JULY, 1971 Vol. 34 P. 329-388 No. 7

Journal of

MILK and FOOD TECHNOLOGY,

58TH ANNUAL MEETING

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Official Publication

International Association of Milk, Food and Environmental Sanitarians, Inc.



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number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association,

ILK and FOOD

INCLUDING MILK AND FOOD SANITATION Official Publication

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

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Blue Ridge Rd., P. O. Box 437, Shelbyville, Ind. 2nd Class postage paid at Shelbyville, Indiana 46176.

EDITORIAL OFFICES: Dr. Elmer H. Marth, Dept. of Food Science, University of Wiscon-sin, Madison, Wis. 53706. H. L. Thomasson, Managing Editor, P. O. Box 437, Shelby-ville, Indiana 46176. Manuscripts: Correspondence regarding man-versity: and others modified model.

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ison, Wis. 53706. "Instruction to Contributors" can be ob-tained from the editor for the use of con-tributors of papers. Page Charge: Effective January 1, 1969 a charge of \$25.00 per printed page will be made for all research papers which are published See Volume 31, issues 10, 11, or 12 for details. Business Matters: Correspondence regarding business matters, advertising, subscriptions, orders for single copies, etc., should be ad-dressed to H. L. Thomasson (address above).

Subscription Rates: One volume per year. Individual non-members. Governmental and Commercial Organization subscription.

1 yr		\$12.00
Public and		
Libraries,	1 yr	\$10.00

Single Copies \$ 1.00

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FIFTY YEARS OF PROGRESS IN THE CHEESE INDUSTRY. A REVIEW

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(Received for publication October 22, 1970)

Abstract

Since 1920 the cheese industry has moved west and south in the United States. Milk production and procurement have changed from simple hand milking and wagon deliveries to machine milking of large herds and bulk handling of milk under supervision of industry and government agencies. Hand labor in factories has been reduced. Sanitary buildings and equipment, use of pasteurized milk, and selected cultures, starter concentrates, bacteriophage-inhibiting media, and improved enzymes have simplified biological control of the processes. Better warehouses and new methods of packaging provide better protection and merchandising.

In the past 50 years the development of process cheese has opened new markets and inspired the development of highly sophisticated methods of packaging and merchandising natural cheese. Movements of cheese on the primary markets from factory to warehouse for curing, and then to dealers, processors, institutions, chain stores, marketing associations, special outlets, and mail order distributors are more orderly. Supervised grading, public marketing organizations, and availability of complete information on market movements, prices, and other market statistics are essentials provided for modern merchandising.

The cheese industry still faces many problems involving production, net income control, utilization of by-products, development of new products, conservation of milk constituents, and methods of merchandising and marketing. Solution of these problems depends upon availability of and supporting facilities for men with training and skill to attack them. Continuing growth during the past 50 years clearly indicates that production of cheese and its related products and by-products is an expanding phase of the dairy industry.

This is a review of changes in the cheese industry in the United States since World War I. That time seems to be the end of the era of the one-man-andwife factory. Some of these changes resulted from providing markets for more dairy farmers; others were caused by pressures of competition, sanitation and health regulations, education, improved techniques and equipment, and the growing knowledge of the bacteriology and chemistry of cheese making.

This discussion will attempt to recall the physical characteristics of the industry of the early period, and then to consider changes in facilities, activities, and functions. Finally, it seems appropriate to mention some of the problems facing industry today, and to imagine possible trends of the industry in the near future. Adjustments and Expansion of the Factory System

In 1920 over 4,000 factories in the United States were making all kinds of cheese. Table 1 shows that numbers of plants in the U. S. 'making common kinds of cheese, other than Italian and Cottage cheese, have declined, yet the total production of important varieties as shown in Table 2 has increased; together they show that output per factory has increased remarkably. Table 3 shows that all areas of the U. S. since 1919 have increased production. Increases of ten-fold or more in the south central and south Atlantic areas were especially notable and indicated the dramatic changes in farming practices in those areas.

Farmers in north Atlantic states in 1920 were changing farm facilities and methods to meet higher standards of quality for city fluid milk markets. Cheese factories, which had originally helped develop these dairy farms by furnishing a market, were trying to adjust to the changing times. Some factories were so remote from city markets that they remained unaffected for years. Others tried to hold patrons by lowering standards of acceptable quality of milk, and paying more than good accounting practices could justify; they disappeared.

Some factories, whose patrons conformed to standards of city boards of health, began to make cheese in summer and ship fluid milk and cream to cities during winter months. Some of these factories were eventually purchased by milk marketing organizations to provide outlets for their own surplus milk. Others ultimately grew to receive several hundred thousand pounds of milk per day. In the flush season they made cheese around the clock with modern, mechanized processes, and did so with seasonal help trained and supervised by production managers who were often makers from the original plants. The milk sheds and milk marketing orders of the '60s were promoting the same type of adjustments and operations.

Some factories met city milk competition by making Italian cheese; they were largely responsible for the increased production of cheese in the early '30s. Still other factories converted to making soft unripened cheese, like Cottage, Bakers, Round and Farmers (Pressed) cheese, and their by-products of sweet and sour cream and cultured buttermilk. Eventually, many of these units merged into multi-product plants making the same and related products with the most modern equipment, laboratory controls, packaging facilities, and fleets of refrigerated trucks to procure milk and move highly perishable products long distances in interstate commerce.

By the late 1920s the areas of cheese production were expanding to the south Atlantic and south central states. State and federal specialists advised communities on factory construction and organization (81). Local business men often financed such ventures to replace the one crop cotton system. By 1930 the economies and future of large scale operations were apparent, and builders of new factories were making full use of them. Trucks, mechanical refrigeration, pasteurization of milk, and low costs of milk production in these developing areas made a profitable venture.

Leading promoters in southern areas were such organizations as Swift, Armour, Cudahy, and the Kraft Cheese Company. The Kraft Company established a strong educational program with fieldmen, and outstanding publications directed to farmers, farm youth, and plant operators. Dairying and cheese making prospered in the South.

Beginning about 1902, the growing needs of population in the far West, and the ideal conditions for dairying in Oregon's Tillamook Valley combined to sustain an outstanding factory system, and, eventually, an enduring cooperative marketing organization with a nationally known brand of high quality cheese, "Tillamook" (23).

FACTORIES

Typical factories in the 1920s seldom had more than 40 patrons, and few of these lived more than 2 miles from the factory. Only about 1 in 20 plants received over 4 million lb. of milk per year. Many closed in winter months (2).

Buildings were commonly built of wood, but most had concrete floors. A few factories still preserved the "kitchen" atmophere of earlier days with hardwood floors, rocking chairs, and window curtains in the make room. The maker and his family usually lived above or adjacent to the factory, and received rent and dairy products as part of his income. Animal pets were often in the make room.

All factories had certain common features. The room for the steam boiler and space for coal and wood fuel was adjacent to the make room. Only a few "self-heating" vats and Swiss kettles with fire boxes or movable fire wagons remained in use. Steam was used for heating and driving machinery. Gas engines were just beginning to replace steam engines.

Milk delivered by farmers was hoisted by a hand winch or lifted by hand to pour into the weigh can on a beam scale. It was sampled for fat testing or measured in gallons and then drained directly to vats or kettles through open conductor troughs at the end of which was usually a strainer or cotton bag. Composite samples were tested for fat by the maker or by a licensed tester at 2- or 4-week intervals. Some factories bought milk by weight or volume without testing.

Cheese vats with capacities of 300 to 500 gal (rarely 1,000 gal) were made of tinned steel or tinned copper and were mounted in wood or galvanized iron jackets. Other principal pieces of equipment included a whey separator, usually steam driven, curd mill, and a continuous pressure press. Power-driven curd mills and agitators were just being introduced in the early 1920s.

In Brick, Limburger, and Munster factories curd was dipped by hand from vats to wooden hoops (tinned steel for Munster) on cloth covered wooden draining tables. Bricks were used to press Brick cheese, which may account for the name. Cheese was salted in brine tanks or with dry salt on tables.

Swiss factories used copper kettles holding up to 300 gal of milk. Whey separators were used to skim a portion of the milk to be used for the cheese. Cheese was commonly made twice a day, which might have accounted for the high suicide rate of makers, according to local opinions. Power stirrers were used in the best equipped Swiss factories, but stirring was more often a hand operation.

Skimmed whey was pumped to tanks or barrels outside the factory, and patrons took it home in unwashed cans. Whey cream was churned to butter for patrons or sold to nearby creameries.

Curing rooms varied according to the cheese variety. American Cheddar factories had drying rooms with shelves, but usually little or no space for curing. Occasionally a connecting ice house with air vents to a storage room cooled it enough for curing. Artificial refrigeration was just beginning to be used by a few American factories.

Factories making American Cheddar cheese were paraffining, but not too skillfully, according to cheese judges. Warehouse operators preferred to receive shelf-dried cheese in boxes so that they could paraffin the cheese themselves for curing. Shipments were made to warehouses once or twice a week or as a load was accumulated.



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	Year				
Variety	1919	1939	1949	1959	1969
		(]	Numbers)		
American	2,206	2,284	1,682	1,059	826
(whole milk)					
Swiss	339	270	277	171	99
Brick & Munster	529	400	224	182	138
Limburger	167	102	44	13	8
Cream and					
Neufchatel ²	61	60	41	31	27
All Italian	64	88	156	182	188
Cottage, Pot					
and Bakers ³	489	1,695			_
Creamed Cottage		_	1,334	1,461	618
Cottage curd		_	1,738	1,407	628
Blue ⁴		-	22	13	12

TABLE 1. CHEESE FACTORIES REPORTING VARIETIES OF CHEESE MANUFACTURED IN THE UNITED STATES.¹

TABLE 2. TRENDS OF PRODUCTION OF IMPORTANT VARIETIES OF CHEESE IN THE UNITED STATES¹

	-	Year				
Variety	1919	1929	1939	1949	1959	1969
			- (Mill	ions lb.)	
American ²	295.1	370.3	537.3	935.0	942.2	1,266.4
Swiss	21.6	19.4	42.6	81.0	112.0	131.6
Brick ³	38.8	31.7	27.9	20.3	24.4	20.9
Munster ³		-	7.0	10.1	23.8	32.5
Limburger	7.9	8.6	9.0	7.2	3.7	2.9
Cream ⁴	5.6	34.4	48.0	58.5	90.5	115.4
Italian	4.4	5.9	20.5	55.1	140.8	360.5
Blue ⁵	_			8.1	12.7	21.0
Creamed						
Cottage ⁶	_		_	286.8	744.2	908.4
Cottage curd ⁶	-		-	283.6	578.2	673.3
Cottage, Pot,				£-		
Bakers	31.6	94.9	166.7			-
Others	11.7	7.5	11.9	27.9	19.1	23.4

¹Sources: Year Book of Agriculture, 1924, Table 466; U.S.D.A., Bur. Agr. Econ. Statistical Bull. 93; U.S.D.A. Statistical Reporting Service, Crop Reporting Board, Annual Report, "Production of Manufactured Dairy Products" Da 2-1(1970) Da 2-1(1960).

²Cream cheese plants only in 1969, 1959, 1949.

³Cottage, Pot, and Bakers reported as Creamed Cottage and Cottage curd after 1939.

⁴— indicates data not available.

Curing rooms in Swiss, Brick, Munster, and Limburger factories needed cool temperatures and high humidity, so these factories were often situated so that "cellars" could be built into a hillside and underground. In these curing rooms cheese was turned, salted, washed, and otherwise cared for until it was sold and moved to storage warehouses for market-Some years later, when ing or further curing. Blue cheese was introduced, it required high humidity and low temperatures for curing. Although refrigeration supplied these facilities in some operations, man-made caves with supplementary refrigeration had high humidity and were very successful (20). Italian cheese manufacturers introduced smoke rooms in factory operations.

EOUIPMENT

Receiving

As factories increased in size, they began to use the labor saving, receiving room equipment developed for the fluid milk industry: conveyors, recording scales, weigh cans, pumps, drop tanks, and samplers. Milk tank trucks delivered more of the milk as patrons adopted bulk milk tanks after World War II.

Receiving vats and holding tanks became necessary in factories to hold milk for treatments before making; they gave time for standardizing, and made factory operations more efficient for starting and stopping round-the-clock operations.

Filters and clarifiers were installed in milk lines

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	CHEESE 1	IN THE	ONTED	DIAILS		
			У	ear		
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			– (Mill	ions lb.)	
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¹Sources: U.S.D.A. Bur. Agric. Econ. Statistical Bull. 93: U.S.D.A. Statistical Reporting Service, Crop Reporting Board, Annual Reports of 1939 to 1969: "Production of Manufactured Dairy Products."

²Whole Milk Cheddar, Colby, Washed Curd, Jack, Monterey, Granular.

³Munster and Brick combined in 1919, 1929.

⁴Reported as Cream and Neufchatel in 1919, 1929 and in other years as Cream Cheese only.

⁵Blue Cheese included in "Others" before 1949.

⁶Reported as Cottage, Pot, and Bakers before 1949.

(7, 16, 34). Separating-clarifiers in the 1920s became standard equipment in the Swiss industry for improving eye formation, and controlling composition (44, 53).

Heat treatments

Pasteurization has been used increasingly since the late 1920s. The holder method was used for smaller operations and for making Brick cheese and certain minor varieties. Flash heating of milk to 165 F for an instant helped to make possible the development of the cheese industry in the southern

TABLE 3. TOTAL POUNDS OF CHEESE PRODUCED IN THE UNITED STATES, NOT INCLUDING FULL SKIM AND COTTAGE¹

	Year						
Area	1919	1929	1939	1949	1959	1969	
			(N	fillions)			
N. Atlantic	110.3	57.8	69.6	103.1	133.8	210.2	
S. Atlantic	0.2	1.4	1.1	2.2	6.5	12.9	
N. Central	333.3	363.5	513.6	899.6	1012.5	1484.8	
S. Central	2	14.2	55.6	114.7	137.2	153.3	
Western	28.7	46.9	68.5	80.4	91.5	124.7	
Total	473.6	483.9	708.5	1200.0	1381.6	1985.9	

¹Sources: U.S.D.A. Bur. Agr. Econ. Statistical Bull. 93; U.S.-D.A. Statistical Reporting Service, Crop Reporting Board, Annual Reports 1939 to 1969: "Production of Manufactured Dairy Products."

²Less than 3 plants per state reporting.



TABLE 4. CHEESE, PROCESSED, CHEESE FOODS, SPREADS AND COLD PACK¹ MADE IN THE U. S. 1957 - 1969².

	Process	ed cheese	Processed of spreads and	cheese foods, l cold pack ³		Total
Year	Plants	Production (1000 lb.)	Plants	Production (1000 lb.)	Plants	Production (1000 lb.)
1969	42	650,161	40	367,195	56	1,017,356
1965	44	631,312	46	228,517	62	859,829
1961	.43	509,333	45	221,727	58	731,060
1957	51	530,826	47	181,559	61	712,385

Processed cheese is made by pasteurizing, emulsifying, and blending natural cheese and contains no other ingredients. The rest of the products contain additional ingredients such as nonfat dry milk, dry whey, and condiments. ²Source: U.S.D.A., Statistical Reporting Service, Crop Reporting Board. Annual Reports, 1957 - 1969, "Production of Manufactured Dairy Products."

³Excludes spreads made from Neufchatel and Cream cheese.

TABLE 5. PROXIMATE IMPORTANCE OF MOVEMENTS¹ OF CHEESE FROM SELLERS TO BUYERS TO CONSUMERS.

		Wholesale b	ouyers				Retail	buyers
Dealer	Mktg. asso.	Proces- sor	Chain store	Retail stòre	Spec- ial	Institu- tions ²	Direct	Mail order
++++	++++	++++	++	++	++	+	++	++
++	+++	+++	++	++	++	+++	0	+
++	+ 2	+++	+++	+++	++	+++	0	++
+	+	++	++++	++++	+++	++++	0	++
0	0	+	0	+	+ -	++	++++ "	+
Õ	0	0	0	0	0	++	++++	+
õ	0	+	0	0	0	0	++++	++++
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Importance of movements: O = none; + = very little; ++ = significant; +++ = extensive; ++++ = large vol-

2"Institutions" includes: Government agencies, military forces, hotels hospitals, restaurants, schools, etc.

