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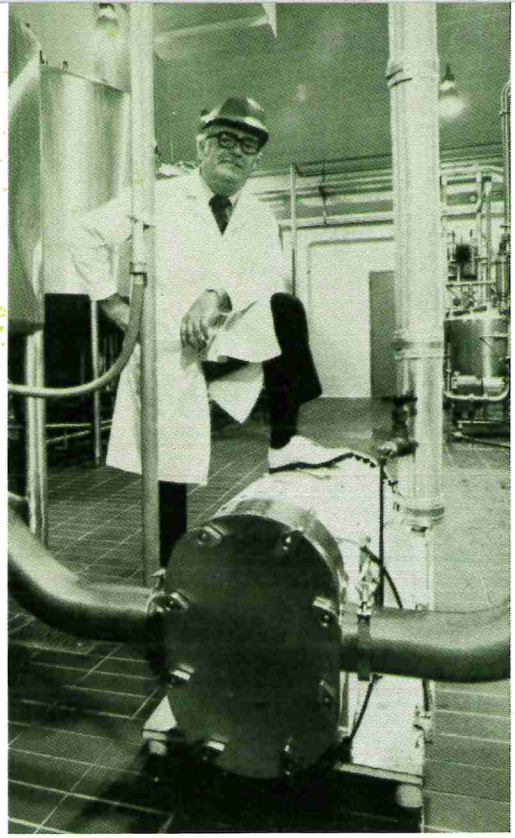

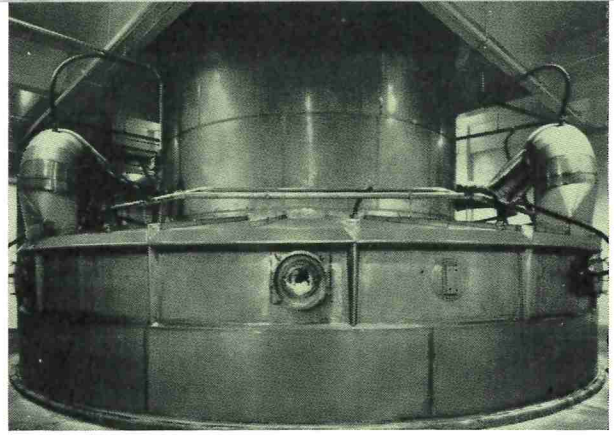

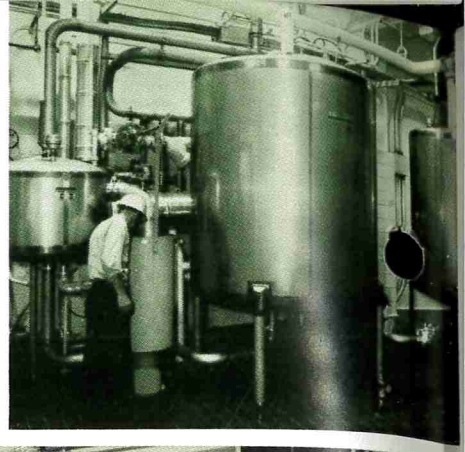

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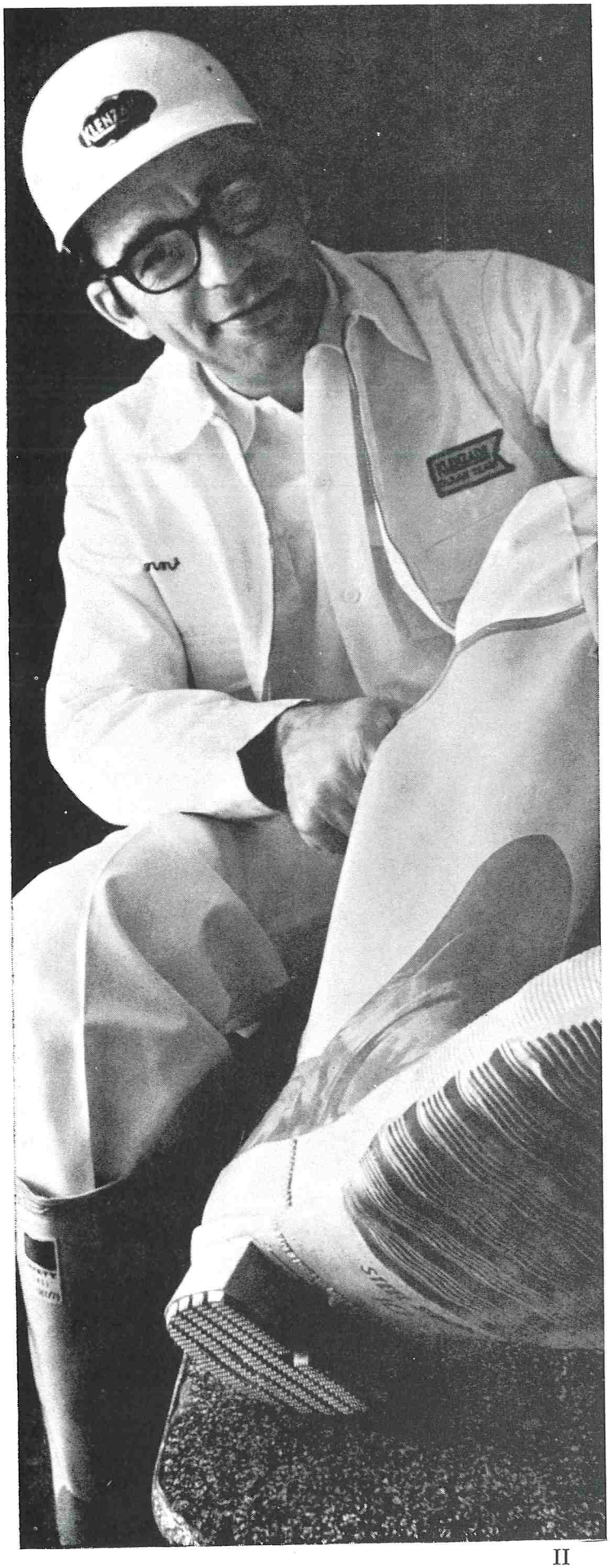

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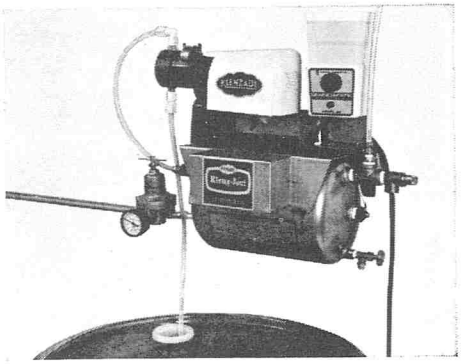

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EXPERIMENTAL PRODUCTION OF AFLATOXIN ON INTACT CITRUS FRUIT[1]

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(Received for publication April 19, 1974)

Abstract

Surfaces of intact citrus fruits were inoculated with spores of *Aspergillus parasiticus* and the fruits were incubated at 24-30 C in an atmosphere with 13-93% relative humidity (RH). With 13-20% RH, no mold growth occurred on limes and growth was not observed on grapefruits until 7 days of incubation. After 14 days, mold growth was noted only on a portion of grapefruits, lemons, and oranges. Although surface areas of fruits without mold growth were dry and brittle, they failed to achieve equilibrium with the atmosphere. When fruit was incubated in an atmosphere of 66-93% RH, fungal growth was considerably faster and more prolific. Generally, one-third to one-half of the surface of each inoculated fruit was covered with mycelium after 4 days of incubation. Sporulation by the mold began at this time. After 7, 10, and 14 days of incubation all fruits were totally covered with mycelium and spores. Relative humidity values between 66 and 93% caused no appreciable difference in amount of aflatoxin present in peels of various fruits. The edible portion (endocarp) of grapefruit contained aflatoxins B_1, B_2, G_1, and G_2 after only 4 days of incubation. In another sample of grapefruit, aflatoxin penetrated 26-33 mm into the endocarp after 7 days. After 7 and 10 days, aflatoxin was recovered from samples of lemon and lime endocarp, respectively. In one lemon, after 7 days, aflatoxin appeared in endocarp 20-26 mm below the surface. The amount of aflatoxin in endocarp became less as the distance from the surface (peel) increased.

Contamination of food by secondary metabolites of molds is an important problem that has been appreciated only during the last 10-15 years. Certain strains of *Aspergillus flavus* and *Aspergillus parasiticus* can produce potent carcinogens, termed aflatoxins *(20, 30)*.

Man, as well as numerous species of animals, can be affected by ingested aflatoxins. Serck-Hanssen *(23)* reported observing aflatoxin-induced fatal hepatitis in a 15-year-old African boy who had daily eaten cassava contaminated with aflatoxin. Recently, Bösenberg *(5)* reported the first direct evidence of acute illness and death in a human after a single dose of aflatoxin.

Aflatoxigenic molds are extremely common and can grow on numerous substrates under many conditions *(21)*. Laboratory studies have demonstrated that these fungi can grow and form aflatoxin on virtually any agricultural commodity, providing temperature, moisture, and aeration are adequate *(10, 18)*. Many such products could be consumed by humans or animals *(14)*. Citrus fruits are prone to fungal spoilage in the field, after harvest, in transit or storage, during processing and marketing, and in the home kitchen *(13)*. Primary factors that hasten mold spoilage of citrus fruits are insect infestation and injury caused mechanically, chemically, or by chilling and freezing *(13)*. The economic loss from fungal spoilage of citrus fruits amounts to millions of dollars per year in the USA *(13)*. Both *A. flavus (26)* and aflatoxin B_1 *(17)* have been recovered from citrus fruit. Earlier work in our laboratory demonstrated that toxigenic aspergilli can produce aflatoxin during growth on or in peels and juice of citrus fruits *(1, 2, 3)*.

Aspergilli are ubiquitous in nature and citrus fruits are often infected with molds. Fungal growth and associated aflatoxin production on these commodities could be a reality, and then would constitute a risk to human health. This study was designated to determine *(a)* if known aflatoxigenic fungi could grow and form aflatoxin on intact citrus fruit, *(b)* if aflatoxins could diffuse from the surface into the edible portion of the fruit, and *(c)* the limiting relative humidity (RH) for growth of *A. parasiticus* on citrus fruit.

Materials and Methods

Organism

A. parasiticus NRRL 2999 was used in this study. It is a known toxigenic strain that was obtained from the Northern Regional Research Laboratory, Peoria, Illinois. Stock cultures were maintained at 5 C on slants of mycological agar (Difco).

Preparation of spore suspension

Mold was grown for 10 days at 28 C on slants of mycological agar (Difco) in 8-oz prescription bottles. Spores were harvested by adding sterile distilled water and a drop of Leconal wetting agent (Laboratory Equipment Co., St. Joseph, Michigan) to the slants.

Preparation and inoculation of fruit

The waxy (peel) surface of grapefruits, lemons, limes, and

[1]Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by Public Health Service Grant FD 00143 from the Food and Drug Administration.

oranges was abraded by gentle sanding to resemble injury and assure fungal growth. Heavy spore suspensions from bottle-slant cultures of the mold were pooled into a previously sterilized 1500-ml beaker in which fruits were placed individually and rotated to afford an even inoculum. Inoculated fruits were placed into individual sterile beakers that were then covered with milk filter discs (Johnson & Johnson, Chicago, Ill.) to allow passage of air and moisture.

Incubation and environmental control

Each beaker containing an inoculated fruit was placed into a humidity cabinet. The chamber was housed in a walk-in incubator and the temperature was monitored by a key-wound thermo-humidigraph, model 4069TH (Bristol Co., Waterbury, Conn.) with a 7-day chart. The percentage of RH was determined with a wet and dry bulb psychrometer. Desired RH values were obtained by utilizing various saturated salt solutions *(29)*. Percentages of RH used in this study were 13-20, 26, 66, 77-79, 82, 85, and 93, and they were obtained with solutions of LiCl, potassium acetate, $NaNO_2$, potassium tartrate, KCl, and NH_4HPO_4, respectively. Samples were incubated for 3-14 days at 24-30 C, depending on the trial. After fruits were removed from the humidity chamber, they were immediately frozen at −35 C.

Sampling

In each trial (except when penetration of toxin into fruit was studied) an area of molded peel (average 27 g) was carefully removed and a section of the endocarp (average 35 g) immediately beneath was excised and subjected to aflatoxin analysis. To determine depth of aflatoxin penetration, frozen fruits were halved, cross-sectionally along the horizontal axis, so that endocarp layers at depths inward from the peel could be removed and tested for aflatoxin.

Aflatoxin analysis

The amount of aflatoxin present in samples (peel or endocarp) was determined as described earlier by Alderman and Marth *(1, 2)*.

RESULTS

Tables 1-3 provide data on the amounts of aflatoxin present on the peel and in the endocarp immediately beneath the moldy peel of citrus fruits. Sometimes aflatoxin appeared to be present on the peel but could not always be quantitated because of naturally occurring fluorescent substances from the peel that interfered with measurement of the toxin. Results from replicates (of the same time period) were not always consistent; this may have resulted from differences in mold growth on the different fruits. Generally, fruits after 4 days of incubation were only partially (one-third to one-half) covered with mycelium and sporulation was commencing, whereas fruits were completely covered with mycelium and spores after 7, 10, and 14 days.

Aflatoxin recovered from grapefruit

Data in Table 1 show that minimal amounts of all four aflatoxins were present in the endocarp of grapefruit after 4 days of incubation. Samples of endocarp at 7 days yielded only aflatoxin B_1 (0.03 and 0.01 μg toxin/g), whereas the peel contained 0.05 and 0.08 μg B_1/g. Ten-day-old samples of grapefruit contained nearly 10 times as much aflatoxins B_1 and G_1 in the peel as in the endocarp. Aflatoxins B_2 and G_2 were also recovered from the endocarp. Some 14-day-old samples contained all four aflatoxins in the peel and endocarp. Up to approximately 62 times as much aflatoxin B_1 was recovered from the peel as from the endocarp.

Aflatoxin recovered from limes

After 4 days of incubation, nearly equal amounts of aflatoxin B_1 appeared in the peel and endocarp of limes (Table 2). Aflatoxins B_2, G_1, and G_2 were present on the peel but were not found in the endocarp. At 7 days, approximately 0.02 μg aflatoxin B_1/g was present in both the peel and endocarp. Other aflatoxins were not detected. Aflatoxins B_1, B_2, and G_1, but not G_2, were recovered from peel and endocarp of limes after 10 days of incubation. The amount of aflatoxin G_1 was greater in the endocarp (0.016 μg/g) than in the peel (0.005 μg/g). In one set of samples incubated for 14 days, no aflatoxin penetrated into the endocarp although the toxin was present in the peel. When a second set of three replicates was sampled after 14 days of incubation, aflatoxins B_2 and G_2 were not detected in the peel nor was aflatoxin G_2 found in the endocarp. At least twice as much aflatoxins B_1 and G_2 was recovered from the peel than from the endocarp.

Aflatoxin recovered from lemons

When surfaces of lemons were inoculated with spores of *A. parasiticus* (Table 3), no aflatoxin G_2 was detected after 14 days of incubation. Only one sample of lemon peel contained aflatoxin B_1 (0.02 μg/g). An equal amount of toxin was present in the endocarp beneath the peel. Aflatoxins B_2 and G_1 occurred in the peel at markedly higher concentrations than in the endocarp of this sample. Samples of endocarp from other lemons contained aflatoxins B_1, B_2, and G_1. Amounts of aflatoxins B_1 and G_1 were equal or nearly equal in these samples.

Penetration of aflatoxins B_1 and G_1 into grapefruit and lemons

Incubated fruits were halved, cross-sectionally across their horizontal axis, so that endocarp at various depths inward from the peel could be examined for the presence of aflatoxin. Data in Table 4 demonstrate that apparently no aflatoxin diffused into the endocarp until after 3 days of incubation. After 7 days, aflatoxins B_1 and G_1 appeared in the top and second layers of one grapefruit sample and in the top layer of one lemon sample. The peel contained the largest amount of aflatoxin and the layers beneath, progressively less. After 10 days of incubation, only

TABLE 1. AFLATOXIN RECOVERED FROM GRAPEFRUIT PEEL AND ENDOCARP AFTER *Aspergillus parasiticus* GREW FOR 4-14 DAYS AT 82-85% RH AND AT 28 ± 2 C

Days	Aflatoxin recovered from Peel				Endocarp			
	B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
	(μg/g)							
4	NM[1]	NM	NM	NM	0.55	0.002	0.02	0.001
4	NM	NM	NM	NM	0.002	0.001	0.02	1.002
7	0.05	NM	NM	NM	0.03	ND[2]	ND	ND
7	0.08	ND	ND	ND	0.01	ND	ND	ND
10	0.01	NM	0.01	NM	0.002	0.003	0.002	ND
10	NM	NM	NM	NM	0.03	0.001	0.02	0.001
14	1.03	0.04	0.22	0.04	0.04	0.002	0.02	0.001
14	0.06	NM	0.12	NM	0.001	0.001	0.03	NM
14	0.19	NM	0.17	NM	0.12	0.006	0.15	0.01
14	0.19	NM	NM	NM	0.03	0.022	0.17	0.01
14	0.22	NM	NM	NM	0.01	0.004	0.16	0.002

[1]Not measured.
[2]Not detected.

TABLE 2. AFLATOXIN RECOVERED FROM LIME PEEL AND ENDOCARP AFTER *Aspergillus parasiticus* GREW FOR 4-14 DAYS AT 82-85% RH AND AT 28 ± 2 C

Days	Aflatoxin recovered from Peel				Endocarp			
	B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
	(μg/g)							
4	NM[1]	NM	NM	NM	0.004	ND[2]	ND	ND
4	0.02	0.01	0.013	0.004	0.013	ND	ND	ND
7	0.02	ND	ND	ND	0.017	ND	ND	ND
10	0.06	0.01	0.005	ND	0.008	0.003	0.016	ND
14	0.02	NM	0.032	NM	ND	ND	ND	ND
14	0.07	0.01	0.032	NM	ND	ND	ND	ND
14	0.11	ND	0.18	ND	0.064	0.002	0.063	ND
14	0.16	ND	0.19	ND	0.066	0.003	0.033	ND
14	0.22	ND	0.50	ND	0.074	ND	0.069	ND

[1]Not measured.
[2]Not detected.

TABLE 3. AFLATOXIN RECOVERED FROM LEMON PEEL AND ENDOCARP AFTER *Aspergillus parasiticus* GREW FOR 14 DAYS AT 82 OR 93% RH AND AT 26 ± 2 C

RH %	Aflatoxin recovered in Peel				Endocarp			
	B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
	(μg/g)							
93	0.02	0.05	0.12	ND[1]	0.02	0.001	0.01	ND
82	ND	ND	0.06	ND	0.03	0.01	0.05	ND
82	ND	ND	0.48	ND	0.01	0.002	0.02	ND
82	ND	ND	0.03	ND	0.08	ND	0.08	ND

[1]Not detected.

TABLE 4. AFLATOXIN RECOVERED ON AND IN GRAPEFRUIT AND LEMON SURFACE INOCULATED WITH *Aspergillus parasiticus* AND HELD AT 66-79% RH AND 27 ± 1 C FOR 3-14 DAYS

Fruit	Sample	Aflatoxin B_1				Aflatoxin G_1			
		3 days	7 days	10 days	14 days	3 days	7 days	10 days	14[4] days
		(μg/g)							
Grapefruit	peel	0.012[1]	0.099	ND		+	0.082	ND	
		+[2]		0.014	+	+	+	+	+
		ND[3]	0.075			ND		+	
	top 18-21 mm layer	ND	0.046	ND		ND	0.007	ND	
		ND	ND	0.001	ND	ND	ND	ND	ND
		ND		ND		ND		ND	
	second 8-12 mm layer	ND	0.003	ND		ND	0.005	ND	
		ND	ND	ND	ND	ND	ND	ND	ND
		ND		ND		ND		ND	
	third 8-12 mm layer	ND	ND	ND		ND	ND	ND	
		ND	ND	ND	ND	ND	ND	ND	ND
		ND		ND		ND		ND	
Lemon	peel	0.004	0.014	0.035		ND	ND		
		0.75	0.43	0.16	0.013	0.15	ND	0.012	0.061
			0.007				0.075	ND	
	top 20-26 mm layer		ND	0.004		ND	ND		
		ND	0.015	0.007	ND	ND	ND	0.005	ND
		ND	ND				0.009	ND	
	second 9-11 layer	ND	ND	0.002		ND	ND	0.006	
		ND	ND	ND	ND	ND	ND	ND	ND
			ND				ND		

[1]Each value (number or ND) represents a separate sample.
[2]Aflatoxin present but not measured.
[3]Not detected.

one grapefruit sample yielded aflatoxin B_1 from the top layer (18-21 mm deep) of endocarp. One-tenth the amount of aflatoxin B_1 in the peel was present in this layer of endocarp. When lemons were incubated for 10 days, aflatoxins B_1 and G_1 were present in all layers of endocarp with the peel containing the highest amounts. This was the only experimental sample with mold spores in the endocarp. Samples of each fruit contained aflatoxins B_1 and G_1 in the peel after 14 days but none were detected in any layers of endocarp.

Relative humidity to limit growth of A. parasiticus

The moisture or RH surrounding a natural substrate is the single most important factor that determines if growth of or aflatoxin production by toxigenic aspergilli will occur *(4)*. To study how RH influenced aflatoxin production on citrus fruits surfaces of grapefruits, lemons, limes, and oranges were inoculated with *A. parasiticus* spores and incubated at various RH values for 14 days at 27 C. At 26% RH, one grapefruit had mold growth with sporulation only on one-half of the fruit, whereas no mold growth appeared on the replicate sample. When no growth occurred on the fruit, its peel was extremely dry and it cracked with handling. Lemons at the same RH were partially (one-half to three-fourths) covered with mold growth, although areas without growth were very dry and brittle. No mold growth occurred on limes at 26% RH. Mold growth and sporulation were present over three-fourths of the surfaces of oranges at 26% RH. In an atmosphere of 13% RH (first 3 days) to 20% RH, the mold did not grow on limes. On lemons and oranges, mold growth was very slow but after 14 days, mycelium and spores appeared on about one-half of the fruit. Growth of mold on grapefruit was very slow; none was observed until 7 days of incubation. Most grapefruit samples exhibited very slight mold growth and a trace of sporulation on part of the fruit at 14 days of incubation.

DISCUSSION

The importance of proper humidity to curtail growth of *Aspergillus* has been noted by several in-

vestigators *(7, 8, 9, 22)*. Generally, these workers used peanuts and corn as substrates and found that RH values near 70% limited mold growth and aflatoxin formation. Data in Tables 1, 2, 3, and 4 indicate that RH values ranging from 66-93% caused no appreciable difference in the amount of aflatoxin present on the peels of various inoculated citrus fruits. Furthermore, when the fruits were inoculated and then incubated at low RH values (13-26%), *A. parasiticus* grew on at least one sample of each fruit, except limes. The only difference between growth in an atmosphere of low RH and one of higher RH (66-93%) was the rate and amount of growth. Citrus peels commonly contain more than 70% water, whereas the endocarp consists of at least 88% water *(28)*. It is apparent that under the experimental conditions, the fruits never achieved equilibrium with the surrounding atmosphere. Brancato et al. *(6)* grew *Penicillium roqueforti* on malt agar in covered and uncovered petri dishes at various RH values from 13.2-98% for 7 days at 25.5 C. These workers reported no difference regarding growth in the covered dishes but the diameter of mold colonies in the uncovered dishes was reduced by one-third and one-fourth at average RH values of 36.1 and 13.2%, respectively. It appears that citrus fruits cannot "safely" be stored at low RH values to inhibit fungal growth. If a given value would indeed inhibit mold growth, the fruit would become physically and organoleptically unacceptable.

