this category fell to 14% at the time of the 1970-71 survey and only 0.6% by the 1973 survey. In the 1967 to 1969 surveys 10% of bulk tankers had residues below 0.02 ppm dieldrin. This rose to 13% in the 1970-71 survey and only 0.6% by the 1973 survey.

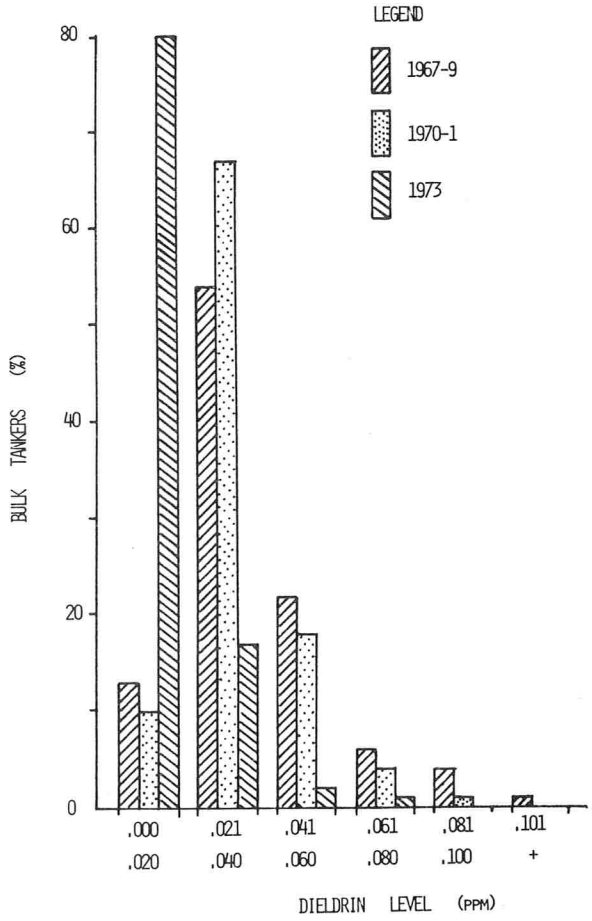

Figure 5. *Distribution of dieldrin residues in butterfat by bulk tankers in the surveys before and after legislative restrictions in 1969.*

1969 surveys 10% of bulk tankers had residues below 0.02 ppm dieldrin. This rose to 13% in the 1970-71 survey and to 80% in the 1973 survey (Fig. 5). There appeared to be a lag of up to two years before a marked decline in residues of dieldrin were observed in milk following its restriction in the field.

The actual quantity of dieldrin resident in the milk supply for the four surveys was 0.68, 0.66, 0.74, and 0.33 kg respectively in the four surveys (Fig. 3). While residues declined slowly after the restriction, the production of milk more than compensated for this decline, hence the actual quantity present in the 1970-71 milk supply was greater than in the previous survey years. The largest milk producing county of Oxford contained almost one quarter of this total amount.

Heptachlor epoxide residues

Heptachlor epoxide was found in milk from only two counties, namely Lambton and Middlesex (Table 6). Residues were extremely low and only occurred in surveys before the restrictions on heptachlor were imposed in late 1969. The use of heptachlor in these counties was largely confined to the treatment of soil insects in the production of rutabagas and the treatment of seed grains. Heptachlor epoxide was detected in only 3.5% of the bulk tankers sampled. Following the restrictions no further residues were detected.

TABLE 6. *Residues of other organochlorine insecticides in the butterfat of milk from the southern region of Ontario.*

County	Weighted mean content in butterfat (ppm)[1]			
	1967	1968-69		1970-71
	Heptachlor epoxide	Heptachlor epoxide	Lindane	Lindane
Brant	ND[2]	ND	ND	0.003
Lambton	0.006	0.005	0.005	ND
Middlesex	0.003	0.002	0.005	ND
Niagara-N.&S.	ND	ND	ND	0.002
Other counties	ND	ND	ND	ND
Southern region	Trace	Trace	Trace	Trace

[1]Analyses also included endrin and methoxychlor (1967-71); the 1973 survey also included chlordane and endosulfan sulfate.
[2]ND = Not detected

Chlordane residues

Chlordane, which came into general use following the restrictions on aldrin, dieldrin, and heptachlor, was not detected in milk fat in any bulk tanker samples analysed in the 1973 survey (Table 3 and 6). Chlordane which contains varying amounts of heptachlor failed to turn up in milk fat as heptachlor epoxide.

Lindane residues

Lindane, while being absent in the 1967 survey, appeared in milk fat from Lambton and Middlesex in the 1968-69 survey and Brant and Niagara in the 1970-71 survey. Residues were extremely low (Table 6). In 1968-69 3.5% of bulk tankers sampled had measurable levels of lindane. By the 1970-71 survey there were only 1.8% of bulk tanker samples with measurable residues of lindane. These residues were associated with the use of lindane around dairy animals or milk handling.

Endosulfan residues

With the restrictions on aldrin, dieldrin, DDT, and heptachlor the use of endosulfan increased markedly in tobacco and vegetable areas of the region. During 1973 a special effort was made to check for endosulfan and its sulfate. However in no instance was this insecticide detected.

Methoxychlor residues

In all four surveys the presence for methoxychlor was checked, but no residues were detected.

Industrial pollutants in milk

Polychlorinated biphenyls (PCB) were detected and measured in the 1970-71 and 1973 surveys and hexachlorobenzene (HCB) was detected and measured in the 1973 survey.

PCB residues

The 1970-71 survey was the first survey in which PCB's were satisfactorily isolated and measured. In the 1967 and 1968-69 survey PCB's were imperfectly isolated and measured in milk fat. In 1970-71 PCB residues in milk fat averaged 0.08 ppm for the region but varied from 0.03 ppm in Oxford to 0.15 ppm in Wentworth. It is note-worthy that heavy industrial centers are associated with Brant and Wentworth counties where the highest residues were observed. Residues in milk from Oxford, Elgin, Kent and Middlesex were lower and these areas are further from the large industrial centers (Table 7).

TABLE 7. *Residues of industrial pollutants in milk fat of the southern region.*

County	Content in butterfat (ppm)				
	1970-1971	1973		1970-71	1973
	PCB	PCB	HCB	Ratio DDT/PCB	
Brant	0.14	0.11	0.011	1.39	0.83
Elgin	0.07	0.07	0.006	1.60	0.76
Essex	0.10	0.17	ND	0.80	0.21
Haldimand	0.12	0.14	ND	1.26	0.29
Kent	0.07	0.13	0.007	0.67	0.17
Lambton	0.08	0.13	0.008	0.71	0.10
Middlesex	0.06	0.09	0.008	1.40	0.36
Niagara	0.10	0.15	0.003	1.46	0.37
Norfolk	0.12	0.08	0.005	2.36	1.18
Oxford	0.03	0.09	0.009	3.37	0.67
Wentworth	0.15	0.10	0.011	1.05	0.70
Southern region	0.08	0.11	0.007	1.53	0.47
			Annual amount in milk supply (Kg)		
			PCB	PCB	HCB
Southern region			1.68	2.33	0.152

The same trend followed in the 1973 survey. Milk from Essex and Niagara counties contained the highest PCB residues, both counties being close to large industrial centers. Milk from the counties of Elgin, Middlesex, and Oxford were low in PCB residues, these counties being more distant from large industrial centers.

In the surveys of 1970-71 and 1973 it was calculated that 1.68 and 2.33 kg of PCB were present in the butterfat of milk produced in the southern region with

the gratest amounts being present in milk from Wentworth and Haldimand.

The ratio of DDT/PCB in milk during the 1970-71 survey averaged 1.53 for the region indicating higher residues of ΣDDT than PCB. By the 1973 survey this ratio had fallen to 0.47 indicating that ΣDDT residues were only about half those of PCB's (Table 7). The increase in PCB residue in milk between 1970-71 and 1973 may have been due to improvements in the procedure for separating and quantifying PCB's in 1973.

In the 1970-71 survey 62% of the bulk tankers samples had measurable levels of PCB. In the 1973 survey milk from all tankers contained measurable levels of PCB's.

HCB residues

The analysis of HCB was first attempted in the 1973 survey. Residues ranged from a high of 0.011 ppm in milk fat from Brant and Wentworth counties to a low of none detected in milk from Essex and Haldimand. Only 0.15 kg was calculated to be present in the entire milk supply for the region. Only 42% of the bulk tankers were analysed for HCB and among these 29% contained measurable residues.

DISCUSSION

Samples of domestic milk collected in 28 cities across the USA and analyzed by the Food and Drug Administration, U.S. Department of Health, Education and Welfare between June 1964 and April 1970 could be compared to the composite samples in this study, (*3,4,5,7,8,14*).

In the six annual U.S. surveys between eight and 10 organochlorine insecticides, one and three organochlorine herbicides and one and two organochlorine disinfectants and pollutants were identified. The organochlorine insecticides identified included DDT and its metabolites DDE and TDE, aldrin and dieldrin, heptachlor and its epoxide, BHC and its isomer lindane, and methoxychlor. In the Ontario study all these compounds were sought but only six were identified and measured. In the U.S. surveys 87 to 100% of the composite samples contained one or more of these organochlorine insecticides. In Ontario milk, all composite samples contained one or more of the six identified components.

In the U.S. survey ΣDDT residues in milk fat declined from 0.835 ppm in 1965-66 and 1967-68 to 0.294 ppm in 1969-70 (*3,4,5,7,8,14*). These residues were more than double the residues in the Ontario survey for a region where the annual application of DDT represented 90% of that used in the Province.

Dieldrin residues in milk fat in the U.S. survey appeared to increase from 0.060 ppm in 1965-66 to 0.092 in 1969-70. On the other hand, levels in milk fat in Ontario declined slightly during the period of 1967 to 1970 from 0.044 to 0.035 ppm. Measurable levels of heptachlor epoxide (0.03 to 0.08 ppm) were found in the U.S. milk survey, while residues on Ontario milk fat were little more than a trace. Methoxychlor, aldrin, heptachlor and BHC were never found or identified as contaminants in Ontario produced milk in any of the four surveys.

In the Ontario milk survey the method of analysis was not designed to identify organochlorine herbicides but was intended to recover polychlorinated biphenyls. In the U.S. survey PCB's were identified at trace level, however measurable levels were found in milk produced in Ontario. The U.S. survey did not report on HCB.

The restrictions placed on the use of DDT in 1970 were reflected in milk fat analysed in the 1970-71 survey when ΣDDT residues dropped by 34% below the 1968-69 level. This decline continued and by 1973 the ΣDDT residue was only 28% of the 1968-69 residue.

The restriction placed on aldrin and dieldrin resulted in a decline of only 15% in dieldrin residues in milk fat by the 1970-71 survey. By 1973 the decline was 63%. Restrictions on heptachlor resulted in no heptachlor epoxide being detected in surveys following the restriction.

The insecticides, chlordane and endosulfan, that were increasingly used in place of the restricted organochlorine insecticides failed to turn up in milk either as the parent compound or as metabolites during either the 1970-71 or 1973 surveys.

Use of persistent organochlorine compounds in a region, can be and often is reflected in the milk fat produced in that region, hence when it is restricted, it is likewise reflected in the milk fat. Restrictions in Ontario resulted in a fairly rapid decline in DDT and its metabolites, but a slightly slower decline in dieldrin.

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(act of October 23, 1962; Section 4369, Title 39, United States Code)

Of The Journal of Milk and Food Technology, published monthly at 413 Kellogg, Ames, Iowa 50010 for December, 1974.

The names and addresses of the publisher, editor, and managing editor are:

Publisher, International Association of Milk, Food and Environmental Sanitarians, Inc., 413 Kellogg Avenue, Ames, Iowa 50010.

Editor, Dr. E. H. Marth, University of Wisconsin, Madison, Wisconsin.

Managing editor, Earl O. Wright, 413 Kellogg Avenue, Ames, Iowa 50010.

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Column one average No. copies each issue during preceeding 12 months—Column two single issue nearest to filing date.

A. Total No. Copies Printed (Net Press Run)	4200	3900
B. Paid Circulation		
1. To term subscribers by mail, carrier delivery or by other means	3900	3585
2. Sales through agents, new dealers or otherwise	45	42
C. Free Distribution (including samples) by mail, carrier delivery, or by other means	79	79
D. Total No. of Copies distributed. (Sum of lines B1, B2 and C)	4024	3706

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Earl O. Wright, Managing Editor

J. Milk Food Technol. Vol. 38, No. 2, Pages 73-75 (February, 1975)

Control of Aflatoxin Production During Fermentation of Wild Rice

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(Received for publication August 9, 1974)

ABSTRACT

The effect of water content of wild rice on aflatoxin production by *Aspergillus flavus* growing in the rice during fermentation, and influences of processing steps as means to reduce aflatoxin contamination were investigated. Aflatoxin was either not detected or rarely observed during 31 days of storage when rice saturated with water or adjusted to contain 33% water was inoculated with spores of *A. flavus* and stored at 25 C. Detectable amounts of aflatoxin developed in rice maintained at 20% moisture. Aflatoxin B_1 content of contaminated fermented wet wild rice was reduced by parching. Aflatoxin analyses of hulls and kernels from parched rice indicated that most of the aflatoxin B_1 remained in the hulls; however, detectable amounts of aflatoxin B_1 were present in hulled rice kernels. When wild rice was sterilized with ethylene oxide, saturated with water, stored at 25 C, and inoculated with *A. flavus*, the mold grew if the rice was mixed daily, but only 10 ppb aflatoxin appeared after 19 days of storage. *A. flavus* did not grow in similarly treated rice that was not mixed.

Wild rice (*Zizania aquatica*) is an annual aquatic grass that grows as a food crop in the upper Great Lakes region of the United States and Canada. Until recently, the main economic importance of this crop was its use as a staple food by Indians and other inhabitants of the area, and as a natural food by game birds (*10*). Wild rice for commercial use was first cultivated successfully in the 1960's and since then production has increased dramaticallly (*1*). In 1973, about 1.5 million lb of processed commercially grown rice were produced in Minnesota, while the harvest obtained for natural stand wild rice was about 500,000 lb (*8*).

Because the industry has grown rapidly, freshly harvested wild rice often must be stored for extended periods before it can be processed. This is the result of economics associated with a short harvesting period and the cost of processing equipment. Processing wild rice involves curing, parching (drying), hulling, and winnowing (separating grain from chaff). When rice is stored (cured) indoors or outdoors before processing, a natural fermentation occurs and involves large numbers of a variety of microorganisms (*3,4*). Many of these microorganisms are subsequently destroyed or removed in the parching, hulling, and final home preparation of the rice (*3*). These steps during processing and home preparation make it unlikely that infectious disease organisms could be carried by the rice. However, the potential remains that toxins produced during storage could survive processing treatments.

Goel et al. (*3*) reported mold counts in unprocessed wild rice of over 10^6/g. It has also been shown that mold growth can occur during storage at outdoor ambient temperatures (*4*). Hence, there has been some concern about the possibility of mycotoxin contamination in the finished product. Goel et al. (*4*) recovered *Aspergillus* spp. from wild rice and the isolates produced aflatoxins in artificial media, but aflatoxin was not recovered from wild rice.

There are two common methods for fermentation and storage of wild rice which could permit mold growth and hence be conducive to aflatoxin production. The first involves storage of rice outdoors in piles up to 3 ft high followed by regular mixing and slow drying of the rice. The second is storage outdoors in piles with mixing, but with regular addition of water to the rice throughout storage. This method is preferred by some processors because it allows for longer storage of rice before it is processed. Conditions used in these experiments were selected to simulate optimum conditions for aflatoxin production that may occur during wild rice storage. Influences of processing steps in reducing the aflatoxin content of the wild rice also were examined.

MATERIALS AND METHODS

Inoculation and incubation of wild rice

Freshly harvested wild rice (Johnson strain) was obtained from a northern Wisconsin processor and held frozen until ready for use. Before inoculation, 1-lb lots of wild rice were placed in 1-gal containers lined with plastic bags. Holes in the containers allowed for aeration and drainage of excess water. Five sample treatments were used: (*a*) 60 ml of water were added daily to keep rice saturated (the excess was drained off) and rice was mixed daily, (*b*) rice was maintained at 33% moisture with daily addition of water and mixing, (*c*) same as (*b*) but rice was not mixed, (*d*) rice was maintained at 20% moisture and mixed daily, and (*e*) one sample of rice was maintained at 33% moisture, not mixed or inoculated, and served as a control.

Rice given treatments (*a*) through (*d*) was inoculated with 10,000 spores of *Aspergillus flavus* NRRL 89717 per gram using a suspension of spores in dry soil. All rice was incubated at 25 C. Samples were collected and analyzed for aflatoxin after 9, 14, 25, and 31 days of storage.

Processing of wild rice

All samples were parched in a laboratory model forced air parcher that dried rice to 4-8% moisture in 25-30 min with air at 120 C. Samples of parched rice were hulled with a Kyowa test huller (Kyowa Agricultural Machine Co., Ltd.). Separation of unhulled rice from hulled rice was done by hand. Moisture values were obtained with a Cenco Moisture Tester (Central Scientific Co., Chicago).

Aflatoxin analysis

A sample of rice from each treatment lot was analyzed for aflatoxin immediately after removal from storage, after it was parched, and after it was hulled (hulls and kernels were analyzed separately). The method of analysis for aflatoxin was similar to that described by Pons and Goldblatt (*7*). Fifty grams of wild rice or 25 g of wild rice hulls and 100 ml of 70% (v/v) aqueous acetone were blended at low speed in a Waring Blendor for 1 min. The resultant slurry was added to a Buchner funnel fitted with Whatman No. 1 filter paper, and filtered with reduced pressure. The filter cake was washed with two additional 50-ml volumes of 70% acetone. The filtrate was transferred to a 500-ml round bottom flask; the suction flask was rinsed with 60 ml distilled water, and this was added to the filtrate. Pigments were precipitated with aqueous lead acetate as described by Pons and Goldblatt (*7*). The precipitate was removed by filtration through a fluted funnel, and rinsed with aqueous acetone. The filtrate was then transferred to a 250-ml separatory funnel and extracted twice with 50-ml volumes of chloroform. These extracts were added to a flask containing 1 g of cupric carbonate (basic powder) and 6 g of anhydrous sodium sulfate, shaken, and allowed to stand 15 min. Treatment with cupric carbonate effected further removal of pigments (*11*). The extract was then filtered and evaporated to near dryness on a steam bath. The concentrated sample was transferred to a 10-ml volumetric flask and was brought up to volume with chloroform rinses.

Chloroform extracts were tested for the presence of aflatoxin with thin layer chromatographic procedures described by Shih and Marth (*9*). A solvent system containing chloroform, acetone, and water (85:15:1.5) was used to develope thin layer plates.

Measurement of O_2 loss in wild rice

Two 2-lb lots of wild rice were placed in containers each lined with a plastic bag having small holes for aeration and drainage of excess water. One lot was maintained at 20% moisture and the other was kept saturated with water. Both lots were inoculated with spores of *A. flavus* and incubated at room temperature with daily mixing. Oxygen tension readings were taken daily in rice receiving either treatment before it was mixed or water was added. A silver-lead oxygen electode with a teflon membrane was placed in the center of the rice the day before the reading was taken. The percent oxygen loss was calculated using the microvolt reading of the electrode in the rice, and the microvolt reading of the electrode in the atmosphere. After 19 days of storage, mold counts and aflatoxin analysis were done on this rice. Mold counts were done by the method of Goel et al. (*3*) using acidified potato dextrose agar (Difco).

Gas sterilization of wild rice

Production of aflatoxin in sterile wild rice (a noncompetitive environment) was determined using rice treated with ethylene oxide gas and inoculated with *A. flavus* spores. Sterilization of rice was done in a 5-gal glass jar equipped with two needle valves. This container also was used to store rice after it was sterilized and inoculated. Before sterilization, rice was air-dried to 20% moisture to achieve the necessary relative humidity for maximum activity of ethylene oxide (*6*). The sterilizing gas mixture was 10% ethylene oxide and 90% CO_2. Rice was flushed with this gas several times over 48 h, and its sterility checked using plate count agar (Difco). Rice was then rehydrated with sterile distilled water to contain 50% moisture and the excess water was removed. An inoculum of 2,000 spores of *A. flavus*/g, was added and rice was incubated at room temperature. Two different incubation conditions were used: (*a*) rice was put on a wire screen platform above one inch of water in the bottom of a closed jar, thus maintaining a relative humidity of 100%; this rice was not mixed but was aerated daily for 1 h with Millipore filter-sterilized air, and (*b*) rice was placed in a similar jar without the screen platform and rice and excess water were mixed daily by rolling the jar on its side; rice was aerated daily as in (*a*).

RESULTS AND DISCUSSION

No aflatoxin was produced in wild rice inoculated with *A. flavus* and kept saturated with water during incubation for 31 days (Table 1). A small amount of aflatoxin B_1 was recovered from wild rice maintained at 33% moisture only in the sample taken after 14 days of storage (Table 1). There was little difference in the amount of aflatoxin produced in mixed and unmixed rice with 33% moisture. Inoculated rice with 20% moisture increased in its content of aflatoxin during the 31 days of incubation, showing that wild rice will support extensive aflatoxin production when the moisture content and incubation temperature are suitable.

TABLE 1. *Aflatoxin B_1 in inoculated wild rice with different amounts of moisture and held at 25 C*

Treatment	Days of storage 9	14	25	31
	(ppb Toxin, dry wt basis)			
Inoculated				
Mixed daily				
Saturated[a]	0[b]	0	0	0
33% moisture	0	4.2	0	0
20% moisture	30.6	238	790	1,310
Not mixed				
33% moisture	0	11.3	0	0
Uninoculated				
Not mixed				
33% moisture	—[c]	—	—	0

[a]Contained between 45-50% water.
[b]None detected.
[c]No data.

Since saturation of wild rice with water may have permitted bacterial growth to the extent that sufficient oxygen in the rice was utilized to prevent aflatoxin production, loss of oxygen from rice between daily mixing was recorded (Table 2). There was little loss of oxygen during storage of rice with 20% moisture and mold growth characteristic of *A. flavus* dominated the mycoflora. Rice saturated with water and mixed daily exhibited up to 9% daily oxygen loss during the first 20 days and 75% loss on the 19th day (Table 2). After mixing, the amount of oxygen in rice was always within 3% of that in the air. Even though the watersaturated rice was inoculated with *A. flavus,* its predominant mycoflora was not characteristic of this species.

TABLE 2. *Percent of oxygen lost from a 2-pound lot of wild rice during storage*

Days of storage	Rice saturated with water	Rice with 20% moisture
	(% O_2 lost)	
2	3	3
3	7	3
4	9	2
5	6	—
8	9	—
9	8	2
10	9	—
11	19	—
15	11	—
19	75[a]	3[b]

[a]Mold count after 19 days was 10^7/g; aflatoxin was not detected.
[b]Mold count after 19 days was 4.6×10^7/g; 185 ppb aflatoxin B_1 was recovered.

In rice that was gas-sterilized, saturated with water, and mixed daily, the amount of *A. flavus* rose from the

initial 2×10^3/g (inoculum) to 5.8×10^6/g after 19 days. In rice that received a similar treatment but was not mixed, mold grew only slightly to 3×10^4/g after 19 days of storage. No measurable amount of aflatoxin was obtained from this sample, although rice that was mixed contained 8 ppb aflatoxin B_1 after 10 days and 10 ppb after 19 days of incubation.

Fate of aflatoxin during processing

Parching more effectively inactivated aflatoxin when the amount in rice was small (30.6 ppb was reduced by 71%) than when it was large (1,310 ppb was reduced by 24%) (Table 3). When parched rice was hulled, 25-39% of the aflatoxin remained with the kernel (80% of the whole rice was kernel). In whole rice containing 11 ppb aflatoxin B_1 or less, parching served to reduce the aflatoxin to an undetectable amount.

TABLE 3. *Aflatoxin B_1 in inoculated wild rice that contained 20% moisture*

Sample	Days of storage before processing			
	9	14	25	31
	(ppb, Dry weight)			
Whole rice				
Unparched	30.6	238	790	1,310
Parched	8.9	51.8	563	1,000
Hulled parched rice				
Kernels	3.9	6.6	410	201
Hulls	23.4	81.6	2,180	2,330

DISCUSSION

Wild rice is a potential substrate for aflatoxin production only when its moisture content drops below (within limits) that present at harvesting (35-45%). Thus, if unprocessed wild rice must be stored at ambient temperatures it should be kept sufficiently moist to minimize the chances of contamination with aflatoxin. Mixing the rice may help to reduce aflatoxin production by preventing the surface of the rice pile from drying.

The smaller amounts of oxygen in wet rice do not appear to be responsible for the lack of aflatoxin production since *A. flavus* has been shown to produce aflatoxin in an atmosphere of 5% oxygen (*2*), which is nearly equivalent to the 75% reduction in oxygen content that we noted in the wild rice (Table 2). However, the apparent failure of *A. flavus* to grow in sterilized, unmixed rice may have resulted from a lack of oxygen since samples were taken from the center of the lot; the lack of aeration from mixing could have caused a greater decrease in oxygen concentration at the center of this lot of rice than in rice that was mixed. The small amounts of aflatoxin recovered from inoculated sterilized rice along with the mold growth that occurred demonstrates that growth of other microorganisms may affect both the amount of aflatoxin found in the rice as well as the growth *A. flavus*. This also may account for some of the difference in aflatoxin production found in the rice containing 20% moisture and that containing 33% moisture. The ratio of moisture to nutrient is known to affect the microflora of grains (*5*).

Parching and hulling will significantly decrease the aflatoxin content of wild rice and hence rice that is slightly contaminated with toxin may be made safe for consumption. However, processing cannot be relied upon to decontaminate wild rice, since the efficiency of decontamination decreases as the amount of contamination increases. Most of the aflatoxin present in contaminated parched rice appeared in the hulls; this was expected since most mold growth occurred on the outside of the hull. However, damaged kernels of rice also have been observed to support mold growth.

In conclusion, contamination by aflatoxin of unprocessed wild rice stored outdoors can be prevented if the rice is kept sufficiently moist. Wild rice containing 20% moisture will support extensive aflatoxin production. Processing of wild rice will decrease the amount of aflatoxin present in the finished product, but cannot be relied upon to make the rice safe for consumption. Even though aflatoxin contamination can be easily prevented during storage of unprocessed wild rice, extensive mold growth can still occur and other mycotoxins may be a problem.

ACKNOWLEDGMENTS

This research was supported by the College of Agricultural and Life Sciences and University of Wisconsin Extension, University of Wisconsin-Madison, the Upper Great Lakes Regional Commission, and the U.S. Department of Agriculture. Presented at the 61st Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, St. Petersburg, Florida, August 12-14, 1974.

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J. Milk Food Technol. Vol. 38, No. 2, Pages 76-77 (February, 1975)

A Direct Microscopic Count Procedure for the Rapid Estimation of Bacterial Numbers on Green-Headless Shrimp[1,2]

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ABSTRACT

A Direct Microscopic Count (DMC) procedure, utilizing the Gram-stain, was used to estimate bacterial numbers on 149 samples of green-headless (shell-on) shrimp. A correlation of 0.876 (significant at $P < 0.01$) existed between the log DMC and log agar plate counts done at 25 C for 2 days. Samples judged questionable or unacceptable organoleptically had DMC's of 10^8 or more.