³"Dealers" are those who buy and sell directly or through brokers, and includes importers and exporters. ⁴"Special" includes roadside cheese stands, cheese specialty shops, gournet stores, mail order and novelty distributors.

states in the late 1920s and following years. Pasteurization was accepted slowly in northern states where buyers opposed it. Tubular "box-tube" heaters and surface coolers were used in a few Wisconsin factories in 1934. Modern, continuous, regenerative pasteurizing units were installed after 1945. "Vacreators" were used in some southern plants, and vacuum chambers were installed in some north-The smaller vacuum chambers were ern plants. used at the end of the holding section of continuous Substandard pasteurizing heat treatpasteurizers. ments of 145 to 150 F for around 20 sec became commonly used when cheese was made for holding at least 60 days at not less than 35 F before shipment in interstate commerce.

In the 1950s, treatment of milk at 125 F with up to 0.05% hydrogen peroxide, followed by addition of catalase to destroy residual peroxide, was accepted by the Food and Drug Administration and used to improve eye formation in Swiss cheese. Its use also was permitted in Cheddar and similar cheeses (76).

Cheese vats

In the late 1920s all-metal, stainless steel vats began to replace the old type tinned steel and tinned copper vats. Capacities of vats increased to 15,000

and 20,000 lb. Eventually, the high-sided horizontal vats and round vertical vats holding 30,000 lb., or more, were adopted after mechanical methods of curd handling in these deep vats were developed.

Wooden rakes disappeared when mechanical agitators, were introduced in the late 1920s. Some stirrers had several fixed stirrers per vat. The Mandel agitator, the prototype of modern machines, was introduced in 1922 (12). It rotated to stir while traveling back and forth lengthwise of the vat. Knives, forkers, and curd pushers were designed to fasten to this machine as mechanization developed. It thus became possible to design and operate the deep, horizontal vats.

The steam jets ("silent heaters") used years ago to move whey from vats to whey tanks and to pasteurize it at the same time, were replaced by pumps.

The wooden whey tanks and barrels for separated whey were replaced by steel tanks, and much of the whey, instead of going back to farms, was sold to condensing or drying plants. When whey began to be used for human food, it was handled with sanitary pumps, piping, milk tanks, and milk tank trucks as it was moved from factories to drying plant or condensery. More and more of the largest factories, in recent years, have begun to operate their



own whey processing facilities.

The finishing vat, a modern version of the ancient curd sink, was developed in the late 40s to use the making vats more often during each day's operation. Such finishing vats hold all the curd from a single vat at the time of dipping. Curd is pumped into the finishing vat for draining and cheddaring in the usual manner before milling and salting; it may be stirred without matting to make granular American. Agitators stir the milled (or granular) and salted curd, move it to the gate end, and push it into a hoop filler.



Figure 1. Walter V. Price, Professor-Emeritus of Food Science, University of Wisconsin.

The counterpart of the finishing vat for Cheddar was patented in the 1950s for Swiss and other kinds of cheese. These pressing vats drain, shape, and press the curd prepared in large vats or vertical tanks (68).

Still other devices to reduce labor in Cheddar making were perfected in the 1950s (66, 67, 77). One machine, the "Ched-O-Matic," received the partially drained curd from the making vat in a rotating, perforated drum which drained and dropped it into a rectangular cheddaring chamber in which it settled, drained, and matted together. It was removed, slice by slice, from the bottom of the chamber to pass through a curd mill, salting bath, and into the cheese hoops (45).

A labor saving Australian machine, "Bell-Siro," which consisted of a curd mill, a salt proportioning and distributing device, and a hoop filler was demonstrated successfully in the U. S. in the late 1950s. The cheddared curd was brought to the curd mill from the making vat so the machine released the vat sooner for the next batch of milk and simplified



Figure 2. P. H. Kasper Cheese Factory, Bear Creek, Wisconsin, 1920. (Courtesy of Marschall Division, Miles Laboratories, Inc.)

the ending operations of cheddar making (9, 10). This, and similar devices since developed ("Curd-A-Matic," "Cheddar-Rite") in the U. S. are being used in some of the largest factories.

Very recently another machine, Stoelting's "Cheesemaker," has been put into use. It receives partially drained curd at dipping, mats, and stretches it in a rotating drum to simulate the effects obtained in hand cheddaring. It delivers the cheddared curd in a long strip to the curd mill for subsequent salting and hooping.

Hoops

Tinned steel hoops with wooden followers of the 1920s have been largely replaced by hoops of stainless steel. The old type Wilson hoops have been redesigned to make blocks of cheese for packaging. Makers now use pre-cut single service bandages and

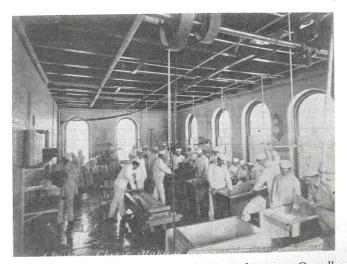


Figure 3. Cheddar cheese making. Laboratory, Cornell University about 1920.



Figure 4. Canned cheese. Cured in 8-oz aluminum-foil wrapped packages in container with gas-venting valve.

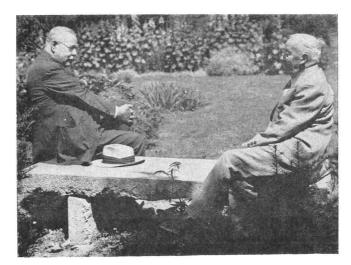


Figure 5. Bacteriologists H. L. Russell (left) and E. G. Hastings.

washing machines to clean press cloths.

Treatments of cheese, like Brick and Limburger, which are drained in hoops, have not changed greatly in principle, but draining tables, formerly made of wood, now are often made of stainless steel and provide temperature control. Stainless steel screens and hoops are replacing draining cloths and wooden hoops.

Cheese presses

The iron and wooden presses of 1920 gave continuous pressure by weights and lever mechanisms. Hydraulic pressure or automatically activated, electric motors provide continuous pressure in today's stainless steel machines. These represent tremendous advances over the sanitary conditions of the older devices.

Presses and draining tables, formerly located close to making vats, today may now be placed in an adjacent room or on a lower level to make better use of space and labor (50, 51).

Vacuum presses can now combine pressing and vacuum treatments of curd. The vacuum treatment of pre-pressed cheese is a new way to improve texture of Cheddar and Swiss (55).

Ripening

Changes in ripening facilities and practices have been made to meet marketing and sanitation requirements. Temperature and humidity controls are accurately and automatically regulated to suit the cheese. Curing storages are carefully constructed and protected to prevent rodent and insect infestations.

In the 1920s Cheddar cheese was made in many cylindrical styles of hoops ranging from 1-lb. midgets up to 75-lb. cheddars. To these forms have been added the 20- and 40-lb. blocks and, still later,

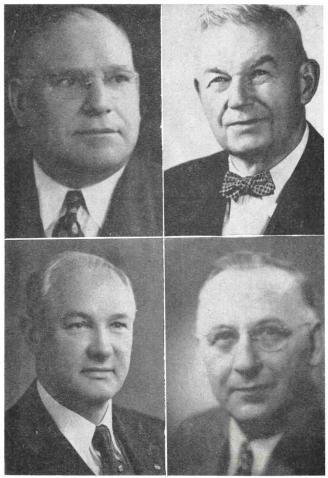


Figure 6. Upper left, N. C. Kraft, innovator; upper right, W. W. Fisk, teacher and author; lower left, E. C. Damrow, engineer; lower right, H. H. Sommer, scientist.

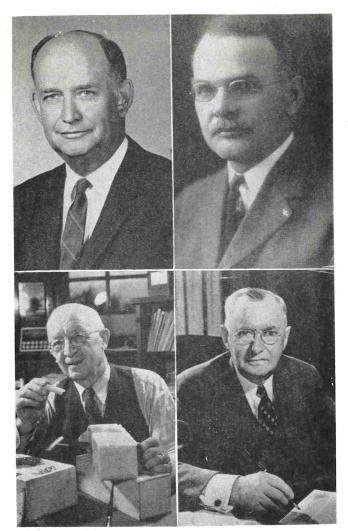


Figure 7. Upper left, J. B. Stine, inventor, director, FAO delegate; upper right, J. L. Sammis, teacher, researcher, counselor; lower left, A. T. Bruhn, judge, cheesemaker, supervisor; lower right, J. L. Kraft, founder.

the "barrels" in which freshly salted curd is packed, drained, settled or pressed, and cured, chiefly for processing (41).

All styles in 1920 were surface dried after they were taken from the hoops, then dipped in scale paraffin at 210 F, and cured in boxes. Now surfaces of cylindrical styles are protected by dipping the shelf-dried cheese in a mixture of paraffin and microcrystalline wax at 230 F to give more flexible, durable coating. The wax may be colored. These mixtures of paraffin and wax are blended to control flexibility and melting characteristics.

Blocks are wrapped in film and boxed for curing. Many small, firm, rounded styles are placed in flexible bags and sealed under vacuum for curing and merchandising. Some Italian varieties, like Provolone, are smoked before waxing; others like Romano, may be shelf cured for drying and smeared with vegetable oil and turned and rubbed frequently to

prevent mold growth. These rubbing procedures resemble the methods of shelf curing cheese practiced to a limited extent in the 1920s.

Curing temperatures may be as low as 29 F for long holding, 35 F for 60-day ripening, 55 F for shelf curing with frequent turning and rubbing (if not wrapped in film), or a combination of high and low temperatures for high flavor and long holding.

Milk Procurement

In 1920, farm facilities for producing milk for cheese were simple. Milk houses were rare. Cans of milk were cooled, if at all, on the barn floor in winter or in a watering trough in summer. "Aeration" over a surface cooler was used occasionally to remove "animal odors."

Milking was usually done by hand. Milking machines were available, but were hard to clean and sanitize. Makers regarded them with suspicion, and justifiably so. Milk was hauled to factories in wagons by farmers. A blanket or piece of canvas protected cans from heat, cold, and dust when necessary. Whey was hauled back to farms in the unwashed cans, and farmers were often given hot water to encourage them to empty and clean cans promptly. Some operators washed a few cans each day by hand scrubbing to give them a "real" cleaning.

CHANGES IN MILK PROCUREMENT

In principal cheese-making areas there have been changes in methods, equipment, and attitudes of personnel which make today's milk supplies far superior to those of 50 years ago. It is only necessary to list the points of improvement to identify these trends: milkers, milking machines, milk houses, cooling facilities, can washers, bulk tanks, transportation, and haulers who are trained to inspect and sample milk.

Improved methods and equipment have been introduced by education in colleges, high schools, short courses, field meetings, and practical on-the-spot demonstrations by educators, fieldmen, and regulatory agents. Far-sighted producers and operators have cooperated with authorities in formulating regulatory standards.

The standards and demands of fluid milk markets, which have opened up to farmers who made these improvements, are powerful challenges to both producers and factory operators as they view future trends of the cheese industry and the competitive status of other outlets for milk.

MILK QUALITY CONTROL

Farmers and cheese makers in 1920 knew that only

PRICE

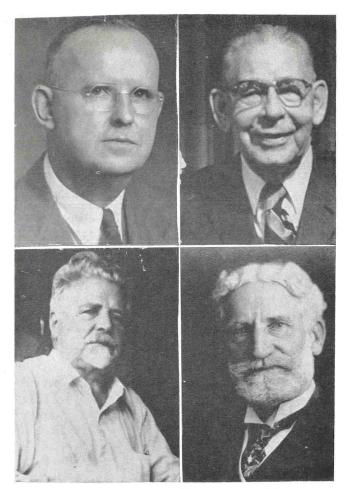


Figure 8. Top left, R. W. Leffler, secretary of the Wisconsin Cheese Exchange; top right, H. L. Wilson, national instructor; bottom left, Carl Marty, Sr., Swiss cheese maker; L. L. Van Slyke, research director.

the best milk would make the best cheese. But all farmers were not careful and all makers were not skillful. Competition for milk between factories often lowered standards of acceptability. The problem was more acute in 1920 because the coming of prohibition had wiped out the recognized outlet for undergrade cheese—the free lunch counters at the corner saloons.

Agricultural colleges held short courses to train farmers and makers. Cheese maker associations discussed the problem and remedies. State departments of agriculture called their inspectors "instructors" and told them to "educate" offenders. Dairy publications publicized these efforts. Cheese dealers sent procurement men to factories to act as trouble shooters. It seemed at times that all these efforts were only attempts to make the best possible cheese out of the worst possible milk.

Measuring quality

Farmers questioned the significance of the "nose test" which makers used at the intake. Some objective tests were available. Makers began to use them. These were the sediment test, curd test, and the methylene blue test.

The *sediment test* produced a pad on which insoluble material in the milk could be shown to the producer. The significance of the visible, as evidence of the invisible soluble material, was not generally appreciated. Better straining was the usual remedy.

Despite its limitations, the sediment test remains the first primitive test to demonstrate gross negligence; it is a part of the most recent definitions of minimum standards of milk for manufacturing (86).

The sediment test acquired added significance with the promulgation of the Food, Drug, and Cosmetic Act of 1939. According to the Act, evidence of filth in a food identified it as adulterated, and penalties were severe. Research disclosed ways to detect, identify, and measure critical materials (filth) in a food product, milk, and cheese (18, 24, 48, 52, 62, 65, 71, 75). Factories had to sign contracts releasing buyers of responsibility for extraneous matter in the factories' cheese. Warehouses established laboratories to test all cheese received. The cheese industry at once asked that cheese imports be required to meet these same standards. Milk clarifiers and filters, mostly the latter, were adopted in most factories.

The curd test was used in various forms before 1920 (61). Operators sampled the milk when it was delivered; they placed it in a clean, pint jar, coagulated it with rennet at 90 F, cut the curd, removed the whey, and held the ball of curd at 90 to 100 F for 12 to 16 hr. If, after that time, the curd had a pleasing acid odor, a curdy consistency, and was free from gas holes when sliced, it was satisfactory. Obviously, milk which contained large numbers of predominantly acid-producing bacteria was readily accepted.

The *acid test* was used by some operators to measure quality in 1920. It was discarded later. Without knowledge of titration values of the fresh milk, the maker penalized the producer of Jersey milk, if most of his producers had Holsteins; and conversely, he accepted low-solids milk with considerable acid development when the rest of his milk supply contained high percentages of milk solids.

The methylene blue test was first used by factories in the early 1920s (30). Some milk tested was so inferior that it decolorized as soon as the dye was mixed with the sample. Makers found that some lots of fast-reducing milk might make good cheese, but that the chances of success were better as the reduction time increased (35, 83). The U. S. D. A., Bureau of Dairy Industry, and the states of Ohio and Wisconsin, demonstrated the test successfully in the Swiss cheese quality program of the 1930s (59).

Makers modified the test by holding the tubes overnight at 98 F to see what fermentation they might expect in their cheese. When long reduction times were followed by gassy, digested curd, it took a lot of educating to convince them that an insignificant number of organisms could make such undesirable changes, and that they were of no importance in their starter-controlled manufacturing operations. This same problem appeared as makers began to see similar effects when curd tests were applied to the long-reducing milks. The methylene blue test was used in a few factories before 1940 to pay differentials for milk of high quality.

The *resazurin test* was introduced in the 1930s. Some makers liked it because it changed from blue to white faster than the methylene blue test (36, 37). Its other advantages were generally disregarded (56).

Both dye reduction tests were approved for use under Wisconsin's mimimum standards in the 1950s. The 1963 minimum standards recommended by federal agencies accepted the resazurin test only for this purpose (46).

Bacterial counts by the plate method have not been used by the cheese industry except in emergencies when they were required. The direct microscopic counts and examinations of milk have been used since 1920 by specialists to find high counts, types of organisms, and locate their probable sources (3, 80).

Coliform counts have been used in a few American Cheddar cheese factories to test pasteurized milk to be made into long-cured cheese. Cottage cheese operators, especially those associated with fluid milk operations, regularly use coliform counts to indicate quality control and shelf life.

Pasteurization and milk procurement

Opponents of pasteurization of milk for cheese argued that it would encourage the use of milk of inferior quality, such as they had seen used in developing cheese areas. When northern factories in the late 30s and early 40s were considering pasteurization, regulatory agencies warned operators that no lowering of standards for milk at the intake would be acceptable. Minimum standards, adopted later, defined these limits in enforceable regulations (85). Today's milk supplies for cheese making are far superior to those generally used by the industry in 1920.

Analyses in the Cheese Industry

Some tests have been added to the methods used in the factories of 50 years ago. Some are remarkably the same.