The amounts of aflatoxin recovered from grapefruit peel (Tables 1 and 4) generally were not as high as those previously reported *(1)*. In an intact fruit, the rind offers resistance to fungal infestation and this resistance decreases progressively from the outside inward *(16)*. The outer rind or flavedo is comprised of oil-bearing sacs *(16)* and citrus oils can exhibit antifungal action *(27)*. The resistance of the flavedo is thought to be, at least in part, mechanical in nature *(16)*. It may result from either cell turgor or the hardness of the walls. The stomata are probably the means of entry *(16)*. The loose spongy nature of white inner rind or albedo is associated with many air spaces, but also contains approximately 30% of its dry weight as soluble and insoluble pectic materials *(25)*. *Penicillium* species can dissolve the pectic substances, thereby softening the walls and gaining entrance into the interior of the fruit *(16)*. It might be possible for aspergilli to do the same, although endocarp from only one inoculated lemon contained mold spores (and aflatoxin) (Table 4).

It is apparent from results of this study that aflatoxins can diffuse from the peel into the edible portion of the fruit. After only 4 days of incubation, aflatoxins B_1, B_2, G_1, and G_2 were detected in grapefruit endocarp (Table 1). After 7 and 10 days, aflatoxin was recovered from lemon (Table 3) and lime (Table 2) endocarp, respectively. Data in Table 4 indicate that after 7 days, aflatoxin can diffuse from the peel as far as 26-33 mm into grapefruit endocarp. In one lemon sample, aflatoxin was detected in the endocarp at a depth of 20-26 mm after 7 days. Other investigators *(10, 17, 24)* have reported that aflatoxin can diffuse from food surfaces into the interior. Data in Table 4 are in accord with those of Lie and Marth *(19)* and Shih and Marth *(24)* who found that the concentration of aflatoxin in cheese became less as the distance from the surface increased. This suggests that a potential hazard might exist if consumers remove only surface mold from citrus fruit, provided that the mold was toxigenic.

Prevention and control of fungal spoilage in the citrus fruit industry are mandatory. Careful handling of the fruit to avoid injuries during all operations from tree to market is important. Injuries and abrasions of fruit that can serve to encourage fungal growth and subsequent decay can originate in picking, handling, washing, sizing, grading, packaging, shipping, and marketing of citrus fruit *(11)*. In the USA, washing and chilling (precooling) are generally the first quality protection precedures to be applied to perishable produce *(15)*. The fungicide, sodium orthophenylphenate, has been suggested for use to control decay in citrus fruits *(15)*. Impregnation of citrus containers with diphenyl has also been suggested to control mold apoilage *(15)*.

REFERENCES

1. Alderman, G. G., and E. H. Marth. 1974. Experimental production of aflatoxin in citrus juice and peel. J. Milk Food Technol. 37:(*In press*).

2. Alderman, G. G., and E. H. Marth. 1974. Production of aflatoxin in concentrated and diluted grapefruit juice. J. Milk Food Technol. 37:(*In press*).

3. Alderman, G. G., C. O. Emeh, and E. H. Marth. 1973. Aflatoxin and rubratoxin produced by *Aspergillus parasiticus* and *Penicillium rubrum* when grown independently, associatively, or with *Penicillium italicum* or *Lactobacillus plantarum*. Z. Lebensm. Unters. Forsch. 153:305-311.

4. Austwick, P. K. C., and G. Ayerst. 1963. Groundnut microflora and toxicity. Chem. Ind. (London) 2:55-61.

5. Bősenberg, H. 1973. Aflatoxinwirkung bei Tier und Mensch. Z. Lebensm. Unters. Forsch. 151:245-249.

6. Brancato, F. P., and N. S. Golding. 1953. The diameter of the mold colony as a reliable measure of growth. Mycologia 45:848-864.

7. Christensen, C. M. 1965. Fungi in cereal grains and their products. pp. 9-14. *In* G. N. Wogan (ed) Mycotoxins in foodstuffs. M.I.T. Press, Cambridge, Massachusetts.

8. Dickens, J. W., and H. E. Pattee. 1966. The effects of time, temperature and moisture on aflatoxin in peanuts inoculated with a toxic strain of *Aspergillus flavus*. Trop. Sci. 8:11-12.

9. Diener, U. L., and N. D. Davis. 1967. Limiting temp-

erature and relative humidity for growth and production of aflatoxin and free fatty acids by *Aspergillus flavus* in sterile peanuts. J. Amer. Oil Chem. Soc. 44:259-263.

10. Diener, U. L., and N. D. Davis. 1969. Aflatoxin formation by *Aspergillus flavus*. pp. 13-54. *In* L. A. Goldblatt (ed) Aflatoxin. Academic Press, New York.

11. Fawcett, H. S. 1936. Citrus diseases and their control. McGraw-Hill Book Co., Inc., New York. 656 p.

12. Frank, H. K. 1968. Diffusion of aflatoxins in foodstuffs. J. Food Sci. 33:98-100.

13. Friedman, B. A. 1960. Market diseases of fresh fruits and vegetables. Econ. Bot. 14:145-156.

14. Goldblatt, L. A. 1968. Aflatoxin and its control. Econ. Bot. 22:51-62.

15. Golumbic, C. 1965. Fungal spoilage in stored food crops. pp. 49-67. *In* G. N. Wogan (ed) Mycotoxins in foodstuffs. M.I.T. Press, Cambridge, Massachusetts.

16. Green, F. M. 1932. The infection of oranges by *Penicillium*. J. Pomol. Hort. Sci. 10:184-215.

17. Hanssen, E. 1969. Schädigung von Lebensmitteln durch Aflatoxin B_1. Naturwissenschaften 56:90.

18. Hesseltine, C. W., O. L. Shotwell, J. J. Ellis, and R. D. Stubblefield. 1966. Aflatoxin formation by *Aspergillus flavus*. Bacteriol. Rev. 30:795-805.

19. Lie, J. L., and E. H. Marth. 1967. Formation of aflatoxin in Cheddar cheese by *Aspergillus flavus* and *Aspergillus parasiticus*. J. Dairy Sci. 50:1708-1710.

20. Marth, E. H. 1967. Aflatoxins and other mycotoxins in agricultural products. J. Milk Food Technol. 30:192-198.

21. Raper, K. B., and D. I. Fennell. 1965. The genus *Aspergillus*. Williams and Wilkins, Baltimore, Maryland. 686 p.

22. Scott, W. J. 1957. Water relations of food spoilage organisms. Advances Food Res. 7:83-127.

23. Serck-Hanssen, A. 1970. Aflatoxin-induced fatal hepatitis? Arch. Environmental Health 20:729-731.

24. Shih, C. N., and E. H. Marth. 1972. Experimental production of aflatoxin on brick cheese. J. Milk Food Technol. 35:585-587.

25. Sinclair, W. B. 1961. Pectic substances. pp. 191-229. *In* W. B. Sinclair (ed) The orange. Univ. of California, Riverside.

26. Stevenson, J. A. 1918. Citrus diseases of Puerto Rico. Puerto Rico J. Agr. 2:43-119.

27. Subba, M. S., T. C. Soumithri, and R. S. Rao. 1967. Antimicrobial action of citrus oils. J. Food Sci. 32:225-227.

28. U. S. Department of Agriculture. 1963. Composition of foods. Handbook No. 8. U. S. Government Printing Office, Washington, D. C. 190 p.

29. Winston, P. W., and D. H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41:232-237.

30. Wogan, G. N. 1966. Chemical nature and biological effects of the aflatoxins. Bacteriol. Rev. 30:460-470.

PFIZER AWARD GOES TO E. M. MIKOLAJCIK

To be eligible for the Pfizer Award in Cheese Research, candidates shall have made an important research contribution pertaining to cheese chemistry, biochemistry, bacteriology, engineering, or technology. Dr. Emil M. Mikolajcik has made outstanding contributions in several of these areas.

The recipient's research contributions pertaining most directly to starter cultures and cheese can be summarized as follows: *(a)* The effects of antibiotics on lactic streptococci and their bacteriophages; this timely work was conducted late in the 1950's and early 1960's when antibiotics constituted a major problem in the cheese industry. *(b)* Differentiation of the lactic group streptococci; his simple test permitted rapid and dependable differentiation of *Streptococcus lactis* and *Streptococcus cremoris*. *(c)* Aerobic sporeforming bacteria; this study delineated the effect of these organisms on the performance of lactic-acid bacteria and the fate of sporeformers in cheese. *(d)* Accelerated cheese ripening; through cooperative effort with other investigators, a process was developed whereby slurried fresh cheese curd could be brought to a full-flavored product in 5 to 7 days. *(e)* Immunoglobulins of milk; he isolated, characterized, and further studied the properties of immunoglobulins in normal and mastitic milk that make them inhibitory to lactic acid bacteria. *(f)* Antimicrobial activity of lactic-acid bacteria; his study resulted in identification and characterization of an antibiotic produced by *Lactobacillus acidophilus*, which is highly toxic against common food-spoilage and toxin-producing microorganisms.

Although Mikolajcik's native state, Connecticut, is not noted as a cheese manufacturing area, his dairy farm background led him to the University of Connecticut where he obtained the B.S. degree (1950) in dairy manufacturing. At Ohio State University, enroute to his M.S. and Ph.D. degrees in dairy technology (1959), he evidently whetted his interest in microbiology. After graduating from Ohio State University, Mikolajcik taught for 8 years at the University of Puerto Rico, Mayaguez Campus. Since then, he has served on the staff of the Department of Food Science and Nutrition at Ohio State University as associate professor. In addition to advising graduate students and research activities, he is a respected teacher of several food-related courses.

Mikolajcik belongs to many professional organizations and honorary societies as well as numerous department and university committees. He is the author or coauthor of over 60 scientific papers, serves on the Editorial Board of the *Journal of Milk and Food Technology*, and is engaged in revision of the 14th edition of *Standard Methods for the Examination of Dairy Products*.

TEMPERATURE AND MICROBIAL FLORA OF REFRIGERATED GROUND BEEF GRAVY SUBJECTED TO HOLDING AND HEATING AS MIGHT OCCUR IN A SCHOOL FOODSERVICE OPERATION[1]

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Abstract

The practices of handling precooked chilled gravy in school kitchens were simulated to determine if they could contribute to outbreaks of foodborne illness. Time-temperature measurements and bacteriological tests were made at intervals during chilling, holding, and heating of gravy. Sixty-six pounds of cooked ground beef gravy were packed hot (158 F, 70 C) in bags, cooled in chilled water for 1 h, and refrigerated for 16 h. The gravy was held for 5 h at 82 F (28 C) and at 42 F (5.5 C) and then it was heated in a compartment steamer for 35 min. After 1 h cooling in chilled water, the mean temperature of gravy was 82 F (28 C) and after 16 h of refrigerated storage, the temperature was 45.5 F (7.5 C). The mean temperature of chilled gravy held at 82 F (28 C) remained about 50 F (10 C) for 4 h and reached 64 F (18 C) at the end of the 5-h holding period. The mean temperature of gravy held under refrigeration for 5 h decreased 2 F, from 45.5 F (7.5 C) to 43.5 F (6.5 C). After 35 min of heating in a compartment steamer, the highest temperature was 136 F (58 C) for gravy initially at 65 F (18.5 C) and 128 F (53.5 C) for gravy initially at 47 F (8.5 C). Bacteriological tests indicated that the greatest increase in the number of total aerobic bacteria in gravy occurred during cooling rather than holding. Although some samples yielded coagulase-positive staphylococci, the numbers changed little during holding or heating. *Clostridium perfringens* was not found in any samples of the gravy.

Changes in the composition of food products or in handling practices in mass feeding systems may increase the potential for outbreaks of foodborne illness. The increasing popularity of precooked, ready-to-serve food has complicated control problems facing the foodservice industry and public health employees who work to prevent foodborne diseases *(2)*. There is an urgent need for information about the safety of precooked chilled foods since their use is increasing in catering and commissary foodservice. It is believed that precooked chilled food is far more important in terms of a public health hazard than other types of food *(5)*.

Use of precooked chilled food has increased rapidly in school foodservice in Finland in recent years *(12)*. Economic benefits derived from centralized preparation and distribution systems suggest that increased use of precooked chilled food is to be expected. The extended interval between food preparation and consumption provides more opportunities for mishandling of food and thus increases the risk that bacteria, including pathogenic types if present, can multiply excessively. Since many people eat food prepared in the same manufacturing plant, questions have emerged about potential health hazards associated with a precooked chilled food system and handling practices used in school kitchens. To date, research has not been reported that evaluated the food holding and heating practices currently used in school foodservice systems.

This study was designed to investigate the effects of two holding and heating treatments as might be used in school foodservice operations on growth of selected bacteria in precooked chilled beef gravy.

Materials and Methods

Preparing the gravy

Meat or combinations of food containing meat and gravy are most often involved in outbreaks of food poisoning caused by *Clostridium perfringens (11)*. Gravy with ground beef was chosen as the experimental food product because it has been incriminated in an outbreak of perfringens food poisoning in Helsinki schools. A formula for ground beef gravy was obtained from a food manufacturing plant in Helsinki and adapted for use in this study. Before initiation of the experiment, pilot studies were made to determine work place layout, types and amounts of ingredients, length of the preparation cycle, and equipment and materials handling procedures required to prepare the gravy in the laboratory where the research was conducted. For each of the two trials of the experiment a gravy was prepared containing: 30 lb. ground beef, 5.13 lb. flour, 7.62 lb. non-dairy creamer, 1.88 lb. dehydrated onions, .75 lb. salt, .50 lb. ketchup, .06 lb. pepper, .03 lb. paprika, .13 lb. brown gravy, and 6 1/2 gal. water. Sixty-six pounds of the cooked gravy were weighed into bags for the chilling, cooling, and holding stages (Fig. 1).

Although operations vary among manufacturing plants from which chilled food is purchased, the basic process is the same. Food is cooked one day before service, chilled immediately, and refrigerated overnight (at 39 F, 4 C). The following day food is transported to school kitchens and held at room temperature until it is reheated. Holding times range from 1 to 3 h but may, in some instances, be as long as 5 h. Food is

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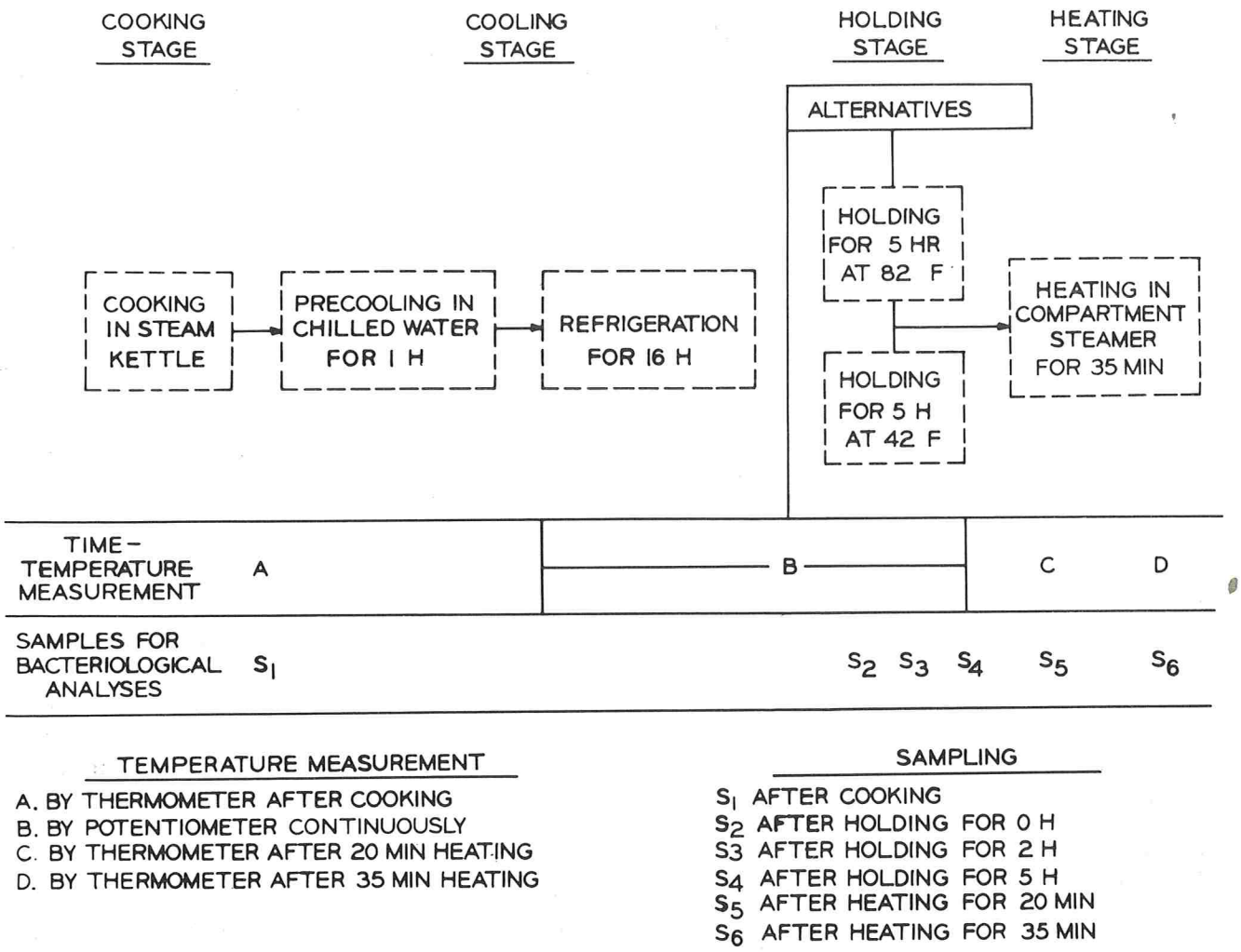

Figure 1. Experimental procedure: Stages of handling for precooked chilled ground beef gravy.

reheated in compartment steamers for 20 to 35 min and served immediately.

Food is usually cooked and packed in aluminum pans (2.5 to 5 lb. in each) in which it is also reheated. If gravy is included in the meal, it is either delivered hot and held in electric warmers or delivered chilled in 2.5 kg. (5.6 lb.) plastic bags within a cardboard case. For heating, three bags of gravy are transferred into one flat stainless steel pan. The pans are covered and heated in compartment steamers.

The experimental procedure simulated the practice in the chilled food system (Fig. 1). After preparation, gravy was allowed to cool until it reached 158 F (70 C). While at this temperature it was packed in 8 × 17 inch plastic bags (polyethylene LT, 0.15 mm). The bags, that each held 5.5 lb. of gravy, were closed with a heavy rubber band and cooled for 1 h in water that was below 50 F (10 C). Bags of gravy were placed in two cardboard cases, six bags in each. Cooling was continued by refrigerating the gravy for 16 h at 42 F.

After refrigeration, the process used in the Finnish schools was simulated. One cardboard case of gravy was placed in an environmental test chamber (Hotpack, Model 1242-4, Philadelphia, Pennsylvania) for holding at 82 F (28 C) for 5 h. The other case remained in the refrigerator at 42 F (5.5 C) for 5 h. These two treatments corresponded to the actual holding practice at room temperature and the recommended holding treatment in the refrigerator. Holding temperatures of the environment were indicated by a recording thermometer. The temperature of 82 F (28 C) was selected for the holding treatment because it was found to be common in institutional kitchens *(8, 9)*. After holding, bags of both refrigerated and unrefrigerated gravy were heated in a compartment steamer (Market Forge Co., Model 3 MTSS, Everett, Massachusetts) for 35 min, the usual heating time used by the food serving units. Two bags of gravy were emptied in one 12 × 10 × 4-inch stainless steel pan. The pans were covered with aluminum foil and heated with steam at a pressure of 7 lb/in². Timing was started when the pressure reached 6 lb/in² in the steamer.

Closing of plastic bags

In the actual processing system, plastic bags are closed by heat sealing. In this work, however, that method could not be used because of the need to insert a thermocouple into the bag and to take samples from the bag during the holding treatment. Therefore, a special device was developed to close the bags. For this purpose, No. 2-1/2 cans were cut 2-1/2 inches in height. Two round holes, diameter of 0.75 and 0.25 inch, were punched in the center of the can. After the bags were cooled in chilled water, a sterilized can was inserted in the opening of each bag and the bag was closed around the can using a heavy rubber band to keep it in place. Thermocouples were inserted through the small hole, and the large hole was closed with a sterile rubber stopper (size 3) until opened for sampling.

Time-temperature measurement

The temperature of gravy in each bag was measured continuously during cooling and holding stages by using copper-constant thermocouples attached to electronic recording potentiometers (Fig 1). Thermocouple wires were threaded through a 13-inch long glass tube, diameter 3 mm, that was sealed around the wire with heat resistant silicone rubber. The thermocouple junction (tip) was left outside the glass tube. Thermocouples were sanitized by dipping in ethyl alcohol and then were inserted into the bags and positioned in the approximate center of the bags. Data from holding gravy at refrigerator temperature were recorded by an Electronic 8-multipoint recorder (Honeywell, Model 153 × 64 – P8H – II – III – 42, Minneapolis, Minn.) and data from holding gravy at room temperature were recorded by an Electronic 12-multipoint recorder (Honeywell, Model 153 × 72 P12 – × – 26, Minneapolis, Minn.)

No continuous temperature recording was made during reheating. Temperature of the gravy was taken with an ethanol-sanitized glass thermometer at the approximate center of the pan, 1-1/2 inches deep, after heating for 20 and 35 min.

Sampling

Samples of gravy for microbiological analyses were taken at six stages (Fig. 1).

When gravy was in cardboard cases, samples were taken from the four corner bags in each case. Samples were taken with a sterile glass tube from the gravy in the approximate center of the bag. Glass tubes (diameter, 1.2 mm; length, 12 to 14 inches) were inserted through the larger hole of the can. About 20 g of gravy were obtained by a pipette equipped with a rubber bulb. A plastic spoon, individually packaged, was used to take the samples from the gravy in pans or kettle. Samples were placed in sterile petri dishes and refrigerated until they were cultured. The time from sampling to plating never exceeded 6 h.

From each sample 11 g of gravy were weighed into 99 ml of sterile 0.1% peptone water, and blended in a sterile jar for 2 min. Decimal dilutions were made with 0.1% peptone water and duplicate plates were made from each dilution on three culture media.

Bacteriological analyses

Tests were done to determine numbers of total aerobic bacteria, coagulase-positive staphylococci, and *C. perfringens*.