In 1953, Lane and Whittaker (*11*) stated, "One of the most urgent needs in the shrimp industry is a rapid and accurate method of testing the quality of iced shrimp. This method must be free from the errors of personal opinion. The industry suffers through the inability of buyers to recognize spoiling shrimp with sufficient accuracy. Lacking objective tests of quality, penalities in the form of lower prices cannot be imposed for poorly handled catches. On the other hand, bonuses cannot be offered careful fishermen or other workers for high quality raw material." Today, over 20 years later, the statement is still basically true. Acid-soluble orthophosphate (*2*), alcoholic turmeric solution (*10*), amino nitrogen (*2, 4, 8, 11*), ammonia (*9*), B-vitamin content (*2*), catechol-ferric cholride (*10*), dimethylamine (*4*), free fatty acids (*4*), glycogen (*2, 11*), hydrogen sulfide (*4*), hydration capacity (*2, 15, 18*), inosine monophospahte (*16*), indole (*4, 7, 9, 11*), iodine titration (*2, 7, 9,*), lactic acid (*2*), methylene blue reductase (*14*), peroxide number (*4*), pH (*2, 3, 10, 11, 18*), phenol red test paper (*10*), photoelectric reflection number (*7*), picric acid (*3*), skatole (*11*), total fat (*11*), total nitrogen (*11*), trimethylamine (*2, 3, 4, 7, 8, 9, 10*), total volatile nitrogen/total nitrogen (*7, 9*), tyrosine (*8*), ultraviolet light-change in fluorescence (*11*), volatile acids (*2, 4, 8*), and volatile nitrogen (*10*) are some of the chemical and physical tests that have been evaluated over the years. None of these are presently used by the industry on a routine basis.

In most chemical tests, results can vary with the age of the shrimp, size, species, area of catch, and handling conditions. Many of the tests only indicate the onset of spoilage thus providing only limited information before that state and have been shown to vary independently of the quality as scored by a sensory panel (*11*). With the exception of pH (*2, 3, 18*) and ratios of volatile nitrogen with total or amino nitrogen (*5, 7, 9*), none of the tests have proved to be rapid, simple, or accurate enough for routine testing of shrimp at unloading or processing facilities. The industry still depends on visual observation, smell, and bacteriological testing for evaluating quality. The purpose of this investigation was to evaluate the use of a direct microscopic count (DMC) as a rapid means of assessing the bacteriological quality of shell-on shrimp tails.

MATERIALS AND METHODS

Green-headless shrimp samples (shrimp tails with shell-on) were either from commercial processing plants along the Texas coast or shrimp held until spoilage under various laboratory storage conditions. Depending on the size of the shrimp, either three shrimp or 50 g were weighed and placed in 100 ml of sterile phosphate buffer. The sample was shaken vigorously and 0.01 ml was transferred with a platinum-rhodium inoculating loop to a fluorescent antibody slide with two etched 10-mm diameter circles. Smears were air-dried in an oven at 50 C for 15-20 min. They were then Gram stained and examined under oil immersion. Twenty fields were examined if the number of bacteria per field was less than 10, 10 if the number per field was 10-50, and five if the number per field was greater than 50. The average number of bacteria per field, dilution factor, and microscopic factor (MF) were used to calculate the direct microscopic count (DMC) per gram. The Microscopic Factor (MF) was determined as prescribed in *Standard Methods for the Examination of Dairy Products* (*1*).

Agar plate count (APC) was determined by spread-plating decimal dilutions of the same sample on Standard Methods Agar (BBL) and incubating plates at 25 C for 2 days. The log numbers of bacteria from agar plate count and direct microscopic count from 149 samples were subjected to standard regression and correlation analysis.

RESULTS AND DISCUSSION

Correlation between the logarithm of DMC and APC (Fig. 1) was 0.876 (significant at $P < 0.01$). A linear relationship Log DMC = 1.79 + 0.82 Log APC was established and the coefficient of variations for DMC and APC were 0.14 and 0.17, respectively.

Lerke and Farber (*12*) utilized a direct bacterial count from scrapings of fish fillets as an indicator of freshness. They substituted a 30-sec staining with safranin for the complete Gram stain because micrococci were the only gram-positive organisms found and then only on very fresh fillets. Since the bacterial flora of freshly landed Gulf of Mexico shrimp consists primarily of coryneforms, *Achromobacter, Flavobacterium,* and *Bacillus* (17), the

[1]*Partially supported through Institutional Grant 04-3-158-18 to Texas A&M University, by the National Oceanic and Atmospheric Administration Office of Sea Grants, Department of Commerce.*
[2]*Presented at the 19th Annual Atlantic Fisheries Technological Conference, Quebec City, Quebec, Canada, September 22-25, 1974.*

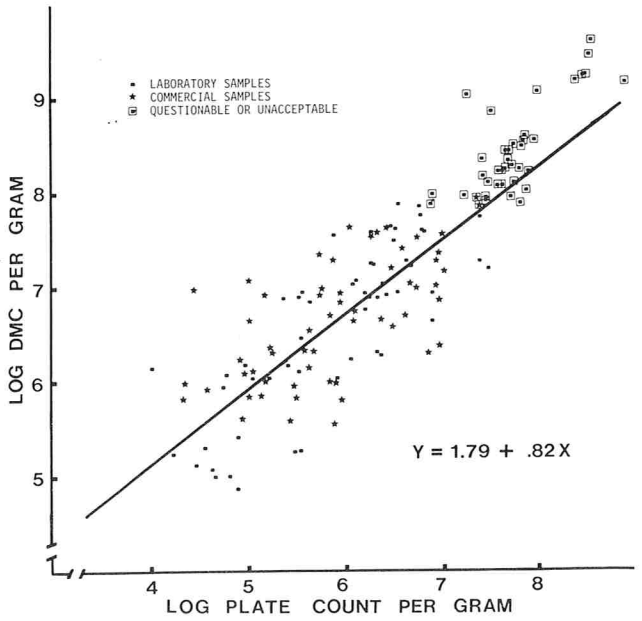

Figure 1. *Direct Microscopic Count (DMC) and Agar Plate Count (APC) of 149 samples of green-headless shrimp.*

entire Gram stain was used in the present study. It proved acceptable and provided useful information as to the types of bacteria present.

It is realized that there are certain inaccuracies inherent to the DMC. Levowitz (*13*) indicated that the direct microscopic count could not be used in estimating the number of bacteria in market grade raw milk because of the small number of cells available. Other investigators (*6*) have used a microscopic counting technique on fish and reported it to be of value only in the later stages of spoilage, when numbers were approaching 10^5 per gram. Bacterial counts below the MF are difficult to estimate because the number of fields examined must be increased and fractions are used to express average number per field. These limitations do not pose a great handicap in using the DMC to estimate the bacterial counts and quality of Gulf of Mexico shrimp because shrimp of poor or questionable quality usually have APC's in excess of $10^6 - 10^7$ per gram. APC's (25 C for 2 days) of commercial shrimp samples in the study ranged from 2.3×10^4 to 3.2×10^7 per gram and DMC's from 2.0×10^5 to 7.3×10^8 per gram. As judged organoleptically only two of the commercial samples were questionable or unacceptable and both had APC's in excess of 10^7 per gram. In addition, onset of spoilage in samples held under laboratory storage conditions was not noted until APC's exceeded 10^7 per gram. Questionable or unacceptable samples in either event had DMC's near or in excess of 10^7 per gram.

High counts may result from bacterial multiplication on shrimp or from contact with heavily contaminated surfaces. The degree of quality deterioration depends to a large extent on the biochemical activity of the microflora upon the chemical constituents of the shrimp. Although the DMC may not separate live from dead cells, high counts are undesirable because they indicate improper handling and/or storage between catch and processing.

The DMC procedure for green-headless shrimp should not be substituted for the agar plate count but does provide a rapid simple means of assessing the storage history and quality of green headless shrimp when used in conjunction with conventional sight and smell evaluations.

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J. Milk Food Technol. Vol. 38, No. 2, Pages 78-83 (February, 1975)

Role of Enterococci in Cheddar Cheese: Free Fatty Acid Appearance and Citric Acid Utilization[1]

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ABSTRACT

Eight lots of Cheddar cheese were manufactured to determine the microbiological response and biochemical effects of two strains each of *Streptococcus faecalis* and *Streptococcus durans* used as supplemental starters in combination with a commercial lactic culture. Each lot consisted of a control vat of cheese manufactured with the lactic starter only and an experimental vat of cheese containing the lactic starter and one of the enterococcus strains. Combinations of two curing temperatures (7.2 and 12.8 C) and two early cooling treatments (air vs. brine cooling) were used for cheeses from each vat to determine environmental effects on the cheeses. Cheeses manufactured with *S. faecalis* had a somewhat lower content of free fatty acids than did control cheeses, possibly because of early conversion of acids to neutral compounds. Cheeses manufactured with *S. durans* showed a fluctuating, but consistent, free fatty acid content among treatments, with overall amounts being greater than in the control cheeses or in cheeses made with *S. faecalis*. Cheeses cured at 12.8 C showed greater free fatty acid liberation, but the effects of early cooling rates were not significant. Citric acid in cheeses made with *S. faecalis* and in control cheeses was utilized most rapidly in 30 days at 12.8 C and extending to 60 days when cured at 7.2 C, after which no more breakdown seemed to occur. Cheeses made with *S. durans* 9-20 followed approximately the same pattern although some utilization took place between 90 and 180 days. In cheeses made with *S. durans* 15-20, however, citric acid utilization was continuous up to 180 days, and in cheeses cured at 12.8 C, citric acid was nearly depleted at 180 days. Cheeses made with *S. durans* 15-20 and cured at 12.8 C exhibited excessive gas production.

In two previous papers (*13,14*) the microbiology of Cheddar cheese containing *Streptococcus faecalis* and *Streptococcus durans* and the effects of these organisms on proteolysis and lactic development were discussed. This paper will evaluate the differences in citric acid (CA) utilization and free fatty acid (FFA) appearance between control cheeses and cheeses manufactured with the two species of enterococci. The fourth and final article in this series will discuss organoleptic results.

MATERIALS AND METHODS

Cheese was manufactured and treated as described in a previous article by Jensen et al. (*13*).

Total FFA were determined by using a modification of the silica gel column method described by Harper et al. (*11*).

Preparation of reagents

(*a*) Chloroform (technical grade) was washed four times by shaking with distilled water to remove traces of alcohol used as preservative.

(*b*) Five percent *n*-butanol was prepared by adding 5 ml *n*-butanol to 95 ml of washed chloroform.

(*c*) Silicic acid (Mallinckrodt No. 2847) was prepared by washing with distilled water, allowing to stand for 10 min. and decanting the supernatant. This was repeated once and followed by drying at 120 C for 48 h.

(*d*) Buffer (pH 6.5) was prepared by mixing appropriate amounts of 2 M KH_2PO_4 and 2 M K_2HPO_4.

(*e*) Alcoholic 0.01 N KOH was prepared in absolute alcohol.

(*f*) Phenol red indicator was prepared by grinding 100 mg phenol red with 0.1 ml 1 N KOH, which was then diluted to 100 ml with absolute alcohol.

(*g*) Silicic acid stock solution was prepared by mixing 5 g of dry silicic acid in 3 ml of 2 M pH 6.5 phosphate buffer and then slurrying with 20 ml of washed chloroform. The stock solution was stored in a tightly stoppered brown bottle.

Preparation of column and extraction of fatty acids

The column consisted of two sections:

(*a*) Lower section: Twenty-five milliliters of well mixed silicic acid stock solution were added to the lower half of the column for each analysis.

(*b*) Upper section: Enough 20.0% H_2SO_4 was added to 5 g of cheese to adjust the pH to 1.7 to 2.0. The final volume was adjusted to 3.0 ml in a 500-ml mortar with distilled water. The cheese was ground thoroughly with the acid and distilled water, then 10 g of dried silicic acid were added, and the mixture was ground again. The sample was slurried with 5.0% *n*-butanol in chloroform and was transferred quantitatively onto the top of the lower section of the column. The column was then attached to a 250-ml suction flask and vacuum was applied so that the solvent would flow through the column at the rate of about 25 ml/min. One hundred-fifty milliliters of eluant were collected.

The total FFA in the eluant were titrated with 0.01 N alcoholic KOH after adding 0.3 ml phenol red solution and 15 ml neutral absolute ethanol.

Citrate was extracted from the cheese as described by Fryer et al. (*9*) and determined colorimetrically using the method of Marier and Boulet (*20*). Percentage of CA was determined by a standard curve prepared by adding known amounts of sodium citrate to distilled water and converting to CA by a gravimetric factor.

RESULTS

Total FFA were determined in this investigation as milliliters 0.01 N alcoholic KOH required to neutralize the FFA in 5 g cheese, and the averages of the eight lots of negative controls are presented in Fig. 1. Although the development of fat acidity in the cheese was somewhat erratic, more FFA were usually observed in those cheeses cured at 12.8 C. This was particularly evident at the end

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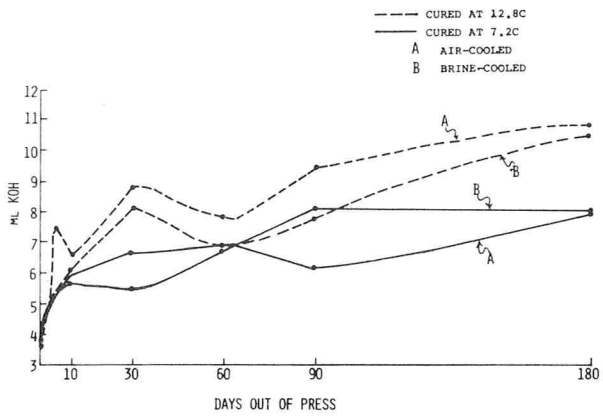

Figure 1. *Increase of free fatty acids during curing of air- and brine-cooled control cheeses.*

of 180 days. These differences resulting from curing temperature are shown in Table 1. Differences due to

TABLE 1. *Analysis of variance; free fatty acids in control cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Lot	7	68.52	7.82	1%
Cooling procedure (C)	1	10.00	1.14	
Curing temperature (T)	1	90.23	10.30	1%
C × T	1	12.75	1.46	
Error	21	8.76		
Days of curing (D)	6	113.20	18.77	1%
C × D	6	1.98	0.33	
T × D	6	8.56	1.42	
C × T × D	6	4.76	0.79	
Error	168	6.03		

early cooling treatment were irregular when observed graphically; statistically, however, no significant difference existed. (Table 1).

Figure 2 shows the average amounts of KOH used to

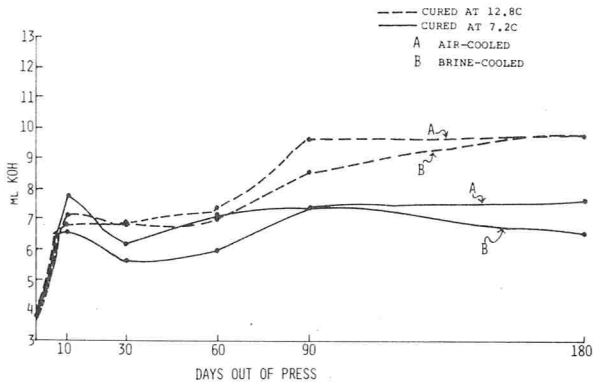

Figure 2. *Increase of free fatty acids during curing of air- and brine-cooled experimental* (S. faecalis) *cheeses.*

neutralize the FFA in the cheeses manufactured with *S. faecalis.* Here, as in the control cheeses, the trends among treatments were the same, with more FFA detected in cheeses cured at 12.8 C than in those cured at 7.2 C. The overall increase, however, was less than that observed in the negative control cheeses. After 10 days, the amount stabilized in cheeses cured at 12.8 C and actually declined in those cured at 7.2 C. Data in Table 2

TABLE 2. *Analysis of variance; free fatty acids in enterococcus cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Species (S)	1	160.48	2.33	
Concentration (CN)	1	2.52	0.04	
S × CN	1	84.99	1.24	
Error	4	68.59		
Cooling procedure (C)	1	1.64	0.30	
Curing temperature (T)	1	76.71	14.17	1%
C × T	1	0.02	0.00	
S × C	1	0.24	0.04	
CN × C	1	8.87	1.64	
S × T	1	7.94	1.47	
CN × T	1	0.04	0.01	
Days of curing (D)	6	115.30	21.30	1%
S × D	6	20.70	3.83	1%
CN × D	6	7.15	1.32	
C × D	6	0.51	0.09	
T × D	6	8.92	1.65	
Pool error	179	5.41		

confirm the differences in FFA appearance resulting from curing temperature with cheeses cured at 12.8 C having the larger amount, the level of significance being 1%. Again, FFA development is not affected by early cooling treatment.

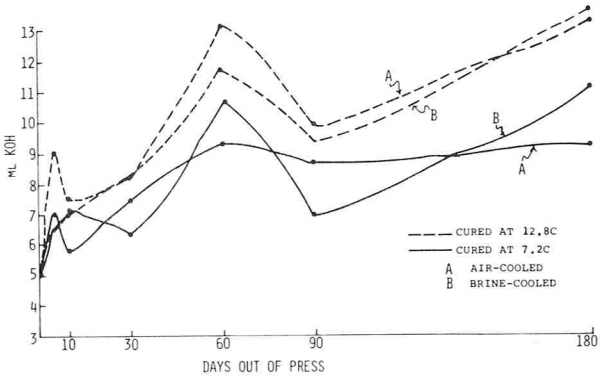

Figure 3. *Increase of free fatty acids during curing of air- and brine-cooled experimental* (S. durans) *cheeses.*

Figure 3 shows the average amounts of FFA in the four lots of cheeses manufactured with *S. durans.* The relationships among treatments were precisely the same as in the negative controls and in the cheeses manufactured with *S. faecalis.* There was, however, an abrupt but continuous increase in fat acidity up to 60 days, at which point cheeses from all four treatments decreased in FFA content and another increase was evident after 90 days. Table 2 reflects in the species × days interaction this difference in FFA appearance when different species of enterococci are used as supplemental starters.

An analysis of variance for the differences between enterococcus cheeses and their respective controls is given in Table 3. This shows that, over the curing period,

TABLE 3. *Analysis of variance; differences in free fatty acids between enterococcus and control cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Species (S)	1	41.15	7.43	
Concentration (CN)	1	4.04	0.73	
S × CN	1	2.59	0.47	
Error	4	5.54		
Cooling procedure (C)	1	3.54	0.60	
Curing temperature (T)	1	0.55	0.09	
C × T	1	11.78	1.98	
S × C	1	1.04	0.17	
CN × C	1	4.42	0.74	
S × T	1	1.24	0.21	
CN × T	1	22.27	3.75	
Days of curing (D)	6	13.80	2.32	5%
S × D	6	15.14	2.55	5%
CN × D	6	9.87	1.66	
C × D	6	1.11	0.19	
T × D	6	4.93	0.83	
Pool error	179	5.94		

enterococcus cheeses contained significantly more FFA than did their controls. Further, the difference between cheeses made with *S. durans* and their controls was significantly greater than the difference between *S. faecalis* cheeses and their controls.

Citric acid utilization

Figure 4 shows the averages of percent CA in the curd

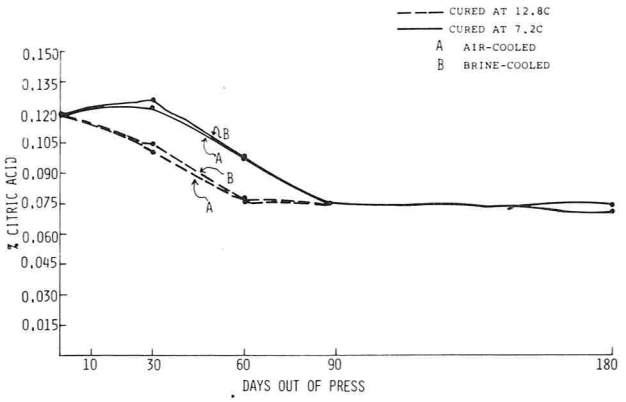

Figure 4. *Citric acid content during curing of air- and bine-cooled control cheeses.*

of the eight lots of negative controls. In nearly all instances the CA content after 30 days was slightly more than that at milling, especially in cheeses cured at 7.2 C. This most likely is due to the difficulty experienced in completely macerating the freshly milled curd, as is required in the extraction. As a result, the citrate in the fresh curd was not quantitatively recovered into aqueous solution for analysis. Because subsequent samples were easily broken up by the extraction, a false increase is observed in most instances. Thus, the values for CA at milling are probably too low. In Fig. 4, a more rapid utilization of citrate was observed in those cheeses cured at 12.8 C. The amount of citrate stabilized after 60 days in cheeses cured at 12.8 C, whereas it took the cheeses cured at the lower temperature 90 days to reach a stable concentration of citrate. The citrate content in cheeses receiving all four treatments stabilized at about 0.075%.

There was no difference in citrate depletion patterns between air- and brine-cooled cheeses cured at the same temperature, as shown in Table 4. The analysis of variance presented in this table also confirms the more rapid utilization of citrate when cheeses are cured at 12.8 C.

TABLE 4. *Analysis of variance; citric acid utilization in control cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Lot	7	0.00598177	12.50	1%
Cooling procedure (C)	1	0.00010400	0.22	
Curing temperature (T)	1	0.00144600	3.02	
C × T	1	0.00008556	0.18	
Error	21	0.00047851		
Days of curing (D)	4	0.01351711	29.73	1%
C × D	4	0.00005380	0.12	
T × D	4	0.00141871	3.12	5%
C × T × D	4	0.00014185	0.31	
Error	112	0.00045469		

The average percentages of CA for the four lots of cheese manufactured with a supplemental *S. faecalis* starter are presented in Fig. 5. The citrate utilization in

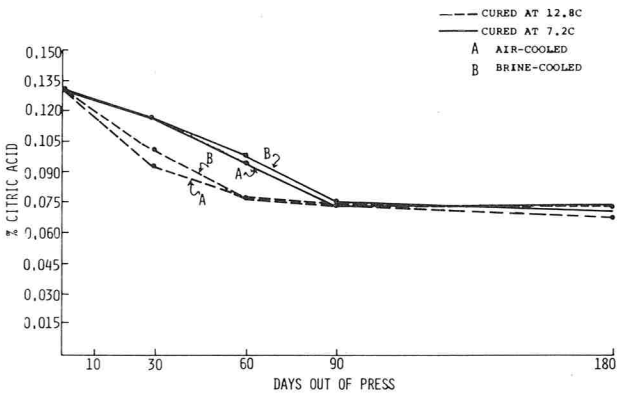

Figure 5. *Citric acid content during curing of air- and brine-cooled experimental* (S. faecalis) *cheeses.*

these cheeses was essentially the same as in the negative controls (Fig. 4). There did seem to be, however, a slight decrease in the citrate level from 60 days to 180 days in cheeses cured at 12.8 C. The significance of the decrease in this period (about 0.01%) is not clear and cannot be ascertained from the statistical information presented in Table 5 since tests were not made between each sampling time, but only over days of cure as a whole.

The patterns of CA utilization in the cheeses made with *S. durans* as a supplemental starter are quite different. In addition to varying from the patterns displayed by the negative controls and by the *S. faecalis* cheeses, each of the two strains of *S. durans* produced vastly different results. Figure 6 represents the average percentages of CA in the cheeses manufactured with *S. durans* 9-20. Although this is somewhat similar to the utilization pattern of *S. facecalis* cheeses, there is a rather slow but steady decrease up to 180 days. Figure 7

TABLE 5. *Analysis of variance; citric acid utilization in entercoccus cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Species (S)	1	0.00020703	0.02	
Concentration (CN)	1	0.01299603	0.96	
S × CN	1	0.00228010	0.17	
Error	4	0.01340419		
Cooling procedure (C)	1	0.00005523	0.25	
Curing temperature (T)	1	0.01173063	52.32	1%
C × T	1	0.00008410	0.38	
S × C	1	0.00000490	0.02	
CN × C	1	0.00002560	0.11	
S × T	1	0.00338560	15.10	1%
CN × T	1	0.00006760	0.30	
Days of curing (D)	4	0.01545118	68.92	1%
S × D	4	0.00239473	10.68	1%
CN × D	4	0.00016535	0.74	
C × D	4	0.00001171	0.05	
T × D	4	0.00090905	4.05	5%
Pool error	125	0.00022419		

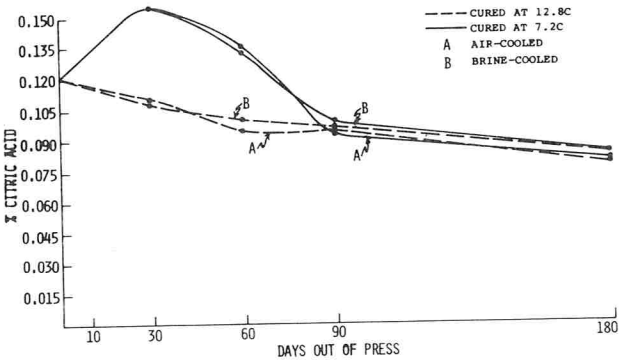

Figure 6. *Citric acid content during curing of air- and brine-cooled experimental* (S. durans *9-20*) *cheeses.*

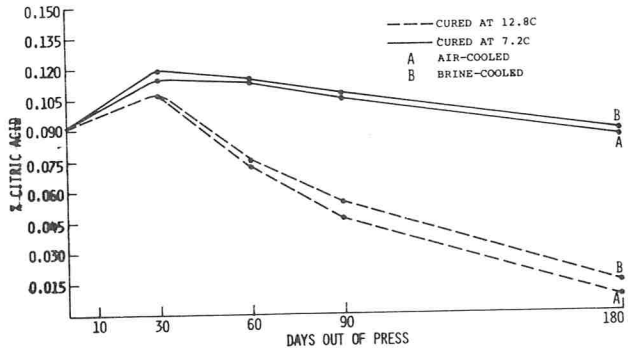

Figure 7. *Citric acid content during curing of air- and brine-cooled experimental* (S. durans *15-20*) *cheeses.*

shows the average response of the two cheeses made with *S. durans* 15-20. In this situation, there was a slow but continuous decrease in CA in the cheeses cured at 7.2 C, but at 12.8 C the CA decreased rapidly, leaving only about 0.01% after 6 months. In the two lots of cheese made with this strain, those cured at 12.8 C exhibited a definite "blowing" effect. Figure 8 shows the excess gas produced in cheeses made with *S. durans* 15-20 compared with a control, both of which were cured at 12.8 C.

Table 5 confirms statistically the differences in CA utilization patterns in enterococcus cheeses when different species and curing temperatures are used. Also,

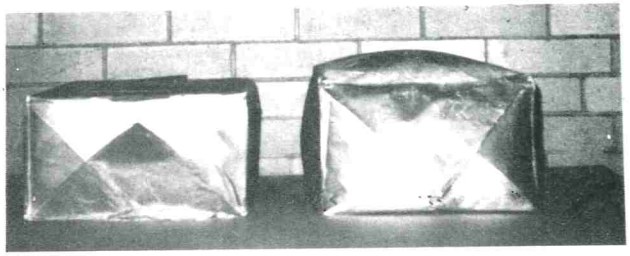

Figure 8. *Excess gas production in cheese manufactured with* S. durans *15-20 and cured at 12.8 C (right), as compared to respective control.*

for the first time, an obvious strain difference has resulted, which is not reflected in the statistical design.