Tests of milk for fat, casein, and specific gravity

(lactometer) remain unchanged. The Mojonnier method for measuring total solids and fat in whey has been useful, but only in commercial laboratories and whey drying plants for control purposes. Casein tests by the Walker method are probably used less than tables for estimating casein in milk from the fat test, when casein/fat ratios are used in standardizing milk.

The rennet test, hot iron test, and acid test have remained unchanged; the first two are used less, the last is more widely used than ever to indicate progress in making, to measure activity of starters, and even to show the presence of high-acid producing organisms in starters for Italian and Swiss cheese.

The rennet test was a practical measure of the time required for 1 ml of rennet to coagulate a "cup" of milk (approximately 700 ml) at the setting temperature of 86 F. Time was measured as "spaces" marked on the inside of the cup from 0 near the top to 5 or 10 (depending on the cup chosen) at the bottom. A 1.5 ml hole in the bottom of the cup determined the rate of flow. Rennet was diluted with 15 ml of 86 F water and mixed with the milk when its level receded to 0 on the scale. When the milk curdled, the spaces exposed gave the desired measure of speed of coagulation. The rennet test was more sensitive to changes in acidity than the titration test so it was a valuable test to measure "ripeness" for setting. By using identical milk in separate tests, it compared relative strengths of different lots of rennet; this was important when shipments sometimes varied widely in strength.

The hot iron test was a practical measure of acidity of curd from dipping to salting in Cheddar cheese making. A handful of curd granules squeezed to form a ball of curd, or a piece cut from matted curd was used for sample. The lump of curd was touched lightly to a clean, smooth iron (pipe or spring steel) hot enough to make the curd stick without scorching. Drawing the curd smoothly and steadily from the iron formed silky threads between the iron and the curd. The length of these threads indicated acidity; the longer the threads, the lower the pH of the curd, and the relation was remarkably close.

The test for pH (hydrogen ion concentration) in cheese attracted attention in the 1930s (5, 78). It proved to be an excellent means of following acid changes in curd and cheese in making and curing. The quinhydrone electrode, and later the glass electrode were applied in a few factory operations to evaluate critical changes in curd and characteristics of cheese. Problems of keeping equipment in perfect operating condition has discouraged many operators who have tried the test in factory practice. Indicator papers have been only reasonably successful since the 1940s.

Analyses of cheese for fat and moisture, and, less often, salt and pH are routine measurements in laboratories of processing plants, warehouses, regulatory agencies, and larger factories. The Mojonnir apparatus and methods are used in some laboratories for fat and dry matter. Less well equipped factories still rely on warehouse laboratories or commercial services for analyses. Fat and moisture (dry matter) are of most interest.

Fat tests by the Babcock method (rather than the Mojonnier test) are essential for operators who are adjusting casein/fat ratios of milk for cheese making (53, 54). Federal regulations promulgated in 1941 permit standardization in Cheddar, Colby and Washed-curd, Swiss, and Italian cheese. Since then it has been accepted in general practice (76). Analyses are essential, however, and problems of accurate analyses may explain in part why the practice is not more general.

Moisture has become the basis of payment for cheese for curing and processing. Premiums increase with dry matter, but only enough to compensate for theoretical loss of yield. The Troy and steam-oven tests (27, 60, 73) of the 1920s for moisture have been replaced by quick approximations made under drying lamps, or rarely by the oil test (26, 27, 33). More elaborate and popular short tests permit weighing samples while they are being dried to "constant" weight (33, 43).

Drying ovens developed in recent years have ranged from a tabletop heater, convection ovens, and vacuum chambers to the accurately controlled, forced-draft ovens with capacities of several hundred samples. Ordinary variations in composition within a cheese and between cheeses of the same lot, as well as differences in equipment, are recognized. Official methods of A.O.A.C. for sampling and testing are accepted as the point of reference for all other equipment and techniques.

Salt analyses are of interest in quality control more than ever before, and began to attract attention about 1940. The indicator papers now available are reasonably good substitutes for the more complicated official methods or titrations of water extracts of cheese (4, 84).

STARTERS

Cheddar and Cottage cheese makers 50 years ago were using lactic starters which they obtained from their neighbors or purchased from commercial laboratories. The laboratories sold them "seed" cultures in liquid or powder form. One-quart milk bottles of fresh starter were also available from commercial laboratories which shipped them in ice by express to users at frequent intervals, sometimes daily. Regular customers for these starters were the makers of soft cheese and fermented milk products sold in the larger cities of the East.

Commercial "seed" starters in 15-20 ml bottles were propagated for a few days in milk held in fruit jars or milk bottles to make "mother" starters. They were then used to inoculate "shot-gun" cans or milk cans of bulk starter for cheese. Propagation of mother starters was sometimes continued day after day in the glass containers, but many operators merely saved a portion of the bulk starter each day to transfer to freshly heated milk for the next day's starter. Some operators claim to have carried the same starters this way for years. Heat treatments of starter milk in hot water or flowing steam varied widely, but generally approximated 175 F to 210 F# depending on the heating facilities. Fifty years ago incubation temperatures of about 72 F were maintained in a water bath or by holding in a place, usually the boiler room, with that approximate temperature.

Improved starter facilities were developed as size of factories increased. Limited access rooms with filtered air under slight pressure were built to isolate cultures and bulk starters from the hazards of airborne contamination and phage infections. Sterilization of milk for cultures was introduced along with pasteurizing tanks, some with flame or steam protected openings. Such precautions were unheard of before the 1940s when phage attacks first began to be recognized in larger U. S. factories, usually those practicing pasteurization and operating long hours each day. Bulk starters were further protected by metering them directly from starter rooms to vats through sanitized piping.

Bacteriophage infections caused severe losses in quality in the 1940s. Better sanitizing helped; so did rotation of cultures, a practice copied from the New Zealand experiences. But introduction of phosphated-milk starter medium, sold in powder form, proved most effective in controlling these losses when the research evidence was translated into commercial production of the anti-phage medium (28, 32).

Concentrates of selected cultures of pure Streptococcus lactis, S. thermophilus and Lactobacillus bulgaricus are now commercially available and distributed in cans held in liquid nitrogen. These cans are available in sizes large enough to inoculate up to 500 gal of prepared starter medium for use in 14 hr. Starter propagation can now be eliminated, if desired. The cultures for concentrates are chosen for proven performance in cheese making and are selected to provide rotations to minimize phage attacks.

Swiss starters, called "lab," were made 50 years ago by incubating pieces of dried rennet vels in warm whey overnight. This uncertain method of providing coagulator and acid culture was improved by the introduction of *Bacillus bulgaricus (Lactobacillus bulgaricus)* and *Bacterium propioni shermanii (Propionibacterium shermanii)* in the mid 1920s, and *Streptococcus thermophilus* starter in the early 1930s (21, 63). Swiss cheese quality showed dramatic improvements partly because of starters and partly because of the excellent, well-rounded, quality improvement program of federal and state specialists. These same cultures now are supplied in deep frozen form by commercial laboratories for use in Italian as well as Swiss cheese.

ENZYMES

Rennet

In 1920 rennet was available as liquid, powder, and tablets. It was made from salted vels of young calves. Unsalted vels, which had been inflated with air and dried, were used in small amounts by Swiss operators for "lab."

Salted vels, obtained generally from U. S. meat packers, were cleaned, dried, and extracted in strong sodium chloride brine. The extracts were blended, standardized, mixed with preservatives and color, and distributed in jugs, bottles, kegs, barrels, and more recently, in plastic containers.

In 1920 shipments of rennet varied in strength, so makers made rennet tests of old and new lots before deciding on amounts to use. Today, regular brands act alike when used at the same rates under comparable conditions; such standard strength came to be known as 100%. Double and triple strength preparations were developed for convenience in setting large vats of milk and to reduce shipping costs.

Rennet powders, made by salting out the proteins of rennet extract and then drying the precipitate, had a limited use in the U. S. but were made and exported until rennet for domestic use became scarce.

Rennet paste made from vels of calves, young goats, and lambs was imported from Italy by makers of Italian cheese to produce flavor as well as coagulation. Today these pastes are made by U. S. manufacturers to meet federal food standards.

When vels were scarce about 1919, pepsin was tried as a substitute for rennet but without too much success. Purified pepsin today is accepted for cheese making. It is usually mixed with an equal volume of rennet when used, and is commonly available in this 50-50 mixture.

Calcium chloride has been used since about 1935 as an aid to rennet coagulation. It is effective, aids in firming the curd, and when used in moderation, not over 0.02% (anhydrous salt) of the weight of the milk, it reduces rennet required without damaging quality, although it is followed by slightly slower rate of breakdown in the body of the young cheese (14). Weak solutions of rennet or pepsin or both have been combined with calcium chloride for use as "coagulators" for Cottage cheese. The "coagulator" became particularly well publicized about 1925 as the "secret" of making the so-called "Sweet-curd Cottage cheese." These solutions were offered first by organizations promoting the manufacture of this type of Cottage cheese. Profits were huge.

Bacterial sources of rennin have been recognized for many years as the cause of "sweet curdling" of some inferior milk. Recently techniques for producing desirable enzyme from cultures of selected organisms have been improved (19, 72). Some of these preparations are being tried with success on a limited scale in the U.S. Problems of uniform rates of coagulation, faulty flavors in the cheese, differences in protein changes, and low yields have been largely overcome in the best products.

Plant sources of milk coagulating enzymes have been tried for cheese in recent years, but the results have been unacceptable for use by critical cheese manufacturers.

Lipases

The preference of Italian makers for rennet paste was based on the mild, pleasing flavor of butyric acid it made in the cheese. Research (15, 29) discovered the lipases in the throat glands of lambs, young goats, and calves. These were concentrated and the preparations sold to duplicate the flavorproducing capacity of the paste. Knowledge of this source of the lipases made the use of rennet pastes obsolete. Blends of these enzymes are now widely used, sometimes mixed with desirable substrates, in Italian cheese and other foods.

Color

Cheese color is made and sold today much as it was 50 years ago. More exact colorimetric methods of standardizing now produce a more uniform product. It was restudied in the late 50s under F. D. A. supervision, as were other synthetic colors, and was approved for making and processing. Cheese color is an alkaline solution of the coloring substance (bixin) on the seeds of annatto (*Bixa orellana*). The colored form of vitamin A was tried about 1960 for coloring cheese, but apparently it could not compete with the less expensive annatto. Color intensity of cheese varies with the market and the variety. American Cheddar cheese with the natural, cream colored shade imparted by the milk fat is preferred in some northern markets, whereas the deep yellow-orange appeals particularly to southern markets. Processed American cheese manufacturers have chosen an intermediate shade between these extremes, as have the majority of manufacturers of Cheddar cheese for widest distribution.

Some kinds of cheese like Swiss and Limburger, are never colored. Other varieties, such as Blue and some Italian cheeses, are permitted by F. D. A. regulations to be made from milk with the natural color of the fat bleached with benzoyl peroxide, providing the vitamin A destroyed by bleaching is restored (76). Sage cheese, commonly made 50 years ago about Thanksgiving time, was usually colored a mottled green to suggest the green of the sage used to give it flavor.

No ways have been found to make annatto color resistant to such faults as acid cut, bleached, and mottled, which may appear in acid-defective or overcured cheese.

PACKAGING

Packaged rindless cheese in the 1920s was limited to a 3-oz unit of "snappy" ground Cheddar cheese wrapped in tinfoil. This was the prototype of today's Club cheese and cold pack cheese foods merchandised in porcelain crocks and as "chubs" in impermeable casing like sausages. Small paraffined prints or 2-lb. minature cheddars were available and convenient, but they were not rindless. Today, 2-lb. miniature cheddars are cut from large blocks, wrapped in a tight film and waxed to make a truly rindless cheese.

Loaf (process) cheese in 5-lb. tinfoil-covered units inspired the search for a "keeping" package for natural rindless cheese. Cans, with and without valves, were successful for curing and keeping but lacked continued sales appeal in market tests (11, 58, 82). Cellophane wrappers on cheese cut in stores or warehouses for daily use attracted buyers, but they molded rapidly.

Rindless natural Cheddar cheese began to be a reality when 20-lb. blocks were wrapped in double layers of clinging Pliofilm, held in a warm room in a pressure-pack box to "start" the fat and carbon dioxide, and then moved to curing storage. The cured cheese was printed, hand re-wrapped tightly in Pliofilm, and replaced in pressure-pack containers for merchandising. Mold-free life depended on many factors; it was acceptable, but variable (38, 39).

Eventually, heat-sealable, durable wrappers made

of laminated layers of cellophane + wax + foil + wax were developed for wrapping blocks of fresh cheese. Actually these most successful wrappers were practically identical to those perfected for process cheese when tinfoil could not be obtained during World War II. Pressure and heat applied to all sides of the film-wrapped cheese gave satisfactory cling and seal when the cheese was held in rigid containers.

Prints of the cured cheese were made and wrapped successfully with similar or transparent heat-sealed wrappers. Elaborate equipment for printing and wrapping in rooms with filtered air under pressure was developed to make and wrap these small units under the most practical aseptic conditions. Flushing of packages with inert gas or sealing under vacuum were more refined treatments developed later to assure keeping quality and mold-free life for successful merchandising. These packaging facilities were operated by large factories with marketing connections, by marketing organizations, cheese mailorder companies, and by processing plants.

The techniques of sealing in oxygen- and moistureimpermeable wrappers has been extended to packaging cheese cut from larger units like cheddars, barrels, and from previously wrapped and cured 90lb. blocks of Swiss. Some of the most successful units have been merchandised after wrapping in less expensive films to protect the surfaces and then covering the film with a heavy layer of flexible wax.

Every kind of cheese has its own special characteristics of shape, size, surface conditions, flavor, consistency, and inherent keeping quality or shelf life. All of these qualities are observed in using the packaging techniques and materials which have been developed during the past 30 years. The range of such characteristics is exemplified by packages of Creamed Cottage cheese in cups, semi-soft Gouda in wax and cellophane, blocks and slices of Cheddar and Swiss in impermeable, sealed wrappers, hard Romano in waxed foil or wax-covered wedges, and grated Parmesan in shaker containers.

Processing

J. L. Kraft in 1916 was granted the first of several U.S. patents covering the blending, grinding, heating, and packaging of cheese (40). The Phoenix Cheese Corporation later originated patents on heating with emulsifying salts to simplify the process (6, 13). The two companies merged. Other manufacturers attempted to produce "Loaf" cheese, as it was then called, but were discouraged, purchased, or absorbed by the patent owners. Eventually, as patents expired, many others entered the field. Production figures in the developing years of the industry

are not available, but Table 4 shows production gradually increasing in recent years, especially the production of cheese foods, cheese spreads, and cold pack (ground, unheated) cheese.

Some cheese manufacturers feared the process cheese industry might overwhelm the natural cheese industry, others welcomed it in the early 1920s. The consumers, however, liked the product. This approval might well have accounted for much of the increase in cheese consumption in the U.S. over the past 50 years.

The fact that the hot melted cheese sealed itself to the tinfoil liner of the boxes in which it was poured, produced a unique and successful package of rindless cheese which did not dry out nor develop mold as long as the seal was not broken. Some manufacturers of natural cheese attempted to coat blocks of original cheese with a thin layer of the processed product to duplicate this keeping seal when the package was covered with tinfoil. The package was not a commercial success.

Consumers liked the mild flavor, consistency, and slicing properties of the new cheese. Maintenance of these characteristics became the goal of the industry. It was achieved by the careful grading and classification of incoming cheese, and the judicious mixing of grades and classes in the blend for the final processing. Composition control was simplified by the use of cheese with low moisture (64).

Pasteurized process cheese manufacturers developed cheese foods and cheese spreads with softer consistencies, differing flavors, and new uses. Combinations of ripened cheese, like Limburger and Blue, with cream, Cream cheese, Neufchatel cheese, spices, pickles, cured meats, and other flavorful foods, provided a family of highly desirable products which have been heavily advertised. Much of the consumer favor can be attributed to the variety and quality of cheese products which were included in the field rations of soldiers during World War II.

Distribution of brand-name process cheese products in cities began with wagons and trucks owned by the manufacturer. Independent distributors were hired in some areas to do this work. All of the cheese products of the manufacturer, along with related food items, were sold from the "wagons." It became common practice for the route salesman to arrange his own display in the store; this was often regarded as unfair by other suppliers. But the system strengthened the market status of the products, and this is a common practice for suppliers of all products of a similar nature in today's highly competitive markets.

Chain stores handled the nationally advertised brand-name products, but frequently contracted with a "custom" process cheese manufacturer to furnish these products in packages bearing the brand name of the stores.