Total aerobic plate counts were determined with Standard Methods Agar (Difco) using the pour-plate method. Inoculated plates were incubated at 37 C for 72 h. Plates having 30 to 300 colonies were counted using a Quebec Colony Counter.

To cultivate coagulase-positive staphylococci, 0.1 ml of each sample dilution was streaked on plates of Mannitol Salt Agar (Difco) that were incubated at 37 C for 48 h. Colonies typical of coagulase-positive staphylococci were picked and inoculated into tubes containing 3 to 5 ml of Brain Heart Infusion Broth (Difco). Tubes were incubated at 37 C for 24 h. Broth in each tube was tested for coagulase reaction with Coagulase Plasma (Difco) using the tube method *(1)*.

Methods described by Shahidi and Ferguson *(10)* were used to enumerate *C. perfringens*. The medium consisted of SFP-agar Base (Difco) combined with egg yolk and two antibiotics, polymyxin sulfate (Antimicrobic Vial P, Difco) and Kanamycin sulfate (Antimicrobic Vial K, Difco). From each dilution of the sample 0.1 ml was streaked on the medium in petri plates. Inoculated but dry plates were covered with 10 ml of SFP-overlay agar made from SFP-agar Base (Difco) and the two antibiotics. Plates were incubated at 37 C for 24 h in anaerobic plastic bags (Gas Pak Disposable Anaerobic System, BBL, Bio Quest, Cockeysville, Maryland) or in screw cap glass jars with a hydrogen and carbon dioxide generator (Gas Pak Hydrogen + CO_2 Generator Envelopes, BBL, Bio Quest) and catalyst (Catalyst Replacement Charges, BBL, Bio Quest) in the jar. Formation of anaerobic conditions was identified by an indicator (BBL).

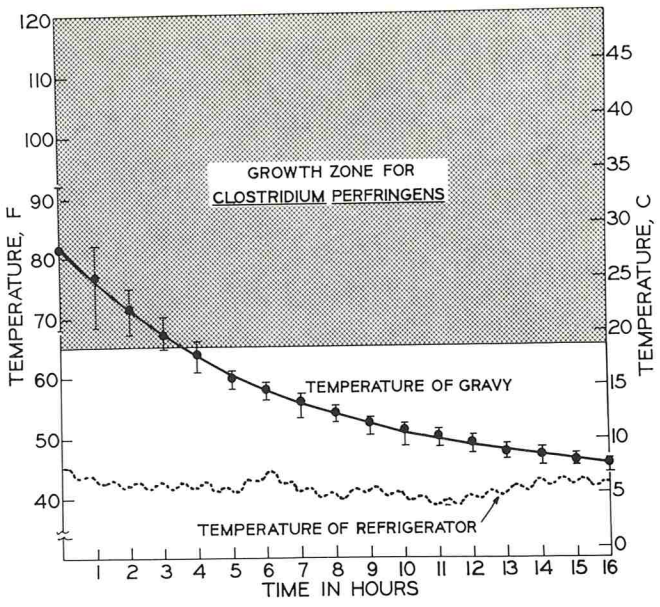

Figure 2. Effect of time on temperature of ground beef gravy during refrigeration: Temperature mean and range for eight samples from two trials.

Black colonies surrounded by distinct white zones of opaque precipitate on incubated plates were counted. Ten colonies from each plate were picked and placed in deep tubes containing 10 ml of Lactose Motility (LM) agar. The tubes of medium had been boiled for 10 min and cooled rapidly before inoculation. A stab was made three-fourths of the distance into the medium in tubes. Inoculated LM-agar tubes were incubated at 37 C for 24 h. An isolate was assumed to be *C. perfringens* when it was non-motile and fermented lactose. Fermentation was indicated by change in color of medium from red to yellow, and formation of gas bubbles.

RESULTS AND DISCUSSION

Cooling of gravy

After cooling in chilled water for 1 h the mean temperature of the gravy was 82 F (28 C) in the center of the bags. In two trials the temperature of gravy in eight bags ranged from 67 F (19.5 C) to 92 F (33.5 C). The range of temperature for rapid growth of *C. perfringens* (88 to 122 F; 31 to 50 C) occurred during the 1-h cooling in chilled water before gravy was held for 16 h at refrigeration.

Results in Fig. 2 indicate that 3 to 4 h of refrigeration were required before the temperature of the gravy in the center of the bags decreased to 65 F (18.5 C), which has been reported as the minimum temperature for growth of *C. perfringens (3, 4)*. The

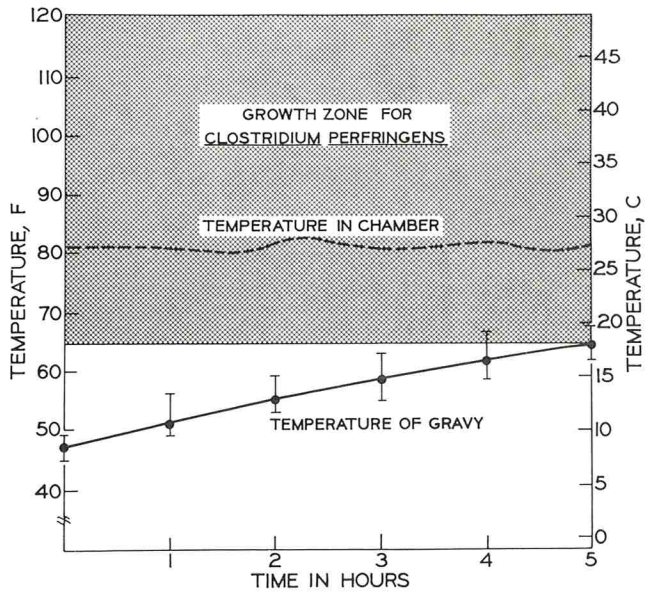

Figure 3. Effect of time on the temperature of ground beef gravy during holding at 82 F (28 C): Temperature mean and range for eight samples from two trials.

rate of heat transfer was reduced as the temperature in the center of gravy approached the temperature of the refrigerator. At the end of 16 h the mean temperature of the gravy was 45.5 F (7.5 C), and the refrigerator temperature was 42 F (5.5 C).

Although some growth is possible, it is doubtful that rapid multiplication of *C. perfringens* could occur during the 4.5 h that gravy was at a temperature within the range for growth of the organism. During refrigeration this was the maximum time that the gravy remained above 65 F (18.5 C), the minimum temperature for growth of *C. perfringens*. However, the gravy always was at a temperature below the optimum temperature for growth of this organism. Substantial populations of *C. perfringens* could develop during the cooling period if the initial contamination in the food product is high. However, the refrigeration process would allow a more rapid increase in number of many other bacteria including some pathogens, if they are present. According to Hall and Angelotti *(6)*, the lowest temperature that permitted growth of pathogenic bacteria in less than one day was 50 F (10 C). The temperature of gravy remained above 50 F (10 C) for 11 h. During the first 2 h of this period gravy was at a temperature in the optimum growth zone for pathogenic bacteria (68 to 113 F, 20 to 45 C) *(7)*.

Holding of gravy

After refrigeration for 16 h, one case with six bags of gravy was held for 5 h at 82 F (28 C). The other case was held for the same time at 42 F (5.5 C). Data in Fig. 3 show that the rate of heat transfer in the chilled gravy held at 82 F (28 C) was slow. In one of the bags, the minimum temperature for growth of *C. perfringens* (65 F, 18.5 C) was reached after 3.5 h of holding. After the 5-h period, the mean temperature in the center of eight bags was 65 F.

Multiplication of *C. perfringens* is not likely in gravy during the holding period, except perhaps in the product very close to the edges of the cardboard case. Multiplication of some other bacteria could occur during the holding stage; the temperature of the gravy at the center of the bags remained above 50 F (10 C) for approximately 4 h when held at room temperature.

During the 5-h period the mean temperature of eight bags of refrigerated gravy decreased 2 F, from 45.4 F (7.5 C) to 43.5 F (6.5 C). Organisms cannot multiply appreciably under these conditions.

Heating of gravy

The temperature of gravy after two periods of heating is shown in Table 1. After 20 and 35 min of heating temperatures at the center of the pan were

TABLE 1. EFFECT OF HEATING TIME IN COMPARTMENT STEAMER ON TEMPERATURES OF BEEF GRAVY

Preceding holding temperature	Temperature of gravy (F)[a]			
		Heating time in min		
	Pan	0	20	35
82 F	1[b]	64	100	118
	2[c]	64	85	98
	3[d]	65	93	136
	4[e]	64	89	124
	Mean	64	92	119
42 F	1[b]	42	69	98
	2[c]	42	58	89
	3[d]	47	89	128
	4[e]	45	77	117
	Mean	44	74	109

[a]All temperatures were recorded with a thermometer, 1-1/3 inch penetration, center of the pan.
[b]Top compartment, trial 1.
[c]Middle compartment, trial 1.
[d]Top compartment, trial 2.
[e]Middle compartment, trial 2.

TABLE 2. NUMBERS OF TOTAL AEROBIC BACTERIA IN GROUND BEEF GRAVY DURING HOLDING (42 AND 82 F) AND HEATING IN A COMPARTMENT STEAMER AT 7 LB/IN^2 PRESSURE

Sampling Stage	Total aerobic plate count (cells/g)[a] in ground beef gravy	
	82 F	42 F
After cooking	50	50
After cooling	320	480
After holding for 2 h	210	410
After holding for 5 h	1530	440
After heating for 20 min	640	510
After heating for 35 min	820	830

[a]Mean counts from two trials, total of six samples after cooking, and total of eight samples at all other sampling stages.

higher in gravy that had been held at 82 F (28 C) than in gravy held at 42 F (5.5 C). After 35 min of heating, there was a mean temperature difference of 10 F between the four pans of gravy held at 42 F (5.5 C) and the four held at 82 F (28 C). The maximal temperature after 35 min of heating was 136 F (58 C) for gravy initially at 65 F (18.5 C). Temperatures lethal for pathogenic bacteria were never attained in the center of the pan during heating. Therefore, this heat treatment would not eliminate the possibility of foodborne illness if excessive numbers of *C. perfringens* were present in gravy.

Some temperature variation occurred in gravy in different pans even though all were subjected to the identical holding treatment (Table 1). The greatest difference in temperatures of gravy among the four pans, after 35 min of heating, was 38 F (3.5 C) for gravy that was held at room temperature before heating. For gravy that was refrigerated, the greatest difference in temperature was 39 F (4 C). The variation in rate of heat transfer may have been caused, in part, by variation in viscosity of gravy and by fluctuations in steamer temperature between the two trials. In addition, variation of temperature may have occurred within the compartments of the steamer.

Bacteriological tests

None of the samples of gravy yielded *C. perfringens*. Coagulase-positive staphylococci were found in some samples, but the numbers did not change markedly during the holding or heating treatments. Although the holding and heating treatments used in these experiments failed to affect the population of staphylococci, it must be remembered that the initial numbers were very low. If a higher number of the organism were present in food, growth of staphylococci might have occurred.

Results in Table 2 indicate that total aerobic bacteria increased most during the cooling phase. No rapid multiplication of bacteria occurred during the 5 h at 82 F (28 C), although a slight increase was observed at the end of this holding period. Some bacteria in the gravy might have reached the logarithmic growth phase if holding had been extended by 1 or 2 h. In this experiment samples were taken only from the center of bags. During holding, multiplication may have occurred in the areas of the bags closer to the edges of the cardboard case. No change in bacterial counts occurred when gravy was held at 42 F (5.5 C) for 5 h. According to data in Table 2 the heat treatment was not sufficient to kill viable bacteria in gravy in the center of the pans. Results of these bacteriological analyses support the findings obtained from time-temperature measurements. The temperature of the gravy at the center of the bags remained above 50 F (10 C) for 11 h during refrigeration and 4 h during holding at 82 F (28 C). The number of total aerobic bacteria in gravy increased during these periods, but the increase was greatest during the cooling rather than the holding phase.

IMPLICATIONS FOR FOODSERVICE OPERATIONS

While a uniform heating time for chilled food in all schools has been suggested, findings from this study indicate that heating for a specific period does not ensure that the minimum temperature of 165 F (74 C) is reached. The adequacy of the heating practice should always be controlled by measuring the final temperature of the food. A pyrometer or quick-read thermometer should be used in every school foodservice operation to check the temperature of food after heating.

Although results of this study show that holding chilled beef gravy at room temperature for 5 h or less did not contribute to excessive bacterial multiplication, this phase still may be critical for the bacteriological safety of some foods. Holding at room temperature may contribute to foodborne disease outbreaks if food has been mishandled before delivery to the schools. If the temperature of food during delivery reaches the minimum for growth of pathogenic bacteria, then holding food for 5 h at room temperature may allow proliferation of such organisms.

Precooked chilled food should be refrigerated during holding whenever possible. When this cannot be done, the environmental temperature should be kept constant and checked regularly. When precooked chilled food is handled, adequate control should include measuring its temperature when it is delivered to the school kitchen and after the holding period before it is prepared for serving. Findings of this study indicate that the maximal safe holding time for chilled ground beef gravy when at room temperature was 3 h. It is suggested that a standard to insure safety should include the requirement that the temperature of chilled food subjected to holding before it is prepared for serving should not exceed 60 F (15.5 C) at the end of the holding period which should not be greater than 5 h. If the temperature of chilled food rises above 60 F (15.5 C) during holding, methods to lower the temperature of the environment or to shorten the holding time should be employed.

Although this study was not designed to evaluate the cooling stage, the experiment showed clearly that this stage is critical in preventing multiplication

of organisms. The importance of rapid cooling must be emphasized. Controlled cooling in chilled water was shown to be effective, but may only be employed when food is packed in plastic bags or other tightly closed containers. It is the responsibility of food processors to evaluate the time-temperature relationships to which food is exposed during such a cooling treatment and to develop adequate techniques for rapid cooling of food.

The safety of the precooked chilled food should be considered at the food processing level and in the foodservice system to prevent outbreaks of foodborne disease.

REFERENCES

1. American Public Health Association. 1966. Recommended methods for the microbiological examination of foods, 3rd ed. Amer. Public Health Ass., Inc. New York.

2. Angelotti, R. 1971. Catered convenience foods—production and distribution problems and microbiological standards. J. Milk Food Technol. 34:227-231.

3. Barnes, E. M., J. E. Despaul, and M. Ingram. 1963. The behaviour of a food poisoning strain of *Clostridium welchii* in beef. J. Appl. Bacteriol. 26:415-427.

4. Duncan, C. L. 1970. *Clostridium perfringens* food poisoning. J. Milk Food Technol. 33:35-41.

5. Foster, E. M. 1972. The need for science in food safety. Food Technol. 26 (8):81-87.

6. Hall, H. E., and R. Angelotti. 1965. *Clostridium perfringens* in meat and meat products. Appl. Microbiol. 13:352-357.

7. Longree, K. 1972. Quantity food sanitation, 2nd ed. Wiley-Interscience, John Wiley & Sons, Inc. New York. pp. 6-30.

8. McDivitt, M. E., and M. L. Hammer. 1958. Cooling rate and bacterial growth in cornstarch pudding. J. Amer. Diet. Ass. 34:1190-1194.

9. Nicholanco, S. 1973. Environmental temperatures within hospital foodservice areas. Unpublished data.

10. Shahidi, S. A., and A. R. Ferguson, 1971. New quantitative, qualitative, and confirmatory media for rapid analysis of food for *Clostridium perfringens*. Appl. Microbiol. 21:500-506.

11. Strong, D. H., and N. M. Ripp. 1967. Effect of cookery and holding on hams and turkey rolls contaminated with *Clostridium perfringens*. Appl. Microbiol. 15:1172-1177.

12. Suomen Kunnallisliitto, Suomen Kaupunkiliitto, Finlands Svenska Kommunforbund, Kouluhallitus, Yksityisoppikoulujen Liitto, and Yleisten Ammattikoulujen Liitto. 1972. Kouluruokailu, Kunnallispaino, Helsinki, 158 p.

HOYT AWARD RECEIVED BY M. S. REDDY

The Richard M. Hoyt Memorial Award is presented each year to a graduate student in recognition of research that has direct application to solution of problems facing the dairy industry. The award was initiated in 1971.

This year's recipient, M. S. Reddy, was born in Uppalapadu, Nellore, A.P., India, in 1945. He was graduated from Andhra-Pradesh Agricultural University in 1967 with the degree of Bachelor of Veterinary Science. The following year he arrived at Iowa State University where he has engaged in graduate study and research in food technology and microbiology. He earned an M.S. degree in 1971 and is nearing completion of requirements for the Ph.D.

Research by Reddy has dealt with various organisms involved in the manufacture of cheese and cultured dairy products. He developed cultural techniques for differentiating and separating various species of streptococci used in starter cultures. Attention then was directed to studies of bacteriophages associated with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. A remarkable series of electron microscope views suggested unique properties of these two phages. He developed a chloroform-based procedure to destroy host and contaminating bacterial cells, thereby eliminating the necessity of filtration when testing for phage. Associative growth studies between *Lactobacillus* species and S. *thermophilus* have shown that stimulation or retardation of acid production in mixed cultures may occur depending on the titer of phage particles present. Additionally, he has investigated sulfadiazine resistance and slime production by several species of propionibacteria. Reddy is senior author of 16 research publications and coauthor of others. Many of these papers have appeared in the *Journal of Milk and Food Technology*.

The industry will benefit directly from this reasearch in that simplified procedures for routine control of lactic group streptococcus cultures now are available. Reduction or elimination of problems related to the presence of rod and coccus bacteriophages should be possible, thus reducing day-to-day variations in acid production in Italian and Swiss cheese plants.

The lack of transport of sulfadiazine into cells of propionibacteria offers a model system for study with sulfa-resistant organisms of importance in mastitis and other disorders. Reddy conducted his research under the guidance of Dr. George W. Reinbold at Iowa State University.

UTILIZATION OF FISH OIL BY *CANDIDA LIPOLYTICA* AND *GEOTRICHUM CANDIDUM*

I. BASAL CONDITIONS

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Abstract

Oil from alewife *(Alosa pseudoharingus)* was oxidized in shake flasks by *Candida lipolytica* Y1094 and *Geotrichum candidum* Y552. Utilization of alewife oil in a basal medium indicated that fish stickwater was as effective a growth supplement as yeast extract or cornsteep liquor. Maximum cell yields of both organisms were obtained when the starting pH was between 7.5-8.2. Nitrogen uptake per gram of cells for both microorganisms was relatively greater during the first 11 h of oil oxidation and declined thereafter. Fish oil concentrations in the basic medium were varied between 0.5 to 10% w/v. The increase in cell yield essentially stopped for both organisms at the 5% oil level. With 5 to 10% oil pH values of media decreased below the optimal range after 22 h without significantly more cell production than in media with 1.0 and 2.5% oil. After 33 h pH values of the media with high oil concentrations decreased to a level where growth was inhibited.

From 1950 to 1970 the world population increased from 2.5 billion to 3.5 billion; it is expected to reach 5 to 6 billion by 2000 *(2)*. As a consequence, it becomes essential to explore all possible avenues for increasing worldwide sources of protein.

The global catch of fish doubled from 1958 to 1968 *(8)*. However, in this same period fish meal production increased 5-fold, fish oil production rose 1.5-fold, and fish for human consumption gained only about 37%. If existing harvest techniques were fully applied, approximately 110 million tons of fish could be taken annually; however, 2 billion tons represent the actual annual harvest potential *(23)*. Future production of fish meal and fish oil should increase accordingly. This means that in the very near future, the annual production of fish oil will reach or exceed 2,000,000 metric tons. Approximately 25% of the world fish oil production is used for nonedible purposes *(10)*. As the fish catch increases, the nonedible fish oil percentage is likely to increase because a greater part of this oil will stem from solvent extracted fish in the production of fish protein concentrate and from fish contaminated by pesticides and insecticides *(5, 9, 15, 16, 27)*.

Because of the growing scarcity of protein supplies, an investigation into ways of utilizing fish oil as a raw material for protein production was instituted. Conversion of fish oil to single cell protein seemed feasible in view of the conversion of hydrocarbons to microbial protein *(4, 26)* and the use of fatty substances as energy sources and growth stimulants in the production of penicillin, streptomycin, and gibberellin *(18, 20, 21)*.

In the oxidation of fish oils by microorganisms, deleterious substances such as solvent, pesticide, or insecticide residues could be decomposed *(11)* or concentrated in the lipoidal portion of the culture media or cell materials whereby they could be extracted from the protein.

This report and subsequent papers *(13, 14)* describe the growth of *Candida lipolytica* and *Geotrichum candidum* on fish oil.

Materials and Methods

Microorganisms

Two types of microorganisms with known lipolytic activity *(17, 19, 22, 28)*, *Candida lipolytica* Y1094 and *Geotrichum candidum* Y552, were obtained from the USDA Northern Utilization Research and Development Division, Peoria, Illinois.

Media

All chemicals were reagent grade, except as indicated.

For stock cultures and intermediate transfers, Bacto-YM agar (Difco) was used. For transfer of slant growth to liquid media, phosphate buffer (0.05 M, pH 6.8) was used.

The composition of the preculture liquid medium was: Fish oil[1], 10.0 g; $(NH_4)_2SO_4$, 6.0 g; KH_2PO_4, 6.0 g; Mg SO_4, 0.5 g; fish stickwater,[2] 4 ml (except as indicated); pH adjusted to 6.0; distilled water to make 1000 ml.

The experimental growth media were the same composition as the preculture liquid medium, except for the variations indicated in the text. All media were sterilized at 121 C for 15 min.

Culture procedure

Stock culture maintenance. The two microorganisms were

[1]Alewife *(Alosa pseudoharingus)* oil was obtained from Schilling fish Co., Green Bay, Wisconsin, and stored in the refrigerator.

[2]The fish stickwater was prepared from frozen alewife, defatted by centrifugation and kept in frozen storage. Stickwater is practically oil free, screened or clarified liquor obtained as a by-product of fish processing, Solids consist of dissolved, or suspended fish proteins, minerals, complex vitamins and unknown factors. Degradation products of proteins such as proteoses, peptones, polypeptides, and amino acids are present.

maintained as stock cultures on YM agar slants, stored in the refrigerator at 3-6 C with transfers every two months.

Preparation for culture. From stock culture slants, sterile YM agar slants were inoculated, kept at 30 C, and transferred every 24 h for 3 days to new slants. On the third day the entire growth from one slant was homogenized into 5 ml sterile phosphate buffer and subsequently added to sterile 50 ml preculture medium in a 225-ml Erlenmeyer flask. The flask was placed on a reciprocal shaker at 27 C with a shaking rate of 125 strokes per minute. At this preculture stage, three transfers were made at intervals of 24 h, each time transferring 2% v/v of inoculum to fresh medium. On the third day, an inoculum of 2% v/v (except as otherwise indicated) was transferred from the preculture medium to 120 ml (except as otherwise indicated) experimental growth medium in a 500-ml Erlenmeyer flask. These flasks were maintained at 30 C on a 250 r.p.m. rotary shaker describing a shake-cycle of 2 inches in diameter. For *C. lipolytica* the 24-h 2% v/v inoculum was equal to 5.6 mg dry cells per 100 ml medium and for *G. candidum* this inoculum was 4.6 mg dry cells per 100 ml medium.