An analysis of variance for differences between enterococcus cheeses and their controls is presented in Table 6. This shows that the difference between enterococcus cheeses and their respective controls cured at 12.8 C is significantly greater than the difference between experimental and control cheeses cured at 7.2 C. Also, the difference in CA utilization between enterococcus and control cheeses is significant throughout the entire curing period.

TABLE 6. *Analysis of variance; differences in citric acid utilization between enterococcus and control cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Species (S)	1	0.00377331	1.13	
Concentration (CN)	1	0.00276391	0.83	
S × CN	1	0.00021391	0.06	
Error	4	0.00333026		
Cooling procedure (C)	1	0.00031081	0.87	
Curing temperature (T)	1	0.00493951	13.90	1%
C × T	1	0.00033931	0.96	
S × C	1	0.00033351	0.94	
CN × C	1	0.00023281	0.66	
S × T	1	0.00000106	0.00	
CN × T	1	0.00050056	1.41	
Days of curing (D)	4	0.00223658	6.30	1%
S × D	4	0.00003038	0.09	
CN × D	4	0.00029848	0.84	
C × D	4	0.00009332	0.26	
T × D	4	0.00115774	3.26	5%
Pool error	125	0.00035526		

DISCUSSION

Free fatty acids

It has been rather firmly established that the products of milk fat hydrolysis are of great importance in the development of typical Cheddar cheese flavor (*1,6,16,17, 18,19,22*). The FFA resulting from this lipolysis have been shown to increase, although erratically, in the control cheeses. These fatty acids may combine with ethanol, shown to be present in cheese, to form esters, or they may be enzymatically oxidized to ketones (*1*). Such reactions may explain the declining trends in the curve. Additional lipolysis would result in another increase. The "dip" seen at the 30-day sampling period in control cheeses cured at 12.8 C may be a reflection of FFA reacting with available ethanol and (or) oxygen (if any) in the curd to form esters and ketones, followed by

continued lipolysis resulting in accumulation of FFA.

Peterson et al. (*23,24*) in their studies of lipase activity during making and ripening of Cheddar cheese, found that milk lipase could play no role in ripening after manufacture because the microenvironment is unsuitable for its stability and pH optimum. They reported that bacterial lipases appear between 5 and 20 days. These workers also found that, during the first 30 days of ripening, caproic, caprylic, and capric acids were absent, whereas *n*-butyric acid had nearly reached its maximum level. In the same period, acetic acid reached about half its maximum (determined after 420 days). The late appearance of caproic, caprylic, and capric acids was believed to be caused entirely by bacterial lipase action. Suzuki et al. (*27*) in a much earlier paper, reported results very similar to those of Peterson et al. (*24*). They observed, however, that acetic acid reached its maximum level at 3 months, after which a decrease was noted, whereas butyric and caproic acid concentrations continually increased. This work was done in 1910 when curing temperatures were probably relatively high, and this may further explain the erratic FFA development in cheeses cured at 12.8 C. Furthermore, *Lactobacillus casei* has been shown to be lipolytic when the cells are lysed. Because this may be the predominant organism in cheeses cured at 12.8 C (*5,13,15*), those cells that autolyse may liberate lipase producing greater amounts of FFA.

Nakanishi et al. (21) examined six strains of lactic acid bacteria and found no appreciable extracellular or intracellular lipase. This further suggests that the lipolysis in the control cheese is mainly due to adventitious types, which proliferate far more readily at 12.8 C than 7.2 C. Besides lactobacilli, it is quite likely that micrococci may play a role in fat hydrolysis.

Very little work has been done on the lipolytic capacities of the enterococci. The investigation of Dovat (*4*) is the primary reference in this area. He found that 50% of the *S. durans* strains he examined produced large amounts of acetic acid in skimmilk and that lactic streptococci consistently produced the lowest amounts of acetic acid. He also found that the enterococci were more active in breaking down tributyrin.

It was expected, therefore, that cheeses made with enterococci would contain considerably more FFA than the controls. However, Fig. 1 and 2 show that cheeses made with *S. faecalis* exhibit patterns very similar to control cheeses. Friedemann (*8*) was able to demonstrate that *S. faecalis* produced about 10 times as much ethanol from glucose as did *S. lactis.* If this is parallel to the lipolytic action of these organisms in cheese, the ethanol in cheeses would react with FFA to form esters. This would reduce the titratable FFA. In Fig. 2, after 60 days, a further increase of FFA is shown in cheeses cured at 12.8 C, which may have resulted from further lipolysis by adventitious flora. Some of the increase of FFA in these high-temperature cured cheeses also may result from further lipolysis by *S. faecalis.* If a larger amount of FFA is being esterified in enterococcus cheeses than in control cheeses, this may explain the increase in flavor intensity in cheeses made with *S. faecalis,* as has been reported by Dahlberg and Kosikowsky (*2*) and Freeman (*7*).

The initial rapid increase in FFA in cheeses manufactured with *S. durans* may be due to the ability of the organism to produce large amounts of acetic acid. Since Suzuki et al. (*27*) noted that acetic acid disappeared after several months of ripening, the simultaneous decrease in fat acidity in all the cheeses is understandable. The more gradual increase in FFA levels between 3 and 6 months is probably a reflection of the continuous increase of butyric, caproic, caprylic, and capric acids. Indeed, *S. durans* may be more lipolytic than *S. faecalis* and the lipase-producing bacteria in the negative controls because, during the period from 3 to 6 months when lipase activity is totally of bacterial origin (*23*), the liberation of FFA in the *S. durans* cheeses progresses at an appreciably greater rate. It should be pointed out that *S. faecalis* may be as lipolytic as *S. durans,* but its more active fermentative capacities may result in production of ethanol, which could subsequently result in formation of esters soon after the release of fatty acids from fat.

Citric acid utilization

Cheese with an excess of CO_2 production often develops slits and gassy openings in the curd, which is considered a defect (*25,26*). It is believed that the presence of citrate-fermenting bacteria, such as *Leuconostoc* sp. and *Streptococcus diacetilactis,* is responsible for excessive and rapid gas production (*9,25*). Robertson (*25*) reported that storage of cheese at lower temperatures usually resulted in retarded CO_2 formation. Assuming that the production of CO_2 is concurrent with utilization of citrate, data presented here agree with this. He also stated that, when CO_2 is formed shortly after manufacture, an open-textured cheese is likely to result. Similar findings have been reported by Dorn and Dahlberg (*3*).

As a result of data presented for control cheeses, two questions arise: first, why is there a more rapid utilization of citrate in cheeses cured at 12.8 C; and second, why does the utilization cease at a certain point rather than continuing until the citrate is depleted? Robertson (*25*) suggested that the gas was produced by lactobacilli. Since these proliferate much more rapidly in cheeses cured at 12.8 C, it is probable that their metabolism during this period is responsible for the greater rate of citrate utilization. The retarded citrate depletion in cheeses cured at 7.2 C is probably a result of slower utilization by the residual lactic streptococci and, to some extent, lactobacilli, which are probably also present but growing much less actively. Fryer et al. (*9*) studied the utilization of citrate in cheese and found that, when *L. casei* was added with the starter, there was no acceleration in the decrease in cheese citrate as compared with the negative control. Thus, the greater utilization may be due to the influence of the warmer curing temperature only.

For all treatments, use of citrate ceased at a certain level. In cheeses cured at 12.8 C, the decrease was noted at 60 days; although in cheeses cured at this temperature, the bacterial counts were generally increasing. Thus it seems that growth itself has little to do with the continued use of cheese citrate. It is likely that the citrate-cleaving enzymes are active in the earlier stages of curing and then become inactive. More extensive proteolysis in the cheeses cured at 12.8 C (*14*) may cause a somewhat more rapid rise in pH in these cheeses. When the pH reaches a critical point, citrate cleavage may be retarded. Harvey and Collins (*12*) illustrated that the citrate permease system of *S. diacetilactis* was pH dependent, with the maximum activity occurring at a lower pH.

Although it has been reported that *S. faecalis* possesses an adaptive CA desmolase (*10*), it seems that its that its action in cheese is nearly identical to the citrate-cleaving system in the negative controls, since the rate of utilization and the point of stabilization are the same.

It does seem, however, that the citratase system of cheeses containing *S. durans* 9-20 may be different than that in the negative controls and in the cheeses containing *S. faecalis.* The persistence of a high population of *S. durans* (*13*) may have required use of a secondary source of carbon. Indeed, this ability to continue utilizing citrate may be why *S. durans* maintained a constant population throughout the 180-day sampling period (*13*). The additional acidity in the *S. durans* (*14*) cheeses may enhance the maintenance of proper pH for citrate permease, thus resulting in continuous depletion of the citrate.

Even more active in citric acid utilization than the cheeses just described are those manufactured with *S. durans* 15-20. Evidently, this strain of *S. durans* possesses a unique, very active citratase that is tolerant of the microenvironment of the curing cheese. Obviously, it is most efficient at the higher temperature. Since acetic acid is a by-product of CA breakdown (*10*), large quantities of acetic acid from the reaction may account for the greater amounts of FFA observed in cheeses made with *S. durans.* Also, it is possible that improper cooling of young Cheddar cheese could encourage growth of enterococci active in CA fermentation, thereby increasing the potential for formation of gas-related texture defects.

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J. Milk Food Technol. Vol. 38, No. 2, Pages 84-86 (February, 1975)

The Fatty Acid, Carbonyl, n-Hydrocarbon, and Phenolic Acid Composition of Wheat and Triticale Flours

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(Received for publication August 2, 1974)

ABSTRACT

A comparison of the chemical composition of wheat- and triticale flours demonstrated that their content of fatty acids, carbonyl compounds, and hydrocarbons is quite similar qualitatively but differed quantitatively. The total phenolic acid fraction from triticale grain, flour, bran, and shorts showed the largest amounts of phenolics in the bran and the smallest amounts in the flour. The predominant phenolic acids in all products were vanillic, ferulic, and p-coumaric acid. No marked qualitative or quantitative differences were found amoung various triticale flours nor when comparing wheat- and triticale flours. The differences in flavor between wheat- and triticale flours and baked products from these flours cannot be explained on the basis of kind and amounts of chemical compounds considered since they did not vary appreciably in these two cereal grains.

This review covers the few published articles about the fatty acid, carbonyl, n-hydrocarbon, and phenolic acid composition of the man-made cereal grain triticale in comparison with wheat. Many of the chemical compounds discussed in this review have a very characteristic odor and taste and could conceivably influence overall flavor, but it still seems almost impossible to predict what the addition of one aromatic compound to another or to a complex will yield (*12*).

Many investigators in the field of flavor believe that a substance which appears in relatively large quantities in a food contributes to the overall flavor of that food. Stating the importance of specific substances, however, may be much more difficult. The complexity of the problem has been expressed repeatedly.

FATTY ACID COMPOSITION

The proximate analyses and the major fatty acid composition of triticale- and wheat varieties, grown in the same location in Colorado are presented in Tables 1 and 2, respectively (*9*). Myristic, palmitic, stearic, oleic, linoleic, and linolenic acid were identified. The percentage GLC composition of the flours was quite similar. The differences, as shown in Table 2, seem to be too small to explain differences in flavor between these two cereal grains.

TABLE 1. *Proximate Analyses of Triticale and Wheat Samples*

Sample	Grain Protein[a] (%)	Flour protein[a] (%)	Flour ash[a] (%)
Spring Triticales			
6-TA-204	14.6	11.3	0.53
6-TA-206	14.5	11.7	0.54
Winter Triticales			
TR-385	12.0	8.7	0.47
TR-386	10.8	8.6	0.47
Wheat:Scout	12.0	11.6	0.43

[a]On a 14% moisture basis

TABLE 2. *Major Fatty Acid Composition of Wheat and Triticale Flours*[a]

	Triticales				Wheat
	Spring		Winter		winter,
Fatty acid	6-TA-204	6-TA-206	TR-385	TR-386	Scout
Myristic (C-14)	1.0	0.9	0.7	0.8	1.4
Palmitic (C-16)	18.0	19.2	19.3	18.2	19.8
Stearic (C-18)	0.6	0.6	1.3	1.1	1.2
Oleic (C-18:1)	10.0	11.7	12.8	11.5	12.4
Linoleic (C-18:2)	62.8	60.6	59.3	62.3	62.5
Linolenic (C-18:3)	6.7	6.6	6.5	5.0	2.3

[a]Expressed as % of total GLC composition

Certain acids individually tend to have somewhat unpleasant odors and could contribute to the flavor complex even when present in only trace amounts. Hunter et al. (*5*) reported a pleasant smell for a mixture of organic acids isolated from a brew, which did not resemble the flavor of bread. On heating, however, this mixture gave a strong aroma reminiscent of bread. Hunter et al. (*5*) also postulated that the higher fatty acids might function in bread flavor development by hindering the evaporation of lower-boiling component. Johnson et al. (*6*) reported that taste and aroma were directly proportional with acid production and decrease in pH, all of which would indicate that the fatty acids, which are originally present in wheat and triticale, contribute to overall flavor.

TABLE 3. *Short Chain Carbonyl Composition of Wheat and Triticale Flours*[a]

	Triticales				Wheat
	Spring		Winter		winter,
Carbonyl compound	*6-TA-204*	*6-TA-206*	*TR-385*	TR-386	Scout
Ethanal	6.9	16.2	7.3	15.0	5.9
Propanal	2.1	7.5	2.3	9.0	4.9
Acetone	22.4	16.0	19.8	12.0	12.5
Butanal	5.2	8.7	11.4	13.8	7.2
2-Butanone	15.6	12.7	19.4	17.0	29.0
Pentanal	32.0	23.6	5.1	15.1	9.8
2-Pentanone	10.0	3.4	6.8	6.6	12.1
Hexanal	3.8	8.0	10.3	7.6	10.8
Heptanal	2.0	3.9	17.6	3.9	7.8

[a]Expressed as % of total GLC composition

The most common decomposition products of unsaturated fatty acids are carbonyls which possess their own characteristic flavors or serve as precursor for other flavor related compounds.

CARBONYL COMPOSITION

The short-chain carbonyl composition of the wheat-and triticale flours, reported in Table 1, are presented in Table 3. Wheat- and triticale flours were found to have the same qualitative carbonyl composition but differed slightly quantitatively. The spring triticale fours were lower in 2-butanone and heptanal, but higher in pentanal in comparison with the wheat flour. The winter triticale flours were lower in 2-butanone than the winter wheat flour. Since the contribution of individual carbonyl compounds to baking quality and final baked product characteristics is not completely understood, an explanation of the significance of some of the differences in carbonyl composition was not attempted (*9*). It is, however, generally accepted that aldehydes and ketones are very important in production of flavor. Wiseblatt (*15*) reported that total aldehyde content has a direct correlation with the flavor and taste of bread. However, the flavor of baked products is derived not only from the ingredients of the formulation, but also from the compounds formed under the constantly changing conditions of moisture, pH, and temperature of the baking process (*13*).

While some researchers are of the opinion that bread flavor owes very little directly to the organic compounds already present in the raw material (*8, 14*), others feel that these compounds do contribute to overall flavor, but cannot agree as to the contribution of individual compounds even though they may be present at levels considerably above their threshold levels.

n-HYDROCARBON COMPOSITION

The n-hydrocarbon composition of the wheat-and triticale flours, given in Table 1, are presented in Table 4. Wheat- and triticale flours were found to have the same qualitative n-hydrocarbon composition but differed slightly quantitatively. The spring triticale flours showed a higher percentage of short-chain n-hydrocarbons (C-7 to C-11) than the wheat flour (*9*). The C-16, C-17, and C-18 n-hydrocarbons amounted to more than 30% of the total distribution in both the triticale- and the wheat samples. Comparing total GLC peak areas of n-hydrocarbons of wheat-and triticale flours, it was concluded that total n-hydrocarbons of triticale flours approximately equal those of wheat flours, which were reported to be present at 0.0036% in wheat flours (*16*).

TABLE 4. *n-Hydrocarbon composition of Wheat and Triticale Flours*[a]

	Triticales				Wheat
	Spring		Winter		winter,
Compound	6-TA-204	6-TA-206	TR-385	TR-386	Scout
C-7	6.4	4.6	3.7	2.8	4.5
C-8	5.4	3.8	4.9	2.6	5.3
C-9	6.6	5.5	4.3	3.3	4.4
C-10	4.2	4.1	4.1	3.8	3.3
C-14	6.9	6.2	6.6	8.2	7.9
C-15	5.5	7.8	7.0	8.2	8.0
C-16	9.7	10.9	11.7	12.4	12.4
C-17	14.3	15.3	13.2	19.2	12.9
C-18	11.7	12.8	14.8	11.4	12.2
C-19	6.0	9.5	6.4	9.0	10.8
C-20	7.7	6.3	8.5	5.2	6.8
C-21	5.4	4.6	6.2	6.1	4.1
C-22	4.2	2.7	4.9	1.9	1.9

[a]Expressed as % of total GLC composition

PHENOLIC ACIDS

Occurrence of phenolic acids in wheat and wheat milling fractions has been reported (*2, 4, 7*). Rye and triticale grains have also been found to contain these acids (*1, 3, 10*). Only the study by Maga and Lorenz (*10*) presents both a qualitative and quantitative distribution of phenolic acids in wheat- and triticale samples.

Phenolic acids have been characterized as possessing sour, bitter, astringent, and phenol-like flavors. The taste threshold values of the acids indicate that they can contribute to the flavor of flour and other milling fractions (*11*).

The phenolic acid composition of wheat- and triticale flours are given in Table 5. The same phenolic compounds which were found in wheat flour were also detected in triticale flours. There were no marked differences among types and amounts of phenolic acids comparing the two cereal grains. Predominant phenolic acids included vanillic, ferulic, and p-coumaric acid. Among triticale milling fractions the greatest amount of phenolics was reported to be in the bran and the smallest amount in the flours. The whole grains and the shorts milling fraction contained these phenolics in amounts in between those found for the flours and the bran fractions (*10*).

TABLE 5. *Phenolic Acid Composition of Wheat and Triticale Flour (ppm)*

	Triticales				Wheat
	Spring		Winter		Winter
Phenolic	6-TA-204	6-TA-206	TR-385	TR-386	Scout
p-Hydroxybenzoic	12	10	10	7	6
Salicylic	4	5	5	5	4
Vanillic	34	35	42	37	39
p-Coumaric	31	36	36	28	23
o-Coumaric	7	8	12	10	8
Iso-ferulic	4	4	6	5	4
Ferulic	27	30	25	26	32
Sinapic	10	12	8	9	15
Syringic	5	5	6	5	5
Chlorogenic	6	5	4	4	5

In an effort to postulate as to the possible flavor contribution of the phenolic acids found in wheat and triticale samples, Table 6 is presented. As can be seen, vanillic acid was found at levels higher than its reported taste threshold. Near threshold levels of p- and o-coumaric acids were found in both triticale- and wheat

TABLE 6. *Threshold Values and Possible Flavor Contributions of Phenolic Acids in Wheat and Triticale Flours*

Compound	Taste threshold (ppm)[a]	Wheat flour	Triticale flour
p-Hydroxybenzoic	40	–	–
Salicylic	90	–	–
Vanillic	30	+	+
p-Coumaric	40	(+)	(+)
o-Coumaric	25	(+)	(+)
Ferulic	90	–	–
Caffeic	90	–	–
Syringic	240	–	–

[a]From:Maga and Lorenz (*11*).
–: Present significantly below taste threshold
(+): Present near taste threshold
+: Present at or above taste threshold

flours. It is also necessary to realize that combinations of phenolic acids can drastically lower their individual taste thresholds (*11*). Equal concentrations of vanillic and p-hydroxybenzoic acid had a taste threshold of 10 ppm, whereas individually their taste thresholds were 30 and 40 ppm, respectively. Other examples of reduced threshold levels are reported by combining two or more phenolic acids which indicates that these acids singly or in combination can significantly contribute to the overall flavor of wheat- and triticale flours.

CONCLUSIONS

Reports in the literature indicate that the fatty acid, carbonyl, n-hydrocarbon, and phenolic acid composition of wheat- and triticale flours do not vary appreciably from each other. Many of the reported compounds have a characteristic odor and flavor and some of the compounds are present in these cereal grains at concentrations higher than their odor or taste thresholds. The difference in flavor between wheat and triticale flour and the baked products from these flours, however, cannot be explained on the basis of kind and amounts of chemical compounds considered in this review, since they simply did not vary appreciably in these two cereal grains, qualitatively and quantitatively.

ACKNOWLEDGMENT

This paper was presented in part at a symposium on triticale held during the 34th Annual Meeting of the Institute of Food Technologists, 1973.

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J. Milk Food Technol. Vol. 38, No. 2, Pages 87-88 (February, 1975)

A Membrane-Filter Technique to Test for the Significance of Sublethally Injured Bacteria in Retail Pasteurized Milk

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(Received for publication July 5, 1974)

ABSTRACT

A membrane transfer procedure previously described was used to study the possible role of sublethally injured bacteria in the keeping quality of retail pasteurized milk. Trypticase soy broth (TSB) was used as nonselective medium and TSB plus NaCl at pH 6.0 (TSBS 6.0), as the selective medium inhibitory to injured organisms. In pasteurized milk at early stages of storage, colony counts on the latter medium were much lower than on the former. Subsequent transfer of the TSBS 6.0 filters to fresh TSB and further incubation usually increased the counts to about the initial TSB range. Generally the organisms presumed injured and subsequently recovered were the same types as those considered uninjured. They were mainly streptococci and micrococci that produced only slow changes in litmus milk at 5 C, so they are of doubtful significance in the shelf life of retail milk at refrigerator temperatures.

It has been suggested that quality deterioration in certain processed foods could arise from recovery and growth of injured bacteria not initially detected by usual culturing procedures (*4,5*). In pasteurized milk, usually it is considered that poor keeping quality arises from post-pasteurization contamination with psychrotrophic species. It might be questioned if in some instances poor keeping quality is due to the subsequent recovery and growth of heat-injured organisms.

A membrane filter technique was shown to be useful in enumerating and isolating sublethally, heat-injured bacteria (*2*). The original procedure used broth cultures of a mixed flora from milk which were incubated at 32 C. Reported here is a study to test the procedure for investigating heat-injured bacteria in retail pasteurized milk.

METHODS

Sources of samples

Various brands of pasteurized homogenized milk were purchased at local retail stores at different times. "Pull" dates on cartons were used to obtain as fresh samples as available. Samples were taken promptly to the laboratory, held refrigerated, and examined the same day.

Membrane filtering procedure

The procedure used to examine milk samples was the same in priniciple as that previously reported (*2*). The fat content of homogenized whole milk limits the quantity that will pass through bacterial membrane filters (pore size 0.45 μm). Surfactant filtering aids are not satisfactory because they inhibit growth of heat-injured bacteria (*3*). In the method used, 10 ml of warm sterile water were added to the filter suction funnel with filter in place. One milliliter of the appropriate dilution of milk sample (usually 1:10) was added and vacuum applied. After filtering, the lower wall of the funnel was flushed with 10 ml of sterile water drawn through the filter. Occasionally the 1:10 dilutions of milk added to the 10 ml of water in the funnel (approximating 1:100 dilution) were difficult to filter, apparently because membrane porosity varied. Higher decimal dilutions caused no problem. Quadruplicate filters were prepared from each sample.

Trypticase soy broth (TSB) was used as the nonselective medium expected to support growth of uninjured and injured organisms. Prior tests showed that pH 7.5 gave higher counts on filters with pasteurized milk than did pH 7.0. As the selective medium to suppress growth of injured cells in pasteurized milk, TSB plus 2% NaCl at pH 6.0 (TSBS 6.0) was used after testing various medium modifications.

The media were added to absorbent pads in separate 47-mm plastic petri dishes (1.6 ml per dish). After the diluted milk sample was filtered, one pair of the quadruplicate filters was placed in TSB plates and the other pair in TSBS 6.0 plates. Both pairs were incubated at 25 C for 4 days. Plate counts with Standard Methods agar (*1*) also were made with incubation of 25 C for 4 days to provide supporting information on general count level. Although this medium may support growth of both injured and uninjured organisms, it will not differentiate between them. The 25-C incubation temperature was used to permit growth of both psychrotrophs and mesophiles and to avoid longer incubation at lower temperatures.

After colonies on each medium were counted, in some trials, those on TSBS 6.0 were marked by perforating the filters near each one with a sterile needle. The filters then were transferred to fresh TSB plates and incubated four additional days and colonies were counted again.

Types of bacteria

In several trials, after the second incubation, marked and unmarked colonies were picked into litmus milk and incubated at 5 C and room temperature. Cultures were observed for litmus milk reactions and examined for morphology and gram stain.

RESULTS AND DISCUSSION

Fourteen lots of retail pasteurized milk were tested as described, with the results shown in Table 1. From purchase to "pull" date varied from 4 to 10 days. Plate counts at 25 C were generally low, showing no particular relation with time until "pull" date. Most samples had a cooked flavor when tested.

Colony counts on TSB filters tended to be lower than the corresponding plate counts, but agreed reasonably well considering differences in techniques and media. Counts on TSBS 6.0 filters were much lower than on TSB filters. After transfer and further incubation the former counts increased considerably and usually approached the same range as the TSB counts. It should be noted that the TSB and TSBS 6.0 filters are duplicates but are not the same filters. Hence some variation can be expected between initial TSB and transfer counts.

TABLE 1. *Inhibition and recovery of bacteria in retail pasteurized milk by the membrane-filter procedure*

Sample	Days before "pull" date	Plate counts/ml	Membrane filter counts[a]		Transfer counts
			TSB 7.5	TSBS 6.0	
1	8	< 100	8	3	7
2	6	2000	125	85	165
3	5	800	73	17	76
4	4	800	68	36	63
5	10	1900	93	48	—
6	6	500	22	10	34
7	4	1200	68	8	67
8	—	—	86	30	79
9	5	1000	53	24	59
10	—	4300	TNTC	10	75
11	7	1000	50	18	58
12	10	100	15	10	10
13	9	850	56	37	54
14	—	1700	86	30	79

[a]Milk dilution, 10^{-1}. Each value is the average colony count from two filters.

Most colonies on the different filters were small and often difficult to see except with a microscope illuminator. Those on TSBS 6.0 tended to be smaller than those on TSB. These occasionally increased in size after transfer. New colonies after transfer also were usually small and appeared to be the same types as on the TSB and TSBS 6.0 filters. In bacterial populations with thermal resistance at about milk pasteurization temperature some organisms should survive uninjured while others of the same type might be sublethally injured.

It might be questioned if the TSBS 6.0 inhibited uninjured bacteria. As no medium is likely to equally inhibit, at the marginal level, various species in pasteurized milk, TSBS 6.0 may have surpressed some uninjured organisms. However, in three samples subsequently stored for varyious periods and that increased considerably in plate counts, counts on TSB and TSBS 6.0 were about equal (Table 2), which suggests

TABLE 2. *Effect of extended milk storage and bacterial increases on colony counts obtained on two filter media*

Sample	Days after "pull" date	Plate count/ml	Membrane filter counts	
			TSB 7.5	TSBS 6.0
1	6	15,000	146[a]	145[a]
2	3	20,000	150[a]	150[a]
3	0	80,000	105[b]	125[b]

[a]Milk dilution 10^{-2}. Each value is the average from two filters.
[b]Milk dilution 10^{-3}. Each value is the average from two filters.

no inhibition of active cells. Later observations indicated that the increased numbers developed from psychrotrophic contamination during processing and were different types than those detected initially in the low count samples.