Processing takes a large share of the surplus cheese which is now purchased by the government and redistributed by the government in school lunch programs and for other purposes. The processors bid for the contract to do this work according to government specifications.

Processors of cheese eventually became leading distributors of all kinds of natural cheese in packages, and so extended and made full use of the facilities of their procurement, grading, curing, and marketing systems.

Leading processors have encouraged and supported cheese quality programs and research projects for the past 30 years. They have acted separately or joined with other industry members in supporting these programs when they have been sponsored by their trade association, the National Cheese Institute.

Processing of cheese has changed from its humble beginning with a steam-jacketed kettle and a delivery wagon to the complicated and highly technical facilities of today's manufacturing, packaging, and marketing operations.

PRODUCT MOVEMENT TO CONSUMERS

Sales of American cheese in the 1920s at the factory level were made by the factory owner or by a salesman acting for a farmer-owned factory. The buyer, who might act for himself, or as an agent for others, expected to cure the fresh cheese or move it on to a secondary market. Prices were based on sales reported on the Plymouth Board, or prevailing prices in New York City, or actions of local boards in centers of cheese production. Local boards were in general merely meeting places for buyers and sellers who frequently bargained privately and who might or might not announce the price agreed upon. Buying and selling periods were sometimes preceded by informal meetings at which trade conditions and production trends could be discussed. Prices of Swiss and minor varieties were arrived at by similar bargaining of individuals or committees of buyers and sellers.

The Wisconsin Cheese Producers' Federation was organized before World War I. It was a cooperative of factories which were either cooperatively or independently operated (85). It was formed to market the cheese of its members, who believed the returns would be greater than those achieved through the dealers who were performing the marketing function and who were guided by sales on the Plymouth Board. The Federation was endorsed by the Wisconsin Department of Markets, the Wisconsin College of Agriculture, the Wisconsin Dairymens' Association, the Wisconsin Livestock Association, and the Farm Bureau Federation.

The Federation expanded rapidly and successfully during the 1920s. It developed a reputation for high quality cheese, and controlled it by careful grading. Prizes were awarded to farmers, who delivered milk of high quality as measured by the methylene blue test, and to makers, who consistently produced cheese of the top grade. The Federation established warehouses in centers of cheese production, adopted a brand name-"Mello-Creme"-which it advertised. Eventually it extended its operations to include foreign types of cheese. It tried to perform the functions of the buyer, warehouse operator, and merchandiser. Overexpansion and large storage holdings during the period of falling prices in the lean years of the 1930s caused financial problems, reorganization and, eventually, association with the Land-O-Lakes Creameries, Inc. The cooperative marketing of cheese continues in the modern activities of several large cheese--producing cooperatives which participate in the cheesemerchandising program of the Land-O-Lakes organization.

The Plymouth Board became a dominant factor in cheese marketing. The boards in smaller areas, despite their rules to govern actions of buyers and sellers, gradually disappeared. In 1921 a Farmers' Call Board was organized in Plymouth, Wis. to compete with the Plymouth Board, and did so for 20 years. The Plymouth Board was reorganized in 1938 and became the Wisconsin Cheese Exchange. It is now operated according to rules devised, and revised many times, and constantly and critically scrutinized by state and federal marketing specialists. So, despite the historical criticisms through the years of collusion between buyers and sellers, conflicting interests, limited representations of producers, and sometimes small volumes of sales, the Exchange, which is now located at Green Bay, Wis., now provides an orderly method of recording public sales. It does reflect the play of forces of supply and demand, although it is unavoidably biased at times by the price support policies of the federal government (42).

The Wisconsin Cheese Exchange today is one of the sources of values on which milk prices are calculated for city fluid milk. It is commonly accepted practice to use the evidence of sales on the Exchange to aid in establishing prices, agreed upon privately, between factories and buyers in all parts of the U.S. for the common benefits of each contractor (49).

The cheese industry now receives daily, weekly, and monthly reports from the U.S.D.A. Statistical Reporting Service, Crop Reporting Board. These re-

ports include such information as prices, markets, production, storage holdings, transactions in leading markets and on the Wisconsin Cheese Exchange, as well as prices paid by factories to milk producers. These and other statistics provided by the same agency help to maintain fair and orderly marketing, a great improvement over the trade reports and gossip of the 1920s.

Regulations of sales of cheese were limited in 1920 to compliance with composition requirements. Grading regulations for American cheese were established in Wisconsin in 1924 in cooperation with the U.S.D.A. Modifications of these regulations still are used, although they have been broadened and revised according to trade practices (22). Grades now cover marketing of other varieties. The U.S.D.A. also has defined grades of cheese to assist in marketing on a national basis. The U.S.D.A. also may supervise actual production in order to certify grades for organizations which elect to use this voluntary service on a fee basis. Wisconsin's Department of Agriculture licenses graders at receiving points and checks their work by supervising graders, without charge. Wisconsin, and some other states, require identifying marks on cheese to show grades, age, factory, and identity.

Table 5 suggests the trend of cheese movements from factory to consumers, and the inter-relations of men and organizations who buy and sell as the product moves from factory to consumers.

CHEESE AS A FACTOR IN NUTRITION AND HEALTH

It is beyond the scope of this discussion to review the extensive evidence of the high nutritive valve of cheese that has been developed since 1920. The publications of the Food and Nutrition Board, National Research Council provide frequent revisions of recommended dietary allowances which provide basic information on the nutritive value of this healthful food (79).

Cheese has not been a serious frequent cause of food-borne illnesses since 1920, but it has not been blameless either. The epidemics of typhoid fever traced to fresh cheese in Indiana and California in 1944 raised questions which inspired other studies of pathogens (8, 17, 25, 47, 57). The industry generally accepted the necessity of pasteurization or aging of cheese as reasonable precautions to observe in addition to the supervision of the general health and techniques of employees. Gastroenteritis outbreaks in 1958 were traced to the presence of toxin of *Staphylococcus aureus* in Colby cheese (1, 31). The significance of S. *aureus* became a matter of concern to the industry as well as public health officials (69, 70). The necessity of proper acid development in manufacturing after the application of pasteurization to destroy the organisms before appreciable amounts of the heat-stable toxin could be produced was indicated (74, 87).

The healthfulness of our present milk supplies is better than ever. Pasteurization or its equivalent is more generally used today. Dairy farmers are more aware of the preventive methods to control mastitis and the dangers of indiscriminant use of antibiotics to control it. Bovine tuberculosis is also forgotten. Bang's disease is no longer widespread. Mastitis is commanding the corrective actions of sanitarians, herd managers, and veterinary doctors. The past record of cheese as a health hazard is not perfect, but today this valuable food is controlled and supervised better than ever before to keep it wholesome and healthful and to protect it on the way to the consumer.

PROGRESS AND PROBLEMS

Problems of the industry have developed with the years. It will only be necessary to point out some of them to show that they vary with the men, the factory, the environment, and the location of the business. Each individual who views the industry will see different problems and view many problems differently.

Milk

The farmer, who expects a fair return on his labor and investment gets lower returns when his milk is sold for cheese than for fluid milk. Sanitarians are working for production of milk of one quality—a quality good enough for any use. Cheese factory patrons who meet these standards, therefore, expect returns to be equal to those from any other outlet. This is a challenge to factory efficiency and marketing skill.

Men

Demands for greater efficiency in the industry must be met by technological improvement in methods and machines. Men to devise, develop, and use these improvements need technical and scientific knowledge. Management must know the principles involved to evaluate new and novel improvements. Today the training of men in operations and management is being left more and more to industrial personnel. Is this adequate?

Costs

Total costs of making cheese and moving it from factories through all stages of handling to the consumer are critically high. When the price of cheese equals or exceeds the price of meat, people in this country eat meat. Where can costs be cut: manufacturing, mechanization, better milk solids utilization, curing speed, packaging, merchandising?

Environment

Conditions inside as well as outside of factories can be changed to benefit employees and the community. Factories need safer floors, better sanitary practices, cleaner air, better humidity and temperature control, and lower noise levels. The industry needs more efficient ways to use water. The community needs a reduction of volume and B.O.D. content of wastes and effluents, and greater efforts by industry to make the factory a desirable, rather than a tolerated part of the total community environment.

FUTURE TRENDS

Those phases of the industry under stress today move inevitably to re-establish stability. This is the only reason for attempting to guess what those moves will be. I have chosen to look at these phases:

Milk

Fewer, larger farms, which are being established in areas of dairying, are more highly mechanized and are managed by more knowledgeable agri-business men. These agri-business men will increasingly challenge the industry's ability to buy their milk at a price to return costs of production plus profit. These men will not hesitate to change their own operations to achieve this goal.

Profit margin

Two courses of action are open to the cheese industry to increase its net income: (a) reduce costs of operation; and (b) increase income. The alternative is to move the industry to less highly organized, less competitive areas and so postpone the inevitable necessity of facing the cost squeeze. It is a fact that the industry today is exercising this alternative by continuing to move west and south. It could move north to locate in the wheat lands of Canada, just as it displaced the wheat farming of Wisconsin.

Reducing costs. Mechanization and automation will reduce costs only if total savings in costs of production exceed total costs of owning and operating equipment. I have seen some mechanized operations which I would guess do not achieve this objective. But there are enough operations which can be mechanized and automated in the industry to look to this possibility hopefully. This prospect is not so bright for factories with limited volume.

Economies obtained by cutting costs of supplies, re-use of coagulating enzymes, and speeding up making and ripening operations are possible, and in the process of development at this time.

Costs of pre-packaging cheese are now greater than

the costs of converting milk into cheese. The tail begins to wag the dog. A new system of preparing cheese for distribution is a reasonable point to attack. This may begin as a change in manufacturing to eliminate steps of the packaging routine, or a change in method of protecting the product as it moves to the consumer, or a change in merchandising practices at the point of consumer contact.

Finally, the ideas advanced in cheese seminars held in Wisconsin in recent years, are convincing evidence that more imaginative, efficient, lower-cost marketing and merchandising methods can and will increase returns to the industry.

Increasing income. History shows that some eastern factories have survived vigorous competition by making new products, accepting new methods of operation—even to becoming a part of the fluid milk marketing system.

In areas where milk can be converted into different products in flexible plants, the cheese industry faces problems of surpluses when returns from cheese making are based mainly on the price of cheese. Consequently, the stability and profitability of the industry must depend on its specialized ability to manufacture efficiently and to use by-products more profitably than the flexible plant.

The income from existing outlets for whey and whey products has reached a plateau for the most efficient manufacturers. New outlets will be needed to increase use of whey and income per unit. Such outlets will probably be for human food and drink. A most promising project would seem to be the perfection of a fermentation process to make proteinrich human foods from the carbohydrate-rich whey. Many others have been proposed. Regardless of the processes or projects chosen, the ultimate goal must be more complete utilization of the 50% of the solids of milk left in the whey after making cheese.

The diversification of cheese factory operations to produce protein analogs from soybeans may seem interesting because such processes have some features common to cheese manufacturing and marketing. It seems doubtful, however, that the cheese industry can now use its equipment and personnel more efficiently for producing such foods than other branches of the food industry that are already in advanced stages of production and test marketing in this field.

U.S. Milk Products Laboratory

There have been several attempts to establish a U.S. Milk Products Laboratory comparable to the U.S. Forest Products Laboratory. Now is the time to do it.

The need for information on milk, its constituents and its manufactured products and by-products is very evident today. The need to conserve and use all of the potential nutritive values of milk is clearly understood and is increasing.

Research efforts in nutrition, manufacturing, utilization of by-products, development of new products, and better ways to use existing products, conservation of food fractions, and more efficient waste disposal, are all important in today's economy. Now these research efforts, such as they are, are scattered among many institutions and agencies. There is little or no coordination and, consequently, a loss of efficiency and effectiveness.

The establishment of a U.S. Milk Products Laboratory can bring together and coordinate the activities of outstanding scientists from the U.S. and abroad. It can provide a place for training young men at the upperclass and graduate levels. It can be a source of information for adult education and extension service to encourage the use of research information and to keep the public informed as knowledge develops. It can maintain close ties with industry and encourage industrial financial support of special projects such as studies of processes, equipment, products, economics, and market acceptability.

The establishment and operation of a U.S. Milk Products Laboratory can strengthen the whole milk industry in its efforts to serve and feed the people of this country and the world.

Despite its problems in milk procurement, manufacturing, and merchandising, the cheese industry in the United States has used increasing amounts of milk in the past 40 years. In 1930 it took 11.5% of all milk used in manufactured dairy products; in 1969 it used 30%. Each year, for the past 6 years, the amount of milk used for cheese has set record highs, and this does not include the increasing amounts of milk used for Cottage cheese.

Cheese is made because it concentrates and preserves the most valuable milk constituents in a variety of food products which are chosen increasingly by the people of the United States. We can only look ahead with enthusiasm and confidence to a bright future for the cheese industry in the United States.

Acknowledgement

I gratefully acknowledge the inspiration, suggestions, and other help of Dr. Elmer Marth, and the generosity of my many friends who have supplied the illustrations.

References

1. Allen, V. D., and W. D. Stovall. 1960. Laboratory aspects of staphylococcal food poisoning from Colby cheese. J. Milk Food Technol. 23:271-274.

2. Bakken, H. H. 1930. American cheese factories in Wisconsin. Univ. Wis., Agr. Expt. Sta. Res. Bull. 100.

3. Breed, R. S. 1926. The microscopic appearance of

market milk and cream. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull. 120.

4. Breene, W. M., and W. V. Price. 1961. Dichlorofluorescein and potassium chromate as indicators in a titration test for salt in cheese. J. Dairy Sci. 44:722-725.

5. Brown, L. W., and W. V. Price. 1934. A study of the relationship between hydrogen ion concentration, titratable acidity, and quality in Cheddar cheese. J. Dairy Sci. 17:33-45.

6. Carpenter, L. E., and E. E. Eldredge. 1921. Cheese and process for sterilizing same. U. S. Pat. 1,389,577. Sept. 6.

7. Combs, W. B., W. H. Martin, and N. A. Hugglar. 1924. Clarification of milk for cheese making. J. Dairy Sci. 7:524-529.

8. Connelly, C. B., L. A. Black, and K. H. Lewis. 1964. Occurrence of coagulase-positive staphylococci in Cheddar cheese. Appl. Microbiol. 12:311-315.

9. Czulak, J. 1962. Cheese curd fusing machine. U. S. Pat. 3,060,575. Oct. 30.

10. Czulak, J., N. H. Freeman, and H. L. C. Chappan. 1958. Mechanization of milling, salting and hooping in Cheddar cheese manufacture. Australian J. Dairy Technol. 13:18-22.

11. Dahlberg, A. C., and J. C. Marquardt. 1942. Ripening cheese in cans. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull. 265.

12. Damrow Bros. Company. 1922. Advertisement of the Mandel cheese vat agitator. Butter Cheese Egg J. 5 April: 38.

Eldredge, E. E. 1921. Process of sterilizing cheese.
 U. S. Pat. 1,374,141. Apr. 5.

14. Ernstrom, C. A., W. V. Price, and A. M. Swanson. 1958. Effects of reducing rennet and adding calcium chloride on the manufacture and curing of Cheddar cheese. J. Dairy Sci. 41:61-69.

15. Farnham, M. G. 1950. A cheese modifying enzyme. U. S. Pat. 2,531,329. Nov. 21.

16. Fisk, W. W., and W. V. Price. 1923. The clarification of milk for cheese making. N. Y. (Cornell) Agr. Expt. Sta. Bull. 418.

17. Foley, A. R. 1932. Epidemic of typhoid fever in the Saint Maurice Valley. 11th Ann. Rept., Prov. Bur. Health, Quebec. p. 25-27.

18. Food and Drug Administration, Federal Security Agency. 1944. Microanalysis of food and drug products. Food and Drug Circ. No. 1. Supt. of Doc. Govt. Printing Off., Washington, D. C.

19. Food and Drug Administration. 1969. Title 21, Food and Drugs Chap. 1, Part 19. Certain cheeses, identity, standards. Order regarding use of additional safe, suitable milk-clotting enzymes. Fed. Reg. 34:14596.

20. Frazier, J. 1960. A treasure in Blue cheese. Milk Prod. J. 51 (1):7-9, 15.

21. Frazier, W. C., H. F. Long, and W. T. Johnson, Jr. 1936. The bacteriology of Swiss cheese. V. The use of *Streptococcus thermophilus* in ripening milk for Swiss cheese. J. Dairy Sci. 19:535-539.