The cover used on the shake flasks to prevent contamination consisted of a double layer of Johnson & Johnson "Rapid Flo" 6-1/2 inch single gauze-faced milk filter disks held in place on the flask by means of a rubber band. With this closure good sterility could be maintained without impairing the air exchange between shake-flask and atmosphere.

At intervals of 11, 22, 33 and 48 h, 20-ml samples from each flask were taken for analysis of pH, nonprotein nitrogen [$(NH_4)_2SO_4$] in the supernatant fluid of media, and cell dry matter.

Analytical methods

Residual nitrogen in the supernatant fluid of media was measured by mixing 5 ml centrifuged cell-free supernatant with 5 ml 10% trichloroacetic acid. To determine the quantity of N present, 2 ml of the clear supernatant fluid of this mixture were distilled by the AOAC-semi-micro-Kjeldahl distillation method *(1)*, recovering the distillate in boric acid, and titrating with 0.2 M HCl.

Nitrogen uptake per gram of dry cells was calculated by subtracting the residual percent nitrogen in the supernatant fluid of media from the percent nitrogen content of fresh media and dividing the difference by the cell dry weight obtained from 100 ml of the same sample.

The pH was measured in the untreated sample with a Beckman-Zeromatic pH meter.

The dry cell weight was obtained by heating the cell suspension to 75 C, acidifying it with 6 N HCl to pH 1.5, then adding 0.4% Triton X-100, mixing vigorously, and centrifuging at 2150 *g* for 10 min. This treatment was essential to remove oil adhering to the cells. The supernatant fluid, when needed, was repeatedly rewarmed, shaken, and centrifuged until the supernatant fluid and the residual oil layer were clear. The sediment was washed twice with an amount of distilled water equal to the original sample volume and recentrifuged. The sediment was transferred into a predried, tared 6-cm wide aluminum pan and dried to constant weight at 78 C in a forced air drying oven.

Residual oil was extracted from the fermentation mixture by shaking and centrifuging the entire sample three times each with 20 ml Skellysolve B. The solvent-oil phase was collected in predried, tared Erlenmeyer flasks, the solvent was removed at 60 C on a hot plate, and the oil was subsequently dried to a constant weight at 78 C.

Dry cell total nitrogen was determined by the semi-micro Kjeldahl method *(1)*. Crude protein was estimated by multiplying total nitrogen by 6.25.

RESULTS AND DISCUSSION

Growth on basal medium and media supplemented with growth factors

The purpose of the following experiments was to determine optimum conditions for microbial growth with the simplest medium possible. The quantities of $(NH_4)_2SO_4$, KH_2PO_4 and $MgSO_4$ were established previously with oxidations of glycerol and fatty acids by the same strains of organisms used in this study.

TABLE 1. GROWTH ON BASIC MEDIUM AND MEDIA SUPPLEMENTED WITH GROWTH FACTORS

Supplement added to media	Doubling time (h)		Specific growth rate (h^{-1})	
	Candida lipolytica	*Geotrichum candidum*	*Candida lipolytica*	*Geotrichum candidum*
None	10.24	13.20	0.0676	0.0525
Yeast extract	3.64	3.94	0.1903	0.1758
Fish stickwater	3.88	3.95	0.1786	0.1756
Cornsteep liquor	3.81	3.87	0.1818	0.1791

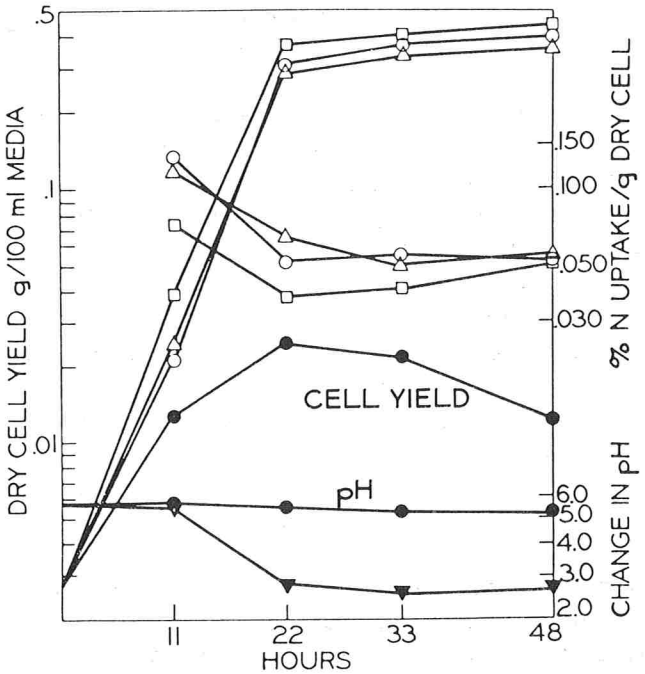

Figure 1. Growth of Candida lipolytica on basic medium and media supplemented with growth factors. No supplement, closed circles; Cornsteep liquor, open circles; Yeast extract, open squares; Fish stickwater, open triangles; pH change of supplemented media, closed triangles.

In the utilization of hydrocarbons, a starting pH of 5.7 was reported for *C. lipolytica (24, 25)*. A starting pH of 5.7-6.1 was reported for *G. candidum (3)* for growth in potato-dextrose broth. Therefore, in

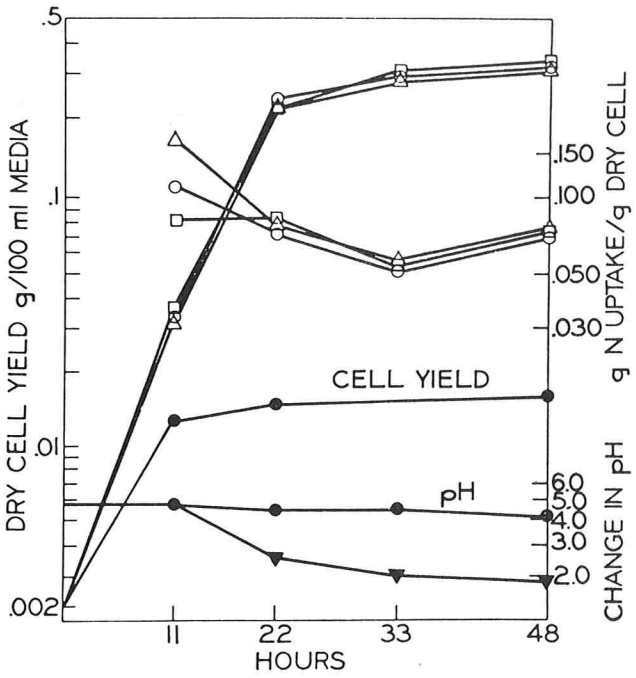

Figure 2. Growth of *Geotrichum candidum* on basic medium and media supplemented with growth factors. No supplement, closed circles; Cornsteep liquor, open circles; Yeast extract, open squares; Fish stickwater, open triangles; pH change of supplemented media, closed triangles.

the present experiments, a starting pH of 5.8 would support growth of the test organism and also inhibit growth of many of the possible bacterial contaminants.

Growth was initiated with 1% v/v inocula, and three media were supplemented with 150 mg total solids (TS) per liter of yeast extract or cornsteep liquor or fish stickwater. A control medium contained no supplement. Fish stickwater was included in these experiments because it is a readily available, low cost by-product of fish oil production. If it produces microbial growth comparable to yeast extract or cornsteep liquor, it could be used as a source of growth factors in the microbial utilization of fish oils. Figure 1, Fig. 2, and Table 1 illustrate the marked effect created by 0.015% TS of supplement added to the media. The three supplements differed little in their growth-promoting characteristics; therefore, fish stickwater was used as an organic growth supplement in subsequent experiments. Visual inspection indicated that in none of these fermentations was the available oil completely utilized. Figure 1 and Fig. 2 indicate that microbial growth stopped as the pH values of the media decreased below pH 3.0. This suggests insufficient buffering capacity of the media and that a higher starting pH might promote better cell yields.

Growth as affected by starting pH of medium

Media were adjusted to starting pH values between 3.0 and 8.8 with 15% NaOH or 10% HCl solutions to study the effect on biomass production. Figures 3-6 and Table 2 indicate that cell-yield and growth rate were a function of the starting pH of each medium and the minimum pH reached during growth. For both organisms maximum cell-yields were obtained between a starting pH of 7.5-8.2.

Figures 3-6 also indicate that for optimum cell-yields the pH during fermentation should not decrease below 6 for both organisms. Furthermore, at starting pH values of 7.5 and 8.5, cell yield became limited due to exhaustion of the energy source. With *C. lipolytica* the maximum specific growth rates were correlated with the 48-h cell yields, whereas with *G. candidum* the 48-h cell yield increased as the growth rate leveled off. This may have been due to the lower pH attained by *C. lipolytica* at the end of the exponential growth phase (pH 2.5-2.7), compared to *G. candidum* (pH 3.0), indicating that low pH inhibition for *G. candidum* was gradual and affected by a zone about 0.4 pH unit wide. For *C. lipolytica* this zone had a spread of only about 0.1 to 0.2 pH unit and, therefore, its growth stopped more

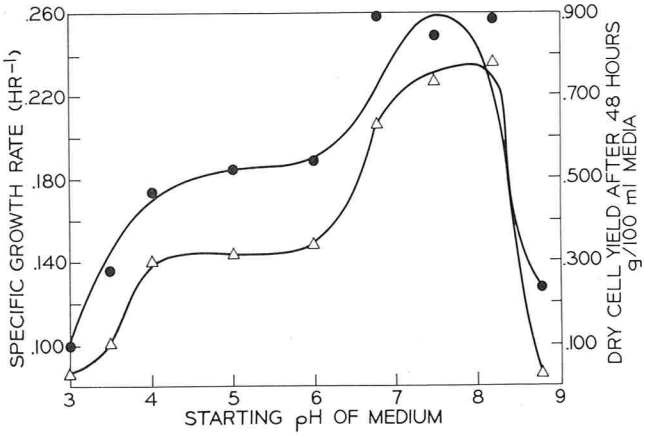

Figure 3. Specific growth rate (h^{-1}), closed circles, of *Candida lipolytica*, and dry cell yield after 48 h, open triangles, as a function of initial pH of the medium.

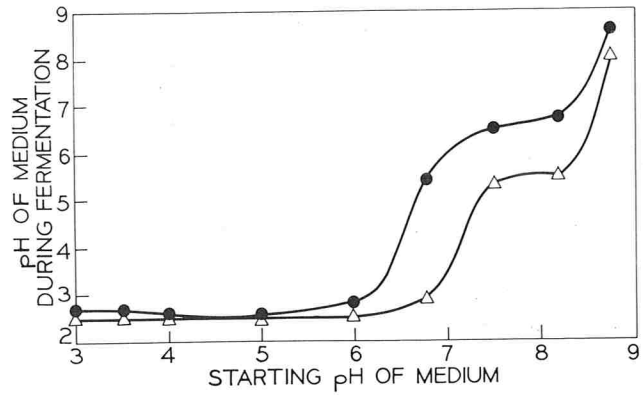

Figure 4. pH of medium at the end of exponential growth period, closed circles, for *Candida lipolytica*, and pH after 48 h of oxidation, open triangles, as a function of initial medium pH.

TABLE 2. NITROGEN UPTAKE BY MICROBIAL CELLS AS A FUNCTION OF pH AND CULTURE TIME

pH of media	*Candida lipolytica* Culture time (h) 11	22	33	48	Doubling time (h)	*Geotrichum candidum* Culture time (h) 11	22	33	48	Doubling time (h)
	(g N uptake/g dry cell)					(g N uptake/g dry cell)				
pH 3.5	–	0.075	0.079	0.081	5.09	0.125	0.127	0.148	0.139	5.82
pH 4.0	–	0.080	0.083	0.083	3.95	0.075	0.063	0.092	0.084	4.03
pH 5.0	0.035	0.068	0.065	0.097	3.73	0.153	0.059	0.092	0.083	3.97
pH 6.0	0.092	0.066	0.082	0.087	3.65	0.147	0.061	0.093	0.078	3.72
pH 6.8	0.117	0.056	0.060	0.073	2.67	0.143	0.057	0.082	0.069	3.66
pH 7.5	0.092	0.056	0.060	0.060	2.77	0.162	0.072	0.085	0.070	3.62
pH 8.2	0.096	0.062	0.059	0.063	2.70	0.148	0.039	0.082	0.067	3.49
pH 8.8	–	0.217	0.272	0.264	5.45	–	–	0.359	0.630	9.37

TABLE 3. EFFECT OF OIL CONCENTRATION ON UPTAKE OF NITROGEN BY MICROBIAL CELLS

Media (% Oil w/v)	*Candida lipolytica* Propagation time (h) 11	22	33	48	Doubling time (h)	*Geotrichum candidum* Propagation time (h) 11	22	33	48	Doubling time (h)
	(g N uptake/g dry cell)					(g N uptake/g dry cell)				
0.5%	0.480	0.122	0.107	0.108	3.98	0.210	0.095	0.110	0.092	4.00
1.0%	0.340	0.096	0.083	0.083	3.41	0.140	0.084	0.083	0.086	3.55
2.5%	0.410	0.089	0.082	0.079	3.34	0.180	0.078	0.078	0.084	3.29
5.0%	0.570	0.083	0.079	0.078	3.31	0.630	0.097	0.084	0.084	3.30
7.5%	0.500	0.085	0.078	0.079	3.20	0.460	0.112	0.089	0.077	3.46
10.0%	0.390	0.089	0.085	0.081	3.38	0.690	0.112	0.108	0.090	3.46

abruptly as the pH of the medium reached this crucial zone.

Nitrogen uptake tabulated in Table 2 shows that during the first 11 h of fermentation relatively more nitrogen was consumed than during the later phases. This could be explained by the cells' need to increase their content of reproductive nitrogen compounds at the beginning of growth *(12)*. On the basis of the 48-h data, the dry cell crude protein content varied from 37.5-60.6% for *C. lipolytica* (average: 0.078 g N taken up/g dry cell = 48.7% crude protein) and 42.0-87.0% for *G. candidum* (average: 0.0844 g N taken up/g dry cell = 52.6% crude protein) (N uptake values at pH 8.8 are not included).

Growth as affected by concentration of fish oil

Concentrations of fish oil in the basic medium were varied between 0.5 and 10% w/v. The pH was adjusted to 7.5 at the beginning of each experiment. Figures 7 and 8 show a pronounced increase in growth rate up to an oil concentration of 2.5% but no further increases with higher concentrations for *G. candidum* and only a slight rise to a maximum at a concentration of 7.5% oil for *C. lipolytica*. Even so, the concentration of other nutrients was high enough to promote a cell yield of 1.6 g/100 ml media at 0.08 g N uptake/g dry cells. The increase in cell yield essentially stopped for both organisms at the 5% oil level, showing only a slight increase at high concentrations for *G. candidum*. These changes in cell yield and growth rate were parallel with changes in the pH of the media as illustrated in Fig. 7 and 8. At a concentration of 5.0-10.0% oil the pH values of the media decreased below the optimal range after 22 h without significantly more cells having been pro-

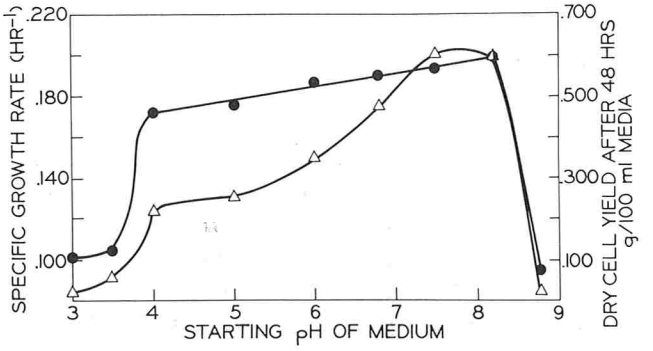

Figure 5. Specific growth rate (h^{-1}), closed circles, of *Geotrichum candidum*, and 48 h dry cell yield, open triangles, as a function of initial pH of medium.

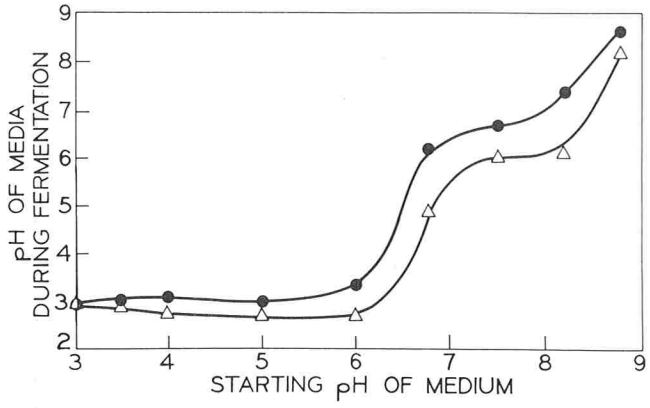

Figure 6. pH of medium at the end of the exponential growth period, closed circles, for *Geotrichum candidum* and pH after 48 h of oxidation, open triangles, as a function of initial pH of medium.

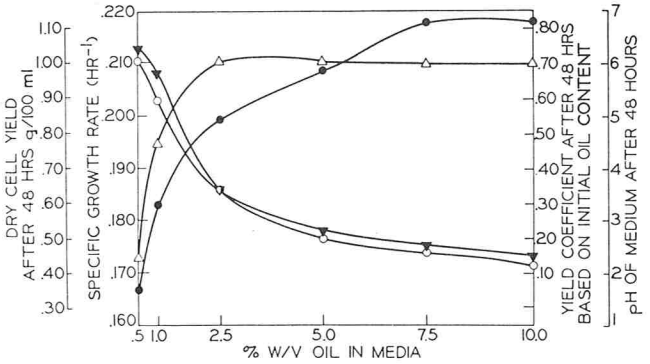

Figure 7. Effect of fish oil concentration on biomass production of *Geotrichum candidum*. Dry cell yield g/100 ml after 48 h, closed circles; Specific growth rate (h^{-1}), open triangles; Yield coefficient, open circles; pH of media after 48 h, closed triangles.

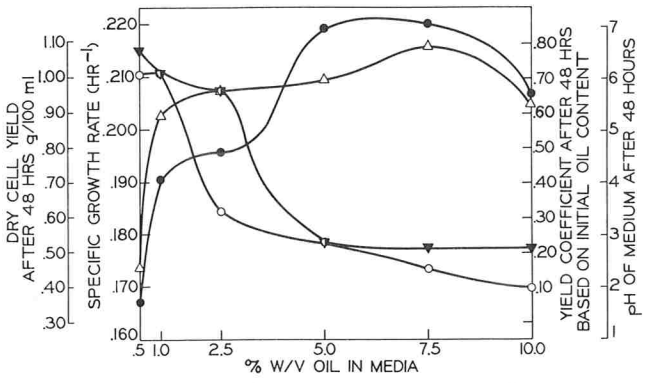

Figure 8. Effect of fish oil concentration on biomass production by *Candida lipolytica*. Dry cell yield g/100 ml after 48 h, closed circles; Specific growth rate (h^{-1}), open triangles; Yield coefficient, open circles; pH of media after 48 h, closed triangles.

duced compared to the 1.0 and 2.5% oil media. After 33 h the pH values of media with high concentrations of oil decreased to a level where growth was inhibited. These data indicate that fermentation at high oil concentrations is limited by the extent of pH changes taking place. We can, therefore, expect that at a constant pH the specific growth rate would reach its maximum at a higher oil concentration and also the dry cell yields would be markedly higher at the higher oil concentrations used in this experiment. This would be true to a point because at higher oil concentrations the crowding effect of the oil droplets on the other nutrients becomes more effective and limits the accessibility of other nutrients to the cell surfaces, thus constituting a major obstacle in cell growth paralleling the increase in oil concentration *(6, 7)*. Oil utilization, based on initial oil concentration, was maximum in the 0.5% oil medium. This can be explained in terms of maximum emulsification of the small amount of oil due to the other ingredients present, and also because at higher dilution the oil droplets do not coalesce and aggregate easily. Also, it may be that the ratio of oil droplets adsorbed onto the cell surfaces to the oil droplets freely suspended in the continuous phase is favorable for a complete uptake of oil by the cells. As Table 3 indicates, nitrogen uptake was fairly constant after 11 h of fermentation; however, a much higher uptake of nitrogen was evident during the first 11 h of fermentation.

ACKNOWLEDGMENTS

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REFERENCES

1. AOAC. 1970. Official methods of analysis. Tenth edition. Association of Official Analytical Chemists. Washington, D. C.

2. Brown, L. R. 1968. World food problems. pp. 11-26. In R. I. Mateles and S. R. Tanenbaum (ed.). Single cell protein. M.I.T. Press, Cambridge, Mass.

3. Butler, E. E., and J. W. Eckert. 1962. A sensitive method for the isolation of *Geotrichum candidum* from soil. Mycologia 54:106-109.

4. Champagnat, A., C. Vernet, B. Laine, and J. Filosa. 1963. Biosynthesis of protein-vitamin concentrates from petroleum. Nature 197:13-14.

5. Committee on Oceanography. 1971. Chlorinated hydrocarbons in the marine environment. National Academy of Science, Washington, D. C. 42 p.

6. Coty, V. F., and R. I. Leavitt. 1971. Microbial proteins from hydrocarbons. pp. 61-71. In E. D. Murray (ed.). Developments in industrial microbiology. Vol. 12. American Institute of Biological Sciences. Washington, D. C.

7. Erickson, L. E., L. T. Fan, P. S. Shah, and M. S. K. Chen. 1970. Growth models of cultures with two liquid phases: IV. Cell adsorption, drop-size distribution, and batch growth. Biotechnol. Bioeng. 12:713-746.

8. FAO. 1969. Yearbook of fishery statistics. Vol. 29. Food and Agricultural Organization of the United Nations. Rome, Italy.

9. Finch, R. 1970. Fish protein for human foods. CRC–

Critical reviews in Food Technology 1:519-580.

10. Finneberg, H., and A. G. Johanson. 1967. Industrial use of fish oils. pp. 222-238. In M. E. Stansby, (ed.). Fish oils. AVI Publishing Co., Inc., Westport, Conn.

11. Henzell, R. F. 1969. Degradation of commercial DDT in silage. J. Sci. Food Agr. 20:499-502.

12. Herbert, D. 1961. The chemical composition of microorganisms as a function of their environment. Microbial reaction to environment. Symposium of the Society for General Microbiology 11:391. Cambridge University Press.

13. Hottinger, H. H., T. Richardson, C. H. Amundson, and D. A. Stuiber. 1974. Fermentation of fish oil by *Candida lipolytica* and *Geotrichum candidum*. II. Optimization. J. Milk Food Technol. 37: (In press).

14. Hottinger, H. H., T. Richardson, C. H. Amundson, and D. A. Stuiber. 1974. III. Fish stickwater as a nitrogen source in the fermentation of fish oil by *Candida lipolytica* and *Geotrichum candidum*. In preparation.