Colony types

Most colonies recorded in Table 1 were small and white or faintly yellow, resembling streptococci and micrococci. Morphologically, most were gram-positive cocci with a few gram-positive rods. Occasionally a few larger, colored colonies were present.

Colonies picked into litmus milk caused only slow changes at room temperature. Some were acid-proteolytic. At 5 C little change was evident in two to three weeks and most changes suggested streptococci and micrococci. Some of the species are known to be relatively heat resistant and might be expected to be the types in low-count, pasteurized milk. They also grow slowly at refrigerator temperatures and are rarely associated with defects in retail milk.

In several samples that subsequently developed defects by "pull" date or soon after, the dominant flora differed from types present on initial examination. The organisms were gram-negative rods that caused rapid deterioration in litmus milk at room temperature. They also were heat sensitive, failing to survive laboratory heating at 55 C for 10 min.

Since there are literature reports of psychrotrophic sporeformers in pasteurized milk causing spoilage in some cases, surveillance was maintained for these types. Although some spore types were observed occasionally in this study, they showed little if any increase in numbers during refrigerated storage and did not cause defects at that temperature.

Additional studies with the membrane transfer procedure are in progress to follow changes in bacterial flora and quality during retail milk storage.

CONCLUSIONS

The membrane-filter procedure offers possibilities in detecting sublethally injured bacteria in low-count pasteurized milk. There are problems with milk filtration and with selecting the appropriate medium for marginal inhibition of injured bacteria in a mixed flora.

In early stages of storage the bacterial types considered to be revived, injured organisms generally were the same as those presumed to be uninjured. They were mostly gram-positive streptococci and micrococci. They produced little change in litmus milk at 5 C. From the samples studied, the revival and growth of sublethally injured bacteria seem of doubtful importance in quality changes in refrigerated retail milk.

ACKNOWLEDGMENTS

Contribution No. 899, Department of Dairy and Poultry Sciences, Kansas Agricultural Experiment Station, Manhattan.

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J. Milk Food Technol. Vol. 38, No. 2, Pages 89-93 (February, 1975)

Some Functional Properties of Succinylated Proteins from Fish Protein Concentrate

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ABSTRACT

Fish protein concentrate (FPC) prepared from hake extracted with hot isopropanol was partially solubilized by succinylation with succinic anhydride followed by heating at pH 9 for 30 min. The yield was 73% of the FPC, and the degree of succinylation of this protein isolate was 90%. FPC was also extracted at a high pH of 11.5; the yield was approximately 50%. Racemization was not detected in either the succinylated protein or the protein extracted at high pH. Pancreatin digestion of protein isolates resulted in release of 4.1 and 41.5% of the total lysine from succinylated fish protein and fish protein extracted at high pH, respectively, indicating a low apparent digestibility for the succinylated protein. The isoelectric point of succinylated fish protein was about 4, whereas that of fish protein extracted at high pH was about 5. Furthermore, fish protein extracted at high pH was relatively soluble below pH 4. Succinylated fish protein generally had a greater emulsion capacity and stability than did fish protein extracted at high pH which in turn had a greater emulsion capacity and stability than did gelatin. Addition of carboxymethyl cellulose decreased stability of the emulsion, but increased the emulsion activity. Succinylated fish protein formed a strong cheese-like curd upon quiescent acidification, whereas fish protein extracted at a high pH formed a very fragile curd. With addition of calcium ion, the curd tension of succinylated fish protein decreased, and the texture of the curd was smoother. Calcium ion precipitated the fish protein extract at high pH.

Fish protein concentrate (FPC) prepared by hot solvent extraction has few functional properties and has received intensive study, but mostly with regard to nutritional evaluation. In general, incorporation of FPC into foods has met with limited success in developing products with desirable characteristics (*2, 10, 15*).

Chemical derivatization of FPC or its proteins might be useful in favorably modifying their functional properties. Succinylation is a simple and effective means of modifying proteins (*11*). Reaction of protein alpha and epsilon amino groups with succinic anhydride yields a product with a much higher net negative charge at pH 7 since carboxyl groups have been substituted for amino groups. This substitution should result in a change in the functional properties of the protein which are dependent on quantity and distribution of charged groups.

Acylation of food proteins has been used to alter their functionality. Modification of egg white with 3,3-dimethylglutaric anhydride resulted in increased heat stability (*7*). Succinylated egg yolk proteins have been found to be useful in the production of mayonnaises and salad dressings (*6*). Acylated soybean proteins used to prepare coffee whiteners result in a product with good flavor, odor, and dispersion characterisitics (*11*). Plant proteins have been modified with succinic anhydride, among other acylating reagents, to yield products with improved functional properties (*3*). Recently, Groninger (*8*) prepared succinylated fish myofibrillar protein from undenatured myofibrillar protein. This modified fish protein hydrated fairly rapidly to yield viscous aqueous dispersions. It had good heat stability, relatively high emulsification capacity, and bland odor and flavor. The degree of succinylation was related directly to functional properties such as emulsification capacity. The protein efficiency ratio for succinylated fish myofibrillar protein was 2.86 compared to 3.64 for casein.

Unlike the myofibrillar protein used by Groninger (*8*), FPC prepared by hot solvent extraction of fish is comprised largely of denatured proteins and, as such, retains little functionality. It was the purpose of the research reported in this paper to improve the functionality of the denatured proteins in FPC by succinylation.

MATERIALS AND METHODS

Fish protein concentrate (FPC), prepared by hot isopropanol extraction of hake (*Merluccius merluccius*), was kindly supplied by Mr. John Spinelli, National Marine Fisheries Service, Seattle, Washington. All reagents were analytical reagent grade or the best grade available.

Preliminary treatment of fish protein concentrate

Approximately 50 g fish protein concentrate (FPC) were blended dry at high speed in a Waring Blendor for 3 min. Blended material was sieved through no. 25, no. 80, and no. 170 sieves. This treatment served to separate bone and scale particles from the finer protein particles. Bone and scale bits retained on sieves of the larger sizes were discarded, and the sieved protein was used for solubilization.

Extraction of fish protein at high pH

One method of solubilizing the denatured proteins of FPC is alkaline extraction (*17*). Thus, fish proteins extracted from FPC at high pH served as a basis for comparing their functional properties with those of succinylated fish proteins. A modification of the method of Tannenbaum et al. (*17*) was used to extract the fish protein from FPC. Sieved FPC was prepared as a 5% suspension (w/v) in distilled water. The pH was adjusted to range from 11.5-12.0 with 1 N NaOH. This was followed by heating the suspension in a 100 C water bath for 25 min. During this time the pH slowly decreased to around 10. The suspension was then centrifuged, and the supernatant was acidified to pH 4.5 with 1 N HCl to precipitate the protein. The precipitated protein was resuspended in water and dialyzed against distilled water for 48 h at 4 C and was lyophilized. Yields from this procedure were about 50% of the FPC.

Optimal conditions for solubilization of fish protein by succinylation

The scheme for solubilization of fish protein from FPC by succinylation is shown in Fig. 1. Variables studied to optimize the solubilization of fish protein by succinylation included (*4*):

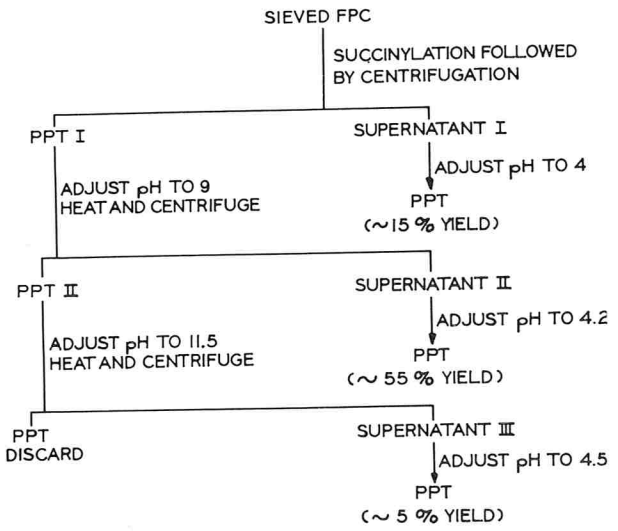

Figure 1. *Scheme for solubilization of fish protein from FPC.*

concentration of FPC, effect of heating of ppt. I on yield, effect of heating time, effect of pH when heating, effect of pH on succinylation, and effect of succinic anhydride (Aldrich) concentration. The precipitated protein was redissolved at pH 7, dialyzed, and lyophilized.

Chemical characteristics of succinylated FPC

Amino acid composition. The amino acid compositions of FPC extracted at high pH and succinylated FPC were determined using the technique of Spackman et al. (*16*). A Phoenix M7800 analyzer was used in the analyses.

Degree of succinylation of FPC. Succinylated FPC preparations were reacted with fluoro-2,5-dinitrobenzene (Eastman) (*12*). The dinitrophenylated proteins were hydrolyzed in 6 N HC1 for 24 h, and the hydrolysates were analyzed for lysine using the Phoenix M7800 analyzer. The degree of succinylation was estimated from lysine analyses of protein before and after dinitrophenylation.

Enzymic digestibility of succinylated FPC. Succinylated FPC, FPC, and whole egg protein were subjected to pancreatin (Nutritional Biochemicals) digestion using a modification of the method of Akeson and Stahmann (*1*). Pepsin was not used in the digestion procedure, and sulfosalicylic acid (Fisher) (3.5%) was used as a protein precipitant. The relative digestibility was calculated from the lysine released during treatment of the FPC preparations and total lysine in each protein preparation. Egg protein was also used for comparison.

Racemization. The method of Larson et al. (*9*) was used to determine the extent of racemization of the fish protein as a result of exposure to high pH values at high temperature. Portions of protein extracted at high pH and succinylated protein were hydrolyzed in 6 N HC1 at 110 C for 22 h under nitrogen. Two milliliters of hydrolysate adjusted to about 1 μmole/ml were each incubated with D-amino acid oxidase (Worthington). Catalase (Worthington) (8800 units) was incorporated into the system to destroy H_2O_2 and generate O_2. Tetracycline (Rachelle Lab.) (1000 ppm) was added to prevent microbial growth. Sulfosalicylic acid (3.5%) was used to precipitate the enzymes. The supernatants were subjected to analysis for L-amino acids.

Physical characteristics of succinylated FPC

Solubility. The relative solubilities of succinylated fish protein and fish protein extracted at high pH were determined by stirring protein suspensions maintained at various pH values at 25 C for 30 min. The suspensions were centrifuged, filtered and the absorbance of the filtrate was determined at 280 nm after appropriate dilution with sodium bicarbonate buffer (0.1 M, pH 8.0). Protein concentrations were determined from standard curves prepared from the appropriate protein.

Emulsifying activity. The technique of Wata and Ishii (*18*) was used to study the emulsion activity of protein extracted at high pH and succinylated proteins. Solutions of gelatin, succinylated proteins, and protein extracted at high pH were prepared at concentrations of 0.1, 0.5 and 1%. These solutions were used to emulsify various amounts of Mazola corn oil using a 50-ml Potter-Elvehjem tissue grinder as the homogenizer. The system was subjected to emulsification for 5 min at ambient temperature. The emulsions were centrifuged at 1,300 × *g* for 5 min in a calibrated conical centrifuge tube, and the volume of emulsion and total volume were measured to derive the emulsion activity by dividing the emulsion volume with total volume. The emulsion stability was determined after heating the emulsions at 80 C for 30 min followed by cooling the emulsions in tap water for 15 min. From the emulsion activity after heating, the emulsion stabilities were calculated as follows:

$$\frac{\text{Emulsion activity after heating}}{\text{Emulsion activity before heating}} \times 100$$

Curd formation. Glucono-delta-lactone (Pfizer) was used as an acidogen (*5*) to acidify 250 ml of 3% protein solutions prepared from protein extracted at high pH and succinylated protein. In addition, calcium chloride at a final concentration of 0.05 M or 3% corn oil were each incorporated into separate 3% protein solutions to observe their effects on curd strength. When the pH decreased to the desired values as a result of lactone hydrolysis, relative curd tension was measured with a Brookfield viscometer (Model RVF) counted on a Brookfield Helipath stand, with a T-C spindle at a speed of 20 rpm, at 20 C (*14*). Raw skim milk was treated with a 1 to 50 dilution of calf rennet at 20 C for 1 h to prepare a curd for comparative purposes.

RESULTS AND DISCUSSION

Blending and sieving the FPC resulted in a yield of protein particles of 86.4 ± 2.8%. Blending did not reduce the size of the scales or bone fragments appreciably, and the sieving effectively separated these particles from the crude protein.

For practical purposes the following conditions were selected for extraction of succinylated protein from FPC: a 10% FPC suspension was succinylated with 10% (w/w) succinic anhydride at pH 8 for 60 min at ambient temperature. The suspension was then heated at 100 C for 30 min at pH 9.0. After centrifugation of the reaction mixture at 1000 × *g* for 15 min, the supernatant was adjusted to pH 4.2 to precipitate the protein. The precipitated protein was redissolved at pH 7, dialyzed, and lyophilized. The yield using this procedure was 72.9% ± 1.6.

The sieved FPC can be completely dissolved in water by heating it at a pH higher than 12.5. The unsieved FPC formed a very viscous suspension, and the yield of recovered protein was only about 50%, similar to that observed by Tannenbaum et al. (*17*). Extraction of the protein at such a high pH resulted in generation of malodorous compounds which were not evident in the succinylation procedure. Furthermore, amino acid analyses of proteins extracted at high pH indicated complete destruction of cysteine.

The protein contents (N × 6.25) of the various preparations in this study were 85.7 ± 2.0% for crude FPC, 88.7 ± 2.9% for succinylated fish protein and 90.3 ± 5.8% for fish protein extracted at high pH. The solubilities of the protein extracted at high pH and succinylated fish protein as a function of pH are shown in Fig. 2. The marked differences in solubilities at acidic pH values presumably reflected the substitution of

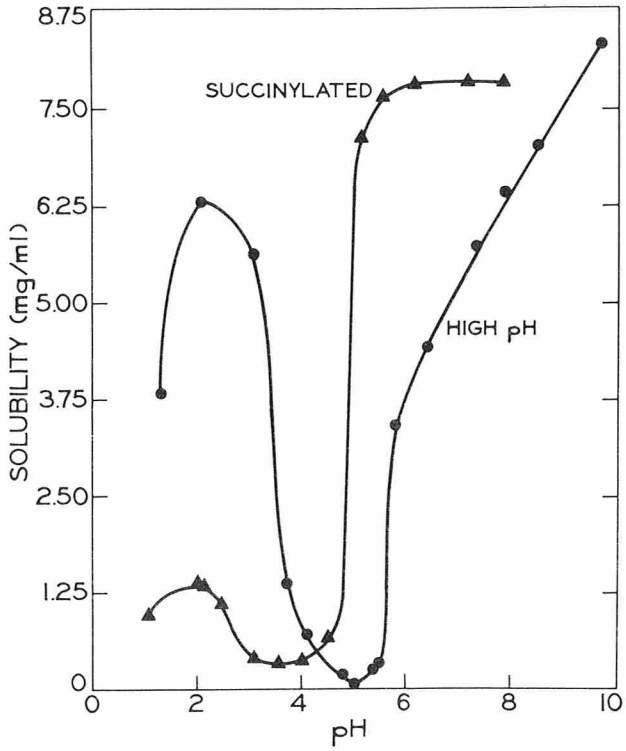

Figure 2. *The solubilities of fish protein extracted at high pH and succinylated fish protein as a function of pH.*

carboxyl groups for amino groups as a result of the succinylation. Minimum solubilities were at pH values of 4 for the succinylated protein and 5 for the protein extracted at high pH. The succinylated protein solubilized rapidly in the pH range of 4.5 to 5.5 and was relatively insoluble below pH 4. By comparison, the solubility of the protein extracted at high pH was quite high below pH 4 and increased less drastically above pH 6.

The degree of succinylation of the fish protein was 90.1 ± 2.4% by the fluoro-2,5-dinitrobenzene method. This extent of succinylation to maximize solubilization of the proteins might have a detrimental effect on the digestibility of the succinylated fish proteins. The apparent digestibility of succinylated fish protein was compared to FPC and whole egg protein by measuring the percentage of the total lysine released by digestion and pancreatin. Only 4.1 ± 0.4% of the total lysine was released from succinylated fish protein compared to 41.5 ± 1.2% for FPC and 18.7 ± 2.9% for whole egg. Apparently enzymes that could remove the succinate group or cleave the bonds adjacent to the succinylated lysine in the protein were not present in the pancreatin. By comparison, Groninger (*8*) reported a protein efficiency ratio of 78.5% compared to casein for succinylated fish myofibrillar protein. However, the myofibrillar protein was succinylated only 30-40% compared to the 90% in the present study. It is likely that the 90% succinylation to maximize solubility would be highly undesirable from a nutritional point of view. Perhaps the fish proteins of FPC could be succinylated to a lesser degree, but extracted at a higher pH to maximize the yield.

Although Tannenbaum et al. (*17*) reported 16% racemization in fish protein extracted at high pH from FPC, reacemization was undetectable in either the succinylated fish protein or the protein extracted at high pH using D-amino acid oxidase. However, the present extraction of fish protein at high pH was less rigorous than that used by Tannenbaum et al. (*17*). In a D, L alanine control, 50% of the alanine was destroyed by the D-amino acid oxidase. Although D-amino acid oxidase will not oxidize D-cysteine, D-aspartic acid, D-glutamic acid, D-lysine and D-asparagine, any racemization should be detectable in the remaining amino acids.

The emulsion stabilities and activities of succinylated fish protein, protein extracted at high pH and gelatin under a variety of conditions are listed in Table 1. In general, when the protein concentration increased, the emulsion activity increased at every level of oil. At 0.1% protein, the emulsion stability decreased as the oil content increased. This was not unusual since there was probably not enough protein to form a film on the surface of the oil droplets to maintain an emulsion. At a 0.1% concentration, gelatin was a very ineffective emulsifying agent. At protein concentrations above 0.5%, all emulsions were relatively stable. Protein extracted at high pH at a 0.1% level had a similar emulsion activity up to 50% oil, but a lower emulsion stability than that of the succinylated protein. The maximum emulsion activity for the succinylated protein was 57% compared to 52% for the protein extracted at high pH. At 0.5% protein concentration, when the oil content was above 40%, succinylated protein had a higher emulsion activity than protein extracted at high pH. By comparison at 0.5% protein the maximum emulsion capacity for protein

TABLE 1. *Emulsion activites and stabilities of succinylated fish protein, fish protein extracted at high pH and gelatin*

		High pH		Succinylated		Gelatin	
% Protein (w/v)	% Oil (v/v)	Activity	Stability	Activity	Stability	Activity	Stability
0.1	20	—	—	20.7	101.1	(oil separated	
	30	31.5	84.6	30.4	99.0	in all cases)	
	40	41.0	86.2	41.6	99.6		
	50	37.3	58.6	51.2	72.7		
	60	—	—				
	max. capacity	52		57			
0.5	10	—	—	—	—	10.5	84.4
	20	24.2	99.2	22.8	99.3	20.5	68.3
	30	34.0	99.0	33.8	100.1	31.2	56.8
	40	44.7	101.1	45.6	99.9	40.0	60.0
	50	54.1	101.7	56.4	100.4	49.8	63.0
	60	66.4	100.1	68.8	99.1		
	max. capacity	57		67		66	
1.0	10	12.0	99.1	—	—	10.6	97.7
	20	23.2	90.5	25.0	102.1	22.3	89.5
	30	35.7	92.2	36.6	104.8	33.6	83.3
	40	44.4	96.4	50.8	104.6	42.0	75.4
	50	51.3	99.0	67.7	102.8	52.6	73.3
	60	70.5	99.0	83.1	101.1		
	max. capacity	67		72		60	

extracted at high pH, succinylated protein and gelatin were 57, 67 and 66%, respectively.

At 1% protein concentration, the succinylated protein had a higher emulsion activity than the protein extracted at high pH had at any level of oil content. Furthermore, the maximum emulsion capacity was 67% for the protein extracted at high pH compared to 72% and 60% for succinylated protein and gelatin, respectively. These results might be due to highly negative charges on the succinylated protein resulting in a more expanded film.

TABLE 2. *Emulsion activities and stabilites at various pH values and and in the presence of 0.127* M Ca^{++} *and 0.254* M Na^{+}*(1% protein plus 50% corn coil)*

	High pH		Succinylated	
	Activity	Stability	Activity	Stability
	(%)		(%)	
pH 7.2	51.3	99.0	66.7	102.8
pH 4.6	59.7	109.6	64.7	101.4
pH 3.6	58.4	105.8	57.7	105.3
Ca^{++} pH 7.2	59.3	111.0	59.2	113.2
Na^{+} pH 7.2	55.1	92.3	57.6	98.4

Table 2 lists the emulsion activities and stabilities of protein extracted at high pH and succinylated protein as a function of pH and in the presence of calcium or sodium ions. Both emulsion activity and stability increased with protein extracted at high pH at the lower pH values, but the activity decreased in succinylated protein undergoing the same treatment. The amino groups of the succinylated protein were substituted by carboxyl groups, thus decreasing the positive charges on the protein.

Succinic acid has a pK of about 4.6. When the pH was decreased, the net charge on the succinylated protein also decreased thus reducing repulsive force. This apparently resulted in a lower emulsion activity even though emulsion stability stayed the same.

Addition of calcium ion caused increases of both emulsion activity and stability with the protein extracted at high pH, but increased only the stability with succinylated protein. On the other hand, addition of sodium ion decreased both values. The divalent calcium ion might have acted as a cross-linking agent holding the protein molecules around the suface of the oil droplet, thus increasing the strength of the protecting film. Sodium ion could have neutralized the charges on the proteins, decreasing the repulsive forces and causing the oil droplets to clump together, thus breaking the emulsion.

Data in Table 3 for succinylated protein indicate that small amounts (0.1%) of carboxymethyl cellulose (CMC) (Hercules-type 9 M 31F) decreased the emulsion activity and stability, but at higher levels, CMC increased the viscosity, so that the emulsion activity was increased. At the same time the emulsion stability tended to decrease.

Curd-forming properties

It appeared that the protein extracted at high pH was composed of two major fractions of protein. One fraction was more readily soluble, and the other fraction tended to aggregate and form a fibrous structure when the pH

TABLE 3. *Effect of carboxymethyl cellulose gum (CMC) on emulsion activities and stabilities (1% succinylated protein plus 30% v/v corn oil)*

CMC (w/v)	Emulsion activity	Emulsion stability
(%)	(%)	
0.0	36.6	104.8
0.1	34.5	98.9
0.2	38.8	97.9
0.3	39.7	98.2
0.4	41.9	91.6
0.5	46.3	95.8
0.6	53.6	86.4

was slowly decreased. When small amounts of glucono-delta-lactone were added to 3% protein (extracted at high pH) solution as an acidogen, the decrease in pH was slow, and two fractions tended to separate with the fibrous fraction settling to the bottom. As the pH decreased further, the fibrous fraction tended to dissolve again. This behavior would be expected from the solubility characteristics of the protein extracted at high pH as shown in Fig. 2. Thus the amount of glucono-delta-lactone had to be controlled very carefully to form a curd-like structure. The curd tensions determined for curds formed from 3% solutions of protein extracted at high pH and succinylated protein under various conditions are shown in Fig. 3. Curd

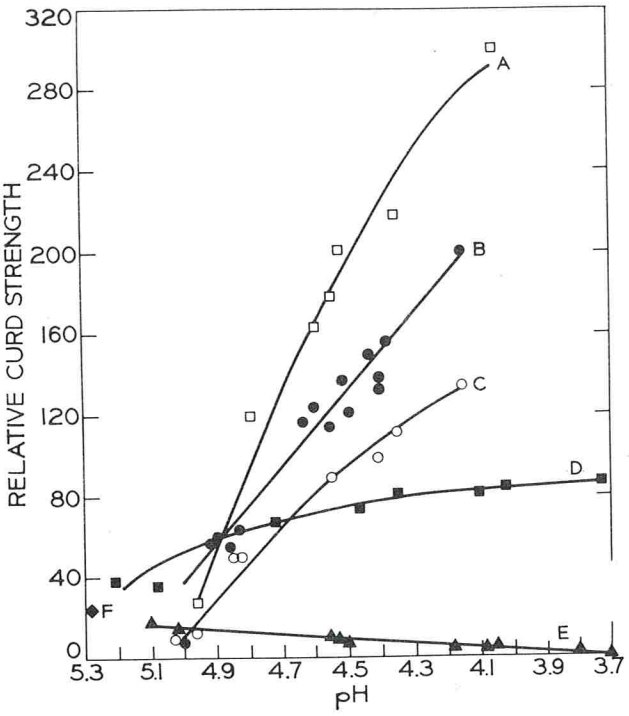

Figure 3. *Relative curd strength of gels prepared from 3% solution of protein extracted at high pH, succinylated protein at various pH values and raw skim milk. A. Succinylated protein + corn oil. B. Succinylated protein. C. Succinylated protein plus* Ca^{++}*. D. High pH protein plus corn oil. E. High pH extracted protein. F. Raw skim milk curd prepared with rennet. Relative curd strength values on the abscissa are Brookfield viscometer readings.*

tension for raw skim milk treated with rennet at pH 6.5 and 20 C is shown for comparative purposes. Since the solubility of protein extracted at high pH increased when the pH decreased below 5.0 (Fig. 2), the curd tension of this protein decreased with the pH.

With the addition of calcium ion (final concentration 0.05 M) to a solution of the protein extracted at high pH, a precipitate formed rapidly at a pH value of 7.2. This precipitate was not soluble at a pH value of 9 and was not suitable for curd formation.

By comparison, the succinylated protein yielded a very strong curd as the pH was reduced toward 4.0 by the addition of glucono-delta-lactone. However, the addition of calcium ions (0.05 M) resulted in a decrease of the curd strength. It may have been that the calcium ion concentration was too high yielding proteins with net positive charges resulting in molecular repulsion and poor curd properties. In this regard, an optimum calcium ion concentration may be necessary for proper curd formation. It is interesting that the incorporation of an amount of corn oil equal in weight to the protein (3%) resulted in stronger curds for both the succinylated protein and the protein extracted at high pH. The reason for this increased curd tension is unknown at this time.

In general, our data indicate that fish proteins of FPC can be solubilized by succinylation without evident racemization of the amino acids. Both fish proteins extracted at high pH and succinylated fish protein were good emulsifiers and better than equivalent quantities of gelatin. Furthermore, as the pH values of solutions of succinylated proteins were decreased by the acidogen, glucono-delta-lactone, a strong curd was formed. A product analogous to cheese can be visualized whereby emulsions of milk fat and succinylated protein in cheese whey can be inoculated with *Streptococcus lactis.* Fermentation of the lactose in the whey should decrease the pH and form the curd. However, concern must be expressed for low digestibility of the succinylated protein which may result from the acylation.

NOTE ADDED IN PROOF

Korschgen and Baldwin (*J. Food Sci.* 38:178-180, 1973) isolated fish proteins from fish protein concentrate by an alkaline extraction process. The soluble proteins were evaluated in the stabilization of mayonnaise-type emulsions.

ACKNOWLEDGMENTS

This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison and by a grant from the Cooperative Research Service, U.S. Department of Agriculture.