22. Fryhoffer, C. W., and R. C. Potts. 1923. Handbook for use in the inspection of whole-milk American cheese under the Food Products Inspection Law. U.S.D.A., Office of the Secretary, Circular 157.

23. George, J. S. 1950. The Tillamook cheese plant. Food Ind. 22 (7):44-47, 102-105.

24. Gibson, A. L. 1947. Extraneous and other matters in cheese. Can. Dairy Ice Cream J. 26 (4):40-42.

25. Gilman, H. L., A. C. Dahlberg, and J. C. Marquardt. 1946. The occurrence and survival of *Brucella abortus* in Cheddar and Limburger cheese. J. Dairy Sci. 29:71-85. 26. Gould, I. A. 1937. A comparative study of methods of determining moisture content of Cheddar cheese. I. The modified Mojonnier method and olive oil methods. J. Dairy Sci. 20:625-635.

27. Gould, I. A. 1938. A comparative study of methods of determining moisture in Cheddar cheese. II. The steam oven method at high pressure and the olive oil methods. J. Dairy Sci. 21:379-383.

28. Hargrove, R. E., F. E. McDonough, and R. P. Tittsler. 1961. Phosphate heat treatment of milk to prevent bacteriophage proliferation in lactic starter. J. Dairy Sci. 44:1799-1810.

29. Harper, W. J., and I. A. Gould. 1955. Lipase systems used in the manufacture of Italian cheese. I. General characteristics. J. Dairy Sci. 38:87-95.

36. Hastings, E. G., and M. Mansfield. 1926. The methylene blue test. Its value in cheese factory practice. Univ. Wis., College of Agr., Ext. Serv. Circ. 204.

31. Hendricks, S. L., R. A. Belknap, and W. J. Hausler, Jr. 1959. Staphylococcal food intoxication due to Cheddar cheese. I. Epidemiology. J. Milk Food Technol. 22:313-317.

32. Henning, D. R., W. E. Sandine, P. R. Elliker, and H. A. Hays. 1965. Studies with a bacteriophage inhibitory medium. I. Inhibition of phage and growth of single strain lactic streptococci and leuconostoc. J. Milk Food Technol. 28:273-276.

33. Hoeflin, M. E., and J. J. Sheuring. 1960. Comparison of the Cenco, Ohaus, and Mojonnier methods for determining total solids of dairy products. J. Dairy Sci. 43:848 (M42).

34. Hood, E. G., and I. Hylnka. 1946. Clarifying milk for cheese making. Natl. Butter Cheese J. 37 (1):38 and 37 (2):39.

35. Horral, B. E., and E. E. Kihlstrum. 1958. A study of clean milk for aged Cheddar cheese. J. Milk Food Technol. 21:246-252.

36. Johns, C. K. 1942. The behavior of resazurin in milk. Can. J. Res. 20:336-346.

37. Johns, C. K., and R. K. Howson. 1940. A modified resazurin test for the accurate estimation of milk quality. J. Milk Technol. 3:320-325.

38. Jones, L. H. 1946. Factory phases of natural cheese packaging growing more important. Cheese Reporter 1 Nov.: 3.

39. Jones, L. H. 1947. Cheddar type cheese cured under pressure in air-free state in container lined with thin, limp, moisture-proof and oil resistant material. U. S. Pat. 2,424,693. July 29.

40. Kraft, J. L. 1916. Process of sterilizing cheese. U. S. Pat. 1,186,524. Reissue 14,777. 23 Dec., 1919.

41. Krueger, G. E., H. F. Mohr, and O. C. Huber, Jr. 1967.

Cheese manufacturing apparatus. U. S. Pat. 3,355,805. Dec. 5. 42. Leffler, R. W. 1954. The Wisconsin Cheese Exchange. J. Dairy Sci. 37:909-912.

43. Livak, C. W., and F. J. Doan. 1945. The determination of total solids in dairy products by drying with forced, heated air. I. Results with milk, skimmilk, cream, condensed milk, condensed skimmilk and evaporated milk. J. Dairy Sci. 28:711-720.

44. Matheson, K. J. 1923. New developments in the manufacture of Swiss cheese. Proc. World's Dairy Congress. Vol. 1: 292-296.

45. McAdam, W. W. 1963. Method for making cheese. U. S. Pat. 3,078,169. Feb. 19.

46. Meister, H. E. 1970. Federal-State quality program for manufactured milk. J. Milk Food Technol. 33:277-279.

47. Mickelsen, R., V. D. Foltz, W. H. Martin, and C. A. Hunter. 1961. The incidence of potentially pathogenic staphylococci in dairy products at the consumer level. II. Cheese. J. Milk Food Technol. 24:342-345.

48. Miersch, R., and W. V. Price. 1944. Testing for extraneous matter in cheese. J. Dairy Sci. 27:881-895.

49. Miller, A. H. 1949. Pricing American cheese at Wisconsin factories. Univ. Wis. Agr. Expt. Sta. Res. Bull. 163.

50. Miollis, R. 1943. Methods and apparatus for making cheese. U. S. Patent 2,324,636. July 20.

51. Miollis, R. 1950. Cheese making method. U. S. Pat. 2,505,984. May 2.

52. Murphy, F. D., and C. K. Johns. 1960. Testing cheese for extraneous matter. Can. Dairy Ice Cream J. 39(10):28, 30, 32, and 34.

53. Price, W. V., and G. C. North. 1935. The mechanical control of the fat content of Swiss cheese. J. Dairy Sci. 18: 149-161.

54. Price, W. V., and H. E. Calbert. 1956. Standardizing milk for cheese making. Univ. Wis. Agr. Expt. Sta. Ext. Serv. (Revised.) Circ. 408.

55. Price, W. V., N. F. Olson, and A. Grimstad. 1965. Vacuum treatments of cheese. Wis. Agr. Expt. Sta. Spec. Bull. 10.

56. Ramsdell, G. A., W. T. Johnson, Jr., and F. R. Evans. 1935. Investigation of resazurin as an indicator of the sanitary condition of milk. J. Dairy Sci. 18:705-717.

57. Rice, T. B. 1944. Typhoid epidemic in northern
Indiana. Ind. State Bd. Health Monthly Bull. 47 (2):29.
58. Rogers, L. A. 1932. Ripening cheese in a sealed

package. J. Dairy Sci. 15:185-189.
59. Rogers, L. A., R. E. Hardell, and F. Feutz. 1939. The relation of the quality of milk to the grade of Swiss cheese.
J. Dairy Sci. 22:43-53.

60. Sammis, J. L. 1917. The moisture test in the cheese factory. Univ. Wis. Agr. Expt. Sta. Ext. Serv. Circ. 81.

61. Sammis, J. L. 1941. Cheese making. 4th ed. Cheese Maker Book Company, Madison, Wis., p. 82 (17B).

62. Scott, D. B. 1954. The identification of dung and plant fragments in dairy products. J. Ass. Off. Agr. Chem. 37:177-181.

63. Sherman, J. M. 1923. The use of bacterial cultures for controlling the fermentation in Emmental cheese. Proc. World's Dairy Congress. Vol. 1: 287-290.

64. Sommer, H. H., and H. L. Templeton. 1939. The making of processed cheese. Univ. Wis. Agr. Expt. Sta. Res. Bull. 137.

65. Spicer, D. W., and W. V. Price. 1938. A test for extraneous matter in cheese. J. Dairy Sci. 21:1-6.

66. Stine, J. B. 1950. Cheese manufacture. U. S. Pat. 2,494,637. Jan. 17.

67. Stine, J. B. 1950. Manufacture of American type cheese. U. S. Pat. 2,494,638. Jan. 17.

68. Stine, J. B. 1950. Emmenthaler cheese. U. S. Pat. 2,494,636. Jan. 17.

69. Takahashi, I., and C. K. Johns. 1959. *Staphylococcus aureus* in Cheddar cheese. J. Dairy Sci. 42:1032-1037.

70. Thatcher, F. S., R. D. Comtos, Doreen Ross, and I. E. Erdman. 1959. *Staphylococcus* in cheese: Some public

health aspects. Can. J. Public Health 50 (12):497-503.

71. Thatcher, F. S., W. Simon, and C. Walters. 1956. Extraneous matter and bacteria of public health significance in cheese. Can. J. Public Health. 35 (6):234-243.

72. Tsuogo, T., K. Taniguchi, K. Yoshino, A. Ozawa, and K. Arima. 1964. Studies on cheese making using milk clotting enzyme *Mucor pusillus* Lindt. II. Cheese making experiment. Jap. J. Zootech, Sci. 35 (4):229-237 (Dairy Sci. Abstr. 27: No. 929. 1965).

73. Troy, H. C. 1917. A cheese moisture test. N. Y. (Cornell) Agr. Expt. Sta. Ext. Bull. 17.

74. Tuckey, S. L., M. E. Stiles, Z. J. Ordal, and L. D. Witter. 1964. Relation of cheese-making operations to survival and growth of *Staphylococcus aureus* in different varieties of cheese. J. Dairy Sci. 47:604-611.

75. Turner, A. W., B. Rogers, and V. Conquest. 1942. Orthophosphoric acid as a cheese solvent. J. Dairy Sci. 25:777-778.

76. United States Department of Agriculture, Consumer and Marketing Service. 1966. A summary of laws and regulations affecting the cheese industry. Agriculture Handbook No. 265.

77. Walter, H. E., A. M. Sadler, J. P. Malkames, and C. D. Mitchell. 1957. Method of manufacturing cheese. U. S. Pat. 2,796,351. June 18.

78. Watson, P. D. 1927. Use of quinhydrone electrode for following changes of pH in Swiss cheese. Ind. Eng. Chem. 19:1272-1275.

79. Watt, B. K. and A. Merrill. 1950. Composition of foods-raw, processed and prepared. U.S.D.A. Bur. Human Nutrition and Home Econ., Agr. Res. Admin., Agr. Handbook No. 8.

80. Whiting, W. A. 1923. The relation between the clumps of bacteria found in market milk and the flora of dairy utensils. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull. 98. 81. Wilson, H. L. 1928. Points to consider in establish-

ing a cheese factory. U.S.D.A. Misc. Publ. 42.

82. Wilson, H. L. 1940. Packaging sliced Cheddar and Swiss cheese in cans for sandwich dispensers. U.S.D.A. Misc. Publ. 386.

83. Wilson, H. L., S. A. Hall, and L. A. Rogers. 1945. The manufacture of Cheddar cheese from pasteurized milk. J. Dairy Sci. 28:187-200.

84. Wilster, G. H., W. V. Price, A. J. Morris, E. F. Goss, and G. P. Sanders. Amer. Dairy Sci. Ass. Sub-committee on cheese analysis. 1937. Determination of fat, moisture, and salt in hard cheese. J. Dairy Sci. 20:27-30.

85. Wisconsin Department of Farms and Markets. 1925. The history and accomplishments of the Wisconsin Cheese Producers' Federation. Wis. Dept. Farms and Mark. Bull. Vol. VI, No. 5.

86. Wisconsin State Department of Agriculture. 1960. Wisconsin Administrative Code, Chapter Ag. 30. Minimum standards for manufacturing milk. p. 49-56c.

87. Zehren, V. L., and V. F. Zehren. 1968. Relation of acid development during cheese making to development of staphylococcal enterotoxin A. J. Dairy Sci. 51:645-649.

A Research Note

THE EFFECT OF RETORT COOKING PRESSURE AND BREADING TECHNIQUE ON YIELD AND PER CENT BREADING OF BROILER PARTS

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(Received for publication February 8, 1971)

Abstract

An experiment was conducted to determine the effect of breading techniques and retort cooking pressure on broiler parts. The cooking pressures used were 0 and 0.36 kg/cm². There was a slight increase in cooking yield of thigh parts when the cooking pressure was increased. The parts gave significantly different cooking yields. One-half of the parts were given a single breading and the other one-half were double breaded. The second breading increased the breading on the parts by 6.9%. Double breaded parts showed an increase of 6.4% product yield over the single breaded parts.

There are many questions still unanswered about the most economical methods for preparing precooked poultry parts. Hale (1) reported that the yield after breading of precooked parts averaged 90-110% depending on the amount and type of precooking and the viscosity of the batter. Yingst (7) found that as the retort cooking pressure of breaded parts was increased from 0 to 1.05 kg/cm2 there was a decrease in cooking yield from 92.0 to 88.6%. Cooking chicken wrapped in aluminum foil in an autoclave at 1.05 kg/cm² for 20 min resulted in an average cooking loss of 24.4% (4). Winter and Clements (6) obtained similar results from 10-week old Delaware \times New Hampshire cross broilers. The leg and thigh parts lost more weight (24.5%) followed by breasts (23.8%) and wings (17.4%). Webb and Goodwin (5) found the breast retained a higher percentage of batter than did either the thigh or drum.

The purpose of this study was to compare the effect of cooking pressure and single versus double breading on the yield of various broiler parts.

MATERIALS AND METHODS

The cooking pressures used were 0 (atmospheric) and 0.36 kg/cm^2 . The breading techniques studied were single versus double breading. Fifty broilers were obtained from the cut-up line of a local processor. The parts, consisting of breasts, drums, thighs, and wings, were ice packed and held in a 3 C walk-in cooler until they were cooked. The parts were weighed and breaded using a commercial wheat breading. The breading was applied using a drum type breading machine and then the parts were reweighed. They were then placed on cooking racks in a preheated autoclave. Ten pieces of each part were cooked together and each treatment was replicated five times. The parts were

	Cooked yield (per cent)	Product yield (per cent)	Breading (per cent)
Grand mean	93.5	101.6	21.9
Cooking pressure			
0 kg/cm^2	93.0a	101.2a	22.3a
0.36 kg/cm^2	94.0b	101.9a	21.4a
Part			
Breast	95.6c	106.0c	24.6d
Drum	89.7a	95.2a	19.1a
Thigh	93.1b	101.0b	22.9c
Wing	95.6c	104.0c	20.9b
Breading technique			
Double	94.1a	104.8a	25.3a
Single	92.8b	98.4b	18.4b

TABLE 1. TREATMENT MEANS¹

¹Means within the same column and factor not followed by the same letter differ significantly (P <.05).

Table 2. Part \times pressure interaction for cooking yield¹

		Pressure
Part	0 kg/cm^2	0.36 kg/cm ²
Breast	95.3c	95.9c
Drum	89.6a	89.7a
Thigh	91.6b	94.6c
Wing	95.4c	95.7c

¹Means followed by different letters differ significantly (P < .05).

TABLE 3. ANALYSIS OF VARIANCE FOR TASTE PANEL EVALUATION .

Treatment	DF	Batter adhesion MS	Texture MS	Flavor MS	General accept- ability
Cooking method (A)	1	0.06	0.04	0.009	0.00
Breading (B)	1	0.80**	1.33**	1.40**	1.47^{**}
$A \times B$	1	0.06	0.07	0.008	0.00
Error	12	0.03	0.02	0.07	0.07

Significance

**P <.01

cooked to an internal temperature of 85 C. Preliminary work indicated that at atmospheric pressure, 20 min cooking time was required to reach the desired end point temperature as measured by a themister probe inserted in the thickest area of the breast. When parts were cooked utilizing 0,36 kg/cm² of pressure, steam was shut off after 12 min of cooking time had elapsed, and the fast exhaust valve was opened for 2 min for a total cooking time of 14 min in the autoclave. After cooking, the parts were weighed to determine cooking loss. Also, one-half of the parts were given a second breading immediately after they were taken out of the autoclave. The parts were then placed in polyethylene bags and blast frozen at -20 C. They were held in a household type freezer until further tests were conducted.

An eight member taste panel evaluated 10 parts from each treatment. The parts were browned in hot oil at 163 C for 5 min prior to panel evaluation. The breasts were cut into bite size portions to be tasted and the remaining parts were displayed for evaluation of breading characteristics. The panel was asked to evaluate the pieces for texture of breading, adhesion of breading, flavor, and general acceptability using a five-point scale.

The per cent breading was determined on the remaining 15 pieces in each treatment by the method described by May et al. (2). Statistical procedures were factoral analysis of variance and Duncan's new multiple range test as described by Steel and Torrie (3).