15. Jensen, S. 1970. Chlorinated hydrocarbon in fauna and flora. Grundfoerbattring 23:81-84.

16. Maceck, K. J., and S. Korn. 1970. Significance of the food chain in DDT accumulation by fish. J. Fish Res. Bd. Canada. 27:1496-1498.

17. Marks, T. A., J. C. Quinn, I. Sampugna, and R. G. Jensen. 1968. Studies on the specificity of a lipase system from *Geotrichum candidum*. Lipids 3:143-146.

18. Muromtsev, G. S., J. S. Rakoskii, L. P. Dubovega, T. V. Temnikova, and A. N. Fedchenko. 1968. Study of sucrose and fat as a carbon-source for the synthesis of gibberellins. Appl. Biochem. Microbiol. 4:295-303.

19. Nelson, W. O. 1952. Some characteristics of the lipase of *Geotrichum candidum*. J. Dairy Sci. 35:455-462.

20. Pan, S. C., S. Bonanno, and G. H. Wagman. 1959. Efficient utilization of fatty oils as energy source in penicillin fermentation. Appl. Microbiol. 7:176-180.

21. Perlman, D., and G. H. Wagman. 1952. Studies on the utilization of lipids by *Streptomyces griseus*. J. Bacteriol. 63:253-262.

22. Peters, I. I., and F. E. Nelson. 1951. Vitamin requirements for lipase production by *Candida lipolytica*. J. Bacteriol. 61:591-593.

23. Snyder, D. G. 1966. Discussion—Edible fish concentrate. pp. 49-51. In R. F. Gould (ed.). World protein resources —Advances in chemistry series, 57. American Chemical Society. Washington, D. C.

24. Tsugawa, R., T. Nakase, T. Kobayashi, K. Yamashita, and S. Okumura. 1969. Fermentation of n-paraffins by yeast. Part I. Agr. Biol. Chem. 33:158-167.

25. Tsugawa, R., and S. Okumura. 1969. Fermentation of n-paraffins by yeast. Part II. Agr. Biol. Chem. 33:676-682.

26. Villenchich, R., and W. Akhtar. 1971. Microbiological synthesis of proteins. Process Biochemistry 6:41-44.

27. Westoo, G., and N. Koidu. 1970. Levels of organochlorine pesticides and polychlorinated biphenyls in fish caught in Swedish water areas or kept for sale in Sweden. 1967-1970. Var. Foeda. 22:93-146.

28. Wouters, J. T. M. 1966. Vetsplitsende activiteit van *Geotrichum candidum* en enkele andere microorganism. Thesis of the University of Amsterdam.

DeMANN RECEIVES DAIRY RESEARCH AWARD

The recipient of the Dairy Research Incorporated Award for 1974 is characterized by his distinguished research ability, organizational skill, remarkable leadership qualities, and limitless energy. Dr. John M. DeMann has published over 100 scientific papers, establishing himself as a national and international authority in at least two main areas: fat chemistry and food rheology. His studies on fat show many innovations in analytical flavor evaluation, new knowledge on light induced oxidation in dairy products, and practical methods for improving the spreadability of butter by removal of a high melting glyceride fraction. Food rheology is a relatively new field lacking appropriate methods of measurement. DeMann has successfully devised several new methods and applied them to measuring texture of cottage cheese, butterfat hardness, ice cream texture, textural properties of proteins, and many other products.

He has contributed to the organization of two university departments, served as president of an Institute of Food Science and Technology, founded a new scientific journal, and continues to serve as its editor. He is a rare administrator who serves as chairman of his department and carries on an exemplary research role. Currently, he supervises three graduate students, two post-doctoral persons, and two technicians.

DeMann was born in Rotterdam, Holland, received his Ph.D. in dairy science from the University of Alberta, was research chemist for Unilever, and since 1969 has served as professor and chairman of the Department of Food Science at the University of Guelph.

CHARACTERIZATION OF TENACIOUS RESIDUE OF ALKALINE CIRCULATION CLEANING[1]

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Abstract

Circulation cleaning presents many opportunities for advancement in liquid food handling systems, but the effectiveness commonly must be evaluated by means other than visual inspection. To study this problem of evaluation, a laboratory system was developed to simulate the soiling and cleaning process of a commercial high-temperature short-time pasteurizer unit. Proof of similarity was obtained by determining quantity of soil at various cleaning intervals and through qualitative examination of the soil with thin-layer chromatography, gas liquid chromatography, and infrared spectroscopy. Lipoidal materials were found to be most resistant to alkaline cleaning. Material removed near the end of the cleaning cycle and material removed by ether rinse of the equipment after the cleaning cycle were similar to milkfat in the milk used for soiling and to the residues recovered from early intervals of cleaning. The removal process for lipoidal material was not attributable to saponification, because little saponification occurred with temperature and alkali concentration associated with circulation cleaning. Data in this paper indicated directions for further work toward criteria of cleaning efficiency and effectiveness.

Liquid food handling systems are commonly cleaned in place (CIP) and have limited access for visual inspection. Understanding the mechanism of cleaning and development of control procedures, therefore, are of major importance.

During the introduction of CIP, regulatory acceptance was based upon evaluation of effectiveness in comparison to hand cleaning of takedown equipment. In later work, cleaning of dairy welded pipeline systems was shown to be equivalent or superior to takedown CIP systems *(7, 9)*. Takedown systems, however, were particularly susceptible to harborages at pipeline joints *(5)*. The process of cleaning harborages is no doubt different from cleaning smooth surfaces *(2)*. In neither process of cleaning, however, has either the rate of soil removal or the nature of the most tenacious residue been fully elucidated. Many factors have been reported to be involved in the deposition and removal of soil arising from heat processing of milk *(3)*. Arnold and Maxcy *(1)* found the most tenacious residue in a milk processing system with a plate-type pasteurized to be lipoidal. More recently, Maxcy *(6)* showed that the residue contributing to a yellow "milkstone" film on equipment after cursory cleaning was phospholipid. Previous publications indicated lipoidal materials were important considerations in the cleaning system.

The present work was undertaken to develop a better understanding of the nature of the tenacious residue, factors influencing cleaning rates, and the mechanism of the chemical cleaning process. The ultimate goals are more effective cleaning, methods for evaluation of cleaning procedures, and useful methods for assurance of compliance with standards of cleanliness.

Methods

Laboratory circulation system

Stainless steel tubing (approximately 5 mm ID × 2 m), a peristaltic pump, glass reservoir, connecting Tygon tubing, and an indirect heating arrangement were assembled to simulate soiling from heating of milk containing approximately 3.3% milkfat and the flow of cleaner in a high-temperature, short-time pasteurizer. Using a single pass system, milk was heated to 75 C by immersion of the stainless steel tubing in 90-95 C water. The rate of milk flow for soiling was approximately 1 ft/sec. Soiling time was 1 h. Cleaning was at 75-80 C with a flow rate of 2 ft/sec, which provided turbulent flow. The primary cleaning compound was 1% sodium hydroxide. In certain experiments, the sodium hydroxide concentration was reduced to 0.9% by substitution of 0.1% sodium hexametaphosphate or sodium gluconate. A fresh cleaning solution was used for each 2 min of circulation cleaning to provide for analyses of the cleaner for stepwise progression of soil removal. Five cleaning steps were considered giving a total cleaning time of 10 min.

Analyses for lipoidal material

Samples were obtained by extraction of alkaline cleaner with organic solvents as applied in a previous study *(1)*. Observations on the identity of the lipoidal material were made with thin-layer chromatography (TLC) and with infrared spectroscopy. Details of the general methods for study of lipoidal materials have been presented *(1)*. Methyl esters were prepared from the extracts with boron trifluoride reagent according to the procedure of Morrison and Smith *(8)*. Methyl esters were then subjected to gas liquid chromatography (GLC) analyses. The instrument was a Varian Aerograph 1700 with a flame ionization detector. The column was 375 cm × 3.2 mm OD stainless steel packed with 7% diethylene glycol succinate (DEGS) on 60-80 mesh Chromosorb P. The operating parameters were: injection port, 210 C; column, 180 C isothermal; detector, 210 C; carrier gas (N_2) 50-55 ml/min; range 10^{-11}; and attenuation × 1 to × 128. Details of the procedures and identification of the equipment have been given *(1)*.

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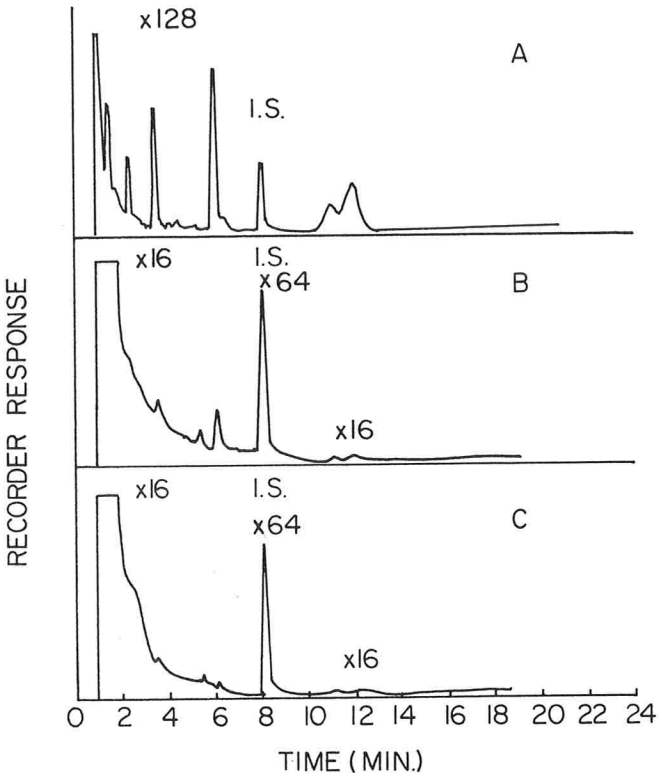

Figure 1. Chromatograms of methyl esters of fatty acids from extracts of cleaning solutions at intervals with progression of the cleaning process. "A" presents results of a first two-minute period; "B" presents results of a third two-minute period; and "C" presents results of a fifth two-minute period. The internal standard (I.S.) was methyl heptadecanoate.

Analyses for proteinaceous material

Proteinaceous material was determined by the method of Lowry et al. *(4)*.

Surface phenomena and saponification

To determine the extent of saponification in a cleaning process, the rate of saponification was studied by suspending milkfat in alkaline solutions of various concentrations for various times and at various temperatures. The nature of the suspension was altered by agitation, additives (trisodium phosphate or sodium gluconate), or the presence of stainless steel wool to increase the surface area of exposure of milkfat in the alkaline solution.

The degree of saponification was determined by acidification of the alkaline solution, extraction with organic solvent, and subsequent titration of the fatty acids in the extract. Extracts of the lipoidal material were also observed with thin-layer chromatography to substantiate data on extraction as well as on saponification.

RESULTS

Applicability of a laboratory soiling and cleaning system

Mixed raw Grade "A" milk was heated to soil the equipment, which was subsequently cleaned by circulation of alkaline solution (1% NaOH). The general results indicated the soiling and the cleaning processes were similar to those reported for a typical commercial operation *(1)*. The laboratory system and the commercial system had the following common characteristics: *(a)* flow rates, *(b)* cleaning pattern as shown by rate of removal of residue, *(c)* similar chemical composition of the cleaning effluent with progression of cleaning, and *(d)* lipoidal material in the various stages of cleaning was similar to the milkfat in the milk for the soiling process. Typical examples of chromatograms shown in Fig. 1 exemplify the results with respect to the lipoidal fraction. Additional data are given in Fig. 2.

Nature of the tenacious residue

Indirect evidence of the nature of the most tenacious residue was obtained by comparing the rate of removal of proteinaceous and lipoidal material. Analyses of the cleaning solutions obtained at intervals during the progression of cleaning indicated the proteinaceous material was removed at a more rapid rate than lipoidal material (Fig. 2). In the cleaning solution for the second interval (2 to 4 min) there was approximately 5% as much protein as in the first cleaning interval. The cleaning solution for the third interval contained only a trace of protein. Neither the proteins nor the lipoidal material of the third, fourth, and fifth cleaning intervals appeared to be significant in comparison to the high values of the earlier intervals as shown in Fig. 2.

Attempts to determine trace quantities of pro-

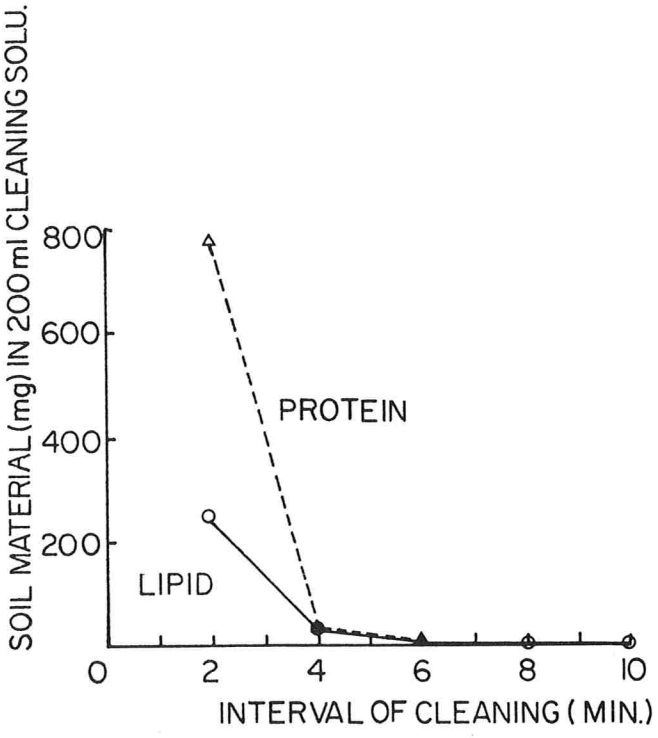

Figure 2. Comparitive rate of removal of proteinaceous and lipoidal material with progression of circulation cleaning.

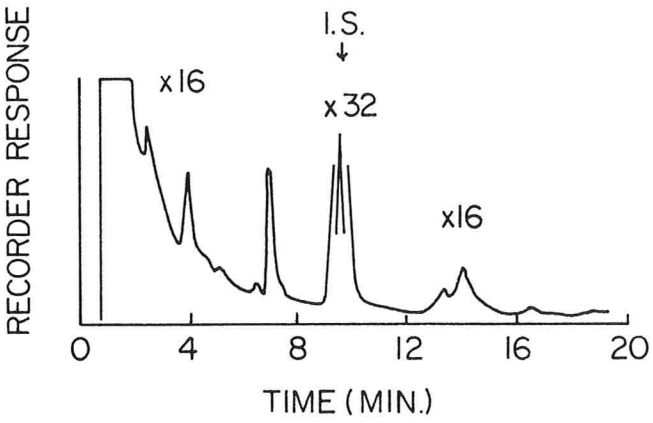

Figure 3. Chromatogram of methyl esters of fatty acids from residue obtained by ether wash of circulation cleaned equipment. The internal standard (I.S.) was methyl heptadecanoate.

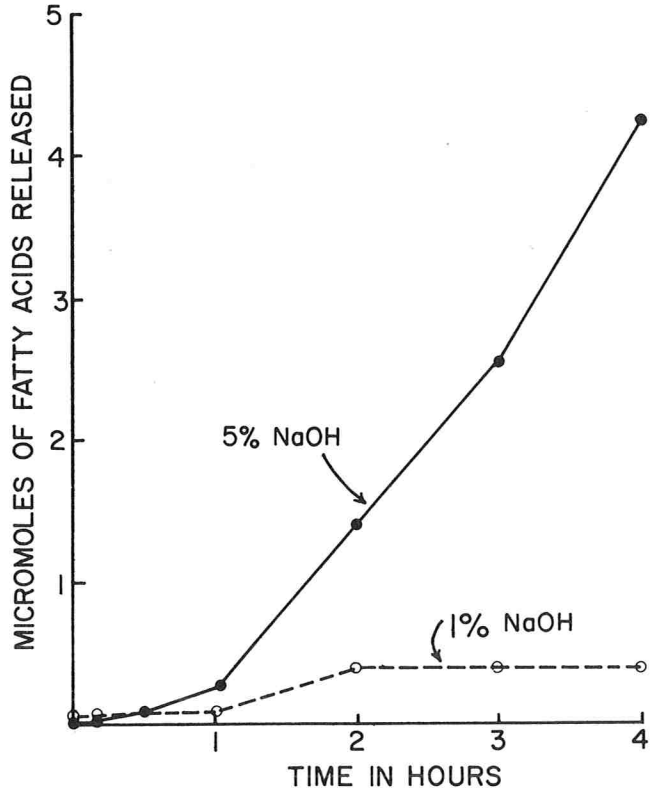

Figure 4. Relationship of time and alkali concentration on the rate of saponification of milkfat at 77 C.

teinaceous matter on equipment surfaces or in cleaning solutions after 6 min of cleaning were unsuccessful with any methods applied.

Another approach to evaluating the tenacious residue was to wash the inner surfaces of circulation cleaned stainless steel tubing with ether. Detectable quantities of lipoidal material were recovered and studied further. Chromatograms of the extracts indicated lipoidal materials with a fatty acid pattern similar to residues recovered from earlier intervals of cleaning and qualitatively similar to the milkfat in the milk used for soiling (Fig. 3).

Saponification in the soil removal process

Saponification was studied under conditions comparable to common chemical reactions in cleaning processes. Various concentrations of sodium hydroxide with 0.5% suspension of milkfat were held at 77 C in a laboratory shaker providing constant, gentle agitation. The degree of saponification was determined by titration. Figure 4 shows that saponification was time and alkali concentration dependent. With 1% sodium hydroxide, less than 1% of the fat was saponified at 77 C in 4 h. Only approximately 19% of the fat was saponified in 5% sodium hydroxide at 77 C in 4 h.

It was assumed that the degree of dispersion might influence the rate of saponification. Two methods of increasing dispersion, therefore, were studied. Loosely placed stainless steel wool to fill the reaction flask above the reaction mixture of fat and sodium hydroxide was included. A reaction time of 2 h at 77 C gave approximately double the saponification of a control without stainless steel wool. Similar increased saponification was obtained without stainless steel wool, but with the addition of 0.25% trisodium phosphate or sodium gluconate to the alkali.

Thin-layer chromatograms of the acidified extracts of the saponification mixture indicated significant saponification had occurred only after 2 to 4 h in 5% sodium hydroxide at 77 C (Fig. 5).

DISCUSSION

Data collected in the study with the laboratory tubular system showed cleaning rate and nature of tenacious residue to be similar to that found for a small commercial high-temperature, short-time pasteurizer. Thus, data obtained with the laboratory system should be meaningful in understanding commercial operations.

The flexibility of the laboratory tubular system allowed observations on the residue recovered in ether washing of the apparently clean equipment. Finding the ether from such washing contained lipoidal material similar to that removed in earlier stages of cleaning and similar to the milkfat used for soiling indicated the tenacious material was not unique. With progression of cleaning there was increasing difficulty in removing the remaining soil. These results are in agreement with the observations of Bourne and Jennings *(2)*.

Common commercial recommendations and practices suggest the mechanism of removal of lipoidal ma-

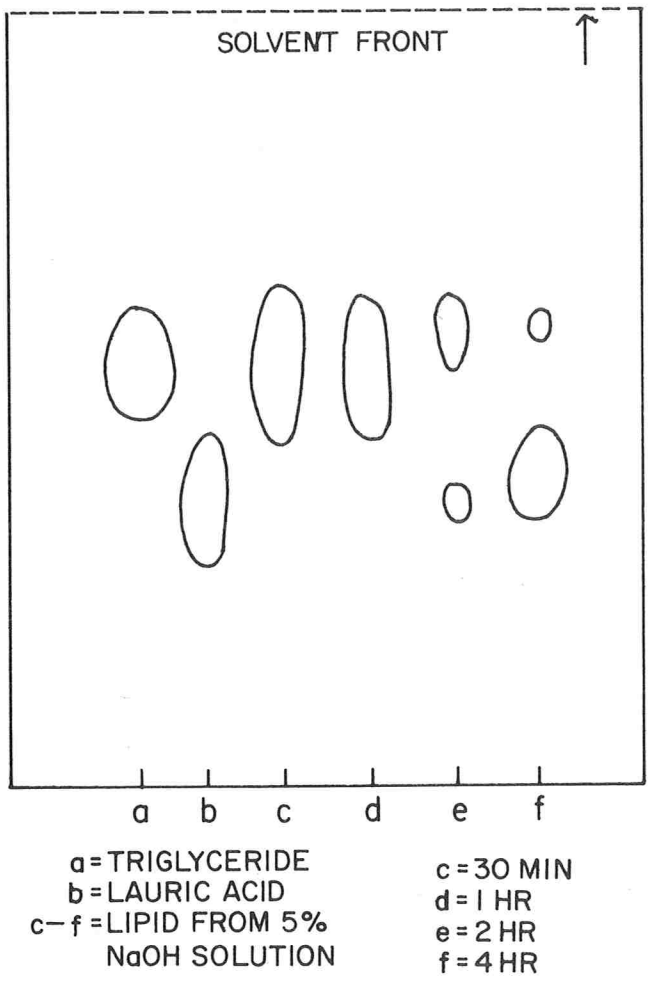

Figure 5. Thin layer chromatogram of milkfat recovered from 5% NaOH solutions.

terial is through saponification to change its physicochemical nature. Our data, however, indicate saponification during common CIP operation is of minor influence in cleaning. An explanation of the phenomenon of removal of lipoidal material from surfaces must be sought in another mechanism(s).

The data in this paper have theoretical interest in understanding cleaning processes. More important, however, is that the data provide a basis for further work toward criteria of "good" cleaners, time required for cleaning, and relationship between cleaner use and depletion, and redeposition of soil. When these factors can be defined in precise terms, the entire cleaning process can be designed to maximize efficiency and to assure perfection in cleaning.

REFERENCES

1. Arnold, R. G., and R. B. Maxcy. 1970. Evaluating circulation cleaning by analysis of soil depletion from surfaces. J. Dairy Sci. 53:1540-1544.

2. Bourne, M. C., and W. G. Jennings. 1961. Some physico-chemical relationships in cleaning hard surfaces. Food Technol. 15:495-499.

3. Burton, H. 1968. Reviews of the progress of dairy science. Section G. Deposits from whole milk in heat treatment plant - a review and discussion. J. Dairy Res. 35:317-330.

4. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193:265-275.

5. Maxcy, R. B. 1966. The relation between residual soil and microbial growth after circulation cleaning. J. Milk Food Technol. 20:123-126.

6. Maxcy, R. B. 1973. Nature and cause of a yellow film occurring on dairy equipment. J. Dairy Sci. 56:164-167.

7. Maxcy, R. B., and K. M. Shahani. 1961. Cleaning and sanitization of welded line systems for handling milk. J. Milk Food Technol. 24:122-127.

8. Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoridemethanol. J. Lipid Res. 5:600-608.

9. Olson, H. C., G. O. Brown, and J. B. Mickle. 1960. A study of welded lines for processing milk. J. Milk Food Technol. 23:86-93.