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J. Milk Food Technol. Vol. 38, No. 2, Pages 94-99 (February, 1975)

Air Injection by Self-Aspirating Impeller in Aerobic Fermentation

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(Received for publication March 1, 1974)

ABSTRACT

A novel fermentor is described in which aeration is achieved by air induction through a hollow impeller system: no air pump is required. Air flow into the vessel increased with rotor speed, impeller diameter and height of liquid above the impeller. Although longer impellers increased the air flow rate, oxygen transfer was favored by shorter impellers. The presence of a draft tube in the vessel creates a vertical flow pattern in the medium which increases gas hold-up and, therefore, oxygen transfer. Biological tests on the system using a mold and a yeast showed that performance compared favorably to conventional fermentor systems using separate aerators and agitators.

In recent years, much attention has been given to development of novel food production processes independent of agricultural land use. Processes which seem to attract most interest are those in which yeasts or fungi are used to manufacture an edible protein from the aerobic fermentation of various substrates such as hydrocarbons and waste carbohydrates (*6, 11*). The success of commercial applications of various fermentation processes depends not only on availability of a low cost substrate but also on the capital cost in fermentor design and the economical operation of the system (*5*).

The objective of this research was to develop a fermentor in which two essential mechanical needs for the process, aeration and agitation, were met by a single moving part, thus decreasing the capital investment while maintaining good gas exchange for efficient operation.

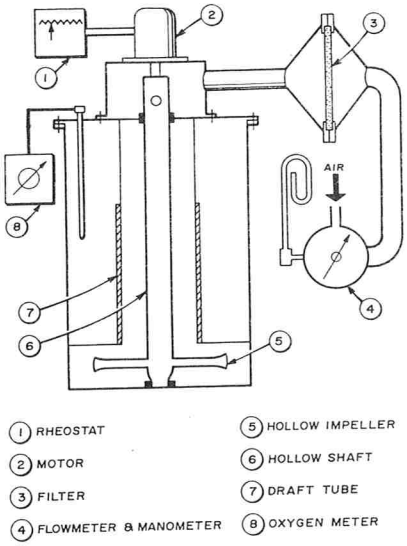

Figure 1. *Schematic of developed fermentor.*

DESCRIPTION OF THE FERMENTOR

The laboratory fermentor (Fig. 1) is a cylindrical stainless steel tank 20.4 × 15.2 cm, fitted with a lid from which extend three plastic bars that support the draft tube and the rotor. The lid is secured to the tank by means of screw bolts so that it would be easy to disassemble for purposes of cleaning, repairs and sterilization.

The rotor consists of the hollow shaft and agitator/injector system. The hollow shaft is equipped with orifices which are located at its upper end in the air-tight chamber which is sealed by means of a Teflon® bushing. The agitator/injector system is made up of four hollow impellers which are screwed tightly to the lower end of the shaft and are curved in the form of arcs at right angles to the cylinder. The agitator/injector system is located at a depth of 12.7 mm from the bottom of the tank to ensure the aeration of the lower part of the fermentor.

The design makes use of the agitator/injector system and a draft tube to provide a forced vortex condition in the fermentor as well as creating a pump action which ensures thorough mixing of the medium (*3, 9*). The comination also helps to control the foaming tendency of the broth. Foam control is very important as resistance created by foam to outflow of gases can cause enough back pressure to reduce the airflow rate considerably (*7*). Air filtration is achieved by means of cotton wool held between two perforated plates.

The simplicity of the design is centered around the fact that there is only one rotating part which: (*a*) draws in the air, (*b*) acts as a fine grain sparger, and (*c*) thoroughly mixes the aerated medium.

AIR AND LIQUID FLOW IN THE FERMENTOR

Air intake into the fermentor can be pictured in terms of two phenomena, namely: (*a*) negative head produced by the rotor and (*b*) vorticity in the fermentor. At sufficiently high speed, the pressure developed by the rotor owing to hydrodynamic forces at the impeller openings becomes so low that air is forced into the medium from the atmosphere. The minimum angular velocity to introduce air by suction for different diameters of impellers was measured as given in Table 1.

TABLE 1. *Minimum angular speed to introduce air into the fermentor*

Depth (mm)	Rotational diameter (mm)	Rotor speed (rpm)
101.6	101.6	300
101.6	82.55	380
101.6	50.8	600

Initially, the hollow shaft is filled with liquid. As soon as it starts to rotate, there will be a relative flow of liquid around the impeller profile and a centrifugal force on the liquid in the impeller. Owing to the roughness of the hollow rotor blades, eddies will develop at their ends. The total effect will be that of creating a negative head behind them. A pressure difference will then be established at the interface of the blade orifices with the medium. The liquid in the rotor will first flow out

through the orifice and air is then "drawn" in from the atmosphere so that a column of air is established from the atmosphere to the rotor. At this point, the pressures acting at the air/liquid interface at the orifice are in equilibrium (Fig. 2) so that

$$P_L + P_A = 0 \qquad \ldots\ldots\ldots (1)$$

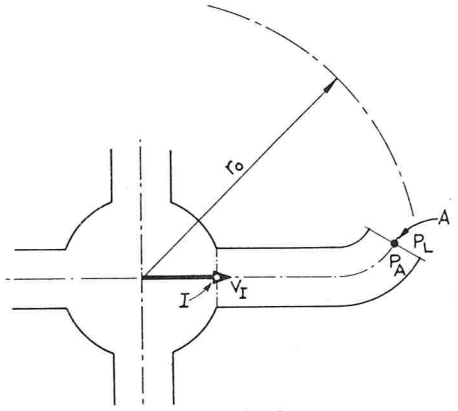

Figure 2. *Pressure equilibrium at point A.*

Ignoring the effect of rotation on the flow pattern by assuming: (*a*) impeller stationary and liquid moving in Newtonian streamline flow, and (*b*) non-compressible fluid, Bernoullis equation can be written for points I and A in Fig. 2:

$$P_A = P_{I\,stat.} + \frac{v_I^2 \cdot \gamma_A}{2g}(1 - f_A) \qquad \ldots\ldots\ldots (2)$$

The flow of liquid in the fermentor is designed to achieve: (*a*) homogeneous distribution of dissolved oxygen in the medium, (*b*) a forced vortex within the fermentor. The impeller and the draft tube produce a pump action similar to that of a centrifugal pump and the entire content of the fermentor is moved through the draft tube in a very pronounced downward current. This process and the impeller action cause a thorough mixing effect.

Rotation of the liquid creates a vortex, so that the pressure at any point along the streamline described by the rotational path of the impeller orifice, neglecting friction, is given (*3*) by

$$P_L = \frac{\omega^2 \cdot r^2 \cdot \gamma_L}{2g}(1 - f_L) - \gamma_L z \qquad \ldots\ldots\ldots (3)$$

where r is measured from the axis of rotation.

Equations 3 and 1 can be combined to give

$$\frac{v_I^2 \cdot \gamma_A}{2g} = \frac{\omega^2 r^2 \gamma_L}{2g}(1 - f_L) - \gamma_L \cdot z - \frac{v_I^2 \gamma_A}{2g} \cdot f_A - P_{I_S} \qquad \ldots\ldots\ldots (4)$$

The expression on the right hand side represents the dynamic pressure at orifice A and can be calculated from the data taken with the air flow meter. The maximum differences in total pressure recorded by the water manometer are given in Table 2.

TABLE 2. *Effect of impeller length on pressure development*

Rotation diameter (mm	Impeller speed (rpm)	Total pressure difference (mm H_2O)
50.8	1122	5.08
82.55	1032	8.89
101.6	1008	14.93

The airflow rate can be expressed in terms of angular velocity of the rotor. From Figures 4 and 5, it can be deduced that

$$Q_2 = Q_1 \left(\frac{N_2}{N_1}\right)^K \qquad \ldots\ldots\ldots (5)$$

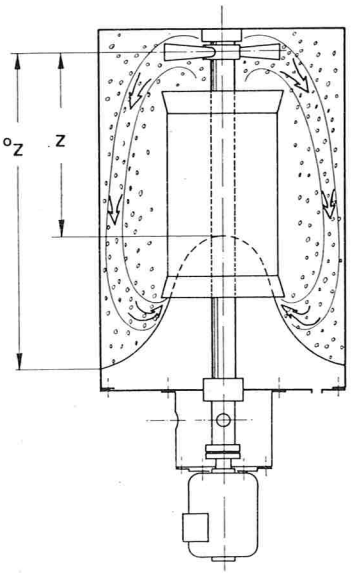

Figure 3. *Illustration of liquid flow pattern.*

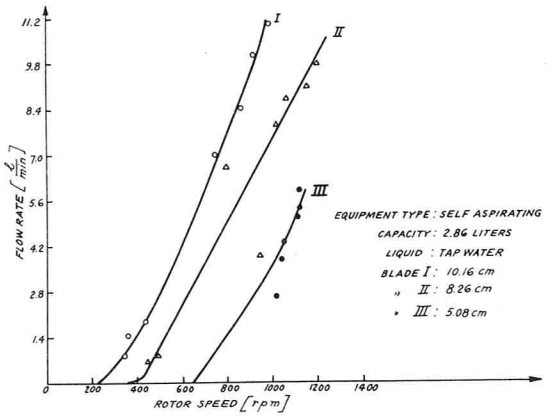

Figure 4. *Air intake vs. speed of rotation of rotor.*

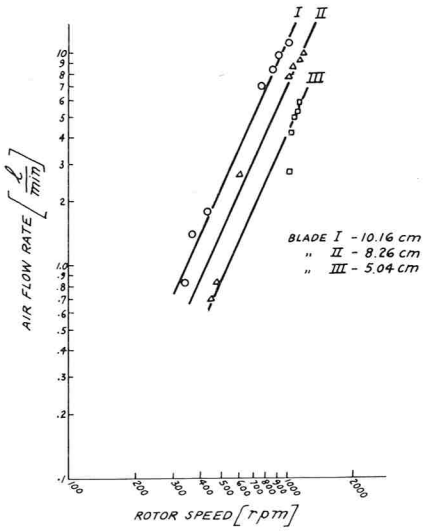

Figure 5. *Log-log plot of airflow rate vs. rotor speed.*

Equation 5 implies that the airflow rate Q can be expressed as

$$Q = K_0 N^K \qquad \ldots\ldots\ldots (6)$$

The condition of the free surface of the liquid (Fig. 3) can be approximated as (3)

$$Z = Z_0 + \frac{\omega^2 r^2}{2g}(f_3 - 1) \quad \ldots\ldots\ldots (7)$$

A friction factor f_3 is introduced in equation (7) to correct for losses due to friction and reduction in liquid momentum caused by the draft tube support. It is to be noticed from equation (7) that $(Z_0 - Z)$ is a measure of vortex depth which is probably directly proportional to air intake into the fermentor. If the path of the paraboloid lies close to the horizontal axis of the hollow impeller then Z = O, and

$$Z_0 = \frac{w^2 r^2}{2g}(1 - f_3) \quad \ldots\ldots\ldots (8)$$

This represents the maximum vortex and possibly the maximum air intake is approximated at this point. Airflow rates were measured at various angular velocities, rotational diameters of the impeller and under varying heads of liquid above the impeller by means of a very low resistance flow indicator. The airflow rate increased with increase in any of the items mentioned above (Fig. 4, 6 and 7). Correlating increase in airflow rate with height of liquid above the impeller should be viewed with caution because the fermentor was only of laboratory scale. It is, however, clear that air flow rate increases with the depth of vortex so that, if the angular velocity is not limiting, an increased liquid height will furnish a greater vortex depth and consequently higher airflow rate.

Data mention above were obtained with the fermentor charged with tap water. In the actual fermentation runs, the airflow rate decreased with time as the biomass accumulated (Fig. 9). The decrease could be due to an increase in drag force because of increased viscosity. It is evident that the drag force retards the generation of air bubbles and their subsequent movement to the liquid surface and, at the same time, the rotor experiences resistance to motion through the medium. A number of forces, some of which retard and some of which facilitate bubble formation, have been identified as (8):

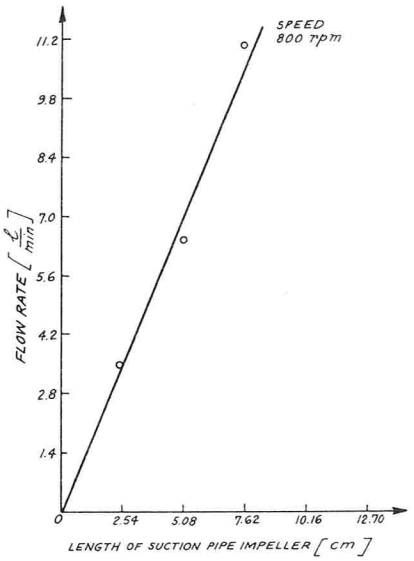

Figure 6. *Air intake vs. impeller length.*

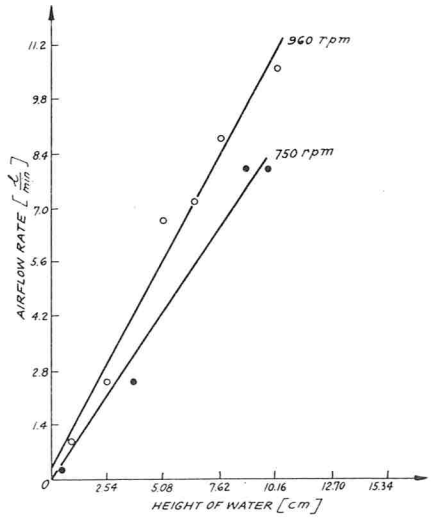

Figure 7. *Air intake vs. height of water above impeller.*

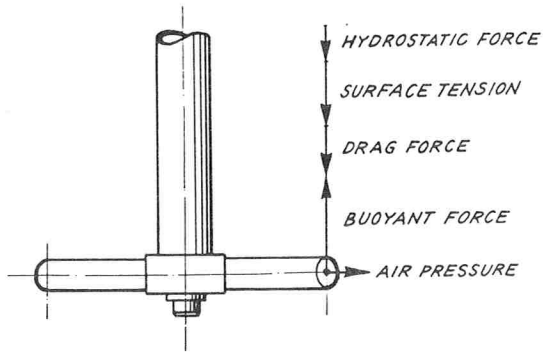

Figure 8. *Total forces acting on air bubble at impeller opening.*

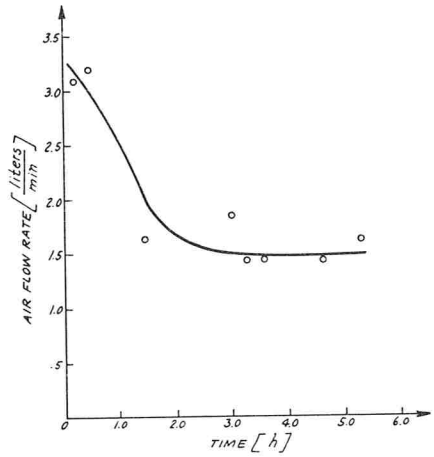

Figure 9. *Air flow rate vs. time during fermentation of whey with* Kluyveromyces fragilis.

(*a*) buoyant force = $A_b \gamma_L z$

(*b*) pressure force = $\frac{\gamma_a \omega^2 r^2 A}{2g}$

(*c*) dynamic force with respect to gas flow at the impeller opening

$$= \sigma \pi d_b$$

(*d*) surface tension = $V_b(\gamma_L - \gamma_a)$

(*e*) hydrostatic force = $A_b P_b$

(*f*) drag force = $CA_b V_t$

A force balance at the impeller opening is illustrated in Fig. 8. The resultant path of the bubble at the orifice is described by

$$V_b(\gamma_L-\gamma_a)+A_b P_b+\frac{\gamma a\omega^2 r^2 A}{2g}-\sigma\pi d_b-A_b\gamma_L z-CA_b V_t=M_b\frac{dV_t}{dt}\quad\ldots\ldots(9)$$

In a stagnant liquid, it is assumed that the pressure force in the bubble is equal to the hydrostratic pressure (*10*) which implies that the bubble volume increases as the bubble rises through the liquid according to gas law. If this notion is applied to the above equation, the pressure force cancels with the hydrostatic force so that

$$V_b(\gamma_L-\gamma_a)+\frac{\gamma a\omega^2 r^2 A}{2g}\sigma\pi d_b-CA_b V_t=M_b\frac{dV_t}{dt}\quad\ldots\ldots(10)$$

$$V_0=\frac{1}{M_b}\int_0^t\left[V_b(\gamma_L-\gamma_a)+\frac{\gamma a\omega^2 r^2 A}{2g}-\sigma\pi d_b-CA_b V_t\right]dt\quad\ldots\ldots(11)$$

where V_0 represents instantaneous bubble velocity at the orifice. At the same time, oxygen is absorbed from the bubble into the liquid and carbon dioxide is transferred from the liquid into the bubble. Absorption efficiency for a low solubility gas sparged in a gas-free liquid is given (*10*) by the following equation

$$\frac{Q_{in}-Q_{out}}{Q_{in}}=1-e^{-ch}\quad\ldots\ldots(12)$$

that is

$$\frac{Q_{out}}{Q_{in}}=e^{-ch}\quad\ldots\ldots(13)$$

Analysis of bubble movement to the liquid surface is very complex, especially when wall effect, liquid flow, bubble interactions with each other, suspended particles and chemical properties of the medium are also taken into consideration. A detailed analysis of this was not undertaken since the emphasis was on production of biomass.

It is interesting to note that the airflow rate vs. time as obtained in whey fermentation seemed to be an exponential function (Fig. 9). This coincides with the change in liquid properties as effected by the exponential growth rate of the organisms. Mention must be made of the curved portion of the hollow impeller. A careful design of this is called for, to minimize the resistance to flow. In fact, a considerable difference in airflow rate was observed by using a more gentle curvature.

OXYGEN TRANSFER IN THE FERMENTOR

The oxygen transfer characteristics of the fermentor were investigated at various impeller diameters. Water was deoxygenated using sodium sulphite catalysed by cobalt chloride. Aeration was started and the dissolved oxygen measured with time (Fig. 10). Since good flow and good mixing was obtained in the fermentor, it can be assumed that the oxygen concentration throughout the tank was uniform so that the increase in the average oxygen concentration of the liquid in the fermentor per unit time was given (*4*) by an unsteady state form of absorption equation

$$\frac{dX_L^{O_2}}{dt}=K_L^a\left(X_S^{O_2}-X_L^{O_2}\right)\quad\ldots\ldots(14)$$

K_L^a was evaluated from a plot of $X_S^{O_2}-X_L^{O_2}$ against time on a semi-logarithmic paper. Thus

$$a=\frac{6H}{d_b}\quad\ldots\ldots(15)$$

It was observed that a higher oxygen transfer coefficient was obtained by a combination of short impellers and high rotor speed rather than with long impellers and low rotor speed (Fig. 11, 12 and 13) although long impellers favor increased air intake.

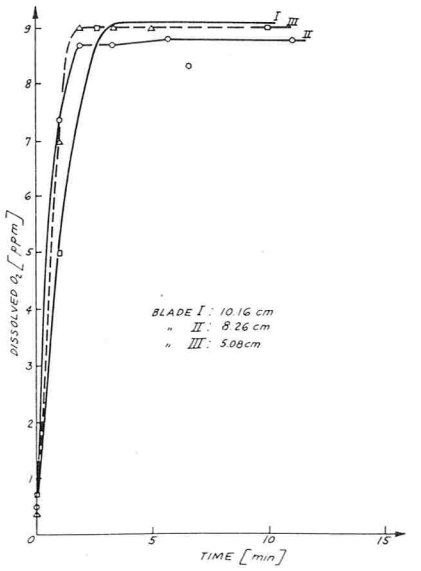

Figure 10. *Dissolved oxygen vs. time.*

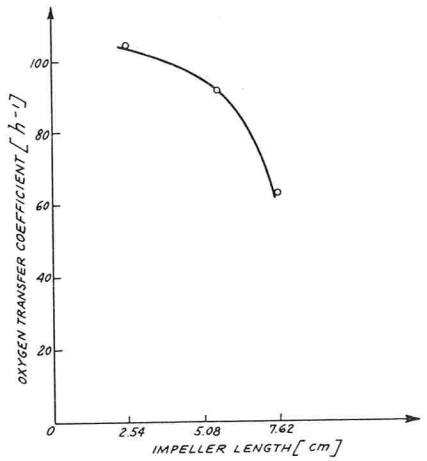

Figure 11. *Oxygen transfer coefficient vs. impeller length.*

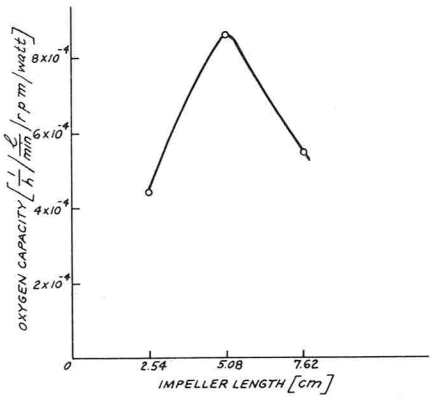

Figure 12. *Oxygen capacity vs. impeller length.*

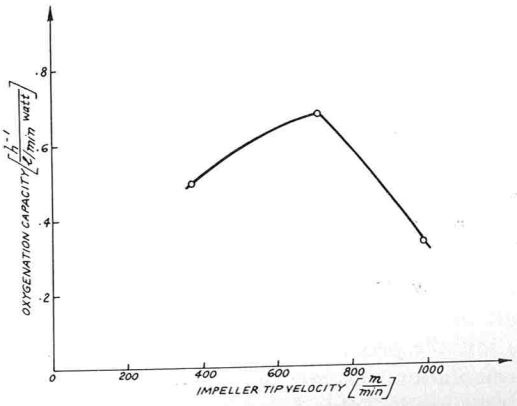

Figure 13. *Oxygenation capacity vs. impeller tip velocity.*

It must be mentioned that oxygen has very low solubility. Water at 20 C holds only nine parts of oxygen per million. The solubility further decreases with increasing temperature and added salts. Efficient ways must, therefore, be sought to make use of this limited absorption capacity. In this fermentor, the effect of multiplicity of orifices and retention of a high percentage of air in the liquid is combined to achieve a large specific interfacial contact area. This area is determined (2) as

$$K_L^a = \frac{\ln (X_S^{O_2} - X_L^{O_2})_{t_1} - \ln (X_S^{O_2} - X_L^{O_2})_{t_2}}{t_2 - t_1} \quad \ldots\ldots\ldots (16)$$

Equation 16 indicates that a high gas hold-up and small air bubbles favor a large specific contact area which increases the overall transfer coefficient. A large gas hold-up can, however, cause pronounced foaming if surface active agents are present in the liquid. The high gas hold-up originates from the fact that part of the air is recycled by the suction action of the impeller, thus giving the gas a chance to equilibrate with the liquid in the fermentor.

Despite the decrease in airflow rate with time during a fermentation run, a very high level of dissolved oxygen was maintained throughout any experiment (Fig. 14).

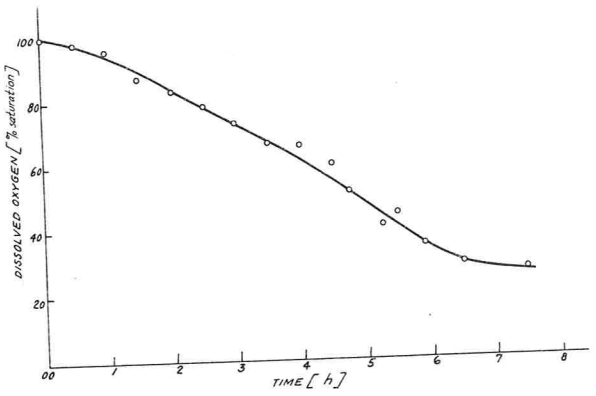

Figure 14. *Dissolved oxygen vs. time during fermentation of whey with* Kluyveromyces fragilis.

FERMENTATIONS

The performance of the fermentor was tested on two substrates: whey and cassava. For preparation of whey from skim milk, the casein was precipitated by acidification with lactic acid to a pH of 4.6 – 4.4 and removed by filtration. Whey was sterilized for 15 min at 121 C. The coagulated serum proteins were not removed. Fermentor trials were done with 2 liters of sterilized non-deproteinized whey, supplemented with 5% corn steep liquor, 0.5% $(NH_4)_2HPO_4$ and 0.1% $FeNH_4(SO_4)_2 \cdot 12 H_2)$. A 3% inoculum of *Kluyveromyces fragilis* consisting of the harvested cell paste of a 24-h old culture propagated in lactose broth was added. The pH of this fermentation medium was 5.2 – 5.4. During fermentation, the temperature was maintained between 28 and 32 C. The rotor speed was 1200 rpm. Foaming was controlled by antifoam Wyandotte "X," a food-acceptable antifoam agent (S. F. Lawrason's and Co. Ltd., 180 Adelaide St. S., London, Canada). After 8 h, all of the lactose initially present in the whey was converted and the fermentation was considered completed. Results of three trials are in Table 3.

TABLE 3. *Biomass yield in whey fermentation*

Experiment no.	Aeration v/v/min	Biomass yield (% weight of initial lactose)	Fermentation period (h)
1	1	42.5	8
2	1	39.5	8
3	0.5	40.5	8

One other substrate, which was used to test the performances of the fermentor, was cassava. The composition of this substrate is given in Table 4.

TABLE 4. *Composition of cassava substrate*

Compound	Concentration (g/1)
Cassava (freeze-dried)	30
Urea	2.86
KC1	0.5
$MgSO_4 \cdot 7H_20$	0.5
KH_2PO_4	1.0
$Fe_2SO_4 \cdot 7H_20$	0.01

In some experiments, 5 ml of corn steep liquor were included per liter of medium. To prepare the medium, ground freeze-dried cassava was suspended in distilled water and heated until the mixture thickened. Salts were then added, and the pH was adjusted to 3.5 with dilute HC1. The medium was sterilized at 121 C for 15 min. Urea and corn steep liquor were sterilized separately and added to the sterile medium bringing the final volume to 2 liters. The inoculum consisted of 300 ml of an 18-h shake flask culture of *Aspergillus oryzae* I.M.I. 44242 grown on a substrate of identical composition to the main medium.

Impeller speed was 1200 rpm, the average rate of induced airflow 3 liters/min and the total fermentation time was 24 h at 30-32 C.

A summary of the yields observed in these fermentations is given in Table 5. At no time during the fermentations in the self-aspirating fermentor did the dissolved oxygen concentration fall below 35% of saturation. It was significant that, despite the viscous nature of the filamentous mold culture, good mixing and adequate aeration were maintinaed in the self-aspirating fermentor. The only adverse observation was clogging of the smaller impeller air outlets with mold growth. This problem was significantly reduced when wide bore outlets were used and was resolved in the pilot scale model.

TABLE 5. *Fermentation yields from* Aspergillus oryzae *I.M.I. 44242 grown on cassava in the developed fermentor*

Suspended solids (g/1)	8.92	11.35[a]
Nitrogen content of suspended solids (% dry matter)	7.36	7.00[a]
Nitrogen yield (g/1)	0.66	0.80[a]

[a]Yields when corn steep liquor was added.

Tables 3 and 5 indicate that the system is capable of giving good yields of biomass. With whey fermentation, the period required for total conversion of lactose was comparatively short, and more interesting, perhaps was

the ability of the fermentor to maintain high level of dissolved oxygen at low airflow rates.