RESULTS AND DISCUSSION

The cooking yield was calculated by dividing the cooked weight by the precooking weight. Cooking method, part, and breading technique all had a significant effect (P < 0.01) on the cooked yield. The parts cooked using 0.36 kg/cm² averaged 94.0% yield while the 0 pressured parts averaged 93.0% yield. There also was a significant pressure \times part interaction (Table 2). The thigh part alone accounted for the higher mean cooked yield of the parts cooked at 0.36 kg/cm². The reason for this result is unknown at this time. The breast (95.6%) and wing (95.6%) gave the highest yield followed by the thigh (93.1%) and drum (89.7%) (Table 1). Parts with thinner muscle tissue would reach a higher temperature when cooked for the same length of time as parts with thicker muscle tissue, resulting in more cook out of juices from the thinner parts. The breading on the parts absorbs some of the juices during cooking. Yingst (6) reported higher cooked yields from breaded as compared to unbreaded parts. The breast picks up the most breading and thus could retain a higher percentage of the juices cooked out of the muscle and skin tissues.

The parts which were breaded both before and after cooking averaged 94.2% cooked yield and the single breaded parts averaged 92.8%. The parts that were given the second breading were weighed and rebreaded before the other parts were reweighed after cooking. Approximately 10 min were required to weigh and rebread the parts. Thus, this difference is caused by evaporation and drip loss. Whether or not this loss could be prevented by immediate cooling or freezing after cooking is not known at this time.

The per cent breading was calculated as follows:

Prewashed weight - Washed weight \times 100 Prewashed weight

10

Part and breading techniques had a significant effect on per cent breading. There were no significant interactions. The breast had the highest per cent breading (24.6%) followed by the thigh (22.9%), wing (20.9%), and drum (19.1%). The surface area and cavities of the various parts are different and account for the differences in batter uptake. The double breaded parts averaged 25.3% breading while the single breaded parts averaged 18.4% (Table 1). When the parts were removed from the autoclave, they were very moist and sticky. Thus, when the second breading was applied there was a considerable increase in per cent breading (6.9%). On the breast pieces there was some problem with doughiness as the result of too much breading. Double breading the wing and drum parts while single breading the breast and thigh parts would produce desirable products. This procedure would give a comparable per cent breading on all of the parts.

The product yield was calculated by dividing the final weight of the part by the raw weight. Part and breading method had a significant effect on product yield. The second breading increased the yield by an average 6.9%. The breast (106.0%) and wing (104.0%) gave the highest yield followed by the thigh (101.0%) and then the drum (95.2%) (Table 1).

The taste panel preferred the double breaded parts to the single breaded parts in all of the traits studied (Table 3). These results indicate that double breaded parts were superior to single breaded parts, except possibly for the doughiness that resulted in some of the breast pieces.

References

1. Hale, K. K., and T. L. Goodwin. 1968. Breaded fried chicken: Effects of precooking batter composition and temperature of parts before breading. Poultry Sci. 47:739-746.

2. May, K. N., A. J. Farr, and J. P. Hudspeth. 1969. Estimating breading content of battered and breaded poultry products. Food Technol. 23:1088-1090.

3. Steel, R. G. D., and J. N. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, Inc.

4. Tadle, J., M. N. Lewis, A. R. Winter, and R. G. Jaap. 1955. Cooked, edible meat in parts of chicken. Part I. Broilers. J. Amer. Dietetics Ass. 31:597-600.

5. Webb, J. E., and T. L. Goodwin. 1970. Precooked chicken: Effect of cooking methods and batter formula on yields and of storage conditions on 2-thiobarbituric acid values. British Poultry Sci. 11:171-177.

6. Winter, A. R., and P. Clements. 1957. Cooked edible meat in ready-to-cook poultry. J. Amer. Dietetics Ass. 33: 800-802.

7. Yingst, L. D. 1970. Processing techniques and their effects on yield, tenderness and chemical composition of commercial broiler parts. M, S. Thesis, University of Arkansas.

PRODUCTION OF AFLATOXIN IN PRE-PACKAGED LUNCHEON MEAT AND CHEESE AT REFRIGERATOR TEMPERATURES

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(Received for publication February 19, 1971)

Abstract

Information is lacking on the ability of the common mold contaminant, *Aspergillus flavus*, to grow and produce aflatoxin in perishable foods at normal refrigerator temperatures. Because of public health interests we investigated the possibility of certain perishable foods contaminated with the mold developing an aflatoxin concentration under conditions of refrigeration.

Cheddar cheese and luncheon-meat samples were inoculated with *A. flavus* ATCC 15517 and were refrigerated for 12 days at 4.4 or 7.2 C or incubated at 25 C for 12 days. Uninoculated cheese and meat samples were handled in the same manner. All samples were quantitatively analyzed by thinlayer chromatography for presence of aflatoxin.

All samples, except those inoculated and incubated at 25 C, were negative for aflatoxin production. This indicated the mold would not produce detectable aflatoxin in the tested foods when kept at normal refrigeration temperatures for 12 days.

Since the discovery that certain strains of Aspergillus flavus produce aflatoxin (1), the role this organism might play in our food chain relationship has been extensively studied (5).

Because human pharmacological data is lacking, it has not been possible to establish tolerances for this common environmental contaminant; however, toxicological and clinical findings in nonhuman primates have been extrapolated to man. Cuthbertson et al. (2) found the no-effect level for aflatoxin B¹ in monkeys to be 0.3 mg/kg of diet, or the equivalent of a daily consumption of 0.015 mg of aflatoxin/kg of body weight. Tulpula et al. (10), who studied subacute toxic effects in the primate, found that monkeys fed either 0.5 or 1.0 mg of aflatoxin B¹ for 18 days and 1.0 mg daily thereafter had primary lesions of portal inflammation and fatty change in their livers.

Pregnant animals are highly susceptible to aflatoxin. Fetal development has been retarded in the mother stressed with aflatoxin $B_{1^{\mu}}(6)$. Elis and Di Paola (4) noted teratogenic effects caused by aflatoxin. Long-term ingestion of foods contaminated with aflatoxin possibly could adversely affect the human fetus, which lends support to Kraybill's (5) statement that any demonstrable level of aflatoxin should be considered proof of contamination.

To determine the ability of *A. flavus* to produce aflatoxin at household refrigerator temperatures, Van Walbeek et al. (12) inoculated strains of the toxinproducing mold on solid and liquid yeast extract sucrose (YES) media and incubated them at 7.5 and 10 C. All cultures produced significant amounts of aflatoxin.

This study was initiated to determine whether *A*. *flavus* would produce aflatoxin in certain foods held in household refrigerators.

MATERIALS AND METHODS

A. flavus cultures

Cultures of A. *flavus*, ATCC 15517 (American Type Culture Collection, Rockville, Maryland) were used to inoculate samples of Cheddar cheese and luncheon meats. Cultures were incubated at 25 C for 2 weeks by the method of Davis et al. (3) and were then placed at -4 C until used.

To determine culture viability and to quantitate aflatoxin production, a slant was removed from the freezer and allowed to thaw to room temperature. Ten milliliters of sterile YES medium (2% yeast extract plus 26% sucrose in water) was added to the slant. A spore suspension, made by gently agitating the growth, was transferred to a culture slant of Sabouraud dextrose (Difco Laboratories, Deerfield, Michigan) agar with a sterile 0.01 ml wire loop. Following incubation at room temperature for 8 days, aflatoxin concentration was quantitatively > a 10 μ l standard containing aflatoxins in amounts of 1.0 μ g B₁/ml and 1.0 μ g G₁/ml.

Aflatoxin assays of meat and cheese samples

Extractions. Extraction of aflatoxin was accomplished with the method described by Pons et al. (8). After they were reduced to dryness and 0.5 ml of chloroform added, the extracts were placed in tightly sealed vials and stored at 4.4 C until quantitative assays could be performed by thin-layer chromatography (TLC).

Thin-layer chromatography. Precoated TLC Silica Gel sheets on aluminum (E. Merck A G Darmstadt, Germany,

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distributed by Brinkman Instruments, Inc., Westbury, New York) were used for quantitative assay. The sheets were placed in a 121.1 C oven for 30 min to ensure dryness prior to plating the samples. Five-and $10-\mu l$ sample portions were spotted, 2 cm from the bottom of the sheet, using a calibrated automatic pipette (Oxford Sampler, Oxford Laboratories, San Mateo, California). A 10 µl portion, made from a 20 µl portion, made from a 20 μ l equal combination of sample and standard, was spotted on the same sheet. Five-and 10-µl portions of the standard were also spotted. Plates were developed at room temperature in a chromogram chamber (Distillation Products Inc., Rochester, New York), with 50 ml of acetone and chloroform, 1:9 v/v, as the developer. The sheets were air dried and viewed under long-wave ultra violet light (Mineralight Model UVSL 25, Ultraviolet Products, Inc., San Gabriel, California).

Aflatoxin quantitation was made by fluorescent comparison of the unknown to the standard, using the formula: μg aflatoxin/kg = (SxYxV)/(XxW); where S = μl aflotoxin standard equal to corresponding sample spot, Y = concentration of specific aflatoxin in standard solution in $\mu g/ml$, V = volume in μl to which sample extract is diluted for TLC, X = μl sample extract spotted giving fluorescence equal to aflatoxin standard, and W = grams of sample originally used times 0.6.

The quantitative standard was prepared from a sealed ampoule containing 5 ml of chloroform solution containing 5.0 μ g B₁, 1.5 μ g B₂, 5.0 μ g G₁, and 1.5 μ g G₂/ml (Supplied by Dr. Leo A. Goldblatt, A.R.S., USDA, New Orleans, Louisiana). When diluted for analytical purposes the working standard contained: 1.0 μ g B₁, 0.3 μ g B₂, 1.0 μ g G₁, and 0.3 μ g G₂/ml. The working standard was stored at 4.4 C in a tightly sealed vial wrapped in aluminum foil until used for TLC spotting.

Equipment preparation. To simulate household conditions, samples were stored in two refrigerators (Frigidaire, Div. of General Motors, Detroit Michigan) for 12 days; the temperature of one was set at 4.4 C and the other at 7.2 C. A maximum-minimum thermometer (Taylor Instrument Companies of Canada Limited, Toronto, Canada) was placed in each refrigerator and monitored for 2 weeks prior to inoculating the samples.

Experimental samples and procedures. Cheddar cheese and variety packs of luncheon meat containing salami, New England loaf, and bologna were selected as samples. The cheese was purchased in 1-lb blocks from a local cheese factory, the luncheon meat from a local grocery store.

To simulate normal refrigerator opening and closing during daily use, each refrigerator was opened three times daily (morning, noon, and evening) for 3 min throughout the 12day incubation period. Refrigerator temperatures increased an average of 8° prior to closing the door. Both refrigerators regained their holding temperatures 15 to 20 min later.

Fifty eight cheese samples were sliced into blocks weighing 16-22 g each. Fifty-eight meat samples were sliced into portions weighing 4-7 g each.

A suspension of A. *flavus* spores was made as previously described. Forty-two cheese and 42 meat samples were inoculated on their top surfaces in an enclosed hood (Isolator/Lab., Fischer Scientific Co., Pittsburg Pennsylvania). A single swath was made on each surface with 0.01 ml wire loop. Sixteen cheese and 16 meat samples served as controls. Each sample was loosely wrapped in aluminum foil.

The inoculated samples were divided in four groups, the first three containing 12 meat and 12 cheese samples per group and the fourth containing six cheese and six meat

samples. Each of the first three groups was divided in two subgroups, each containing six cheese and six meat samples. Group one of each subgroup was inoculated and immediately placed in either a 4.4 or a 7.2 C refrigerator. Group two was inoculated and held at 25 C for 2 hr prior to refrigeration. Group three was incubated and held for 4 hr before refrigeration. The fourth group was inoculated and held at 25 C for the 12-day incubation period.

Control samples were divided into four groups, each consisting of four cheese and four meat samples. Each of the first three groups was divided into subgroups, each consisting of two cheese and two meat samples. The fourth group was not divided. Each group and each subgroup was handled as previously described but was not inoculated.

All samples were incubated for 12 days and then placed in a freezer at -4 C until assayed.

RESULTS

The samples with visible mycelial growth were positive for aflatoxin B_1 and G_1 but G_1 was pressent in concentrations of less than $1 \mu g/kg$.

Table 1 shows the results of quantitative aflatoxin assay of the six inoculated cheese samples left at 25 C for 12 days. Each sample had visible mycelial growth on the surface of the cheese and each was found to contain a B₁ aflatoxin concentration that ranged from 10.8 to 13.7 μ g/kg with a mean of 11.9 μ g/kg.

Table 1 also presents the results from the inoculated luncheon meat placed at 25 C for 12 days. Visible mycelial growth was slight on each sample. Bi aflatoxin was found in trace amounts in two samples, but the concentration in the remaining four samples varied from 7.1 to 10.0 μ g/kg (mean 8.4 μ g/kg).

Quantitative assays performed on cheese and meat samples that had been inoculated and immediately refrigerated, and on those held at 25 C for 2 or 4

TABLE 1. AFLATOXIN CONTENT OF SAMPLES INOCULATED AND NOT REFRIGERATED

		NOT P	EFRIGER	ATED.		
Cheese						
Sample #	1	2	3	4	5	6
Wt. in g	22	18	23	22	20	21
B ₁ μg/kg content	11.3	13.7	10.8	11.3	12.5	11.9
Meat						
Sample #	1	2	3	4	5	6
Wt. in gm.	1	7	6	7	5	6
${f B_1\ \mu g/kg}\ content$	٥	7.1	8.3	0	10.0	8.3

*Recorded as a trace

hr prior to refrigeration were all negative for mycelial growth and had no detectable aflatoxin concentration. The 32 meat and cheese samples not inoculated were also negative for mycelial growth and had no detectable aflatoxin concentration.

DISCUSSION

Aspergillus flavus grew well at 25 C, closely correlating with the optimal growth range of 30 to 40 C found by Raymond (9). The aflatoxin yields in this study were less than those obtained by Van Walbeek et al. (12); but they used malt-yeast agar and broth as a medium, which probably enhanced the production of toxic metabolites.

Because pharmacological data on man are lacking, it is not possible to determine whether the amounts of aflatoxin recovered are sufficient to cause human aflatoxicosis. Van Welbeek et al. (11), however, reported illness in a child who had consumed spaghetti containing 12.5 μ g aflatoxin/kg. Leistner and Ayres (7), who studied potentially toxinogenic molds commonly associated with country-cured hams and fermented sausages, recovered molds of the genus Aspergillus from 90% of the hams and 33% of the sausages surveyed. Because of the highly toxinogenic capabilities of certain strains of Aspergillus, the hazards of potential aflatoxin production could outweigh the benefits molds may contribute to cured meats. Further work in this area is warranted.

Samples refrigerated immediately following inoculation, and those incubated at 25 C for 2 and 4 hr prior to refrigeration, failed to yield detectable concentrations of aflatoxin. This indicated that although food might become contaminated with A. flavus when left at 25 C for periods of up to 4 hr, if the food is thereafter refrigerated at 4 to 8 C, there is little danger of aflatoxin production within 12 days.

CONCLUSION

Aspergillus flavus will grow and produce aflatoxin on Cheddar cheese and luncheon meat at 25 C. Re-

frigerating these perishable foods prevents detectable aflatoxin growth and production for at least 12 days, even though the food may have been previously contaminated with A. *flavus*.

Acknowledgments

Appreciation is extended to Mrs. Shyrll Hoffman, Medical Technician at the Comparative Toxicology Laboratory, for assistance with methodology; and also to Mrs. Jean Heikes and Mrs. Eva Graybeal of the Veterinary Public Health Laboratory and to Dr. Lucille Wakefield, Head of Foods and Nutrition, for their assistance.

The author is indebited to the Office of the Surgeon General, Department of the Army, for selecting him for this course of study at Kansas State University.

References '

1. Blount, W. P. 1961. Turkey "X" disease. J. Brit. Turkey Fed. 9:55-58.

2. Cuthbertson, W. F. J., A. C. Lawsen, and D. A. H. Pratt. 1967. Effects of alfatoxin in monkeys. Brit. J. Nutr. 21:893.

3 Davis, N. D., U. L. Diener, and D. W. Eldridge 1966. Production of aflatoxins B_1 and G_1 by Aspergillus flavus in a semisynthetic medium. Appl. Microbiol. 14:378-380.

4. Elis, J., and J. A. DiPaola. 1967. Teratogenic effects of aflatoxin. Arch. Pathol. 83:53.

5. Kraybill, H. F. 1969. The toxicology and epidemiology of mycotoxins. Trop. Geogr. Med. 21:1-18.

6. Le Breton, E., C. Frayssinet, C. Lafarge, and De Recondo. 1964. Effects of aflatoxin on the foetus. Food Cosmet. Toxicol. 2:675.