MICROBIAL QUALITY OF BARBECUED CHICKENS FROM COMMERCIAL ROTISSERIES

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ABSTRACT

The microbial quality of 121 barbecued chickens sold by 11 establishments was examined. Approximately twenty-six percent of 121 chickens had total bacterial counts of 10^6/g or more and/or presence of pathogens or potential pathogens. No salmonellae were isolated, but coagulase-positive staphylococci were found in four samples and *Clostridium perfringens* in three. Prolonged storage after barbecuing proved to be a major factor in high levels of contamination. However, neither the time of year nor the weight of the chickens seemed to affect the microbial quality of the barbecued product. Recommendations, based on the findings and intended to improve the quality of the product on sale, have been offered.

Commercial poultry rotisseries were introduced into Israel a few years ago, when food poisonings associated with the consumption of barbecued chicken sold by retailers had already been reported from the U.S.A. *(1, 8)*, Canada *(6)* and England *(11)*. These reports and our isolations of salmonellae, staphylococci, and *Clostridium perfringens* from raw packaged poultry sampled randomly in supermarkets, prompted us to investigate the microbial quality of chickens barbecued in commercial rotisseries and sold in retail food establishments.

MATERIALS AND METHODS

Sampling

Public Health inspectors purchased barbecued chickens late in the morning from four to five different retail food establishments on any given sampling day. Sampling days were varied during the week to allow for differences of supply, storage, and production practices. Each chicken was handled and wrapped by the vendor in his customary manner.

Because of the possible climatic effects on the microbial quality of perishable food-stuffs we conducted the survey in two stages: during the winter, from October 1970 to February 1971, 67 samples were collected; in the summer of 1971, from July to September, 54 samples were taken.

Handling practices at the food establishments

At the time of sampling the inspectors questioned the vendors about date of supply, procedures of handling, conditions of storage before and after barbecuing, and time and temperature of grilling. This information could not be checked and was entered as offered in a standard questionnaire form. From information so collected emerged a picture of great variability in all aspects of handling in any given establishment and among different establishments.

Raw chickens were bought from several commercial slaughter houses. Barbecuing took place anywhere from immediately to one week after receipt of the chickens at the retailers. When chickens were not barbecued on the day of receipt they were stored in a refrigerator.

The usual broiling time was 90 min, although four of the samples, bought from three different establishments, had been barbecued for 120 min. Most chickens in all rotisseries were barbecued at 150-155 C, some at 170-175 C, and three in three different establishments at 200 C. No explanation was offered for the variations in time and temperatures, and they were not related to the size of the chickens.

After barbecuing, chickens were sometimes left on the spits with the heat in the oven turned off. At other times they were taken off the spits and placed in a tray in the lower part of the oven. Temperatures measured in the barbecue ovens at the time of removal of the chicken varied from room tmperature to 170 C. When the barbecued chickens were not sold on the day of preparation, they were placed in the refrigerator in the evening after having cooled slowly in the oven or outside it. The next day the chickens were usually removed from the refrigerator and either rewarmed on spits, or again placed in the holding tray in the lower part of the oven, or just put on the sales counter. Sometimes they were left in the refrigerator and sold cold. They were kept unwrapped until sold.

Of the 121 samples obtained, 56 had been barbecued on the day of purchase, 60 on the day before purchase, and five between two and four days previously.

Specimens

A total of 121 whole barbecued chickens was obtained. One hundred and fifteen of these were purchased from eight establishments and the additional six samples came from three other stores that did not operate daily.

The specimens were brought to the laboratory within 1 to 2 h from the time of purchase and were examined immediately. Chickens that were collected from cold storage were brought to the laboratory in an ice box.

Laboratory methods

Preparation of samples. Each sample was divided aseptically into two sub-samples: *(a)* skin and meat from the outer surface of the whole chicken; and *(b)* meat from the inside of the chicken, obtained after the carcass was split open. Both sub-samples were examined in identical fashion.

pH. pH was measured using a Metrohm glass electrode pH meter.

Bacteriological examinations. Twenty grams of meat were homogenized in a blender with 180 ml of 0.1% peptone water and suitable dilutions were prepared. Counts for total number of bacteria and coliforms were done according to standard procedures *(2)* using plate count agar and VRB agar, respectively. For salmonellae, lactose broth was inoculated for preenrichment (incubated at 41 C for 20 h), tetrathionate broth served for enrichment and BG agar for plating (the last two media were incubated at 35 C for 24 h). Suspicious colonies were picked for standard diagnostic examinations. For isola-

TABLE 1. BACTERIAL FINDINGS IN BARBECUED CHICKENS FROM ELEVEN RETAIL FOOD ESTABLISHMENTS

Establishment	No. of samples	No. of samples, TC/g			Coag.+ staphyloc.	C. perfring.	Entero-cocci	No. of grossly contaminated samples[1]
		10^3 & 10^4	10^5	10^6 or more				
1	19	14	4	1	–	–	–	1
2	17	10	6	1	–	2	–	2
3	16	14	2	–	–	–	1	1
4	15	12	–	3	–	–	1	3
5	14	4	3	7	1	–	4	8
6	13	4	1	8	2	1	1	8
7	12	3	5	4	–	–	–	4
8	9	5	1	3	–	–	1	3
9	3	3	–	–	–	–	–	–
10	2	–	1	1	1	–	2	2
11	1	1	–	–	–	–	–	–
Total	121	70	23	28	4	3	10	32
%	100	57.9	19.0	23.1	3.3	2.5	8.3	26.4

[1]A sample was considered grossly contaminated if it contained 10^5 or more bacteria per gram and/or if pathogens or potential pathogens were isolated from it.

tion of staphylococci mannitol salt agar was used (incubated at 35 C for 48 h). Human blood from the local blood bank served for the coagulase test; production of enterotoxin was determined according to the method of Casman et al. *(3)*. For *C. perfringens* thioglycollate broth was inoculated (incubated at 30 C for 48 h) followed by streaking on neomycin blood agar. Confirmation tests were done according to the procedure of Seligmann *(9)*. For isolation of enterococci KF-agar was used (incubated at 35 C for 48 h). Enterococci were identified by the following characteristics: catalase activity, survival at 60 C for 30 min, growth at 45 C, at pH 9.6, and in 6.5% NaCl broth.

RESULTS AND DISCUSSION

Bacterial findings

The bacterial findings of 115 specimens collected from eight establishments and of six specimens from three additional stores are outlined in Table 1.

No salmonellae or coliforms were isolated. The 17 positive cultures of hazardous organisms were obtained from 15 chickens, and included coagulase-positive staphylococci from four and *C. perfringens* from three samples. Only two isolations of staphylococci were tested for production of enterotoxin and both were positive for enterotoxin C.

The total bacterial count proved a very useful indicator of hazardous organisms (Table 2).

Results in this table show that no hazardous organisms were found at total bacterial counts of less than 10^4/g. Positive findings of hazardous bacteria increased to 5% in samples with total bacterial counts of 10^4/g and to 9% in samples with total bacterial counts of 10^5/g. In samples with total bacterial counts of 10^6/g or more the incidence of contamination with pathogens or potential pathogens rose to 39%. We therefore considered all samples with total bacterial counts of 10^6/g or more to be grossly contaminated.

Duration of storage after barbecuing

Pivnick et al. considered bacterial counts of 10^5-10^7/g in barbecued chickens indicative of storage at improper temperatures *(5)*. In our study prolonged storage after barbecuing (at ambient temperatures) seemed to be the most common characteristic of contaminated samples.

The association between duration of storage after barbecuing and microbial quality was calculated by the x^2 test. The data are presented in Table 3.

The result indicates a significant correlation between duration of storage and microbial contamination.

A certain pattern in the microbial quality of the products of different establishments was discernible. Of 52 chickens from establishments No. 1, 2, and 3, four (8%) were grossly contaminated (Table 1). On the other hand, of 27 samples from retailers No. 5 and 6, 16 (59%) were grossly contaminated. These latter two establishments contributed 50% of all the contaminated specimens found in the survey.

Comparing the three establishments that supplied relatively few contaminated samples with the two which contributed 50% of all the grossly contaminated chickens in the survey, interesting differences in selling practices were found. Of the 52 barbecued chickens bought from the first three establishments, 27 (52%) were prepared on the day of purchase (0 days storage) and only one (2%) was barbecued two days before sale. In contrast to this, only five (19%) of the 27 samples from the other two establishments were sold on the day of preparation and four (15%) were offered for sale two to four days after barbecuing. (Not surprisingly, one chicken that had been stored for four days, was found to be foul-smelling and moldy when cut open in the lab-

oratory.)

Season and contamination

Gross contamination was found in 12 (18%) of the 67 barbecued chickens examined during the winter and in 20 (37%) of the 54 examined during the summer. In spite of the seemingly large seasonal percentile difference in contamination, the x^2 test did not show this difference to be significant: $x^2 = 3.1$ $p < 0.1 > 0.05$.

Weight of chickens and contamination

The weight of the chickens ranged between 550 and 1400 g (median 800 g). Sixty-two samples (51%) weighed between 700 and 899 g. No correlation was found between the weight of the chickens and contamination: $x^2_{(5)} = 3.18$ $p < 0.7 > 0.5$.

Comparison of outer and inner sub-samples

Outer and inner meat sub-samples from the same chicken often showed differences in pH values and bacteriological results.

pH values. The pH of the 121 outer sub-samples ranged between 5.5 and 6.8 (median 6.3) with 117 values between 6.0 and 6.7. In 65 samples (54%) the pH was 6.2-6.3. For the inner sub-samples the pH ranged between 5.9 and 7.3 (median 6.5) with 116 values between 6.2 and 6.9. In 64 samples (53%) the pH was 6.4-6.5.

In the vast majority of the samples (106) differences of 0.1-0.3 pH unit were found between outer and inner sub-samples. In four chickens the pH values of outer and inner sub-samples were identical while in another eleven the difference ranged between 0.4 and 1.0 pH unit. Differences in pH values of up to 0.3 unit are frequently encountered in meat and are not considered significant *(4)*. However, differences of pH in outer and inner subsamples in our series can hardly be attributed to chance since they invariably occurred in the same direction, the outer .sub-sample having a lower pH than the inner one. Examination of raw chickens at the poultry processing plant immediately after slaughter revealed the same shift in pH values.

No correlation was found between the pH value of the sample and its microbial quality although the two samples with the highest pH (7.2 and 7.3) contained more than 10^6 bacteria/g and 2000-5000 enterococci/g.

Bacteriological results. The cultures of outer and inner sub-samples of 32 barbecued chickens yielded 45 bacterial findings indicative of gross contamination (Tables 1 and 2). Table 4 shows the distribution of these findings in outer and inner sub-samples.

In 24 chickens, outer and inner sub-samples showed identical findings indicative of contamination. High bacterial counts were found in 10 inner sub-samples without corresponding contamination of the outer sub-sample but occurred only once in an outer subsample when the inner sub-sample showed no similar contamination. *C. perfringens* was isolated from three chickens; in two of these only the inner subsample yielded a positive culture. Positive findings from the inner part of the chickens alone might be interpreted as the result of a heat-gradient with temperatures too low to kill sporeformers and other heat-stable organisms inside the chicken. Examination of raw chickens obtained from poultry slaughter houses showed no difference in the degree of contamination of outer and inner sub-samples *(10)*. Visceral contamination therefore does not seem to be involved. Findings from the outer meat alone should be understood as post-barbecuing contamination. The number of positive findings is small and more work is necessary for confirmation of our tentative interpretation. The time-temperature relationship for barbecuing (90 min at 150-155 C) that was reported for

TABLE 2. NUMBER OF BACTERIA/G (TC) AND PRESENCE OF HAZARDOUS ORGANISMS

TC/g	No. of chickens	Coag.+ staphyloc.	C. perfring.	Entero-cocii	Chickens containing hazardous organisms	
					No.	%
10^3	30	0	0	0	0	0
10^4	40	0	0	2	2	5
10^5	23	1	1	1	2[1]	9
10^6 or more	28	3	2	7	11[1]	39
Total	121	4	3	10	15	12

[1]From one chicken both coagulase-positive staphylococci and enterococci were isolated.

TABLE 3. DURATION OF STORAGE AND CONTAMINATION, x^2 TEST

Days of storage	No. of samples	Grossly contaminated samples	
		No.	%
0	56	9	16
1	60	19	32
2-4	5	4	80
Total	121	32	26.4

$x^2 = 8.331$ $p_{(2)} < 0.02 > 0.01$

TABLE 4. BACTERIAL FINDINGS IN OUTER AND INNER SUBSAMPLES OF GROSSLY CONTAMINATED BARBECUED CHICKENS

Findings	Unsatisfactory sub-sample		
	Outer	Inner	Both
Total bacterial count, 10^6/g or more	1	10	17
Coag. + staphylococci, 10^3 and 10^4/g	2	1	1
C. perfringens, 10^1/g	–	2	1
Enterococci, 10^3 and 10^4/g	5	–	5
Total	8	13	24

most of the samples seems appropriate to prevent the survival of food poisoning organisms present in raw chicken *(7)*.

RECOMMENDATIONS

Recommended procedures for preparation and sale of barbecued meats were clearly outlined by Todd et al. *(12)*. Unfortunately, many of the small and crowded retail food establishments, operating a single rotisserie, lack the facilities to fulfill these recommendations. For these small establishments we propose the following guidelines:

(*a*) Storage after barbecuing should be limited not to exceed 48 h at most.

(*b*) If storage (up to 48 h) is unavoidable, barbecued chickens should be cooled rapidly, refrigerated, and sold cold. They should not be rewarmed at the establishment.

(*c*) Barbecued chickens, purchased cold, should be kept refrigerated until used and then be eaten cold or immediately after reheating.

(*d*) Total bacterial counts of 1×10^5/g or more should be considered above the minimum standard for barbecued chickens.

REFERENCES

1. Ager, E. A., J. Allard, S. B. Werner, H. H. Trayner, and R. Olson. 1966. *Salmonella typhi-murium* contamination of barbecued chickens-Spokane, Washington. Salmonella Surveillance Report 53:6, USDHEW/PHS.

2. American Public Health Association. 1967. Standard methods for the examination of dairy products. 12th ed. Amer. Public Health Ass. Inc., New York.

3. Casman, E. P., R. W. Bennet, A. E. Dorsey, and J. E. Stone. 1969. The micro-slide gel double diffusion test for the detection and assay of staphylococcal enterotoxins. Health Lab. Sci. 6:185.

4. Hofman, K. 1973. Die Feststellung des pH- Wertes in der Praxis. Fleischwirtschaft 53:187.

5. Pivnick, H., I. E. Erdman, S. Manzatiuk, and E. Pommier. 1968. Growth of food poisoning bacteria on barbecued chickens. J. Milk Food Technol. 31:198.

6. Pivnick, H., T. R. B. Barr, I. E. Erdman, and J. I. Pataki. 1968. Staphylococcus food poisoning from barbecued chicken. Can. J. Public Health 59:32.

7. Roberts, D. 1972. Observations on procedures for thawing and spit- roasting frozen dressed chickens and post-cooking care storage, with particular reference to food-poisoning bacteria. J. Hyg. Cambridge 70:565.

8. Rogers, E. A., K. Maier, B. Aserkoff, W. Woodward, and W. Martin. 1967. A large outbreak of salmonellosis following a turkey barbecue. Salmonella Surveillance Report 67:2, USDHEW/PHS.

9. Seligman, R. 1971. *Cl. welchii* as a potential cause of food poisoning. Harefuah 81:556. (English summary).

10. Seligmann, R. Public Health Laboratory, Haifa, Israel. Unpublished data.

11. Semple, A. B., G. C. Turner, and D. M. O. Lowry. 1968. Outbreak of food-poisoning caused by *S. virchow* in spit-roasted chicken. Brit. Med. J. 4:801.

12. Todd, E., I. Erdmann, and H. Pivnick. 1974. Recommended procedures for preparation and vending of barbecued meats cooked in rotisseries. J. Milk Food Technol. 37:157.

NEW INSECT CONTROL BOOKLET AVAILABLE

No insect is welcome in food handling or processing plants. State and federal sanitary codes require a virtually insect free environment in and around places where food is processed, stored or handled.

A special booklet designed to help sanitarians in food handling and processing establishments learn more about insect pests is now available from the University of Wisconsin-Extension (UWEX).

The booklet is designed to strengthen the cooperative effort between sanitarians responsible for insect control and the professional pest control services they usually employ, according to W. L. Gojmerac, UWEX entomologist. Gojmerac prepared and assembled the information along with W. E. Burkholder and J. E. Gorman of the U. S. Dept. of Agriculture, Stored Product Insects Laboratory.

Control measures depend on the problem and the pest, Gojmerac says. In some situations, accurate identification is essential—for other problems, it's not necessary. The more those persons responsible for sanitation know and understand about the pests involved, the more accurately they will be able to assist pest control firms in doing their jobs. And the more confidence they will have in making the decisions necessary to achieve adequate insect control, Gojmerac feels.

The booklet contains a bulletin on pest control in food processing plants. It also contains fact sheets on many other stored product and common household pests because, as Gojmerac points out, basic insect control principles are quite similar whether insects are found in the household, food processing plants, canning factories or restaurants.

The booklet is available from local UWEX offices. Out-of-state purchasers may order it from the Agriculture Bulletin Building, 1535 Observatory Dr., Madison, Wis. 53706. Ask for publication A2571, "Insect Control in Food Handling and Processing Establishments." The price is $1 plus postage.

HOLDERS OF 3-A SYMBOL COUNCIL AUTHORIZATIONS ON AUGUST 20, 1974

"Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y-Treas., 413 Kellogg Ave., P. O. Box 701, Ames, Iowa 50010."

01-06 Storage Tanks for Milk and Milk Products As Amended

116 Jacob Brenner Company, Inc. (10/ 8/59)
450 Arlington, Fond du Lac, Wisconsin 54935

28 Cherry-Burrell Corporation (10/ 3/56)
575 E. Mill St., Little Falls, N. Y. 13365

102 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Streets, Chester, Pennsylvania 19013

1 Chicago Stainless Equipment (5/ 1/56)
555 Valley Way, Northbrook, Illinois 60062

2 CREPACO, Inc. (5/ 1/56)
100 C. P. Ave., Lake Mills, Wisconsin 53551

117 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

76 Damrow Company (10/31/57)
196 Western Avenue, Fond du Lac, Wisconsin 54935

115 DeLaval Company, Ltd. (9/28/59)
113 Park Street, So., Peterborough, Ont., Canada

109 Girton Manufacturing Company (9/30/58)
Millville, Pennsylvania 17846

114 C. E. Howard Corporation (9/21/59)
9001 Rayo Avenue, South Gate, California 90280

127 Paul Mueller Company (6/29/60)
P. O. Box 828, Springfield, Missouri 65801

197 Paul Mueller (Canada), Ltd. (9/ 9/67)
84 Wellington St., South St. Marys, Ont., Canada

233 Stainless Steel Craft Corporation (4/13/72)
4503 Alger St., Los Angeles, Calif. 90039

21 Technova, Inc. Gosselin Division (9/20/56)
1450 Hebert c. p. 758
Drummondville, Quebec, Canada

31 Walker Stainless Equipment Co. (10/ 4/56)
Elroy, Wisconsin 53929

02-03 Pumps for Milk and Milk Products as Amended

214R Ben H. Anderson Manufacturers (5/20/70)
Morrisonville, Wis. 53571

212R Babson Bros. Co. (2/20/70)
2100 S. York Rd., Oak Brook, Ill. 60621

29R Cherry-Burrell Corporation (10/ 3/56)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52406

63R CREPACO, Inc. (4/29/57)
100 C. P. Ave., Lake Mills, Wisconsin 53551

205R Dairy Equipment Co. (5/22/69)
1919 So. Stoughton Road, Madison, Wis. 53716

180R The DeLaval Separator Co. (5/ 5/66)
Dutchess Turnpike, Poughkeepsie, N. Y. 12602

65R G & H Products, Inc. (5/22/57)
5718 52nd Street, Kenosha, Wisconsin 53140

145R ITT Jabsco, Incorporated (11/20/63)
1485 Dale Way, Costa Mesa, Calif. 92626

26R Ladish Co., Tri-Clover Division (9/29/56)
9201 Wilmot Road, Kenosha, Wisconsin 53140

236 Megator Corporation (5/ 2/72)
125 Gamma Drive, Pittsburgh, Pa. 15238

241 Purity S.A. (9/12/72)
Alfredo Noble #38, Industrial Pte. deVigas
Tlalnepantla, Mexico

148 Robbins & Myers, Inc. (4/22/64)
Moyno Pump Division
1345 Lagonda Ave., Springfield, Ohio 45501

163R Sta-Rite Industries, Inc. (5/ 5/65)
P. O. Box 622, Delavan, Wisconsin 53115

72R L. C. Thomsen & Sons, Inc. (8/15/57)
1303 53rd Street, Kenosha, Wisconsin 53140

219 Tri-Canada Cherry-Burrell Ltd. (2/15/71)
6500 Northwest Drive, Mississauga, Ont., Canada L4V 1K4

175R Universal Milking Machine Div. (10/26/65)
National Cooperatives, Inc.
First Avenue at College, Albert Lea, Minn. 56007

52R Viking Pump Div.
Houdaille Industries, Inc. (12/31/56)
406 State Street, Cedar Falls, Iowa 50613

5R Waukesha Foundry Company (7/ 6/56)
Waukesha, Wisconsin 53186

04-03 Homogenizers and High Pressure Pumps of the Plunger Type, As Amended

247 Bran and Lubbe, Inc. (4/14/73)
2508 Gross Point Road, Evanston, Illinois 60201

87 Cherry-Burrell Corporation (12/20/57)
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404

37 CREPACO, Inc. (10/19/56)
100 C.P. Ave., Lake Mills, Wis. 53538

75 Gaulin, Inc. (9/26/57)
44 Garden Street, Everett, Massachusetts 02149

237 Graco Inc. (6/ 3/72)
60-Eleventh Ave., N.E., Minneapolis, Minn. 55413

256 Haskon Inc. (1/23/74)
2285 University Ave., St. Paul, Minnesota 55114

05-11 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service, As Amended

131R Almont Welding Works, Inc. (9/ 3/60)
4091 Van Dyke Road, Almont, Michigan 48003

98R Beseler Steel Products, Inc. (3/24/58)
417 East 29th, Marshfield, Wisconsin 54449