In conventional, commonly-used submerged fermentation, two factors contribute to the power consumption: air compression and injection, and liquid agitation. These two components of power consumption are usually of the same order of magnitude (*10*). The use of an air compressor is eliminated in the new design which combines the features of vortex, Waldhof (*9*) and Cafflesi tank systems. Vortex aeration is unpopular (*1*) owing to low oxygen transfer. Literature sources seem to agree that although the Waldhof fermentor has good foam controlling qualities (*1*), it appears only to be successful in the production of food yeast (*1*). The self-aspirating fermenter used in this study, however, was successful in yeast and mold fermentations. The fermentor has been scaled up to pilot size (140 liter) and it is anticipated that, with more research, the system will be adapted to commercial application.

ACKNOWLEDGMENTS

The research was supported by the Canadian Commonwealth Scholarship and Fellowship Plan, the Nation Research Council of Canada and the International Development Research Center.

NOTATION

P_L = Total liquid pressure at orifice A
P_A = Total air pressure at the orifice A
P_I = Pressure at inlet station
v_1 = Air velocity at inlet station
v_A = Air velocity at the orifice
f_1, f_3 = Frictional factors
A = Density of air
L = Liquid density
Z = Liquid elevation
g = Acceleration due to gravity
w = Angular velocity
r = Radius
Q = Air flow rate
N = Rotor speed
K_o,k,c = Constants
V_b = Bubble volume
A_b = Bubble area
P_b = Bubble pressure
O = Surface tension
A = Cross sectional area of impeller opening
d_b = Bubble diameter
V_t = Bubble velocity
M_b = Bubble mass
h_t = Liquid height
E = Absorption efficiency
$X_S^{O_2}$ = Saturation concentration of oxygen
$X_L^{O_2}$ = Dissolved oxygen concentration
t = Time
K_L = Overall mass transfer coefficient
a = Specific area
H = Gas hold up
C_L = Dissolved oxygen concentration
δ_A = Specific weight of air

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J. Milk Food Technol. Vol. 38, No. 2, Pages 100-104 (February, 1975)

Enteroviruses and Potential Bacterial Indicators in Gulf Coast Oysters

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(Received for publication June 4, 1974)

ABSTRACT

Bacteriological and virological examinations of Louisiana and Texas Gulf Coast oysters were done over a 12-month period. Oysters taken from approved waters sometimes contained detectable enteroviruses: echovirus 4 and poliovirus 1 were in one each of 17 samples from Texas, and poliovirus 3 was in one of 24 samples from Louisiana. Neither the coliform MPN for the water from the shellfish beds nor the coliform MPN, *Escherichia coli* MPN, or aerobic plate counts for the shellfish meats was clearly indicative of the presence of virus. Two of the 24 oyster meat samples which contatined *Vibrio parahaemolyticus* also yielded virus; however, virus was present in one of 17 samples in which *V. parahaemolyticus* was not detected. Poliovirus 1 was also found in frozen, shucked oysters imported from Japan.

There are several reasons for concern that viruses may be transmitted to man by oysters and other bivalve molluscs. First, these molluscs grow in waters which are increasingly subject to pollution with human sewage. Second, the molluscs feed by a filtration process which enables them to concentrate viruses from their environmental waters (*17*). Third, though the viruses evidently do not multiply in the molluscs (*5*) and are more or less confined to the digestive organs (*13, 15*), the alimentary tract of the molluscs and its contents, unlike that of most other edible animals, is usually eaten together with the other soft tissues of the body (*14*). Fourth, the molluscs are frequently eaten raw or with minimal cooking (*12*), and they appear to protect viruses against heat to an unusual degree (*8*).

The record of human outbreaks and cases of infectious hepatitis which have resulted from eating contaminated shellfish has been reviewed extensively elsewhere (*6, 7*) and need not be retold here. Suffice it to say that shellfish are the vehicle implicated in a plurality of food-associated hepatitis outbreaks. Enteroviruses or reoviruses have been detected in oysters taken from a contaminated estuary (*15, 16*), and some of the mussels purchased in an Italian market had enteroviruses in them (*3*). Moreover, crabs become contaminated by feeding upon enterovirus-contaminated clams under experimental conditions (*9*).

The association of shellfish with human intestinal viruses is clearly demonstrated. This does not mean that all molluscs contain human intestinal viruses nor that viruses are the only hazard to human health related to shellfish. The judgment that shellfish from a given source are likely to cause human disease is based, at present, upon counts of coliform bacteria, of *Escherichia coli,* or of organisms detected by a standard plate count. These might be applied either to testing the shellfish or the water in which they have been growing.

The present report describes the occurrence, at low frequency, of enteroviruses in oysters taken from approved waters. Bacteriological studies of the water and shellfish were done in the hope of discovering a relationship between the results of these and the presence of viruses. Whereas the required bacteriological methods are largely standardized (*1, 2*), the method for detecting viruses in the oysters was based upon one of several which are available (*11*). Though there may now be an authentic laboratory host for the infectious hepatitis virus, there are no methods yet for detecting the agent in foods.

MATERIALS AND METHODS

Oyster sampling

Oysters were collected during September, November, January and February, and April, so that all four seasons were represented. Samples were taken (with the exceptions noted) from approved growing areas, under favorable hydrographic conditions as judged from data for the Galveston Bay and Louisiana furnished by the U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Climatic Center, Asheville, North Carolina.

Thirty oysters were taken from each of seventeen pre-selected areas of Texas' Galveston Bay and six Louisiana (near the Mississippi River delta) Gulf Coast oyster beds. In each instance samples were obtained through the respective state health department agencies responsible for shellfish harvesting and shipped under dry ice to the Dallas District laboratory for analysis.

Seventeen samples were examined from Texas and 24 samples from Louisiana during one calendar year of oyster dredging. The oyster harvesting season in Texas' Galveston Bay is November 15 to April 30 for public reefs; harvesting during closed season is negligible. For Louisiana, there is no harvesting season and harvesting is permitted from all areas during the entire year.

Figure 1 illustrates the general area of Galveston Bay, Texas, where the majority of Texas oysters are harvested. The collection points are numbered from 1 to 17. Samples from points 7, 8, 9, and 10 were from oyster growing areas that were classified as "closed areas" and were included for comparative purposes. Figure 2 is a semi-schematic illustration of the Louisiana oyster harvesting area.

In addition to the field samples just described, oysters imported from Japan were tested. These had entered the port of Houston as frozen, shucked oysters (species not stated) on November 23, 1970. A grab sample of 1 gal from this lot was collected December 1, 1970; a single 200-g portion of this sample was tested for virus contamination.

Bacteriologic tests

Oyster growing waters were tested by the respective state health

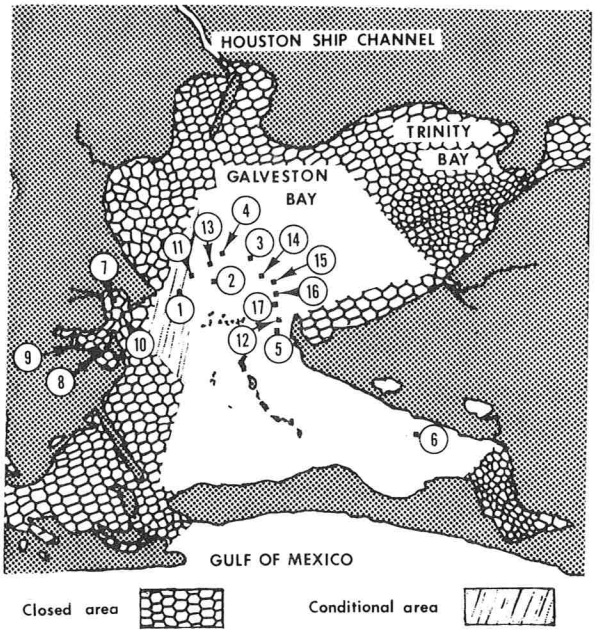

Figure 1. *Areas sampled for oysters in the Galveston Bay, Texas*

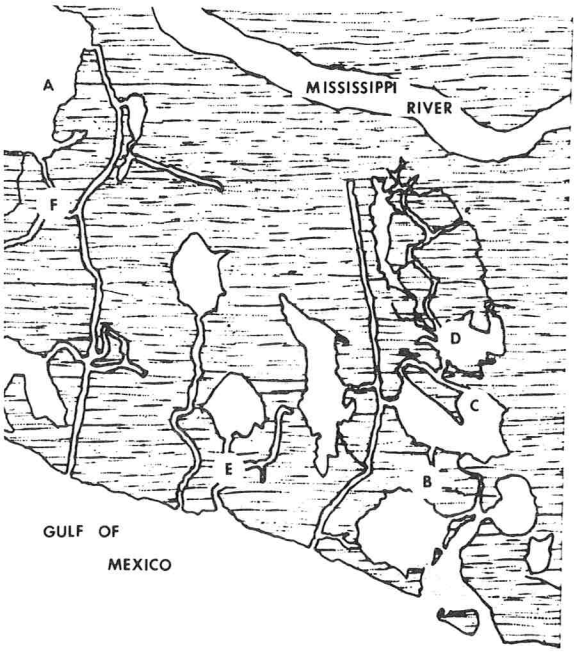

Figure 2. *Areas sampled for oysters on the Louisiana Gulf Coast.*

departments, using standard methods (*1*). Ten oysters per sample were used to determine coliforms, *E. coli,* and standard plate count according to APHA recommended procedures (*2*). Six to eight oysters per sample were provided to Morris Fishbein and Warren Landry for *Vibrio parahaemolyticus* isolation studies (*19*); their findings were reported to us as positive or negative, without Kanagawa reactions.

Virus extraction

The procedure used to extract and concentrate the viruses from the oysters was essentially method "SBF" of Herrmann and Cliver (*11*). It was scaled up to accommodate 15 oysters (from the sample of 30) at a time. The oysters were shucked aseptically, weighed, and homogenized in a blender with 100 ml of glycine-NaOH (GN) buffer (pH 8.8), 10 ml agamma calf serum, 20 g $MgCl_2 \cdot 6\ H_2O$, 10 g bentonite, and 100 ml Freon TF (Dupont) per 25 g of oyster meats. After centrifugation of the homogenized mixture for 30 min at 8000 × *g*, the supernatant fluid was sampled, concentrated, and sampled again in an effort to detect viruses.

Volumes of supernatant fluid that were obtained in these experiments were approximately 800 ml: this necessitated a preliminary concentration step in which the fluid, in dialysis tubing, was placed in metal pans and covered with polyvinylpyrrolidone K-90 (Matheson Coleman and Bell). After about 24 h, the remaining volume of fluid was small enough to allow placing it on the outside of a dialysis tube loaded with a "slug" of polyethylene glycol and imbibing fluid inward (*11*). After 12 to 14 h at room temperature, this process was ended; and the fluid was transferred to ultracentrifuge tubes. The pellet formed in 5 h at 105,000 × *g* was collected in 3.5 ml of Hanks' BSS for virus detection.

Virus detection

The actual detection of the viruses was done in tissue cultures. Virus plaque formation was observed in primary African green (*Cercopithecus aethiops*) monkey kidney (PMK) cells or in the Vero established line of African green monkey kidney cells grown in 6-oz (180 ml) screw cap prescription bottles. Virus cytopathic effects (CPE) were seen in tube cultures of the BSC-1, KB, and FL established lines. All but the last, which came from Microbiological Associates (Bethesda, Maryland), were obtained from Flow Laboratories (Rockville, Maryland). The PMK cells were grown in equal parts of Eagle's minimal essential medium (MEM) and Liebowitz' (L-15) medium plus 10% heat inactivated fetal calf serum and penicillin (100 units/ml), streptomycin (100μg/ml), neomycin (200μg/ml), and nystatin (1μg/ml). The L-15 was omitted from the growth medium for the established cells.

In the plaque procedure, each cell monolayer was inoculated with 1 ml of the concentrated oyster extract and held for 60 min of 36 C before receiving 18-20 ml of agar overlay medium. The overlay medium comprised MEM in Earle's solution without phenol red (pH 7.2 to 7.6) plus 0.92% Ionagar No. 2 (Colab Laboratories, Chicago Heights, Illinois), 0.0015% neutral red, 0.051% Mg $Cl_2 \cdot 6H_2O$, 0.01% DEAE-dextran (Mw 2×10^6; Pharmacia, Uppsala, Sweden; *10*), 2% heat inactivated fetal calf serum, and 1% sterile whole milk. After the medium had solidified, cultures were inverted and incubated at 36 C in the dark. Cultures remained in the cell-side-up position so that serotypically pure virus could be harvested from a plaque (*18*). Plaque counts were recorded by the procedures of Berg et al. (*4*).

Virus CPE were detected by inoculating 0.2 ml of the concentrated oyster extract into two tubes of each of the three cell strains. These were maintained at 36 C and were observed after 48 h and daily thereafter. Three cell culture passages were done before a sample was considered negative, and positive specimens were passed several times to increase the virus titer before "plaque-purification."

The "plaque-purification" procedure consisted of subculturing virus from a discrete plaque, to ensure homogeneity before serotyping was attempted. Identification and characterization of the viruses were done at the National Center for Disease Control, Atlanta, Georgia.

RESULTS

The Texas samples were taken from "approved" (sampling points 2 to 6 and 12 to 17), "conditional" (sampling points 1 and 11), and "closed" (sampling points 7 to 10) oyster beds. The microbiologic findings are summarized in Table 1. Results of the six observations for a given sampling point are rather weakly correlated. The coliform numbers in the oyster meats tend to exceed those in the overlying waters, but the oysters that are highest in coliforms do not necessarily come from the waters that are highest in coliforms. The first four measurements are intended to serve as bacteriologic indices of sanitary quality, but are not clearly related to the occurrence of *V. parahaemolyticus*

TABLE 1. *Microbiological examination of Texas oyster meats and overlying water*

Sampling		Water	Oysters				
Date	Point	Coliforms (MPN/100ml)	Coliforms (MPN/100g)	*E. coli* (MPN/100g)	Aerobic plate count/g	*V. parahem-olyticus*	Virus[a]
4/15/69	1	110	230	20	27,000	–	–
	2	70	330	330	28,000	–	–
	3	33	230	–	14,000	–	–
	4	110	230	–	18,000	–	–
	5	1.8	<18	–	1,200	–	–
	6	2	<18	–	1,500	–	–
9/9/69	7	5	80	80	34,000	+	–
	8	33	490	110	17,000	+	–
1/21/70	9	1,600	1,600	–	17,000	–	–
	10	2	20	20	31,000	+	–
	11	1.8	230	–	13,000	+	–
	12	5	230	–	13,000	+	–
	13	1.8	–	–	1,600	+	–
4/22/70	14	11	2,300	<18	15,000	+	–
	15	33	330	<18	11,000	+	–
	16	46	78	45	7,900	+	Po-1
	17	2	7,900	20	9,000	+	EC-4

[a]Po-1 is poliovirus type 1; EC-4 is echovirus type 4.

TABLE 2. *Microbiological examination of Louisiana oyster meats and overlying water*

Sampling		Water	Oysters				
Date	Point	Coliforms (MPN/100ml)	Coliforms (MPN/100g)	*E. coli* (MPN/100g)	Aerobic plate count/g	*V. parahem-olyticus*	Virus[a]
9/8/69	A	1.8	<18	–	11,000	+	–
	B	1.8	2,300	50	50,000	+	–
	C	49	4,900	110	56,000	+	–
	D	79	2,200	170	48,000	+	–
	E	33	2,300	130	39,000	+	–
	F	13	110	110	38,000	+	–
11/3/69	A	2.0	<18	–	22,000	+	–
	B	6.8	2,300	20	15,000	+	–
	C	22	2,300	–	46,000	+	–
	D	21	3,300	330	38,000	+	–
	E	4.5	<18	–	10,500	–	–
	F	1.8	<18	–	14,000	+	–
3/17/70	A	1.8	<18	–	1,700	–	–
	B	26	<18	–	3,100	–	–
	C	33	1,300	–	22,000	–	–
	D	49	80	49	26,000	+	–
	E	7.8	1,300	130	26,000	–	–
	F	23	<18	–	1,300	–	–
4/27/70	A	4.5	<18	–	2,800	–	–
	B	350	<18	–	3,400	–	Po-3
	C	110	180	–	6,100	–	–
	D	110	<18	–	3,600	+	–
	E	79	230	17	3,500	+	–
	F	23	<18	–	5,000	–	–

[a]Po-3 is poliovirus type 3.

or of enteroviruses in the oysters. These isolations of *V. parahaemolyticus* were confirmed in Galveston Bay oysters tested 3 years later by Vanderzant et al. (*21*). This organism is significant as a pathogen in its own right.

The results obtained with samples from the six Louisiana sites are shown in Table 2. Here seasonal variations can be seen, but the correlations among microbiologic indicators are still poor. There was no sampling point from which oysters always had or always lacked *V. parahaemolyticus,* but the vibrio was not detected in the only sample of oysters in which virus was found. The water from which the virus-contaminated Louisiana oyster sample came showed the highest coliform level of the entire Louisiana series. This appears to have been fortuitous: the Texas samples did not show the same association.

The Japanese oysters were not accompanied by a water sample. The coliform level in the oyster meats (MPN/g) was 2.0, with no *E. coli* detected. The aerobic plate count

was 450 to 900/g. No *V. parahaemolyticus* test was performed, but poliovirus type 1 was detected.

All of the polioviruses were found to be "vaccine-like" in that they were unable to multiply in tissue cultures at 40 C and that they showed closest relation to the vaccine strains in the intratypic serodifferentiation test. These are, of course, still authentic viruses of the human intestines; but the "vaccine-like" strains are relatively unlikely to cause disease in the consumer. Since there is no vaccine for echovirus type 4, this isolant must be regarded as "wild-type."

DISCUSSION

The presence of enteroviruses in oysters taken from approved waters may be significant to consumer health, even though the frequency of virus detection was low. Enteroviruses are quite stable in oysters, whereas *V. parahaemolyticus* may decline, and the aerobic plate count increase under some conditions, during storage of the shellfish (*21*). Coliform MPN in approved oyster-growing waters should be below 70 per 100 ml, with not more than 10% of samples exceeding 230 per 100 ml; shellfish meats should not exceed a fecal coliform MPN (more-or-less comparable to the *E. coli* data) of 230 per 100 g and an aerobic plate count of 500,000 per gram. The low frequency with which enteroviruses were detected did not permit valid statistical measurement of the correlation between virus incidence and results of the bacteriologic tests. Inspection of the data shows that none of these tests was consistently high in numerical value, where applicable, or positive when virus was detected.

It is noteworthy that the samples were taken only under favorable hydrographic conditions. In the case of Galveston Bay, this relates principally to wind direction, for a wind from the north or northwest carries highly polluted water from the Houston Ship Channel to the oyster beds. The samples from Texas were taken during a southeast wind, but the oysters may have acquired their virus when the wind was from another direction. Assuming that, say, *E. coli* and the enteroviruses were present at the same time, the bacteria may have been eliminated more rapidly than the viruses when the quality of the water was improved by a change of wind direction.

There is ample discharge of raw or inadequately treated community sewage into the Trinity and Mississippi rivers, especially during periods of rainfall, to account for the presence of *E. coli* and of the enteroviruses. Insofar as water pollution is related to weather, there may have been some significance to the fact that the positive samples were taken within a 5-day period. The numbers of the bacterial indicators may have been somewhat affected by the fact that the oysters had been frozen with dry ice on their way to the laboratory. On the other hand, *V. parahaemolyticus* was at least qualitatively detectable, in oysters which had been frozen previously, at a frequency similar to that found in a subsequent survey of the Galveston Bay (*21*).

The imported oysters are another category of product: they are distributed and stored frozen. The presence of virus in this single sample says little about the general sanitary quality of this kind of item. Taken together with the bacteriologic data, the finding of virus does suggest that the validity of the bacterial indices of sanitary quality is low for shucked oysters distributed or stored frozen for significant lengths of time.

It is unfortunate that none of these bacterial tests is a strong indication of the presence of viruses, for the Gulf Coast has just experienced another major outbreak of oyster-associated infectious hepatitis (*20*). In the absence of direct tests for the presence of infectious hepatitis virus, it may be that the enteroviruses, which are significant unto themselves, are also the best available indicators for the hepatitis agent in shellfish. This hypothesis could be tested only if more extensive surveys were made for enteroviruses in oysters. There are other methods available for extracting enteroviruses from oysters: some of these methods appear to be easier than the one used in the present study. The others may be easier, but we know that this one works because we tried it.

ACKNOWLEDGMENT

A preliminary report of this investigation was presented at the Second International Congress for Virology held in Budapest, Hungary, June 27-July 3, 1971.

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ISI® Announces First Multidisciplinary Index to the Review Literature

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The world's only multidisciplinary guide to reviews, the *Index to Scientific Reviews* will cover review articles from the Physical and Chemical Sciences; Medical and Life Sciences; Engineering and Technology; Agricultural, Biological, and Environmental Sciences; and, to a limited degree, Social and Behavioral Sciences. *ISR* will thus be the first reference tool that permits users to quickly find review articles on subjects from any scientific discipline.

One of the major problems that had to be dealt with in producing the index was the actual identification of review articles to be covered. Although some review articles are easily detected because words like "survey," "review," "state of the art" are included in their titles, others are much more difficult to find. These may have more subtle clues like a large number of references. In producing the *Index to Scientific Reviews,* ISI will use a combination of computer and human selection techniques to assure that the largest possible number of review and "review-like" articles are identified.

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Another feature of the new index is currency. Users will be able to locate reviews within a few months of their publication. A soft-bound, semiannual issue of *ISR* covering the review literature published during the first half of each calendar year will appear every September; a hard-bound, two-volume annual cumulation covering the review literature of the entire year will come out the following April.

The *Index to Scientific Reviews* is expected to be extremely useful to librarians who need reviews to begin literature searches and to students, educators, and researchers who want quick summaries of knowledge in fields unfamiliar to them.

The Institute for Scientific Information provides a variety of services to help professionals of all types make better use of scientific and technical information. It has long recognized the importance of review articles and in recent years has joined with scientists and information specialists who have stressed the need for more and better review articles. Although the Institute for Scientific Information cannot do anything to increase the number of written reviews, Eugene Garfield, ISI president, feels that "making existing review articles more accessible is a step forward."

The first annual issue of *ISR* covering review articles published in 1974 will appear in April 1975. For more information, contact the Institute for Scientific Information.

J. Milk Food Technol. Vol. 38, No. 2, Pages 105-107 (February, 1975)

Farm Tank Temperature Recording: Its Effect on Quality and Marketability of Fluid Milk

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(Received for publication August 15, 1974)

ABSTRACT

The only positive method to assure both producer and processor that farm cooling tank temperatures within legal limits have been achieved and maintained during the holding period is with the use of a recording thermometer. The same instrument records washing time/temperatures, another critical aspect of maintaining product quality and observing safety limitations. Several summaries of random testing by state inspectors of farm cooling tank temperatures with portable recorders indicate a wide variance in compliance with regulations and underline the urgency for tighter control of stored milk temperatures on the farm.

No other industry approaches the standards of quality and sanitary control that dairy processing maintains. Not only are there rigid governmental controls, but it is good business to aspire for the highest quality available. But in even the most sophisticated quality control programs in dairy processing there can be a critical flaw—the possibility that proper holding temperature of milk is not maintained on all producer farms.

Unless there is assurance that milk temperature is reduced quickly from that of the cow's body to a safe storage level, and held at that point until pickup, the quality of the raw product remains in doubt. Absence of control at the farm opens up at least three avenues of profit erosion: (a) risk of a contaminated milk-tanker—possibly the contents of a plant silo tank—often from an unidentifiable source; (b) dollar loss although off-standard milk may be salvaged for manufactured products; and (c) drastically curtailed shelf life due to excessive bacteria counts in raw milk. All this emphasizes the importance of recording thermometers and what effects these monitoring devices have on the overall quality and marketability of fluid milk.

THE NEED TO RECORD TEMPERATURE

From time immemorial, man has savored the refreshing and nutritional value of milk—long before the advent of a recording thermometer. Why now all the clamor for the requirement of recording farm milk tank holding temperatures?

To answer that question it is necessary to examine the changes that have come about in the past few years in the milk processing industry. It is obvious from statistics published by the Department of Agriculture that the number of dairy farms in the country has diminished considerably and that trend is continuing. This has resulted in larger farms, larger herds, larger milk production, and, naturally, larger holding or cooling tanks. As milk volume per farm increases, there is also a danger of failing to increase the milk cooling capacity required for the additional production.

For many years, milk control officials and large milk processors have been concerned about improperly cooled milk from farm storage tanks. Too frequently, tanker loads of milk have been lost, or the value of the milk reduced, due to intermingling of batches of milk which have become unsatisfactory because of improper cooling. This condition can result from a single unidentifiable source of milk received from a producer who has failed to meet the requirements for proper cooling in the bulk storage tank.

Although item 19r of the Grade "A" pasteurized milk ordinance, 1965 recommendations of the U.S. Public Health Service states, "raw milk for pasteurization shall be cooled to 50 F, or less within 2 h after milking and shall be maintained at that temperature until delivered," many areas are requiring a maximum of 45 F.

Under the present method of checking the temperature of milk in a farm storage tank, milk control agencies must rely on the reading (by the farmer or hauler) of a standard indicating thermometer at the time of viewing.

At its best, this is a very sketchy and inaccurate method of determining the efficiency of the cooling capacity of the tank, and it is virtually impossible to determine if the requirements of the code have been met. Since it is uneconomical to post inspectors at every farm at milking time to watch the indicating thermometer and observe the efficiency of the cooling system as required by the code, little, if any, data are available on deficiencies or variations of the time-temperature requirements. Violations of the code in this respect are discernible only if an inspector, by chance, is on the premises at the time the deficiency exists.

The recording thermometer for bulk milk storage tanks has played, and will play, an increasingly important role in insuring that only milk stored under conditions in compliance with the code requirements will find its way to the processor.

As of this date, the states of California and Nevada have adopted regulations which require recording thermometers on all farm bulk milk tanks and voluntary

use is increasing at a rapid rate. Several other states are in various stages of working toward similar regulations and are conducting test programs and evaluating results for drafting of regulations or legislation.

The concept of recording temperatures on the farm tank began in California approximately six years ago. At a recent meeting of Dairy Sanitarians at Oregon State University, Dr. John Bruhn of the University of California told of a California herd heavily infected with *Staphylococcus aureus.* After milking the dairyman had neglected to start the tank refrigeration system. Although the milk passed through a pre-cooler it was not sufficiently cooled to deter rapid microbial multiplication. Consequently after 8 h of ideal incubation temperature the organisms reproduced tremendously and produced an enterotoxin, which is heat stable.

On the next milking, the dairyman noticed his error and turned on the refrigeration system and continued as usual. At pick-up time, the milk temperature had been reduced to approximately 40 F, was picked up, sent to a small processing plant where it was pasteurized, bottled, and distributed.

Within 6 h of distribution, there were reports of food poisoning from *S. aureus* intoxication and the beginning of a very serious outbreak.

The dairy industry received a black eye in the press, even though it was an isolated case. The industry in California immediately began to consider means of controlling such problems, including the use of recording thermometers.

Aware of the need for this type of quality control throughout the industry, the Partlow Company made its instruments available to state agencies for conducting field tests to collect data in various milk sheds.

FIELD TESTS

Results of these field tests have been startling—although many sanitarians claim that recording merely proved what they had long suspected. Considerable data collected have revealed that milk is held for extended periods at an illegal temperature on far too many farms.

What are the reasons for this? From the interesting field tests conducted in such states as Michigan, Wisconsin, Illinois, and Connecticut, a few examples are noted.