7. Leistner, L. and J. C. Ayres. 1968. Molds and meats. Die Fleischwirtschaft. M1:62-65.

8. Pons, W. A., A. F. Cucullu, A. O. Franz, and L. A. Goldblatt. 1968. Improved objective fluorodensitometric determination of aflatoxins in cottonseed products. J. Amer. Oil. Chem. Soc. 45:694-699.

9. Raymond, W. D. 1966. Mycotoxin problems in the United Kingdom. Food Technol. 20:904-910.

10. Tulpule, P. G., T. V. Madhaven, and C. Gopalan. 1964. Effect of feeding aflatoxin in young monkeys. Lancet 1:962.

11. Van Walbeek, W., P. M. Scott, and F. S. Thatcher. 1968. Mycotoxins from food-borne fungi. Can. J. Microbiol. 14:131-137.

12. Van Walbeek, W., T. Clademenos, and F. S. Thatcher. 1969. Influence of refrigeration on aflatoxin production by strains of *Aspergillus flavus*. Can. J. Microbiol. 15:629-632.

A Research Note

FLAVOR CHARACTERISTICS OF NORMAL AND ABNORMAL MILK

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(Received for publication February 26, 1971)

Abstract

Flavor is one characteristic of quality in raw milk. Presence of abnormality, as reflected in somatic cell levels, is considered aesthetically undesirable and may well affect the flavor characteristics of raw milk. Raw milk producer samples were analyzed for flavor, somatic cell concentration, acid degree value, total and coliform bacteria, pH, butterfat, and total solids. There was an inverse relationship between flavor score and acid degree value. Results did not confirm the supposition that somatic cell concentrations may affect the flavor of raw milk.

The importance of high quality milk is well recognized by the dairy industry. The producer is directly affected because he usually gets paid more for good quality milk than poor quality milk. There are many quality indices that can be used. The more commonly used tests for quality of raw milk are flavor, odor, and bacterial content. Screening tests are currently used as a further means to check the milk supply for signs of abnormality caused by mastitis.

Questions often asked are: "How does mastitic milk affect the flavor of milk?" "Is mastitic milk harmful to humans?" Milk, by definition, must come from healthy cows. Since mastitis represents an udder abnormality, the animal is not healthy and hence the milk should not be used.

Boland (2) suggested that the leucocyte count is important enough to be used as an index of quality —even to replace the present bacteria count standard. He recognized the necessity for a standardized procedure before such a test could be used. He also speculated that 'low leucocyte counts in milk would overcome many' of the problems encountered with cultured products.

Singh and Marshall (6) report a direct relationship between the leucocyte concentration and bacterial population of raw milks held 24 hr at 40 F. They observed that control sample counts (free of leucocytes) remained relatively constant, but counts for leucocyte-containing milks decreased rapidly. This phagocytic activity of the leucocytes decreases quite rapidly with an increase in time or temperature

of storage.

1.12

The literature on mastitis is voluminous but very few data are available on the relationship of leucocyte concentration to flavor of milk. This study was undertaken to shed some light on the relationship of flavor to various compositional factors including somatic cell concentration (SCC).

Procedure

Raw milk samples were obtained directly from the farm bulk tanks by the milk hauler. The samples were stored in crushed ice until delivered to the laboratory and their analyses completed.

Flavor evaluations were made as soon as practical, but always within 24 hr of the time of pickup. All samples were scored according to the American Dairy Science Association approved milk score card. Organoleptic evaluations were made by one and in some instances by two experienced judges. Additional tests conducted on all milk samples were as follows: Wisconsin Mastitis Test (WMT), Clemson Catalase Test (CCT), acid degree value (ADV), total bacteria count (TC), coliform count, pH, total solids (TS), and butterfat.

The WMT procedure described in Public Health Service publication 1306 (5) was used except for a minor modification (3). The CCT procedure (4) was used to obtain a quantitative measure of oxygen released under conditions of the test.

The degree of rancidity was measured by a simple titration procedure. The method of Thomas et al. (7) was used for ADV determinations with two modifications; namely, a 0.01 N alcoholic KOH solution was used instead of 0.02 N and the reported ADV's represent the ml of 0.01 N KOH required to neutralize the free fatty acids in 1 g of milk fat.

Standard methods (1) procedures were employed in plating milk samples using standard methods agar and desoxycholate agar. All platings were made within 24 hr of the time of pickup of samples collected in Jeb tubes or plastic sampling bags.

The pH measurements were made on a Beckman, model N pH meter. Total solids were determined using the Mojonnier tester and fat tests using the Babcock procedure.

RESULTS AND DISCUSSION

A total of 286 raw milk producer samples were analyzed for flavor, somatic cell concentration WMT and CCT), acid degree value, total bacteria, coliform count, pH, butterfat, and total solids.

The mean compositional values for raw milks classified according to somatic cell concentration are presented in Table 1. The mean values for somatic cell concentration (WMT, CCT) and ADV for spe-

¹Technical Contribution No. 913, S. C. Agri. Expr. Sta. Published by permission of the Director.

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TABLE 1.	Mean	COMPOS	SITIO	NAL V	ALUES	FOR	RAW	MILKS	
CLASSIFI	ED ACC	ORDING	то	SOMA	TIC CI	ELL	CONT	ENT.	

	Mea	Mean values for SCC1				
Attribute	$<^{500,000}$	500,000-1,000,000	>1,000,000			
Flavor score	36.0	36.7	35.6			
WMT (SCC/ml)	276,000	691,000	1,600,000			
ADV	0.85	0.70	0.99			
$CCT (\% O_2)$	23	35	52			
pН	6.69	6.71	6.73			
B. F. (%)	3.77	3.78	4.76			
T. S. (%)	12.50	12.41	13.40			
Total count/ml	59,000	80,000	61,000			
Coliforms/ml	2,600	3,200	370			
No. samples	162	113	11			

 $^{1}SCC = Somatic cell count$

TABLE 2. FLAVOR SCORES AND MEAN WMT, CCT, AND ADV VALUES FOR RAW MILK.

Flavor score	No.	samples	WMT ¹	CCT ¹	ADV1
			(000)	(% O ₂)	
37.5 - 38.5	÷.,	103	537	31	0.67
36.5 - 37.0		78	486	28	0.71
35.0 - 36.0		59	421	27	0.84
Less than 35.0		45	482	29	1.20

¹Mean values

cified flavor score groupings are presented in Table 2. There appears to be an inverse relationship between flavor score and ADV. This is to be expected. None of the other attributes seemed to have any direct relationship to flavor score.

Statistical analysis (AOV) of the data led to the following conclusions: (a) there was a significant (.01) correlation between flavor score and ADV (r = 0.46); (b) all other compositional factors studied were not significantly different when compared with flavor score; (c) there was a significant relationship between the CCT results and WMT, total bacteria, and total solids (r = 0.70, 0.23, 0.26); and (d) somatic cell concentrations (WMT) did significantly (.01) influence the pH, butterfat, and total solids content (r = 0.25, 0.39, 0.28).

It is recognized that the number of milk samples in the ">1,000,00" group is rather small. However, the results do indicate a downward trend in flavor score. Use of additional judges would possibly have strengthened these observations.

Even though the data do not substantiate the belief that somatic cell concentration may affect the flavor of milk, more work needs to be done to try and determine its possible effect on the shelf-life of the pasteurized product.

References

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

2. Boland, H. 1964. Too much abnormal milk. Amer. Milk Rev. 26:116.

3. Janzen, J. J. 1969. Evaluation of five screening tests used for estimating leucocytes in bulk milk. J. Dairy Sci. 52:329-334.

4. Janzen, J. J., and W. C. Cook. 1967. A simple quantitative catalase procedure for abnormal milk. J. Milk Food Technol. 30:205-207.

5. Public Health Service. 1965. Screening tests for the detection of abnormal milk. Public Health Service Publication 1306. U. S. Dept. of Health, Education and Welfare, Bureau of State Services (Environmental Health), Washington, D. C. 30 p.

6. Singh, B., and R. T. Marshall. 1965. Effect of leucocyte concentration on bacteriological quality of milk. J. Dairy Sci. 48:769.

7. Thomas, E. L., A. J. Nielson, and J. C. Olson, Jr. 1955. Hydrolytic rancidity in milk-A simplified method for estimating the extent of its development. Amer. Milk Rev. 17:50-52.

Erratum

AN ASSESSMENT OF THE FLAVOR QUALITY OF WHOLE MILK AVAILABLE AT COMMERCIAL OUTLETS

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(Received for publication December 10, 1970)

34 (May, 1971). Authors and their affiliations were have been as given above.

This paper appeared on pages 244-248 of volume not listed properly on page 244; the listing should

INOSINE MONOPHOSPHATE (IMP) AND HYPOXANTHINE FORMATION IN THREE SPECIES OF SHRIMP HELD ON ICE

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Abstract

The major pathway for degradation of adenine nucleotides in pink (*Pandalus borealis*), spot (*Pandalus platyceros*), and sidestripe (*Pandalopsis dispar*) shrimp results in formation of inosine monophosphate (IMP) and hypoxanthine. The IMP content of shrimp held on ice increased to 2-3 μ M/g during the first day and decreased to 0.4-0.7 μ M/g during the next two weeks, while 0.8-1.3 μ M/g of hypoxanthine accumulated during this period.

Collins (3) reported that, for optimum machine peeling, shrimp require a controlled aging period of at least 40 hr on ice or in refrigerated sea water to release the meat from the shell. Newer processes, such as the Laitram Model PCA Peeler² (10) and the two-stage precook method (5), precondition shrimp by precooking prior to machine peeling. Canned fresh shrimp, prepared by the two-stage precook method were reported to have more color and better texture and flavor than shrimp which had been held on ice prior to processing.

The relationship of inosine monophosphate (IMP) and hypoxanthine to the flavor and freshness of fisheries products has been examined (7, 12, 13, 14). Tarr and Comer (15) and Arai (2) observed IMP and hypoxanthine in shrimp (*Pandalus* sp.) but did not include shrimp held on ice for prolonged periods.

The purpose of this study is to determine the changes which occur, in the IMP and hypoxanthine contents of pink, spot, and sidestripe shrimp in relation to ice-holding time.

MATERIALS AND METHODS

Samples

Shrimp used in this study were obtained aboard a commercial boat near Wrangell, Alaska, in August 1967. The trawls contained mainly pink shrimp (*Pandalus borealis*) but also sufficient spot shrimp (*Pandalus platyceros*) and sidestripe shrimp (*Pandalopsis dispar*) so that these species could be examined at the same time. Zero time samples were immediately frozen in dry ice and stored at -50 C until analyzed. The remaining shrimp were mixed with crushed ice in perforated fish boxes for shipment to the laboratory. Fresh ice was added daily and duplicate samples for analysis were taken periodically over a period of two weeks.

Extraction of nucleotides

Samples containing 50 g of peeled shrimp tail muscle were homogenized for 1 min with 2 volumes of chilled 0.6 N perchloric acid and filtered using a suction flask. The zerotime samples were peeled and homogenized while frozen to prevent further changes in the nucleotides. The filtrates were immediately adjusted to pH 6.5-6.8 with 10% KOH and held at 0 C for 1 hr. The insoluble potassium chlorate was removed by filtration and the clear filtrates were stored at -50 C until analyzed.

Ion-exchange chromatography

Perchloric acid extract samples representing 5 g of shrimp muscle were placed on 1.0×28 cm columns of Dowex 1×8 (Formate) resin. The colums were washed with water until the effluent was free of material absorbing at 260 m μ . The effluents, which contain nucleosides and free bases, were collected and used in the analysis for hypoxanthine.

The nucleotides were eluted from the formate columns using the formic acid-ammonium formate buffer system described by Porter (11). The effluent from the columns was continuously monitored at 260 m μ using a Vanguard² model 1056 UV monitor and collected in 10 ml fractions. In order to obtain appropriate formic acid-ammonium formate blanks for spectral analysis, the procedure was repeated using a column without nucleotides.

Fractions common to the individual peaks were pooled and identified by comparing their ultraviolet spectra with published spectral data (1, 6, 11). The absorbance of the fractions containing adenosine triphosphate (ATP), adenosine monophospate (AMP), and IMP were measured and the amount of nucleotide present calculated by using published molar extinction coefficients (1, 6).

Hypoxanthine analysis

The effluents from the water washings of the formate columns were analyzed for hypoxanthine by the xanthine oxidase method of Kalcker (9) as modified by Jones et al. (8). This enzyme converts hypoxanthine to uric acid. The hypoxanthine content was then determined from the increase in absorption at 290 m μ due to uric acid formation. The increase in uric acid, rather than the decrease in hypoxanthine was measured, because there was less interference from the other UV absorbing substances found in shrimp (15) at 290 m μ than at 250 m μ .



¹Address after July 1, 1971: Marine Fisheries Center, National Marine Fisheries Service, Box 1638, Kodiak, Alaska 99615. ²Use of trade names is for descriptive purposes only and does not imply endorsement.

		Species						
Time on ice	Pink shrimp (Pandalus borealis)	Spot shrimp (Pandalus platyceros)	Sidestripe shrimp (Pandalopsis dispar)					
0 time	1.8	2.0	1.8					
6 hr	2.8	2.8	2.0					
12 hr	2.6	3.0	2.5					
18 hr	2.2	2.8	2.1					
24 hr	1.7	2.7	1.6					
36 hr	1.5	2.6	1.4					
48 hr	1.4	2.3	1.3					
3 days	1.3	2.0	1.2					
4 days	1.2	1.6	1.1					

TABLE 1. ADENOSINE MONOPHOSPHATE CONTENT OF 3 SPECIES OF SHRIMP HELD ON ICE $(\mu M/G)$

Table 2. Inosine monophosphate content of 3 species of shrimp held on ice $(\mu M/G)$.

		Species	
Time on ice	Pink shrimp (Pandalus borealis)	Spot shrimp (Pandalus platyceros)	Sidestripe shrimı (Pandalopsis dispar)
0 time	1.8	1.0	1.9
6 hr	1.9	1.2	2.4
12 hr	2.6	1.4	2.6
18 hr	2.4	1.8	2.8
24 hr	2.0	2.0	2.8
36 hr	1.6	1.7	2.0
48 hr	1.4	1.6	1.6
3 days	1.2	1.4	1.4
4 days	1.0	1.1	1.2
5 days	0.8	0.9	1.0
7 days	0.6	0.9	0.8
10 days	0.5	0.8	0.5
14 days	0.4	0.7	0.4

TABLE 3. Hypoxanthine content of 3 species of shrimp held on ice $(\mu M/G)$

		Species	
Time on ice	Pink shrimp (Pandalus borealis)	Spot shrimp (Pandalus platyceros)	Sidestripe shrimı (Pandalopsis dispar)
0 time	0.05	0.00	0.00
6 hr	0.10	0.05	0.10
12 hr	0.10	0.10	0.10
18 hr	0.15	0.10	0.15
24 hr	0.20	0.20	0.20
36 hr	0.30	0.25	0.30
48 hr	0.40	0.30	0.30
3 days	0.60	0.35	0.40
4 days	0.80	0.50	0.50
5 days	1.00 ′	0.60	0.60
7 days	1.10	0.70	0.70
10 days	1.25	0.75	0.75
14 days	1.30	0.90	0.80

RESULTS AND DISCUSSION

Pathways for nucleotide degradation

Arai (2) observed that the adenine nucleotides in shrimp may be degraded by two pathways. ATP is

dephosphorylated to AMP with a slight amount of adenosine diphosphate (ADP) formed as an intermediate. AMP may then be deaminated to IMP which is then dephosphorylated to inosine and converted to hypoxanthine. As an alternate, AMP may be dephosphorylated to adenosine which is then deaminated to inosine and converted to hyphoxanthine.

Because the activities of the enzymes involved at each step of these pathways are temperature dependent, Arai (2) observed an accumulation and subsequent decline of IMP in shrimp stored at 19 C but not in shrimp stored at -6 C. At -6 C, the deamination of adenosine to inosine predominates over the deamination of AMP to IMP so that inosine and hypoxanthine are formed without the accumulation of IMP.

The zero-time samples of each of the three species of shrimp contained between 2 and 3 μ M/g of ATP. Less than 0.2 μ M/g of ATP was detected after 6 hr on ice and none was measurable in the subsequent samples.

Tarr and Comer (15) suggested that ATP is degraded during capture. It appears, from the present work, that the ATP remaining after capture is rapidly depleted during ice holding. Significant amounts of ATP will probably be found only in very fresh shrimp.