70R Jacob Brenner Company (8/ 5/57)
450 Arlington, Fond du Lac, Wisconsin 54935

40 Butler Manufacturing Co. (10/20/56)
900 Sixth Ave., S. E., Minneapolis, Minn. 55114

118 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

66 Dairy Equipment Company (5/29/57)
1818 So. Stoughton Road, Madison, Wisconsin 53716

45 The Heil Company (10/26/56)
3000 W. Montana Street, Milwaukee, Wisconsin 53235

201 Paul Krohnert Mfg., Ltd. (4/ 1/68)
811 Steeles Ave., Milton, Ontario, Canada L9T 2Y3

80 Paul Mueller (Canada), Ltd. (11/24/57)
84 Wellington Street, So., St. Marys, Ont., Canada

85 Polar Manufacturing Company (12/20/57)
Holdingford, Minn. 56340

144 Portersville Equipment Company (5/16/63)
Portersville, Pennsylvania 16051

71 Progress Industries, Inc. (8/ 8/57)
400 E. Progress Street, Arthur, Illinois 61911

121 Technova Inc. Gosselin Division (12/ 9/59)
1450 Hebert c. p. 758
Drummondville, Quebec, Canada

47 Trailmobile, Div. of Pullman, Inc. (11/ 2/56)
701 East 16th Ave., North Kansas City, Mo. 64116

189 A. & L. Tougas, Ltée (10/ 3/66)
1 Tougas St., Iberville, Quebec, Canada

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

08-09 Fittings Used on Milk and Milk Products Equipment, and Used on Sanitary Lines Conducting Milk and Milk Products

79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue, Waukesha, Wisconsin 53186

138R A.P.V. (Canada) Equipment, Ltd. (12/17/62)
103 Rivalda Rd., Weston, Ont., Canada

245 Babson Brothers Company (2/12/73)
2100 S. York Road, Oak Brook, Illinois 60521

82R Cherry-Burrell Corporation (12/11/57)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52406

124R DeLaval Company, Ltd. (2/18/60)
113 Park Street, South, Peterborough, Ont., Canada

184R The DeLaval Separator Co. (8/ 9/66)
Dutchess Turnpike, Poughkeepsie, N. Y. 12602

67R G & H Products, Inc. (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140

199R Graco, Inc. (12/ 8/67)
60 Eleventh Ave., N.E., Minneapolis, Minn. 55413

203R Grinnell Company (11/ 7/68)
260 W. Exchange St., Providence, R. I. 02901

218 Highland Corporation (2/12/71)
74-10 88th St., Glendale, N. Y. 11227

204R Hills McCanna Company (2/10/69)
400 Maple Ave., Carpentersville, Ill. 60110

34R Ladish Co., Tri-Clover Division (10/15/56)
2809 60th St., Kenosha, Wisconsin 53140

239 LUMACO (6/30/72)
Box 688, Teaneck, N. J. 07666

200R Paul Mueller Co. (3/ 5/68)
P. O. Mox 828, Springfield, Mo. 65801

242 Purity, S. A. (/12/72)
Alfredo Nobel #39 Industrial Pte. de Vigas Tlalnepantla, Mexico

149R Q Controls (5/18/64)
Occidental, California 95465

227 Stainless Steel Craft Corporation (1/11/72)
4503 Alger Street, Los Angeles, California

89R Sta-Rite Industries, Inc. (12/23/68)
P. O. Box 622, Delavan, Wis. 53155

73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street, Kenosha, Wisconsin 53140

191R Tri-Canada Cherry-Burrell, Ltd. (11/23/66)
6500 Northwest Drive, Mississauga, Ontario, Canada L4V 1K4

250 Universal Milking Machine Division (6/11/73)
Universal Cooperatives, Inc.
408 First Ave. So.
Albert Lea, Mn. 56007

86R Waukesha Specialty Company, Inc. (12/20/57)
Darien, Wisconsin 53114

09-00 Thermometer Fittings and Connections Used on Milk and Milk Products Equipment and Supplement 1, As Amended

32 Taylor Instrument Process Control, Div. Sybron Corp. (10/ 4/56)
95 Ames Street, Rochester, New York 14601

206 The Foxboro Company (/11/69)
Neponset Ave., Foxboro, Mass. 02035

246 United Electric Controls (3/24/73)
85 School Street, Watertown, Massachusetts 02172

10-01 Milk and Milk Products Filters Using Disposable Filter Media, As Amended

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

11-03 Plate-Type Heat Exchangers for Milk and Milk Products, As Amended

20 A.P.V. Company, Inc. (9/ 4/56)
137 Arthur Street, Buffalo, New York 14207

30 Cherry-Burrell Corporation (10/ 1/56)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404

14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets, Chester, Pennsylvania 19013

38 CREPACO, Inc. (10/19/56)
100 CP Avenue, Lake Mills, Wisconsin 53551

120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street, South Peterborough, Ont., Canada

17 The DeLaval Separator Company (8/30/56)
Dutchess Turnpike, Poughkeepsie, N. Y. 12602

15 Kusel Dairy Equipment Company (8/15/56)
100 W. Milwaukee Street, Watertown, Wisconsin 53094

12-04 Internal Return Tubular Heat Exchangers, for Milk and Milk Products, As Amended

248 Allegheny Bradford Corporation (4/16/73)
P. O. Box 264, Bradford, Pa. 16701

243 Babson Brothers Company (10/31/72)
2100 S. York Road, Oak Brook, Illinois 60521

103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street, Chester, Pennsylvania 19013

152 The DeLaval Separator Co. (11/18/69)
350 Dutchess Turnpike, Poughkeepsie, N. Y. 12602

217 Girton Manufacturing Co. (1/23/71)
Millville, Pa. 17846

252 Ernest Loffrandoi (12/27/73)
P. O. Box 455, Ferndale, Calif. 95536

238 Paul Mueller Company (6/28/72)
P. O. Box 828, Springfield, Missouri 65801

96 C. E. Rogers Company (3/31/64)
P. O. Box 118, Mora, Minnesota 55051

13-01 Farm Milk Cooling and Holding Tanks, As Amended

240 Babson Brothers Company (9/ 5/72)
2100 S. York Road, Oak Brook, Illinois 60521

11R CREPACO, Inc. (7/25/56)
100 C. P. Ave.
Lake Mills, Wisconsin 53551

119R Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park, St. Cloud, Minn. 56301

4R Dairy Equipment Company (6/15/56)
1919 S. Staughton Road, Madison, Wisconsin 53716

92R DeLaval Company, Ltd. (12/27/57)
113 Park Street, South Peterborough, Ontario, Canada

49R The DeLaval Separator Company (12/ 5/56)
Dutchess Turnpike, Poughkeepsie, N. Y. 12602

10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846

95R Globe Fabricators, Inc. (3/14/58)
3350 North Gilman Rd., El Monte, California 91732

179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
1261 Industrial Road, Preston, Preston, Ont., Canada

12R Paul Mueller Company (7 /31/56)
P. O. Box 828, Springfield, Missouri 65801

235 Stainless Steel Craft Corporation (4/13/72)
4503 Alger St., Los Angeles, California 90039

249 Sunset Equipment Co. (4/16/73)
3765 North Dunlap Street
St. Paul, Minnesota 55112

216R Valco Manufacturing Company (10/22/70)
3470 Randolph St., Huntington Pk., Calif. 90256

42R VanVetter, Inc. (10/22/56)
2130 Harbor Avenue S.W., Seattle, Washington 98126

16R Zero Manufacturing Company (/8/27/56)
Washington, Missouri 63090

14-00 Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers, As Amended

122R Cherry-Burrell Corporation (12/11/59)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52406

69 G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140

27 Ladish Co. - Tri-Clover Division (9/29/56)
2809 60th Street, Kenosha, Wisconsin 53140

78 L. C. Thomsen & Sons, Inc. (11/20/57)
1303 43rd Street, Kenosha, Wisconsin 53140

16-04 Evaporators and Vacuum Pans for Milk and Milk Products

254 Anhydro, Inc. (1/ 7/74)
130 S. Washington St., North Attleboro, Mass. 02760

132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street, Buffalo, New York 14207

164R Anderson IBEC (4/25/65)
19609 Progress Drive
Strongsville, Ohio 44136

107R C. E. Rogers Company (8/ 1/58)
P. O. Box 118, Mora, Minnesota 55051

186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road, Birmingham, Mich. 48010

259 Pollution Control, Inc. (4/ 5/74)
P. O. Box 208, Wilson Place, South Barre, Vt. 05670

17-00 Fillers and Sealers of Single Service Containers, For Milk and Milk Products, As Amended

192 Cherry-Burrell Corporation (1/ 3/67)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52404

137 Ex-Cell-O Corporation (10/17/62)
P. O. Box 386, Detroit, Michigan 48232

220 Haskon, Inc., Package Equipment Division (4/24/71)
2285 University Ave., St. Paul, Minnesota 55114

211 Twinpak, Inc. (2/ 4/70)
2225 Hymus Blvd., Dorval 740 P.Q.

19-00 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

141 CREPACO, Inc. (4/15/63)
100 C. P. Avenue, Lake Mills, Wisconsin 53551

146 Cherry-Burrell Corporation (12/10/63)
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404

22-03 Silo-Type Storage Tanks for Milk and Milk Products

168 Cherry-Burrell Corporation (6/16/65)
575 E. Mill St., Little Falls, N. Y. 13365

154 CREPACO, Inc. (2/10/65)
100 C. P. Ave., Lake Mills, Wisconsin 53551

160 Dairy Craft, Inc. (4/ 5/65)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

181 Damrow Company, Division of DEC International, Inc. (5/18/66)
196 Western Ave., Fond du Lac, Wisconsin 54935

156 C. E. Howard Corporation (3/ 9/65)
9001 Rayo Avenue, South Gate, California 90280

155 Paul Mueller Co. (2/10/65)
P. O. Box 828, Springfield, Missouri 65801

195 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada

234 Stainless Steel Craft Corporation (4/13/72)
4503 Alger St., Los Angeles, California 90039

165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

23-00 Equipment for Packaging Frozen Desserts, Cottage Cheese and Milk Products Similar to Cottage Cheese in Single Service Containers

174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road, Rockford, Illinois 61109

209 Doboy Packaging Machinery (7/23/69)
Domain Industries, Inc.
869 S. Knowles Ave., New Richmond, Wis. 54017

258 Haskon, Inc. (2/ 8/74)
2285 University Ave., St. Paul, Minnesota 55114

222 Maryland Cup Corporation (11/15/71)
Owings Mills, Maryland 21117

193 Triangle Package Machinery Co. (1/31/67)
6655 West Diversey Ave., Chicago, Illinois 60635

24-00 Non-Coil Type Batch Pasteurizers

161 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N. Y. 13365

158 CREPACO, Inc. (3/24/65)
100 C. P. Avenue, Lake Mills, Wisconsin 53551

187 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846

166 Paul Mueller Co. (4/26/65)
P. O. Box 828, Springfield, Mo. 65601

25-00 Non-Coil Type Batch Processors for Milk and Milk Products

162 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N. Y. 13365

159 CREPACO, Inc. (3/24/65)
100 C. P. Avenue, Lake Mills, Wisconsin 53551

188 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

167 Paul Mueller Co. (4/26/65)
Box 828, Springfield, Mo. 65801

196 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada

202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wis. 53950

26-00 Sifters for Dry Milk and Dry Milk Products

228 J. H. Day Co. (2/28/72)
4932 Beech Street, Cincinnati, Ohio 45202

171 Entoleter, Inc. (9/ 1/65)
Subsidiary of American Mfg. Co.
P. O. Box 1919, New Haven, Connecticut 06510

229 Russell Finex Inc. (3/15/72)
156 W. Sandford Boulevard, Mt. Vernon, N. Y. 10550

173 B. F. Gump Division (9/20/65)
Blaw-Knox Food & Chem. Equip. Inc.
750 E. Ferry St., P. O. Box 1041
Buffalo, New York 14240

185 The Orville-Simpson Co. (8/10/66)
1230 Knowlton St., Cincinnati, Ohio 45223

176 Sprout, Waldron & Co., Inc. (1/ 4/66)
Munsy, Pennsylvania 17756

172 SWECO, Inc. (9/ 1/65)
6111 E. Bandini Blvd., Los Angeles, California 90022

28-00 Flow Meters for Milk and Liquid Milk Products

253 Badger Meter, Inc. (1// 2/74)
4545 W. Brown Deer Road, Milwaukee, Wis. 53223

223 C-E IN-VAL-CO, a division of Combustion Engineering, Inc. (11/15/71)
P. O. Box 556, 3102 Charles Page Blvd., Tulsa, Oklahoma 74101

231 The DeLaval Separator Company (3/27/72)
350 Dutchess Turnpike
Poughkeepsie, New York 12603

226 Fischer & Porter Company (12/ 9/71)
County Line Road, Warminster, Pa. 18974

224 The Foxboro Company (11/16/71)
Foxboro, Massachusetts 02035

29-00 Air Eliminators for Milk and Fluid Milk Products

251 TheDeLaval Separator Company (12/10/73)
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FOURTEENTH ANNUAL MEETING OF NMC

The 14th annual meeting of the National Mastitis Council is scheduled for February 10-12, 1975 at the Radisson Hotel, Minneapolis, Minnesota.

Two International authorities from England will appear on the program. Dr. G. C. Brander is from the Beecham Research Laboratories and will report on the Somerset mastitis control scheme and problems of gram-negative organisms in mastitis control. Dr. James M. Booth from the Milk Marketing Board will discuss mastitis control in the field. Other subjects including teat dips, dry cow therapy and coliform mastitis will be discussed. Reports dealing with Sonatic will be included in the program.

The meeting will start at 8:45 A.M. on February 11 and will adjourn at noon on February 12. Send requests for room reservations directly to the Radisson Hotel, 45 South 7th Street, Minneapolis, Minnesota 55402.

PERFORMANCE AND MORPHOLOGY OF *KLUYVEROMYCES FRAGILIS* AND *RHODOTORULA GRACILIS* GROWN IN COTTAGE CHEESE WHEY

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Oklahoma Agricultural Experiment Station[2], Stillwater, Oklahoma 74074.
Department of Animal Sciences and Industry

(Received for publication February 21, 1974)

Abstract

The overall objectives of this research were to find practical methods of cottage cheese whey disposal, and economical methods of recovering usable products from the whey. The specific purposes of this study were: *(a)* to determine whether *Kluyveromyces fragilis* could reduce the COD of cottage cheese whey more efficiently than in previous trials, *(b)* to attempt adaptation of *Rhodotorula gracilis* to lactose, and *(c)* to describe the morphology of the adapted *Rh. gracilis* culture. *K. fragilis* reached maximum cell numbers in approximately 7 h, with initial inocula of 1×10^8 cells/ml. At this rate of inoculation, the COD of cottage cheese whey was reduced 82 ± 2% in 10-11 h, and 93 ± 2% in 24 h, a greater reduction than reported by most authors. *Rh. gracilis* was adapted to utilize lactose as its sole carbon source by successive transfers on lactose agar. Photomicrographs of this adapted *Rh. gracilis* culture showed morphology similar to that reported in the literature when the yeasts had been grown on other media.

Disposal of cheese whey is a major problem for the dairy industry, since this material has a relatively high biological oxygen demand (BOD). Such whey is not allowed in many municipal sewer systems, and must be disposed of in some other way. Disposal of cottage cheese whey is even more difficult, since this whey is acid, and difficult to dry. In addition much of this whey is produced in relatively small plants and the volume makes drying or other concentration methods unprofitable. Most of these latter methods (reverse osmosis, ultrafiltration, etc.) require at least 100,000 lb. of whey per day to be economical *(2, 8, 9,)*. Over 69% of the BOD in cottage cheese whey comes from lactose. Removal of this sugar simplifies the disposal problem. Knight et al. *(9)*, showed that *Saccharomyces fragilis* — now *Kluyveromyces fragilis (10)* — used lactose and reduced the chemical oxygen demand (COD, a measure of BOD), of cottage cheese whey by 60% or more. This paper reports a continuation of that work.

Most of the yeast literature has been reviewed in four recent books *(10, 13, 17, 19)*. Knight *(8)* and Knight et al. *(9)*, reviewed the recent literature concerning *K. fragilis*, and Halter *(6)* described the morphology of *K. fragilis* and *Rh. gracilis*. Many of the research results about whey fermentation, however, are in the hands of private companies *(16, 18)* or processes are being patented *(5)*. Thus, many of the details needed for commercial yeast production are not available to others.

Of the several microorganisms considered for use in the industrial production of fat, *Rh. gracilis* seems to hold the most promise, since it may yield as much as 50-60% of its dry weight as lipid *(3, 12, 14)*. Such high fat contents could be of value in certain poultry rations. Lundin *(12)* described two growth phases for *Rh. gracilis*. First was a protein-forming phase during which sugar, nitrogen, and phosphorus were utilized. Yeast cell numbers increased rapidly during this phase. When most of the nitrogen was exhausted, the fattening phase began after additional sugar was added. While the growth rate slowed, fat content and cell sizes increased rapidly during this phase.

Since *K. fragilis* reduces the BOD of whey by 80% or more *(2, 8, 9, 13, 19)*, further work with this organism was needed to determine whether it could lower whey COD more efficiently than in earlier trials *(8, 9)*. According to the literature, *Rh. gracilis* does not use lactose. However, Lodder *(11)* mentioned that Rennerfelt had isolated a strain from wood pulp which would "weakly assimilate lactose." No pictures of *Rh. gracilis* grown on cottage cheese whey were found.

In summary, the overall objectives of this research were to find practical methods of reducing the COD of cottage cheese whey and economical procedures to recover usable products from the whey. The specific purposes of this study were: *(a)* to determine whether *K. fragilis* could reduce the COD of cottage cheese whey more efficiently than in previous trials; *(b)* to attempt adaptation of *Rh. gracilis* to lactose; and *(c)* to describe the morphology of the adapted *Rh. gracilis* culture.

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[1]Portions of this publication were taken from theses submitted by Ms. Halter and Knight to Oklahoma State University in partial fulfillment of the requirements for the Master of Science degrees. Present address of Ms. Halter: Department of Home Economics, Lamar University, Beaumont, Texas. 77710.

[2]Journal Article 2778 of the Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma. The microscope used in this work was financed in part with funds from a "Biomedical Research Grant" No. 5-S05-RR-07077-05.

PROCEDURES

K. fragilis (NRRL Y-1156) and *Rh. gracilis* (NRRL Y-1091) cultures were obtained from the U.S.D.A. Northern Regional Research Laboratory. *K. fragilis* was immediately transferred to lactose agar slants (2.5% agar, 2% lactose, 1% peptone, 0.1% yeast extract). The original strain of *Rh. gracilis* was unable to use lactose. However, after eight successive transfers on lactose agar, a technique adapted from Nielsen and Nilsson *(14)*, an adapted culture was obtained. Thereafter both stock cultures were carried on lactose agar.

To prepare inocula for growth trials, a loop of stock culture was transferred to a broth containing 2% lactose, 1% peptone, and 0.1% yeast extract. When the yeast cells were in the log phase of growth, a 30% inoculum (v/v) of this starter broth was added to the whey. The yeast cell counts of these inocula were approximately 1×10^8, which was less than used by some researchers, who had inocula of 5×10^8 or cell weights representing 25% of the sugar in whey *(23, 24)*. Knight had previously determined the optimum growth conditions for *K. fragilis* to be: pH of 4.6-5.0, temperatures of 35 ± 2 C, and airflow rates of approximately one volume per minute *(9)*. The trials reported here were conducted under the same conditions, except that *Rh. gracilis* occasionally was grown at 30 ± 2 C during fattening. Samples of the yeast-whey mixture were taken hourly. One portion was analyzed for composition, another for cell counts, and a third, in *Rh. gracilis* trials, used for photography. Lactose and sucrose were determined by the method of Perry and Doan *(15)*. Protein contents were obtained by Kjeldahl *(4)*, and fat compositions of certain *Rh. gracilis* samples were measured with a method adapted from Steinberg and Ordal *(21)*. On certain samples COD and alcohol were measured *(1, 4, 22)*, as was titratable acidity, calculated as lactic. Yeast cell counts were determined with a microscope and hemacytometer *(8, 9)*, and reported as the average of two determinations. Certain samples of *K. fragilis* trials also were plated on YM agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1.0% dextrose and 2.5% agar) to make certain that the measured cells were alive. The original whey contained 4.5-4.9% lactose, 0.5% lactic acid, 0.5% protein, 0.5% ash, and 0.1% fat. *K. fragilis* trials were continued for 24 h with no supplemental nutrients *(9)*.

Rh. gracilis was grown until the nitrogen in the original whey was exhausted (approximately 20 h). After this, 5% sucrose was added to the yeast whey mixture, and the fattening phase was continued until the yeast had grown 160 h or more. Photographs were taken from wet mounts, stained with 0.01% methylene blue, using a 35 mm "Exacta" and "Panatomic X" film (ASA 32), exposure times of 5 sec with both a neutral density and Zeiss green filter (VG-9) were used. The oil immersion objective (100 ×) and a 10 × ocular were used on the microscope. Yeast cell measurements were obtained from several photomicrographs by comparing them to pictures of a stage micrometer taken through the same microscope under the same conditions. Cell dimensions were then obtained by considering the pictures of the micrometer in relation to those of the cells. Mean (average) cell dimensions were determined, and in certain instances the "standard error of the mean" (S.E.) also was calculated. Some portions of the *Rh. gracilis* samples were stained with "Sudan Black B," a fat stain.

RESULTS AND DISCUSSION

The cell count data (Fig. 1) showed that *K. fragilis* reached maximum cell counts of 4.5×10^8 in approximately 7 h. In most trials the rapid reduction of COD was the principle objective. Fig. 1 shows that 82 ± 2% of the COD had been removed by *K. fragilis* in 10-11 h. Thus, when COD reduction is the objective, there is no reason to grow these yeasts more than 11 h. If, on the other hand, maximum protein production is the objective, one might profitably continue growth under somewhat different conditions *(5 16, 18, 23, 24)*. In the present trial (Fig. 1), when the lactose was removed, the remaining substances of interest were yeast cells, minerals, protein, alcohol, and lactic acid. The alcohol disappeared between 7 and 24 h, and the titratable acidity was lowered from 0.33 to 0.05% in the same period. The primary difference between the trials reported here and those reported earlier by Knight et al. *(9)*, was the size of the original inoculum. Previous inoculations had cell counts of 0.2×10^8 while the inocula for the present trials was approximately 1.0×10^8, an increase of more than five fold.

The growth curve showing cell numbers of *Rh.*

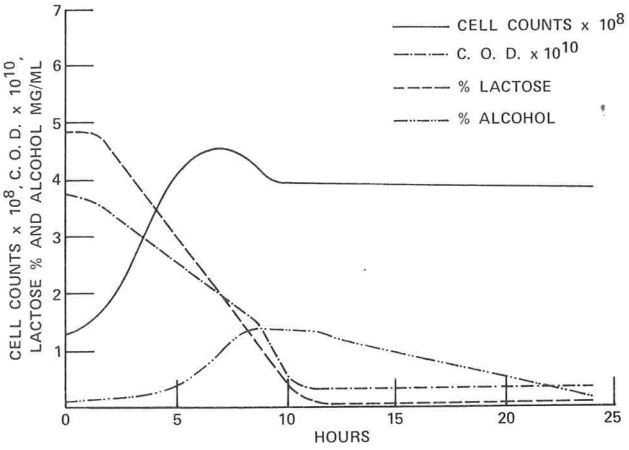

Figure 1. Cell counts, COD, lactose, and alcohol measurements of *K. fragilis* grown 24 h in whey.