When tests were first done in Connecticut, the four farms selected for intial testing were of the highest calibre. Although recording devices proved that the mechanical cooling systems were functioning satisfactorily, one case of over-temperature milk (from human failing) was recorded. The farmer simply forgot to turn on the compressor.

In another state we found the following: The chart marked "John Doe Farm" (farm names will remain anonymous) clearly indicated that milk in the tank was at a legal temperature when the recorder was installed. During a 7:30 P.M. milking, the blend temperature did not exceed 50 F. The overnight storage temperature remained steady and within the prescribed requirements.

However, the chart indicated a deficiency in the producer's procedure which occurred between the 7:30 A.M. milking and the 9:45 A.M. pickup. During that period the temperature of the milk had risen to 59 F at blending and fluctuated between 57 and 59 F when the agitator was engaged for 15 min.

Milk was then allowed to remain in the tank without activating the refrigeration unit until pickup. Milk in this tank was allowed to exceed 50 F, the maximum temperature permitted by the ordinance, and to remain between 50 and 70 F for approximately 1.75 h.

In another test in a midwestern state, the chart clearly indicated a failure in the refrigeration system. This resulted in the temperature of milk rising some 40 F above the code requirement and showed that milk remained at an elevated temperature for a period exceeding the 2 h requirement. The deficiency was corrected and at the time of pick-up the temperature of the milk was within the requirements. This period of deficiency was quite short lived, but demonstrates the problem that could, and does, exist where product is held for long periods at elevated temperatures but at the time of pick-up is at the proper temperature. Without a recording thermometer, it would be impossible to know that the temperature requirement was violated.

On one farm milk in the tank was 54 F at the 6 A.M. milking, and remained there for approximately 0.5 h. While this is not a critical period, nevertheless, the chart again recorded the deficiency.

Another test on a different farm showed that while the storage temperature of the milk was consistently below 40 F, the blending temperature was consistently above 50 F reaching approximately 60 F. This indicated that the compressor was apparently not able to cool the quantity of milk added at blending. What happens when a long power failure occurs in the middle of the night? Without the chart neither the farmer nor the sanitarian would have any knowledge that this occurred. The result is an obvious increase in bacterial population.

The periods of deficiency indicated on the charts demonstrate that problems can and do occur and that they would never be discovered without the aid of a recording thermometer.

Mr. Scott Creach, Manager of Quality Control at Early Dawn Dairy at Veradale, Washington, affectionately refers to the farm tank recorder as "a 24-H field man."

QUALITY CONTROL

Early Dawn is a relatively small independent dairy processor that believes in maintaining the highest standard of quality control. Its quality control program in the past has included direct microscopic counts for leucocyte, fresh and pasteurized plate counts, preliminary-incubation counts, and coliform counts on raw milk. They do antibiotic tests once a month and notify the dairyman as soon as possible of anything in his

milk that might be abnormal. This is a most extensive control program. Yet, Mr. Creach feels they leave out one most important ingredient in a completely thorough quality control program: there must be a continuous temperature check of that raw milk before it reaches the processing plant.

Early Dawn during the spring of 1972 installed recorders on the farm tanks of all their producers. The recorders verified what Early Dawn had suspected for a long time but couldn't prove—that tank truck drivers because of friendly relationships with dairymen they must see every other day, sometimes accept milk that isn't up to standard. With the advent of the recorder the load slips suddenly began to change and reflect the true temperature in the producer's tank.

As a second benefit the driver took on a feeling of responsibility. He was able to convey to the producer the need for more awareness of the quality of his product.

They were also able to account for high temperatures as a result of power shortages, as well as higher acidity, again a result of the higher temperatures.

Mr. Creach reported a recent incident in which a shipper lost two tanks of milk of approximately 30,000lb. each and a week later another 8,000 lb. Most of that milk would have been brought into their plant had it not been for the recording thermometer. Why should the public have to accept less than quality milk?

Mr. Creach goes on to say that, "As a result of recorders, they have done great things to their shelf life in the dairy. They have cut customer complaints practically to zero." He believes the answer to a complete quality control program will never be a guaranteed quality control program until you can control the raw incoming milk on a temperature basis.

"Recorders work 24 h a day, 365 days out of the year," says Mr. Creach. "They are unbiased. Even though they are owned by the dairyman, they are not prejudiced with him. They tell the truth. They tell you what is happening in the field. That's something that no fieldman is able to do because you can't put a fieldman on any of these farms all that time."

He goes on to say that, "His experience with recording thermometers has been excellent. We are now having to buy some outside milk, because of increased volume through our plant. We have suffered tremendously because of this milk. We know it but there is nothing we can do about it, which is why we began to talk to some in this area about going before groups and encouraging processors and co-operatives to put in the farm bulk tank recorder. They are the greatest tool that the dairyman, the processor, and the industry has really ever had to supplement a good quality control program."

THE CLEANING CYCLE

Besides recording the temperatures of the milk, the recorder provides an additional feature of portraying the time and temperature of the cleaning cycle. Many sanitarians believe that the cleaning operation is equally as important in proper quality control as is temperature control. These field tests have confirmed suspicions that sanitarians have held for several years, that many tanks are either not cleaned after every milking or are not cleaned at the prescribed temperature and for the prescribed time.

Does the hauler rinse the tank as required? This short, but important, operation is also indicated on the chart and the presence of a recorder on the farm eliminates this frequent hauler omission.

We have found from this limited testing program that unsatisfactory cooling and washing procedures are present on a scale larger than previously known. One way to effectively insure that proper temperatures are maintained throughout the entire holding cycle is with the utilization of a recording thermometer. It's *inexpensive, accurate, permanent, unbiased* . . . in short, an effective insurance policy that has proven its worth wherever it has been in force.

ACKNOWLEDGMENT

This paper was presented at the 61st Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, St. Petersburg, Florida, August 12-14, 1974.

J. Milk Food Technol. Vol. 38, No. 2, Pages 108-110 (February, 1975)

Collecting and Handling Milk Samples

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(Received for publication August 19, 1974)

ABSTRACT

Accurate quality and compositional test results depend on representative samples and proper analysis. Proper sampling and handling are essential to prevent contamination and to maintain temperatures between 33 and 40 F. Representative samples must be delivered to the laboratory in good condition to insure the reliability of quality and compositional tests.

Sampling programs have several purposes including regulatory, quality assurance, payment, and informational. Laboratories are operated by regulatory agencies, cooperatives, processors, and private companies.

Obtaining a representative sample and subsequent proper handling are keys to reliable results for quality and compositional tests. Recent widespread industry adoption of universal sampling programs represents an important milestone in upgrading present raw milk quality assurance programs. A universal sample is one which may be used for any or all quality or compositional tests. The importance of training and supervising all persons who collect and handle these samples cannot be overemphasized. While this article covers the sampling of raw and pasteurized milk, primary emphasis is placed on methods and equipment currently in use to obtain samples of raw milk from farm bulk milk tanks and pickup truck tanks. It is at these two points that the initial and most critical determinations of composition and quality are made.

FARM SAMPLING

The farm bulk milk hauler is the key person involved in the sampling of individual producer milk. Major effort should be directed toward training, licensing, and supervising haulers who sample milk.

Results of all tests made on samples picked up by the hauler will be reliable only if care is exercised in carrying out each step of the procedure that follows:

(*a*) Regularly check milk temperature with an accurate pocket thermometer to be sure it is 40 F or under. This thermometer should be checked monthly against one of known accuracy. Alert the producer and fieldman when temperature exceeds 40 F.

(*b*) Inspect milk for any characteristics such as odor, sediment, churning, or freezing. Do not sample milk with white flecks, ice particles, or butter granules, as results of fat and other compositional tests may not be accurate.

(*c*) Wash and dry hands before collecting a sample.

(*d*) Agitate milk for at least 5 min before collecting a sample. Tanks over 1,000 gal capacity require at least 10 min of agitation.

(*e*) Legibly identify each sample container with producer number. Use a prepared label or water proof felt-tip pen. Each rack of samples should be marked with date of sampling and hauler's name.

(*f*) Collect a duplicate sample at the first stop to be used as a temperature check from the farm to the laboratory.

(*g*) Use a sterile straw, tube, or a dipper held in sanitizer solution to obtain the sample. The dipper may be carried on the truck or stored at the farm.

(*h*) Open the sample container closure and do not touch the inner surface. If a dipper is used, immerse two times in the milk before taking a sample. Empty the dipper each time to eliminate any sanitizer solution.

(*i*) Fill container no more than three-quarters full to permit subsequent proper mixing.

(*j*) Hold the sample container away from outside edge of bulk tank and sample through the porthole.

(*k*) Immediately close the sample container to prevent contamination.

(*l*) Cool the sample to 33-40 F. When refrigerated with ice water, make certain that the water is in direct contact with the sample container and that the ice water level is as high as the milk level.

(*m*) Rinse and wash dipper and return it to the sanitizer solution.

(*n*) Maintain all samples between 33 and 40 F until delivered to the laboratory.

EQUIPMENT AND HANDLING

Individually wrapped single-service paper or plastic straws are preferred for taking the sample. If a stainless steel dipper is used, it must be kept clean and continually carried in a sanitizing solution. The container of sanitizer solution holding the dipper should be carried into the milkhouse to avoid bacterial contamination of the dipper. The proper strength of chlorine (100 ppm) or iodine (12 ppm) sanitizer must be maintained. The sanitizer concentration should be checked periodically when arriving at the plant and a fresh sanitizer solution should be prepared for each load.

Many types of containers are used for collecting milk samples for compositional and quality testing. Containers include heat sterilized multi-use vials or bottles, sterile single-service plastic bags, sterile single-service glass or plastic tubes, and plastic vials with snap caps.

The size of containers vary from 1 to 16 fluid ounces. However, a 1- fluid ounce container is sufficient for most automated testing. The sediment test may require a 4-ounce sample, but sediment screening may be done with a 1- ounce sample.

Plastic vials with snap caps have been found to have few if any bacteria in them. They are approved for raw milk, if they contain less than one organism per milliliter of capacity.

The sample case should be properly insulated and may be constructed of either rigid plastic, metal, or metal-lined wood. It must be able to maintain sample temperature between 33 and 40 F regardless of the season. If ice water is used as a coolant, racks should be used to hold samples in an upright position. Sufficient ice and water should be provided to maintain proper temperatures without submerging the samples; freezing of samples must also be prevented.

Samples must be maintained between 33 and 40 F at all times and delivered to the laboratory within 36 h. *Standard Methods for the Examination of Dairy Products* requires that samples be tested within 36 h of collection. If a central or regional laboratory does the compositional or quality tests, it may be necessary to operate the laboratory at unusual hours to satisfy the time requirement for doing bacteria and somatic cell tests. Industry and regulatory supervisors should increase their efforts to check sample temperatures.

HAULER REGULATION AND TRAINING

It is imperative that uniform requirements be adopted for training, licensing, and supervising haulers who collect milk from farms. In this regard the efforts of the United States Public Health Service to achieve uniformity are commendable. Hauler training should include instruction from regulatory, extension, or industry personnel. Information on sampling, judging milk quality, measuring, cleaning, sanitizing, sanitary requirements, quality tests, and truck operation should be covered.

All haulers, both regular and relief, should hold a license which is renewable each year. This license should be issued only after initial training and examinations are satisfactorily completed. A written examination should include all regulations and requirements relating to measuring, sampling, handling samples, standards, and the penalty action for violation of quality and composition standards. In addition, periodic refresher training, such as an evening session each year, should be a prerequisite to continued licensing.

The performance of some haulers has been the weak link in obtaining accurate quality test results. This will improve as the United States Public Health Service program is adopted. Surveillance of every licensed hauler will be required at least every 6 months.

Several training aids are available. In Pennsylvania we use a set of 74 - 2 inches × 2 inches color slides and a 20-min script in written form or on cassette tape.

TANK TRUCK SAMPLING

Collection of a representative sample from a tank truck presents a problem. The following alternative practices are being used by haulers and plant personnel to collect samples:

(*a*) Collect a sample through the manhole by aseptic means immediately after loading milk at the last pickup.

(*b*) Drop a high speed mechanical agitator into the manhole and operate for at least 10 min on tanks up to 3,000 gal capacity. For larger tanks the minimum agitation time should be established by interval sampling and testing for a constant fat content.

(*c*) Connect the end of bulk pickup hose to a clean sanitary pipe in the manhole. Extend the pipe below the level of the milk and circulate by pumping for at least 15 min.

(*d*) Install attachment for air agitation which will agitate the milk for 15 min without causing rancid flavor.

(*e*) Unload the entire tank of milk in an empty storage tank and collect a sample through the clean, sterilized sampling valve.

For tanks of 5,000 gal and larger, practices *a, c,* and *e* seem more satisfactory. Some regulatory agencies do not approve of all of the above methods, yet all are in common use.

Proportionate sampling systems to sample milk during the course of delivery from the tank truck into the plant appear to hold significant promise. The reliability of butterfat samples obtained with one such system used for sampling farm tank milk without any prior agitation has recently been demonstrated. The adequacy of such sampling systems for collecting universal or quality test samples is currently under study.

The same precautions are exercised in collecting samples from tank trucks as from farm milk tanks. This includes identification of sample as to date, time, truck and sampler. Bacterial tests should be done before flavor or compositional testing.

PLANT SAMPLING

Samples to be evaluated for plant control purposes may be collected at the HTST balance tank, pasteurized surge tank, and filler bowl. Drip samples have no place in today's operation. In-line sampling using rubber plugs and sterilized needles may be helpful in pinpointing troubles, but care must be taken to prevent contamination.

Milk in storage tanks must be agitated until it is homogeneous. It is necessary to determine for each type and size tank the amount of agitation required to achieve this. With air agitation it is common practice to agitate continually with minimum air pressures and volumes to prevent rancid flavor development.

Sampling valves must be washed manually and sanitized thoroughly before storage tanks are filled. Even then, it is difficult to get a representative sample from a

storage tank sampling valve. Preferably samples should be collected from the balance tank of the HTST pasteurizer.

Unopened retail size containers of dairy product should be submitted to the laboratory for analysis whenever possible. Temperature and processing code or date of the sample should be noted on the form submitted to the laboratory.

Collection of the first filled containers off each filler line as well as other samples during the day is recommended for quality control. Samples for fresh analysis should be kept below 40 F. Duplicate samples should be incubated at 45 F for 7 and 14 days. Analyses of these samples will indicate potential shelf life when evaluated at the end of each period.

When necessary to sample from retail containers, it is important to agitate as well as possible. Containers should be inverted 25 times in 7 sec. When possible, the first milk delivered through the tube of dispenser containers should be collected. After drawing off about one quart of milk, a second sample may optionally be collected to determine the cause of possible contamination.

AUTOMATION AND VARIATION

Automated testing for fat, protein, and somatic cells is increasing. Coded identification of samples using prepared labels is available. Electronic equipment must be under proper calibration at all times to secure accurate results. Use of this instrumentation has reduced the chance of human error and the reporting of incorrect results.

In any milk analysis there may be a variation in the test result when a sample is tested in duplicate or by two technicians or two laboratories. The agreement of tests depends on obtaining representative samples, strict conformance with sampling and testing procedures, and accurate reading and reporting of analytical results.

The two most common causes of unsatisfactory test results are failure to properly agitate milk before sample collection and subsequent mishandling of samples. Obviously some variations must be considered acceptable. Table 1 indicates permissible variations in the results of a single sample tested by two technicians or laboratories and between two samples of the same milk.

TABLE 1. *Acceptable Variations of Some Compositional and Quality Tests*

Test	Same sample tested by two technicians or laboratories	Different sample same milk
Milkfat	± 0.1%	± 0.2%
Freezing point	± 0.001° C	± 0.002° C
Bacteria	± 30%	—

ACKNOWLEDGMENTS

This paper is based on a symposium paper presented at the 1973 Annual Meeting of the American Dairy Science Association and on the Northeast Dairy Practices Council Guidelines for Sampling Fluid Milk.

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J. Milk Food Technol. Vol. 38, No. 2, Pages 111-112 (February, 1975)

A Research Note

Influence of Aeration Rate on Yeast Production in Sauerkraut Brine

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(Received for publication August 16, 1974)

ABSTRACT

The optimal rate of of aeration for production of food yeast (*Candida utilis*) in sauerkraut brine was found to be approximately 24 mM O_2/1/h.

In sauerkraut manufacture, about 29% of the salted, shredded cabbage is discarded as brine (*3*). This discard is high in BOD (biochemical oxygen demand), total acid as lactic, and NaC1 content. It presents a serious treatment problem. Several food yeasts have been shown to grow well in sauerkraut waste without adding nutrients or neutralizing the acids; *Candida utilis* grew most rapidly and gave the highest cell yields (*2*).

Since aeration plays an important role in the manufacture of yeast (*5*), the present study was undertaken to determine the effective aeration levels (the amount of oxygen dissolved in the solution) required for production of *C. utilis* NRRL Y-900 in sauerkraut brine.

MATERIALS AND METHODS

Sauerkraut brine was obtained from a commercial sauerkraut factory; it contained the the following, expressed as milligrams/liter: BOD, 29,000; total acid as lactic, 16,100; Kjeldahl nitrogen, 1,390; total phosphorus, 208; NaC1, 28,500; pH 3.3. Experiments were done at 26 C in a 7-liter fermentor (New Brunswick Scientific Co.) containing 5 liters of sauerkraut brine and 10% (vol/vol) of a 24-h yeast culture. Dow Corning Antifoam A Spray was used to depress foam formation. Rates of effective aeration in the fermentor were measured by the sulfite oxidation method of Cooper et al. (*1*). Methods used to determine the 5-day BOD, yeast dry weight, total acid as lactic, Kjeldahl nitrogen, total phosphorus, and NaC1 were described previously (*3*). All samples were prepared in duplicate and the reported data are average values.

RESULTS AND DISCUSSION

Growth of *C. utilis* is sauerkraut brine as affected by rates of effective aeration is shown in Fig. 1. The optimal rate of aeration was approximately 24 mM 0_2/1/h. At aeration rates below 24 mM 0_2/1/h, the yield of yeast cells decreased sharply. Rates higher than 24 mM 0_2/1/h, however, did not give an appreciable increase in yeast yield. Wasserman and Hampson (*5*) reported that an effective aeration rate of 90 mM 0_2/1/h was required for maximal growth of *Saccharomyces fragilis* in whey. This variation is attributed mainly to differences in the composition of growth media and in the species of yeast used.

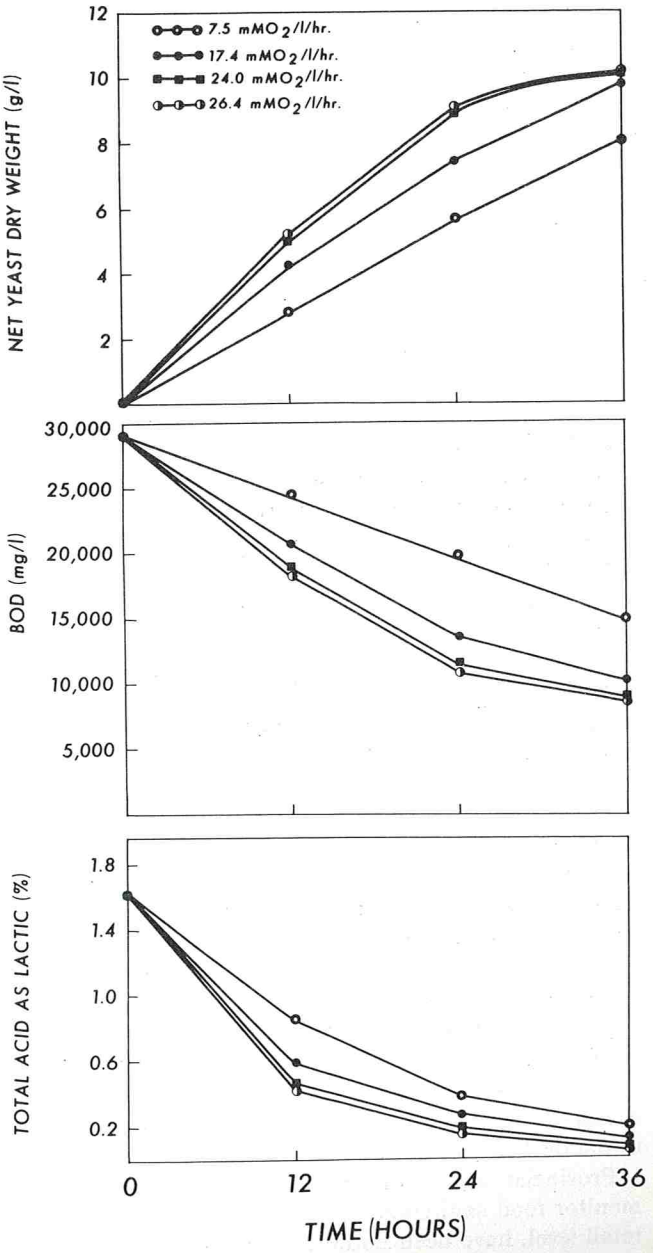

Figure 1. *Effect of aeration on the growth of* Candida utilis *NRRL Y-900 in sauerkraut brine*

Removal rates of the brine BOD and total acid as lactic by the yeast were also dependent on the rates of effective aeration (Fig. 1). As the effective aeration rate was increased, rates of BOD and total acid removal greatly increased. At the optimal aeration rate, Kjeldahl nitrogen and total phosphorus reductions were approximately 52 and 63%, respectively, in 24 h.

There was no appreciable influence of effective aeration on the protein content of the yeast. Thus, the amount of protein in dried yeast grown at the aeration rates of 7.5, 17.4, 24.0, and 26.4 m M 0_2/1/h was 45.5, 44.7, 46.3, and 46.4%, respectively. Singh et al. (*4*) have also noted no effect on the protein content of yeast due to aeration.

Obviously from results obtained in this work, aeration must be sufficient for maximal growth of *C. utilis* in sauerkraut brine and for most rapid removal of BOD and total acid.

ACKNOWLEDGMENTS

Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 2133.

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Preliminary Results on Ground Beef Survey

OTTAWA—Health and Welfare Minister Marc Lalonde today announced the first results of a national survey of the micro-biological quality of ground beef.

Of the first 897 samples of ground beef analyzed, taken from 180 stores in 5 provinces, 18 have been found to be contaminated with salmonella organisms. In all positive samples the level of contamination was low. Although salmonella organisms are readily destroyed at normal cooking temperature, their presence in raw food represents a potential health hazard because of the possibility of transferring such contamination to other food.

There were 7 stores in which positive samples were found.

Each of the stores involved were notified of the results obtained, and issued a warning. If positive samples are found on re-sampling their ground beef, further regulatory action will be taken, under the authority of the Food and Drugs Act. Re-sampling is now underway. Detailed inspection of all aspects of the operations of the retail stores in which positive samples have been found is being conducted, in order to determine if the contamination occured at the retail level or was present in the beef as received by the retailers.

Provincial and municipal health agencies, which monitor food sanitation, storage and processing at the retail level, have been notified of the findings, in order that they also can initiate corrective action as considered appropriate.

The design of the study made it necessary to analyze more than the 1,000 samples on which the study was originally based. Therefore, staff of the Health Protection Branch currently are analyzing a total of 1,340 samples of ground beef, including hamburger, ground chuck, minced beef, minced round and meat-soya protein mixtures, taken from stores in each of the 10 provinces. Each sample is being examined for total bacterial content, salmonella, coliforms and staphylococci. Only the results for salmonella are as yet available for a significant number of samples, but information for the other measures of quality is being produced and evaluated as rapidly as possible.

As a result of the finding of salmonella organisms in some samples of ground beef, the survey is being expanded further in size and scope, to include a total of 2,000 samples, selected from a wider variety of retail outlets in each province. This will permit a more detailed assessment of the overall quality of ground beef on the Canadian market and provide basic information on which to develop proper legal standards for the microbiological quality of ground beef. Plans are being made to undertake a similar survey of prepared meats, such as sausage, bologna, etc.

A series of workshops for retail meat handlers is being planned by the Health Protection Branch to reinforce the importance of proper practices and procedures relating to meat sanitation.

Proposals Requested to Study Methods for Analysis of Milk and Milk Products

The Intersociety Council on Standard Methods for the Examination of Dairy Products, in an attempt to improve analytical procedures, has identified some areas that need further study. The Council has limited funds available to support such studies and is inviting interested investigators to submit proposals for the work.

Proposals should be short (5-7 double-spaced typewritten pages) and should include the following: (*a*) brief review of pertinent literature, (*b*) objectives of the study, (*c*) precedures to be used in the study, (*d*) budget, (*e*) time required for the study and when results can be expected, and (*f*) list of references cited in the proposal. Funds are limited and hence the budget should be largely restricted to supplies. In some instances funds for labor may be available.

ELEVEN COPIES OF THE COMPLETED PROPOSAL SHOULD BE SENT BY APRIL 15, 1975 TO: DR. H. L. BODILY, P. O. BOX 247, MIDWAY, UTAH 84049. Questions about technical matters related to a given proposal should be addressed to the person whose name, address, and telephone number appears with each of the areas to be investigated. Areas of investigation are described in the following paragraphs.

1. *Sanitary Quality of Butter.* Attempt to correlate coliform, yeast and mold, enterococcus (or fecal streptococcus), and Standard Plate counts of butter with the degree of sanitation during manufacture (continuous and batch methods) of uncultured salted and unsalted butter. A series of comparative studies should be done in various plants (Dr. D. W. Mather, Research and Development, Kraftco Corp., Glenview, Ill., 60025; phone: 312-998-2017).
2. *Comparison of Etched Slides With Slides Having Ceramic Rings.* Compare the suitability of the following slides for the direct microscopic somatic cell count: Slides with ceramic rings, slides with black rings (National Mastitis Council slides), and conventional etched slides. Determine the minimum number of fields that must be counted to obtain satisfactory counts of bacteria and/or somatic ceils when the different slides are used. (Dr. W. W. Ullmann, Connecticut Department of Health, P.O. Box 1689, Hartford, Conn. 06101; phone: 203-566-5102.)
3. *Improved Medium for Standard Plate Count.* With the advent of technological change in the dairy industry, the microbial flora in raw and pasteurized milk has changed from primarily mesophilic to psychrotrophic. As evidence of this, many suggestions have been made to change incubation time and temperature for the Standard Plate Count so results more nearly reflect production or processing conditions. Little has been done on evaluating alternative formulations for Standard Methods agar so that results obtained with the medium will be more useful as indicators of farm or factory conditions and practices during handling of milk and milk products. (Dr. W. S. Clark, Jr., American Dry Milk Institute, 130 N. Franklin St., Chicago, Ill. 60606; phone: 312-782-4888.)
4. *Dilution Water System.* Develop a dilution water system that is either nontoxic or only minimally toxic to the microbial flora in dairy products. Several dilution systems may be required to achieve the objective because of variations in composition of distilled water. The specific components of the diluent that will affect its quality must be detailed. (Dr. W. J. Hausler, Jr., State Hygenic Laboratory, University of Iowa, Iowa City 52242; phone: 319-353-5990.)
5. *Preliminary Incubation of Raw Milk.* Develop a standardized procedure for doing the preliminary incubation test on raw milk. Parameters to be studied should include but are not limited to: time and temperature for preliminary incubation, surface to volume ratio of milk during preincubation, time and temperature for incubation of plates inoculated with the incubated milk. Results of the test should be indicative of conditions on the farm when the milk was produced. (Dr. W. W. Ullmann, Connecticut Department of Health, P.O. Box 1689; Hartford, Conn. 06101; phone: 203-566-5102.)
6. *Modification of Resazurin Reduction Test.* This relatively simple test has been used for years to indirectly measure the bacterial content of raw milk. It may be possible to modify the method so it better reflects the largely psychrotrophic population in present milk supplies. The study should include but is not limited to: incubation of the test at a lower (i.e. 30 C) temperature, and combining the test with preliminary incubation of milk. Results of a modified resazurin test should be compared with those from other indicators of milk quality. (Dr. J. L. Dizikes, U.S.D.A., 601 South Canal St., Chicago, Ill. 60609; phone: 312-353-6525.)
7. *Conditions for Coliform Count.* Conditions for the present coliform count may not be optimal and hence studies to include the following should be done: (*a*) use of 30, 32, and 35 C for 24 and 48 h to incubate violet red bile (VRB) agar plates inoculated with dairy products, (*b*) compare productivity of boiled and autoclaved VRB agar when the medium is used to test dairy products, (*c*) productivity of boiled and autoclaved VRB agar after storage under different conditions. Sufficient samples and plates must be used to provide statistically valid results and to represent diversity of coliform types. (Dr. R. T. Marshall, Dept. of Food Science and Nutrition, University of Missouri, Columbia 65201; phone: 314-882-7355.)
8. *Conditions for the Standard Plate Count.* The Standard Plate Count as presently done is believed to inadequately enumerate psychrotrophic bacteria present in raw milk and in pasteurized milk products. Additional data are being sought on the Standard Plate Count when incubation is at 30 C for 48 and 72 h and when incubation is at 32 C for 48 and 72 h. (Dr. W. W. Clark, American Dry Milk Institute, 130 North Franklin St., Chicago, Ill. 60606; phone: 312-782-4888.)
9. *Other Studies.* Although the Intersociety Council has identified the foregoing eight items as worthy of investigation, proposals dealing with other studies relating to standard methods for examining dairy products also will be considered. Such proposals should be sent to Dr. Bodily.

E. H. MARTH
Chairman
Intersociety Council on Standard Methods for the Examination of Dairy Products

News and Events

Walker Appointed to Committee on Environmental Health

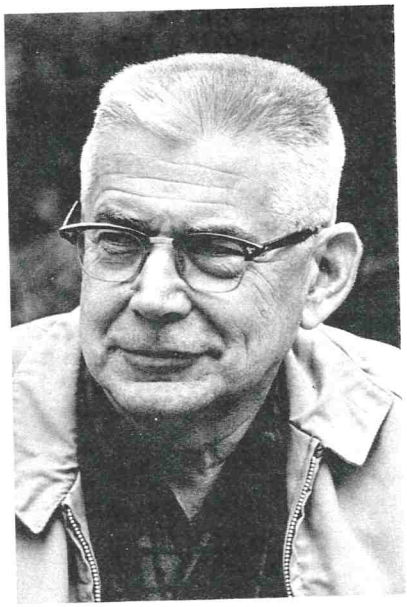
Bailus Walker Jr., director of the District of Columbia Environmental Health Administration, has been appointed to the Committee on Envrionmental Health of the Medical Society of the District of Columbia.

The appointment of Walker to the Committee breaks a longstanding policy of the Society to name only physicians to its committees and boards.

In announcing the appointment, Dr. William Cooper, president of the Society said the appointment of Walker, a highly respected environmental health specialist, to Committee gives new emphasis and expertise to the Scoeity's continuing interest in environmental health.

The Medical Society of the District of Columbia has been instrumental in promoting environmental health services in the Washington metropolitan area.

Research Scientist to Retire



PUYALLUP—Everett Wolford, leader of research in the fruit and vegetable testing laboratory at the Western Washington Research and Extension Center in Puyallup, will retire Dec. 27 after 33 years with the USDA's Agricultural Research Service.

As a microbiologist, Wolford has worked with plant breeders and horticulturists at Washington State University (WSU) to make sure new creations were able to produce a fruit or vegetable economically practical for canning and freezing. A large part of his work has involved super cold food freezing techniques, an area in which he was one of the earlier researchers.

A native of Spokane and a graduate of the University of Washington, Wolford spent his life in the frozen food business, including work as a processing foreman for a Wenatchee firm early in his career.

Wolford was assigned to work with WSU scientists at the Puyallup Center in 1955 as a USDA collaborator. Before being returned to his home state, he served the USDA for 14 years in California, at Albany and Pasadena, where his work included the testing of frozen orange juice.

Some of the biggest changes he has seen, Wolford says, are not so much in the processing of food products themselves but in the production shifts. "So much of the land in this area where the products were once grown is now covered with asphalt. But the processing of fruits and vegetables has improved a great deal through the years. The process has been simplified for better sanitation, among other things. Simple measurements of bacterial counts reflect this."

Besides testing fruit and vegetables, Wolford and his staff have produced some new products as an off-shoot to their research. Tasty, sugar-coated treats with big flavors from such valley grown products as rhubarb, strawberries and raspberries have become personal favorites of many. Some interest from commercial outlets has been shown.

Wolford has been a contributor to a number of scientific publications in his field and is a member of the editorial board of the *Journal of Milk and Food Technology*.

Away from his laboratories, Wolford has been active in civic activities. He is a past district governor of Toastmasters, past president of the Puyallup Lions club and he and his wife, "Willie," have opened their home to American Field Service students from Sweden and Ceylon.

Their own children include Ann, editor of the Potomac News in Woodbridge, Va.; Mary, who is a senior at Whitworth; and Doug, who is attending Central Washington State College in Ellensburg.

The Wolford's plan to continue making their home in Puyallup.

R & DA Meeting

The 29th Annual Spring Meeting of Research & Development Associates for Military Food and Packaging Systems, Inc., will be held 8-9 April 1975, at the Biltmore Hotel, New York, N.Y.

For information contact:

Col. Merton Singer, USA (Ret)
Executive Secretary, R&D Associates
Room 1315
90 Church Street
New York, N.Y. 10007
Tel: 212-264-7612

Mary Jo Garreis

1974 Student Awards Presented

The winners of the Department of Environmental Sciences and Engineering awards, made yearly to outstanding students, were announced at the annual ESE Student-Faculty Banquet in April. Dr. Russell F. Christman, Head of the Department, presented the American Inter-Society Academy for the Certification of Sanitarians Award to Mary Jo Garreis, and the George C. Bunker Award to Richard J. Heggen. The Environmental Achievement Award was given to Lewis T. Kontnik who was not at the presentation.

Ms. Garreis, a student in the Environmental Management and Protection Program Area, will receive a Master of Science in Public Health in May. She received a Bachelor of Arts from the College of Notre Dame of Maryland and has worked for the Maryland Envrionmental Health Administration until her admission to graduate school. The American Inter-Society Academy for Certification of Sanitarians Award is made yearly to honor a graduate student for academic achievement and leadership qualities.

John F. White

John F. White Named Vice President Kraftco Corporation

John F. White, Director of Research and Development, has been elected Vice President of Kraftco Corporation, according to William O. Beers, Chairman of the Board of Directors. Mr. White will continue to direct and coordinate all research and development activities for Kraftco's five operating divisions from the Glenview, Illinois laboratory, pilot plant, and administration complex.

Mr. White has been associated with the laboratory for 21 years: as a food scientist, Assistant Division Research Coordinator for the Kraft Foods Division and in 1966, he took on the assignment of Research Coordinator for three other divisions of Kraftco. He became Technical Assistant to the Director of R&D in 1968 and was named Director in 1973.

A native of Madison, Wisconsin, Mr. White, 45, received his B.S. degree in Food Science from the University of Wisconsin where he became a member of Alpha Zeta honorary agricultural fraternity. He served as a Battalion Supply Officer of the U.S. Army Corps of Engineers during the Korean conflict. He is a member of the Institute of Food Technologists.

The research and development program at Kraftco is directed toward developing new and improved products, building better nutrition in products, extending usable shelf life and protecting product quality over longer periods, designing efficient processing techniques, safeguarding the consumer and environment, and investigating alternate sources of raw food materials.

Kraftco Corporation is a leading producer of processed, packaged foods. Kraftco's operating divisions include Kraft Foods, Sealtest Foods, Breakstone Sugar Creek Foods, Kraftco International and Kra-Pak (HumKo Products, HumKo Sheffield Chemical, Metro Containers, and Universal Packaging).

Report of the Baking Industry Equipment Committee - 1974

This committee has had one meeting with the Baking Industry Sanitation Standards Committee (BISSC) since our last report. The usual Fall BISSC meeting was not held in 1973. In place of the Fall BISSC meeting the Baking Exposition was held in Atlantic City, New Jersey.

In addition to the excellent U.S. equipment shown at the exposition there was equipment shown by manufacturers from Germany, Israel, Japan, and the Netherlands. Equipment bearing the BISSC seal of approval was prominent throughout the arena.

The list of companies manufacturing equipment to BISSC standards and certified to use the BISSC seal continues to grow. The list of companies now totals 53. Many of the 53 companies manufacture two or more pieces of bakery equipment certified to meet BISSC standards.

The revising and updating of BISSC standards continues at an accelerated pace. Ten of the 34 published BISSC standards have been carefully reviewed, revised and approved for publication. This committee's recommendation that metric system measurements be incorporated into the revised standards has been adopted by BISSC.

BISSC informational booklets as well as all BISSC Standards are available without charge to Sanitarians. Copies may be obtained by writing to Mr. Ray Walter, Executive Secretary, BISSC, 521 Fifth Avenue, New York, New York 10017.

VINCENT T. FOLEY, *Chairman,* City Health Department, 21st Floor, City Hall, Kansas City, Missouri 64106

A. E. ABRAHAMSON, City Health Department, 125 Worth Street, New York, New York 10013

LOUIS A. KING, Jr., American Institute of Baking, 400 East Ontario Street, Chicago, Illinois 60611

FRED R. VITALE, Continental Baking Company, Inc., Post Office Box 731, Rye, New York 10580

HAROLD WAINESS, Wainess & Associates, 464 Central Avenue, Northfield, Illinois 60093

The 1975 International Microwave Power Symposium Will Create Worldwide Interest

The 10th Annual International Microwave Power Symposium to be held at the University of Waterloo in Waterloo, Ontario, Canada on May 27th through May 30, 1975 will draw worldwide participation. Scientists, engineers, businessmen and users of microwave energy from the U.S., Canada, Far East, Asia and East European countries will gather to present papers and hold discussions covering the latest technology in microwave heating, microwave power generation, medical and biological effects of microwave energy, microwave chemistry and microwaves in the food industry.

Representatives from Russia, Hungary, Czechoslovakia and Poland have been invited as guest speakers so that attendees may have an opportunity to evaluate the progress of microwave development and the difference in emission standards of these countries as compared to the U.S. and Canada.

Special emphasis will be given in this year's Symposium to the large segment of users of microwave energy in the food industry. A one-day Short Course of a tutorial nature will equip such users who have little or no background knowledge in microwaves to better understand the nature of microwave heating and get a good grasp of the economics and potential use for this unique form of energy. The principles outlined in the Short Course relate not only to food, but also to numerous other applications in such areas as chemical, rubber, textile, agricultural, medical and general industrial processing with microwave energy. A Microwave Cooking Workshop will be highlighted to inform users of the profitable advantages of a microwave kitchen and the innovations of menu that can be created.

Those interested in attending this Symposium can receive a program booklet by contacting the International Microwave Power Institute, P.O. Box 1556, Edmonton, Alberta, Canada.

Food Processing Industry Conference

GAINESVILLE, FL., Dec. 17—"Regulations and Their Cost to the Food Industry" will be the topic of the first Food Processing Industry Conference, to be held March 12 and 13 at the University of Florida at Gainesville.

Sponsored by the Cooperative Extension Service of the University's Institute of Food and Agricultural Sciences and the Florida Food Processors Advisory Council, the conference will provide information on some of the important government regulations that relate to the food processing industries. Speakers from several industries will discuss problems they have experienced with regulations and costs involved.

The conference is primarily designed for management personnel of the food processing industries. However, much of the discussion will relate to the technical problems involved with meeting required regulations. Therefore the technical personnel of the industries should also find the conference very worthwhile.

The conference fee is $35.00. For additional information and registration forms, contact Dr. R. F. Matthews, Cooperative Extension Service, 325 Food Science Bldg., University of Florida, Gainesville, FL 32611.

Scientist Develops Use for Clam By-Products

ITHACA, N.Y.—A Cornell University carbohydrate chemist and the Shelter Island Oyster Company of Greenport are joining forces to find a solution to the food industry's waste disposal problem.

This curious match was drawn together by the same factors that attract other novel teams. The Shelter Island Oyster Company has something that Prof. Robert S. Shallenberger, of the N.Y. State Agricultural Experiment Station, Geneva, wants. Moreover, the Greenport-based firm has excess tons of the commodity in question, "clam bellies" from surf clams, and is more than happy to dispose of them.

Surf clams are familiar to consumers when sliced and fried, or minced into clam sauce.

Shallenberger explained that surf clams feed on small food particles found in their natural aquatic environment that are considered difficult to digest.

"Therefore, these clams would be expected to have an unusual set of digestive enzymes," said Shallenberger, who is working with Sea Grant funding. "These enzymes, normally unavailable from terrestrial sources, can rapidly break down stable carbohydrates which are found in food processing wastes, such as potato peels, beet pulp, apple pomace, and sauerkraut juice."

"Large amounts of these vegetable processing wastes are currently disposed of in lagoons, where breakdown by microbes is slow and relatively ineffective," Shallenberger added. "The digestive enzymes of the surf clam seem capable of much faster degradation of the wastes."

Shallenberger has extracted this important enzyme product from the digestive tract, or "belly" of clams, which is normally discarded during processing and comprises about 20 per cent of the clam's body tissues. The Shelter Island Oyster Company has been providing Shallenberger with buckets of bellies. These donations comprise only a small fraction of the nearly one million pounds of belly wastes that are turned out by the Long Island's surf clam industry annually.

Shallenberger said that he has a limited idea of the volume of enzyme that would be needed by industry, but he believes that a process for extracting the enzyme can meet commercial needs. He also speculated that the enzymes contained in the surf clam extract may break down resistant carbohydrates in food wastes to simple sugars. This could create a new salable product for food processors.

John Plock Sr., president of the Shelter Island Oyster Company, and a 40-year veteran of the clam and oyster trade, said that he was pleased to cooperate in a research venture to find a use for the clam bellies.

"We have approached many people in an effort to make use of them," said Plock. "But until now we have had no luck."

"We have dumped clam bellies into the ocean to feed the fish, or have sold them to fishermen to use as bait. Trade with sports fishermen is reasonably profitable, but very seasonal," he added.

Completion of Shallenberger's project may convert two waste products into valuable resources. Plock's surplus of clam bellies would become a raw source material for powerful degrading enzymes. These chemicals, in turn, may transform wates products of food processors into a potential source of sugar.

Charles M. Fistere
1904 - 1974

Members will be saddened to learn that Charles M. Fistere passed away December 31, 1974 in Washington, D.C.

Charlie, as he was affectionately known, played a major role in the programs of several of the national dairy trade associations. He served as legal counsel of the Dairy Industry Committee, was the architect of the legal underpinnings of the 3-A program, served as legal counsel to the National Association of Food and Dairy Equipment Manufacturers and was an eminent authority on Food and Drug law.

Memorial services are scheduled Saturday, January 4 at St. Albams Church, Massachusetts and Wisconsin Avenues, Washington, D.C.

The family has requested that expressions of sympathy be made to the Charles M. Fistere Scholarship, Dairy Remembrance Fund, 910 - 17th Street, N. W., Washington, D.C. 20006.

Walter F. Snyder Award

Members of the International Association of Milk, Food and Environmental Sanitarians are invited to submit nominations for the 1975 Walter F. Snyder Award for achievement in attaining environmental quality.

This award is made annually by the National Environmental Health Association and NSF (National Sanitation Foundation) to a recipient, chosen by a nominating committee, who has received peer recognition for outstanding accomplishment in the field of environment . . . for notable contributions to the public health and quality of life.

According to Robert M. Brown, president of NSF, any man or woman with suitable qualifications may be nominated by any person or group identified with envrionmental health and safety. Nominations for the 1975 Walter F. Snyder Award close May 1. Nomination forms are available from the National Environmental Health Association headquarters office, 1600 Pennsylvania, Denver, Colorado 80203.

Ralph V. Hussong, 1904-1974

Dr. Ralph V. Hussong died in Deerfield, Illinois on December 2, 1974. Hussong had a distinguished career in research, teaching, and laboratory management.

Dr. Hussong was born on October 31, 1904 in Corwith, Iowa. His family later moved to Ames where Ralph completed his high school education. He then enrolled at Iowa State University where he did his undergraduate and most of his graduate work. One year of graduate work was completed at Cornell University under the supervision of Dr. James M. Sherman. Hussong was awarded the Ph.D. degree by Iowa State University in 1932. His training was in Dairy Bacteriology and Dr. B. W. Hammer served as Hussong's major professor. As a consequence of his graduate work, a close and lasting relationship developed between Hussong and Hammer. Research completed by Hussong for the Ph.D. degree dealt with *Pseudomonas fragi* and this work, for years, served as the basis for classification of this organism in *Bergey's Manual.*

From 1932 to 1935 Hussong was on the staff of Iowa State University, first as an Instructor and later as an Assistant Professor of Dairy Bacteriology. During this time he developed procedures to culture *Penicillium roqueforti* for use in making blue cheese. He also worked out methods to use the microscope for examining butter.

In 1935 Dr. Hussong joined the laboratory of Sugar Creek Creamery, a division of National Dairy Products Corporation (now Kraftco Corporation), in Danville, Illinois. Here he spent nine years doing research on the chemistry and bacteriology of butter.

In 1944 Hussong was appointed Professor of Dairy Bacteriology in the Department of Dairy Husbandry at the University of Illinois. He assumed this position upon the retirement of Professor M. J. Prucha. Hussong remained at the University of Illinois until 1948 when he became Chief Bacteriologist for the Kraft Foods Company in Glenview, Illinois. Later this laboratory was merged with another laboratory operated by National Dairy Products Corporation and Hussong became Manager of the Fundamental Research Laboratory which encompassed bacteriology, chemistry, statistics, and nutrition. Hussong held this position until 1966 when, because of ill health, his responsibilities were lightened. He retired in 1968 and continued to make his home in Deerfield, Illinois until the time of his death. During his professional career, Hussong co-authored a number of papers that appeared in the *Journal of Milk and Food Technology.*

In 1936 Dr. Hussong and Frances Fish of Ames, Iowa were married. They were the parents of two daughters, Mary and Ellen. Mrs. Hussong died in 1944 and two years later Hussong married Harriet Naumann of Greensboro, North Carolina.

Dr. Hussong is survived by Mrs. Harriet Hussong of Deerfield, Illinois; Mary Hussong Richards of St. Louis Park, Minnesota; and Ellen Hussong Vogles of Moraga, California.

NRA Expands

The Membership Services Department of the National Restaurant Association takes on a new posture as the result of staff additions. Operating from a broader and more effective base, it will be able to provide services to all phases of NRA membership.

New staff members include Eileen Goldberg, formerly associated with the Mayflower Hotel in Denver, Colorado, where, following business school in New Jersey, she gained valuable foodservice experience. Ms. Goldberg will be responsible for Organizational and Educational Memberships.

Richard Erwin joins the NRA with most recent experience in European employment as operational and beverage manager at the Cockney Pride Tavern in London, England. Previously, he had been Assistant Food and Beverage Manager for a Northeast Motel Corporation property in Randolph, Massachusetts, and has his Bachelor of Arts degree from Brown University. "Rick" will be responsible for the Allied Membership Services.

Loretta Harlow Tomjanovich, formerly Assistant to the NRA Director of Membership Services, now takes on new responsibilities by working with the Organizational Membership Accounts. Before joing NRA, Loretta had worked with Institutions/Volume Feeding magazine.

Barbara Jacobs, who formerly assisted in the Educational Materials Center, is presently an Assistant to the Membership Services Director. Barbara received a Bachelor of Philosophy in Psychology degree from Northwestern University.

Each of the new staff in Membership Services have valuable backgrounds in foodservice, which will allow them to better meet the needs of NRA members. They are most anxious to have new or present members visit the Department in the NRA offices.

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Letter to the Editor

A change in the Standard Plate Count

DEAR SIR:

I should like to deal with some of the arguments advanced by those opposed to the proposed change in temperature and time of incubation (30 C—72 h) for the Standard Plate Count (SPC).

It has been said that the resultant increase in count is less than the allowable variance between two technicians for the SPC. But the counts by both procedures reported by Roughley et al. (1974) were made by one technician, and were found to show statistically significant differences. It also seems highly unlikely that the technician would consistently err in one direction to give the proposed procedure an advantage.

Much has been made of the increase in cost. Huge increases in incubator and laboratory space have been said to be necessary. To avoid having to count plates over the weekend, many laboratories plate only on Monday, Tuesday, and Wednesday. With the 72-h incubation, no additional space is required if they plate on Monday, Tuesday, and Friday. If platings are made on Monday through Friday, capacity would only have to be increased by one-third. Is this too big a price to pay for a more reliable procedure?

It has been contended that the extra day's delay has an impact on farm calls for quality improvement. Such a delay has not prevented striking improvements in production practices and milk quality where samples have been subjected to Preliminary Incubation (PI) at 13 C (55 F) for 18 h before plating.

It is generally agreed that psychrotrophs are the most important organisms in both raw and processed products, being both indicators of contamination as well as spoilage agents. The present procedure (32 C for 48 h) frequently fails to reflect these organisms adequately; the proposed procedure does much better. This should increase the usefulness of the SPC where PI is employed with raw milk, as well as with Moseley's Keeping Quality Test for pasteurized products, since both are basically dependent upon encouraging the growth of psychrotrophs before plating.

The objection that the proposed changes have no public health significance can be ignored. I am still seeking evidence that the SPC, whichever procedure is used, has any direct public health significance.

Finally, I would again draw attention to what the late Dr. Arthur Fay said in 1960: "The objective of testing (raw milk) is not to detemine how many bacteria there are in a sample but to identify the producer who is doing less than a good job with the tools with which he has to work." And I would urge that additional comparative studies be done in as many areas as possible before a final decision is made regarding the suggested change in procedure.

C. K. JOHNS
Rte. 4, Angler's Paradise, Bonita Springs, Fla. 33923

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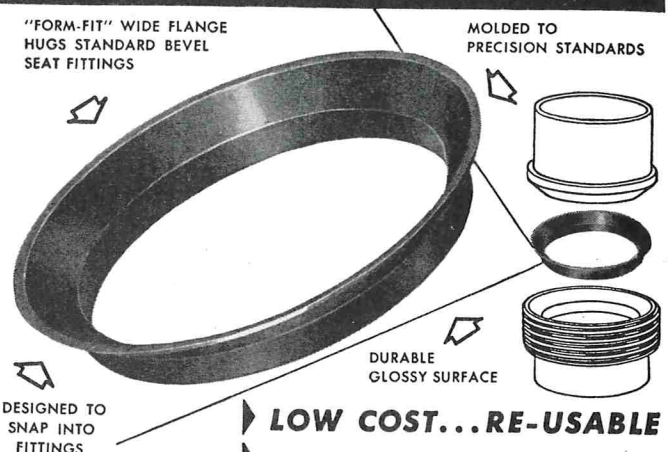

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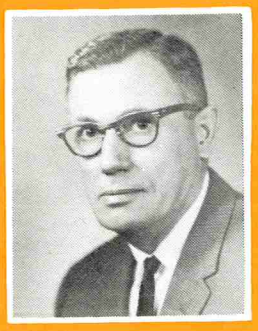
Dairy authorities speak out on better cow milking



Ralph Bonewitz, Extension Specialist
Department of Dairy Science
Kansas State University

What questions should a dairyman ask before expanding or modernizing?

Here are some of the most basic questions I believe dairymen should ask themselves before they begin a program of modernization or expansion:

What about my future in dairying? Will the changes I make be profitable in the long run? Will the changes be feasible in the short run?

Long run profitability refers to a period of eight to ten years or more. It is usually studied through the use of budgets. In the budgeting process, capital expenditures are prorated over the life of the assets by arbitrary depreciation methods.

Short run feasibility refers to the income-generating ability of a business in a short period of time. It is usually studied through the use of a projected cash flow.

Watch your cash flow

Projected cash inflow and outflow during the period are compared, reflecting payment requirements from credit agencies as well as all normal expenditures. Some business changes are capable of being profitable in the long run *but they are not capable of meeting short run demands for cash, particularly when payment requirements for capital expenditures are concentrated in the early years of an investment.*

Management must expand as herds get larger. More failures are due to lack of management than any other one thing. Hours spent thinking and getting facts will pay off and the manager or owner must assume this responsibility. A manager must have the ability to get the right things done at the right time.

Here are some more questions:

Will my expansion or modernization plan improve the chances and ease of producing a higher quality product? Will it increase the ease of the key jobs associated with dairying? Will it increase the efficiency of labor, investment and/or equipment? Will the modernized set-up provide the minimum movement of men, animals and material? Can this modern dairy farm compete with nonfarm occupations for good labor or manpower?

Do you as a manager or owner have a concern for production, people, quality milk and a desire to make the dairy more profitable? Will you develop a good milking program? One that obtains high production per cow, controls mastitis, produces clean excellent-flavored milk and uses a minimum amount of labor?

Check this planning list

Tomorrow's profitable dairy farm can be expected to be a planned operation. Here are some of the things you will want to consider during the planning phase: 1) Size of herd to be accommodated; 2) Dairy breed; 3) Climate conditions; 4) Topograpy of farmstead, slope, drainage and exposure; 5) Size and productivity of the farm; 6) Feeding program as related to feed storage and equipment for feeding; 7) Existing buildings and equipment that will fit into long range plans; 8) Availability of labor; 9) Capital available for investment; 10) Sanitary regulations or codes or present and potential milk markets; 11) Personal preference of the owner; 12) Water and air pollution regulations.

Planning with pencil and paper prior to the start of construction permits you to analyze combinations of equipment and building arrangements without any expenditure. Visit as many farms as possible and incorporate the good points seen at these farms into your facilities. Use university people and private consulting people.

Unless a dairyman regularly tests production, a pipeline milker can be the best friend that a cull cow ever had. The cull cow strolls in with the good producers, gives only enough milk to color the line, eats almost as much feed as the best cows, and then goes her merry way. A dairyman needs profitable production from every cow. You will call the correct signals and build a profit-winning dairy team if you use production records.

Feeding practices important

Constant attention to feeding practices, breeding for production and herd health during expansion are very important. A well designed, properly installed milking system is essential to proper milking. You can't afford an inadequate or poorly maintained milking system.

Productivity must be the key

The productivity of a resource depends on the amount and kind of other resources with which it is combined. As an example, when capital is substituted for labor, it requires fewer hours of labor per cow. However, unless production increases or more cows can be handled, the machinery and equipment cost will offset the savings in labor. Productivity of a resource then depends on the kind and quality of the resource with which it is combined. Modern technology is closely related then to the substitution of capital for labor and land. It increases the demands on management, emphasizes financial management and increases technical requirements of hired labor.

Finally, all of this modernization should help make dairying more pleasant, as well as more productive for you and your family.

This is one of a series of topics developed by noted Dairy authorities. For a complete set write for a free booklet.