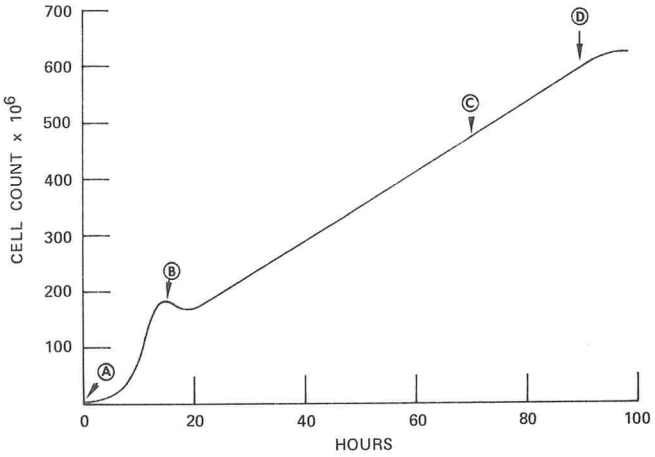

Figure 2. Cell counts of *Rh. gracilis* grown in whey. Letters indicate points of sampling.

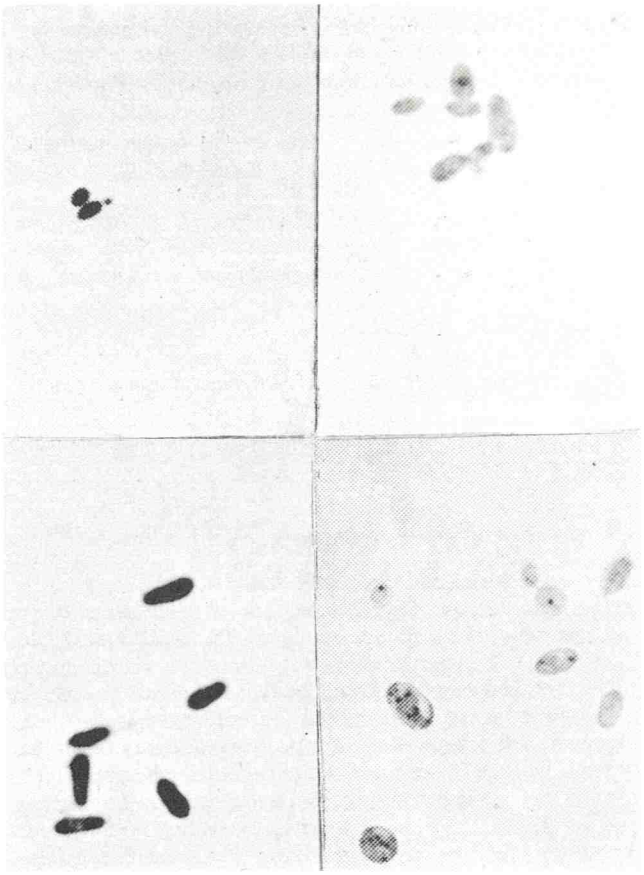

Figure 3. Top, left: Example of *Rh. gracilis* at 0 h, stained with 0.01% methylene blue. Average cell size, 2.0 × 3.4 μ. Picture at 1000× on 35 mm film, enlarged to present proportions. Top right: Example of *Rh. gracilis* at 18 h, stained with 0.01% methylene blue. Average cell size, 3.5 × 6.4 μ. Picture at 1000× on 35 mm film, enlarged to present proportions. The large indistinct spot is debris, or a film imperfection. Bottom left: Example of *Rh. gracilis* at 66 h, stained with 0.01% methylene blue. Average cell size, 3.0 × 5.2 μ. Picture at 1000× on 35 mm film, enlarged to present proportions. Bottom right: Example of *Rh. gracilis* at 90 h, stained with 0.01% methylene blue. Average cell size, 4.2 × 6.4 μ. Picture at 1000× on 35 mm film, enlarged to present proportions.

gracilis vs. time (Fig. 2) is a composite of many trials from data obtained over several months. *Rh. gracilis* organisms reached their first maximum after 18-20 h, when the available protein in the whey was exhausted. When 5% sucrose was added to the medium, the growth rate declined for a few hours, then increased again. Growth continued to increase during the fattening phase, although at a slower rate than originally. The fattening phase continued in these experiments for 90 h or more. After the yeasts used all of the sugar, growth rate again slowed. In the present trials, the amount of lipid formed by *Rh. gracilis* during the fattening phase was only 32% when the organisms were grown at 35 C, but 53% lipid accumulated in the same yeasts grown at 30 C.

Too much time was required to grow *Rh. gracilis* in whey for this organism to be a practical means of reducing whey COD. Still the results were of interest since it had not previously been demonstrated that *Rh. gracilis* could be adapted to use lactose. Pictures of the adapted culture do not appear in the literature. Photomicrographs taken at 0, 18, 66, and 90 h (examples in Fig. 3), showed these yeasts were primarily oval during early growth stages, but grew larger and elongated as the fattening phase progressed. At 90 h the cells contain large vacuoles. A portion of this sample, stained with "Sudan Black B," showed that the vacuoles were filled with lipid. Average cell sizes increased from 2.0 × 3.2 μ at 0 h, to an average of 4.2 × 6.4 μ at 90 hours (S.E. = 0.4 μ)[3]. With increased age, the cell walls of the *Rh. gracilis* became progressively thicker, which was noticeable at 66 and 90 h (Fig. 5 and 6). These findings are in substantial agreement with the reports for these yeasts when grown on other media *(7, 10, 11, 21)*.

REFERENCES

1. Agricultural Handbook 176. 1960. Dairy waste treatment by aeration. U. S. Dept. of Agriculture. Washington, D. C. p. 18.

2. Agricultural Research Service. 1970. Proc. whey utilization conference. U.S.D.A., EU. Publ. No. 3340.

3. Allen, L. A., N. H. Barnard, M. Fleming, and B. Hollis. 1964. Factors affecting the growth and fat formation of *Rhodotorula gracilis*. J. Appl. Bacteriol. 27:27-40.

4. Association of Official Agricultural Chemists. 1965. Official methods of analysis. 10th ed. Washington, D. C.

5. Bechtle, R. M., and T. J. Claydon. 1971. Accelerated fermentation of cheese whey. Developing the system. J. Dairy Sci. 54:1595-1604.

6. Halter, D. M. 1973. The morphology of *Saccharomyces fragilis* and *Rhodotorula gracilis* yeasts grown in cheese whey. M. S. Thesis. Okla. State Univ.

7. Harrison, F. C. 1928. A systematic study of some torula. Royal Soc. Can. Trans. and Proc. 22, sect. 5:187-191.

8. Knight, N. S. 1972. Lactose removal from cheese whey using *Saccharomyces fragilis* yeast. M. S. Thesis. Okla. State Univ.

9. Knight, S., W. Smith, and J. B. Mickle. 1972. Cheese whey disposal using *Saccharomyces fragilis* yeast. Cult. Dairy Prod. J. 17 (2):17-22.

10. Lodder, J. 1970. The yeasts. 2nd ed. North Holland Publ. Co. Amsterdam. pp. 317-378 and 1187-1233.

11. Lodder, J., and N. J. W. Kreger-Van Rij. 1952. The yeasts: A taxonomic study. North Holland Publ. Co. Amsterdam. pp. 181-183, 647-649.

12. Lundin, H. 1950. Fat synthesis by microorganisms and its possible applications in industry. J. Inst. Brewing. 56:17-28.

13. Mateles, R. I., and S. R. Tannenbaum. 1968. Single cell protein. M.I.T. Press. Cambridge, Mass.

14. Nielsen, N., and N. G. Nilsson. 1950. Investigations on respiration, growth, and fat production of *Rhodotorula*

[3]The standard errors quoted in this paper apply to measurements of both the length and width of the yeast cells.

gracilis when cultivated in a media containing different carbohydrates. Arch. Biochem. 25:316-322.

15. Perry, N. A., and F. J. Doan. 1950. A picric acid method for the simultaneous determination of lactose and sucrose in dairy products. J. Dairy Sci. 33:176-185.

16. Powell, M. E., and K. Robe. 1964. Solves whey disposal. Food Process. 25 (2):80-81, 95.

17. Reed, G., and H. J. Peppler. 1973. Yeast technology. Avi Publ. Co. Westport, Conn.

18. Robe, K. 1964. Wheast. Food Process. 25 (2):95-96.

19. Rose, A. H., and J. S. Harrison. eds. 1969. The yeasts. Vol. 1, 2, & 3. Academic Press. N. Y.

20. Ruinen, J., and M. H. Deinema. 1968. Cellular and extracellular structure in *Cryptococcus laurentii* and *Rhodotorula* species. Can. J. Microbiol. 14:1133-1137 and plates 1-8.

21. Steinberg, M. P., and Z. J. Ordal. 1954. Effect of fermentation variables on rate of fat formation by *Rhodotorula gracilis*. J. Agr. Food Chem. 2:873-877.

22. Sunshine, I., and R. Nenad. 1953. A modification of Winnick's method for the rapid determination of ethyl alcohol in biological fluids. Anal. Chem. 25:653-655.

23. Wasserman, A. E., J. E. Hampson, N. F. Alvare, and N. J. Alvare. 1961. Whey utilization. V. Growth of *Saccharomyces fragilis* in whey in a pilot plant. J. Dairy Sci. 44: 387-392.
Sew. Ind. Wastes. 30:913-920.

24. Wasserman, A. E., W. J. Hopkins, and N. Porges. 1958. Whey utilization growth conditions for *Saccharomyces fragilis*.

ASSOCIATION AFFAIRS

CHARLES E. WALTON

1918 - 1974

Charles E. Walton, President of I.A.M.F.E.S. in 1963 passed away July 12, 1974 in Denver, Colorado where he was being treated for a series of illnesses during the past year.

After a three year stint in the Navy during World War II he started his public health career in Pueblo, Colorado. He was City Sanitarian for Laramie, Yymoing from 1957 to 1964. From 1964 to 1968 he served as Chief of the Food and Drug Division of the Wyoming Department of Agriculture. In 1968 he went to Las Vegas, Nevada as Chief Sanitarian for the Occupational Medical Division of the A.E.C. Because of illness he retired from the A.E.C. in February, 1972.

Charlie is survived by his wife "Zip," eight children and ten grandchildren.

DAIRY SCIENTISTS HONOR NORTH FOR DISTINGUISHED SERVICE

Robert H. North, executive vice president of the Milk Industry Foundation and the International Association of Ice Cream Manufacturers and a dairy industry leader for nearly 30 years, is the recipient of the coveted "Distinguished Service Award" for 1974 from the American Dairy Science Association.

The award is given annually to "honor a person who has contributed in an unusual and outstanding manner in the dairy industry." It was presented to Mr. North at the ADSA annual meeting, held recently in Guelph, Ontario, Canada.

In presenting the plaque to symbolize the awrd, Prof W. M. Roberts, ADSA Awards Selection Committee chairman described Mr. North as "a prime mover and active participant in practically every organization and activity that has been concerned with the welfare of the dairy industry. He has worked diligently to improve the market for dairy products, to train the personnel within the dairy industry to perform more effectively, and to serve as a watchdog over governmental agencies that promulgate laws and regulations of importance to the welfare of the dairy industry."

The American Dairy Science Association is a worldwide professional organization of educators, scientists, and industrialists concerned with the welfare of the dairy industry, and with the role the industry plays in fulfilling the economic and health requirements of the world's population.

Associated with the dairy industry in some manner throughout his adult life, Mr. North began as a staff member of the ice cream association in 1945 and was named chief staff executive in 1957. In 1964 he assumed the added responsibility of executive vice president of the Milk Industry Foundation. These two organizations represent the vast majority of ice cream manufacturing and fluid milk processor companies in the United States.

Mr. North also serves as an officer of the American Cultured Dairy Products Institute, the Dairy Training and Merchandising Institute, the Dairy Products Improvement Institute and the Dairy Industry Committee.

Before joining IAICM he was information specialist with the U. S. Department of Agriculture where he was responsible for such World War II food programs as the Eight Point Dairy Production Program, which increased milk production substantially; Crop Goals, to convert thousands of acres of corn to soybeans to provide needed food oils; and the Victory Garden and Home Food Preservation programs.

For three years before his USDA service, Mr. North was administrative assistant to the then Congressman Gehrmann of of Wisconsin.

"I can think of no person active in the dairy industry who has made greater contributions to the success of the total industry than Bob North," Prof. Roberts said in making the presentation.

P. R. ELLIKER GRANTED KRAFTCO TEACHING AWARD

Dr. P. R. Elliker, the recipient of the Kraftco Teaching Award in Dairy Manufacturing for 1974 was born in La Crosse, Wisconsin, on February 12, 1911. His family moved to New Glarus, Wisconsin, where he received his early schooling and was employed on several farms. His father, like his grandfather who had immigrated from Switzerland, was a minister. In 1922 the family of eight moved to Waukon, Iowa, where Elliker attended high school and distinguished himself scholastically and athletically. In 1930 Paul entered the University of Wisconsin where he won varsity letters in football and track. In 1934 he achieved the B.S. degree in bacteriology, writing a thesis on sporeforming lactic acid bacteria. He remained at the University of Wisconsin for graduate training in dairy bacteriology, receiving his M.S. degree in 1935 and his Ph.D. in 1937 under the guidance of Dr. W. C. Frazier and Dr. Walter V. Price.

Elliker's first full-length paper was published in the *Journal of Dairy Science* in 1938 and was entitled "Factors Affecting the Activity and Heat Resistance of Swiss Cheese Starter Cultures." Since then he has authored and coauthored over 200 scientific publications, including one well-known textbook, *Practical Dairy Bacteriology*. Many of Elliker's papers have appeared in the *Journal of Milk and Food Technology*.

Following graduation, the recipient was a Research Fellow at the University of Maryland and then returned to the University of Wisconsin as an instructor. From 1940 until 1947 he was assistant and associate professor of Microbiology at Purdue University; during this time for two years of World War II he was in the Army stationed at Fort Detrick, Maryland, engaged in bacteriological warfare research.

In 1947 Elliker became professor of Microbiology at Oregon State University and was named chairman of the department in 1952, which he holds today. He has been active in many professional organizations, including IAMFES which he has served in numerous ways, including membership on the Executive Board and as president. In 1954 he received the Borden Award for Dairy Manufacturing Research.

At Oregon State University, Dr. Elliker has distinguished himself in every way, receiving acclaim as a research director, administrator, and especially as a teacher, for which he is now being honored. He has insisted over the years on maintaining a close teaching and personal relationship with undergraduate students. His enthusiasm for the profession and his dedication to truth along with his personal attributes of humility, friendliness, and sensitivity to students have made him an outstanding teacher. He has valued his profession above personal gain.

The best way to share Elliker's greatness is to indicate what some of his students have written to support his nomination for this award One said, "He has done many great things for us, for example, asking our ideas on curriculum content and impressions of teachers, helping us to secure industry-related jobs while in college and permanent positions upon graduation, and always being available to help with any problem in our profession, years after we had graduated."

Another said, "He has not only distinguished himself as a fighter to obtain decent facilities for his students, a contender to maintain a balanced program of studies in both basic and applied courses, but also as a tremendous teacher. His teaching abilities begin in the classroom, where he is able to instill a sense of excitement within us for the subject matter at hand. He explains the basics and also illustrates their application so that we really sense a need to learn. But he goes beyond the classroom; his organized field trips are always profitable. He relates to us as individuals, our future is his main concern."

James Russell Lowell once described a great leader as "One who loved his charge but never loved to lead." Great teachers also display this trait of genuine concern for those they teach coupled with professional dedication and humility. Dr. P. R. Elliker is well known for these qualities.

DR. REDDY JOINS LEPRINO CHEESE COMPANY

Dr. M. S. Reddy, a recent graduate of the Department of Food Technology, Iowa State University, has joined the staff of the Research and Development Laboratories, Leprino Cheese Co., Denver, Colorado.

Reddy, a dairy microbiologist, was awarded the American Dairy Science Association Richard M. Hoyt Memorial Award, last June at Guelph, Ontario, Canada. This award recognized his outstanding research efforts in the area of starter cultures used in the production of foreign-variety cheeses and yogurt. He isolated and studied bacteriophages which attack these microorganisms and developed techniques which will enable dairy plant personnel, with relatively modest tools, to monitor the presence of phage in their plants. He, also, has worked extensively with direct plating techniques for studying lactic-group streptococci. As an outcome of these studies, Reddy has published twenty-two scientific papers in leading journals.

A native of Andhra-Pradesh, India, he is a member of the American Dairy Science Association, American Society for Microbiology, Society for Industrial Microbiology, Institute of Food Technologists, International Association of Milk, Food, and Environmental Sanitarians, Gamma Sigma Delta, and Sigma Xi.

His presence will greatly strengthen the current quality assurance and research and development programs of the Leprino Cheese Co.

TRENDS IN PACKAGING

"Trends In Packaging" the 9th Annual Symposium cosponsored by the Western New York Section of IFT and the New York State Agricultural Experiment Station, Geneva, New York. This one-day symposium will be held November 21, 1974 at the Sheraton Gatehouse Motor Inn in Rochester, New York.

The program is as follows:

Aseptic Bulk Storage of Partially Processed Foods –P. E. Nelson, Purdue University.

Aseptic Bulk Filling and Packaging–Lee Lange, Scholle Corporation.

Aseptic Packaging Using a Form, Fill and Seal Container–J. R. Chapman, Mead Packaging Company.

Sterilants for Commercial Aseptic Packaging–R. T. Toledo, University of Georgia.

Preparing Cold Sterilized Fruit Juices in Glass–H. C. Scott, Owens-Illinois.

Retortable Flexible Pouches–Commercial Possibility –K. R. Johnson, Rehmah Corporation.

A New Dimension in Packaging–W. P. Andrews, Reynolds Metal Co.

For further information, contact D. L. Downing, Dept. of Food Science & Technology, NYS Agric. Expt. Station, Geneva, NY 14456.

UNIVERSITY OF MARYLAND SHORT COURSES

November 13, 1974–University of Maryland 30th Annual Dairy Technology Conference, Center of Adult Education, College Park, Md.

January 6-15, 1975–University of Maryland 25th Annual Ice Cream short course, Department of Dairy Science, Animal Sciences Center, College Park, Md.

January 16, 1975–University of Maryland 25th Annual Ice Cream Conference, Center of Adult Education, College Park, Md.

March 12, 1975–University of Maryland Annual Cultured Products Symposium, Center of Adult Education, College Park, Md.

April 16, 1975–University of Maryland Soft Serve Conference, Center of Adult Education, College Park, Md.

For information, contact Dr. Ray King, Department of Dairy Science, College Park, Maryland 20742.

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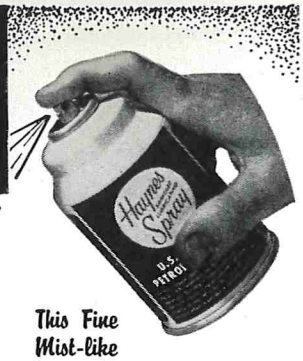

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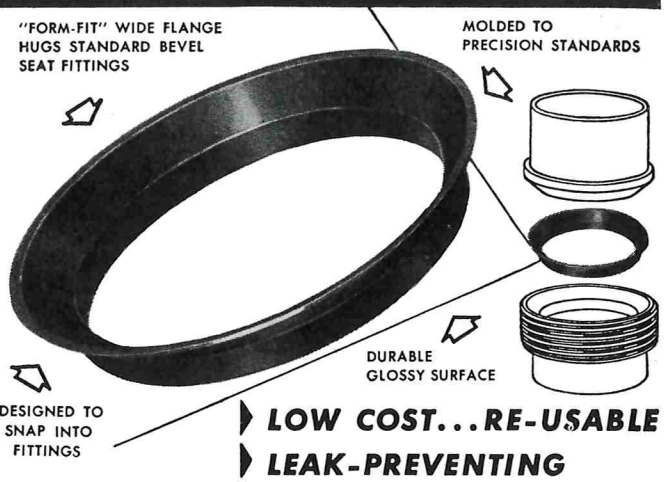

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Dairy authorities speak out on better cow milking

Dr. Elmer H. Marth
Department of Food Science,
University of Wisconsin/Madison

Rapid cooling can help maintain high quality of raw milk

The two most important things a dairyman can do to insure milk quality at his dairy are:

1. Put the cleanest possible milk into the bulk tank.
2. Cool it as quickly and efficiently as possible.

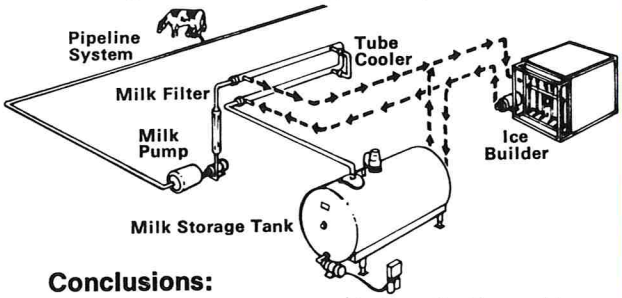
Much has been said about the necessity of sanitary milk handling through the entire milking operation. However, even under the most sanitary conditions, milk from a healthy cow will contain several hundred to several thousand bacteria per milliliter. Certain strains can cause undesirable conditions such as rancidity or other off-flavors unless their growth is retarded.

Follow the rules

The best way to retard bacterial growth is by cooling milk as rapidly as possible, without freezing it.

1. *Milk must be cooled promptly.* Delays result in bacterial growth. Some of the bacteria in milk can multiply in as little as 20 to 30 minutes if the milk is warm.
2. *Cooling should be rapid,* so further appreciable bacterial growth does not occur during the cooling process. Care must always be exercised so that milk does not freeze.
3. *Milk must be cooled to and maintained at a safe temperature.* Cooled milk must be held at a 40° F. and preferably 36-38° F. This temperature must be maintained throughout the storage period. When freshly drawn milk is added to milk already in the bulk tank, the rise in temperature of the initial milk must be minimal and the temperature of all milk in the tank must be rapidly reduced to 36-38° F. (Again, milk must not be frozen in the process.)
4. *Raw milk should not be stored for excessive periods* and should be moved from the bulk tank to the tank truck under conditions which preclude additional microbial contamination.

The refrigerated bulk cooling tank is the most widely used device to cool milk on the farm today. However, it is limited in its ability to meet some of the demands outlined above. New equipment available makes it possible to "pre-cool" milk on the way to the tank. Instant coolers using chilled water from an ice-builder help make rapid cooling practical on the farm. This type of cooling also eliminates the possibility of freezing milk.



Conclusions:

Even though all conditions needed for effective rapid cooling are met, some bacteria can still grow in refrigerated milk. Two points already discussed bear repeating. Be certain that good sanitary practices are followed during production of milk to insure that few bacteria of the kind able to grow at refrigeration temperatures are present. Do not hold raw milk refrigerated for excessive periods.

The successful marketing of milk depends on everyone doing his part at each step along the way. Even though you, as an individual dairyman, may not benefit *directly* from each of your efforts to improve sanitation and milk handling, you most certainly will benefit *indirectly* through greater total consumer acceptance of milk and dairy products thanks to fewer flavor problems and the absence of other negative factors caused by improper handling.

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