The AMP content of each of the three species of shrimp (Table 1) increased to 2.5-3.0 μ M/g during the first 6 to 12 hr and decreased during subsequent storage. Since the maximum IMP content is observed after the maximum AMP content, it appears that the major pathway for the degradation of the adenine nucleotides in shrimp held on ice results in the accumulation of IMP rather than adenosine.

IMP content

The IMP content of shrimp held on ice (Table 2) increased to 2-3 μ M/g during the first day and decreased to 0.4-0.7 μ M/g during the next two weeks. Although the effects of IMP upon the flavor of shrimp were not studied, the presence of a high IMP content suggests that shrimp should be processed during the first day after capture rather than subjected to prolonged periods of ice holding.

After 4-5 days on ice, the IMP content of shrimp falls below 1.0 μ M/g. This IMP level is reached at about the same time as the onset of spoilage, as indicated by a rapid increase in the trimethylamine (TMA) content (4). Since the IMP content of shrimp changes rapidly before any pronounced change in TMA is observed, the IMP content could serve as an early indication of change in quality.

Hypoxanthine content

The hypoxanthine content of shrimp held on ice (Table 3) is lowest immediately after capture and increases steadily to 0.80-1.30 μ M/g over a period of two weeks. The buildup of IMP and subsequent conversion to hypoxanthine occurs somewhat more rapidly in pink than in spot or sidestripe shrimp. The greatest amount of IMP and the least amount of hypoxanthine both occur during the first day on ice.

The hypoxanthine content of shrimp at the onset of spoilage was not definitely established. Since no sharp increase in hypoxanthine content was observed during the two-week period, the rapid increase in TMA content observed after 4-5 days on ice (4) would probably be a better criterion of the onset of spoilage than the slow increase in hypoxanthine.

Spinelli (12) reported that the addition of 3.0 μ m/g of hypoxanthine did not produce detectable flavor differences in petrale sole fillets unless the total bacterial counts exceeded 10⁶/g. Although the effect of hypoxanthine upon the flavor profile of shrimp was not determined, it is unlikely that the relatively low amounts detected in the present study could significantly alter the flavor of shrimp, particularly after the onset of spoilage.

References

1. Anon. 1956. Ultraviolet absorption spectra of 5'ribonucleotides. Circular OR-10, Pabst Laboratories, Milwaukee, Wisconsin.

2. Arai, K. 1966. Nucleotides in the muscle of marine invertebrates. Bull. Jap. Soc. Sci. Fisheries 32:174-180.

3. Collins, J. 1960. Processing and quality studies of shrimp held in refrigerated sea water and ice. Part 1. Preliminary observations on machine-peeling characteristics and

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product quality. Com. Fisheries Rev. 22(3):1-5.

4. Collins, J., H. Seagran, and J. Iverson. 1960. Processing and quality studies of shrimp held in refrigerated sea water and ice. Part 2. Comparison of objective methods for quality evaluation of raw shrimp. Com. Fisheries Rev. 22(4):1-5.

5. Collins, J., and C. Kelley. 1969. Alaska pink shrimp (*Pandalus borealis*): Effects of heat treatment on color and machine peelability. Fisheries Ind. Res. 5(5):181-189.

6. Dorough, G. D., and D. L. Seaton. 1954. A method for the extraction and assay of nucleic acid fragments in tissues. J. Amer. Chem. Soc. 76:2873-2877.

7. Hughes, R. B., and N. R. Jones. 1966. Measurement of hypoxanthine concentrations in canned herring as an index of the freshness of the raw material, with a comment on flavor relations. J. Sci. Food Agr. 17:434-436.

8. Jones, N. R., J. Murray, and (in part) E. I. Livingston, and C. K. Murray. 1964. Rapid estimations of hypoxanthine concentrations as indices of the freshness of chillstored fish. J. Sci. Food Agr. 15:763-774.

9. Kalckar, H. M. 1947. Differential spectrophotometry of purine compounds by means of specific enzymes. I. Determination of hydroxypurine compounds. J. Biol. Chem. 167:429-443.

10. Lapeyre, J. M. 1966. Frocess for peeling pre-cooked shrimp. U. S. Patent 3,276,878.

11. Porter, R. W. 1968. The acid-soluble nucleotides in king crab (*Paralithodes camtschatica*) muscle. J. Food Sci. 33:311-314.

12. Spinelli, J. 1965. Effect of hypoxanthine on the flavor of fresh and stored low-dose-irradiated petrale sole (*Eopsetta jordani*) fillets. J. Food Sci. 30:1063-1067.

13. Spinelli, J., M. Eklund, and D. Miyauchi. 1963. Measurement of hypoxanthine in fish as a method of assessing freshness. J. Food Sci. 29:710-714.

14. Spinelli, J., and D. Miyauchi. 1968. Irradiation of Pacific Coast fish and shellfish. V. The effect of 5'-inosine monophosphate on the flavor of irradiated fish fillets. Food Technol. 22(6):123-125.

15. Tarr, H. L. A., and A. G. Comer. 1965. Nucleotides and related compounds, sugars, and homarine in shrimp. J. Fisheries. Res. Board Canada 22:307-311.

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GROWTH CHARACTERISTICS OF LEUCONOSTOC CITROVORUM IN SKIMMILK FORTIFIED WITH LIVER CONCENTRATE OR PANCREAS EXTRACT

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(Received for publication January 13, 1971)

Abstract

Four strains (CAF-B, Da3, 9, and 14) of *Leuconostoc citrovorum* were grown at 22 and 30 C in sterile skimmilk which contained 0.0, 0.001, 0.01, or 0.1% liver concentrate or 0.0, 0.01, 0.1, or 0.5% pancreas extract. Numbers of leuconostocs were determined with the plate count at intervals during a 30-hr incubation. Smears, prepared concurrently, were stained and examined microscopically to determine numbers of cells in chains of leuconostocs.

Highest numbers (approximately 10⁸ or greater per milliliter) of leuconostocs resulted when highest concentrations of liver concentrate or pancreas extract were used. Lower concentrations of both additives were less' stimulatory or without effect. Incubation temperature had little or no effect on stimulatory activity by either additive. All strains produced markedly higher (0.5 log or greater) populations with liver concentrate but only CAF-B and Da3 responded substantially to pancreas extract. Higher populations of most strains were attained earlier in fortified rather than plain milks. Chains formed by CAF-B and Da3 tended to be longer during the 10-22 hr segment of incubation when milk was fortified with pancreas extract; Da3 showed the greatest increase in chain length. Strains 9 and 14 formed longer chains in skimmilks which contained 0.1 or 0.5% pancreas extract and were incubated at 30C. Differences in chain length caused by pancreas extract were statistically significant, whereas those produced by liver concentrate were without statistical significance.

Organisms in the genus *Leuconostoc* are important in the food industry as spoilage agents (3) and as starter cultures for certain fermented dairy products (5). In addition *Leuconostoc citrovorum* elaborates an antibacterial substance(s) which inhibits some Gram-positive and -negative bacteria (13).

Although the importance of these organisms is well recognized, independent growth of *L. citrovorum* in milk has received little attention even though pure cultures of this organism are sometimes used to produce flavor substances for later use in some dairy products. Goel and Marth (8) noted that some strains of $L_{\rm c}$ citrovorum attained higher populations in skimmilk at 22 than at 30 C, whereas others behaved comparably at both temperatures. Most strains formed slightly longer chains at 30 than at 22 C.

Growth of some bacteria can be enhanced by adding stimulants to the medium (1, 11, 12, 14, 16, 17). Sauberlich and Baumann (15) probably were first to report that crude extracts of liver enhanced growth of L. citrovorum. Later Agren (1) and Kereszlesy and Silverman (10) recovered a substance from liver which stimulated growth of L. citrovorum but which was inactivated by acid. In 1956 McAnelly and Speck (14) reported that an aqueous extract of the porcine pancreas stimulated growth and activity of Streptococcus lactis and L. citrovorum in milk but had little or no effect on growth of Escherichia coli, Pseudomonas fluorescens, and Bacillus subtilis. Subsequently Speck et al. (17) confirmed that pancreas extract stimulated growth of lactic acid bacteria without simultaneously enhancing proliferation of spoilage organisms which might occur in cultured dairy products.

Although there are many reports on use of additives to enhance growth of S. lactis and Streptococcus cremoris, only a few have been concerned with L. citrovorum. Consequently the present investigation was conducted to determine: (a) if different concentrations of liver concentrate and pancreas extract in skimmilk stimulate growth of L. citrovorum at both 22 and 30 C and (b) how the additives affect chain formation by L. citrovorum at the two temperatures. A preliminary report on some of these experiments has been presented (9).

MATERIALS AND METHODS

Cultures

Four strains of *L. citrovorum* were used; their sources were reported by Goel and Marth (8). Strains CAF-B and Da3 formed long chains, whereas strains 9 and 14 produced short chains when grown in skimmilk (8). Cultures were maintained in litmus milk at 5 C and transferred every other week. Before use, cultures were transferred daily, 2 or 3 times, in sterile skimmilk to ensure activity.

Animal by-products

Pancreas extract (Procheez) and liver concentrate for

Published with the approval of the Director of the Research Division of the College of Agricultural and Life Sciences, University of Wisconsin.

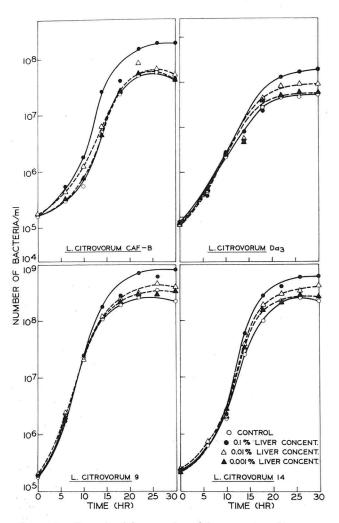


Figure 1. Growth of four strains of *Leuconostoc citrocorum* at 22 C in skimmilk fortified with various levels of liver concentrate.

these experiments were obtained from Wilson Laboratories, Chicago, Illinois.

Preparation of test samples

Skimmilk from the University of Wisconsin dairy plant was autoclaved at 121 C for 15 min and cooled to 22 or 30 C. The sterilized skimmilk was fortified with sufficient sterile additives to contain 0.001, 0.01, and 0.1% of liver concen-

Table 1. Change in generation times of four strains of Leuconostoc citrocorum when grown at 22 and 30 C in skimmilk with added liver concentrate and pancreas extract.

Strain of		iver ate (0.1%)	Panc extract	
I citrovorum	22C	30C	22C	30C
	. <u> </u>	(Change	in per cent) ¹	
CAF-B	- 8.2	-7.3	-17.2	- 4.5
Da3	-18.8	-22.4	+26.2	-20.0
9	-11.1	+17.7	+17.0	- 9.7
14	-32.4	+ 5.2	+ 1.6	-50.0

¹Minus values indicate reduction in generation times from those of controls; plus values indicate an increase in generation time. trate or 0.01, 0.1, and 0.5% of pancreas extract. Skimmilks were then inoculated with 0.25% of a 48-hr old culture of the test strain of *L. citrovorum*. Inoculated milks were held quiescently at 22 or 30 C and were mixed gently and sampled periodically during the 30-hr incubation. Samples were plated in duplicate with Elliker's lactic agar (4) and plates were incubated for 96 hr at 22 or 30 C (depending on incubation temperature of skimmilk) prior to counting. Earlier work by Goel and Marth (7) established that a satisfactory estimate of the population of leuconostocs is obtained with the plate count, especially when higher (e.g. 10^{-6} or 10^{-7}) dilutions are plated since most chains are broken into 2- or 4-cell fragments by dilution and shaking procedures.

Smears also were prepared each time a sample was taken for plating. Slides were stained by the Levowitz-Weber

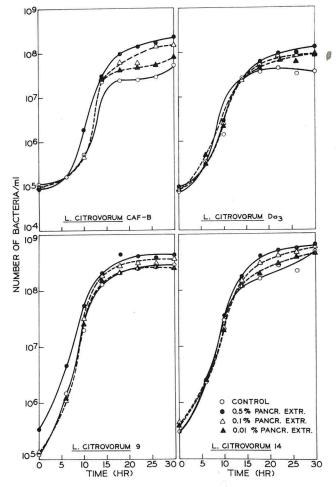


Figure 2. Growth of four strains of *Leuconostoc citrovorum* at 22 C in skimmilk fortified with various concentrations of pancreas extract.

procedure (19) and examined microscopically for numbers of cells in chains of leuconostocs. Cells in 10 chains randomly selected from five microscopic fields were counted and the average chain length was calculated. Results were analyzed statistically by analysis of variance. TABLE 2. CHAIN LENGTH OF Leuconostoc citrovorum when Grown at 22 and 30 C in skimmlik with 0.0, 0.01, 0.1, and 0.5% added pancreas extract.

		ž						Str	ain of L	euconosto	c citrovor	um					
Incubati		-	CA	F-B			D	a3			9				14	1	and the second
Temp.	Hours	0.0%	0.01%	0.1%	0.5%	0.0%	0.01%	0.1%	0.5%	0.0%	0.01%	0.1%	0.5%	0.0%	0.01%	0.1%	0.5%
		1994 Barrier						(Avg.	no. ce	ells/cha	in)						
22C	0	4.8	6.4	4.8	4.8	8.0	7.2	6.8	4.4	3.6	4.2	4.8	3.6	5.2	5.2	5.2	5.2
	6	9.2	10.2	8.2	9.2	11.6	11.2	11.2	13.2	5.6	4.7	4.4	5.4	6.8	6.8	5.6	5.6
	10	11.4	9.8	14.0	10.0	22.4	24.0	21.8	33.6	5.0	4.4	5.0	5.8	3.2	4.6	4.8	6.2
	14	10.4	10.2	16.0	12.8	17.2	34.2	21.0	29.0	4.2	5.3	4.7	4.2	3.0	4.8	4.8	5.8
	18	11.8	12.4	16.8	14.0	15.4	21.6	24.8	25.2	3.2	5.0	6.2	3.6	2.8	3.4	3.8	5.0
	22	17.6	15.4	.13.0	13.2	12.6	13.4	17.0	17.0	2.6	3.4	3.4	3.6	3.0	3.2	3.4	4.0
	26	15.0	14.4	9.8	9.2	12.0	11.8	12.2	10.8	2.4	3.2	3.4	2.8	2.6	3.6	3.2	3.2
	30	10.2	6.2	9.0	9.0	11.0	8.6	11.0	10.8	2.2	2.8	3.0	2.6	2.4	3.4	3.2	3.4
30C	0	4.8	6.3	5.2	4.8	6.0	8.0	6.4	6.4	7.6	3.6	8.0	3.4	6.8	5.2	6.0	8.0
	6	11.4	9.8	10.4	14.6	6.6	24.6	18.4	18.2	8.0	4.0	7.2	9.0	5.4	5.4	7.6	8.8
	10	20.0	24.2	21.6	27.6	16.4	32.2	34.2	41.8	5.4	5.4	8.2	5.6	5.8	6.6	7.6	9.6
	14	28.8	24.8	28.4	27.6	17.6	29.0	26.2	18.4	4.0	6.2	6.4	7.4	5.0	5.4	7.2	9.2
	18	16.4	24.8	22.6	18.5	12.2	30.4	21.8	13.8	3.4	5.0	5.4	5.8	3.6	3.7	6.2	6.8
	22	8.6	15.0	13.4	10.6	11.8	20.0	12.2	10.6	3.2	3.8	4.6	5.6	2.8	4.2	7.2	5.4
	26	6.8	7.4	8.6	8.2	12.0	16.2	12.4	10.0	3.4	3.8	3.8	5.2	3.0	3.8	6.0	6.5
	30	5.4	5.8	7.6	8.0	10.2	11.6	9.2	9.0	2.8	3.2	3.8	4.4	2.8	3.6	5.8	5.2

TABLE 3. ANALYSIS OF VARIANCE OF RESULTS ON CHAIN LENGTH OF Leuconostoc citrovorum during growth in skimmilk Fortified with pancreas extract or liver concentrate.

W W0 I D00	Degrees of freedom-	Mean	square
Source of variation	either additive	Pancreas extract	Liver concentrate
Additive (A)	3	57.84**	5.00
Incubation temeprature (B)	1	186.83**	95.18**
Incubation time (C)	7	350.48**	164.81**
Strain (D)	3	2137.83**	756.14**
$A \times B$	3	15.27	4.14
$A \times C$	21	11.09	3.28
$A \times D$	9	24.92**	2,21
$B \times C$	7	35.88**	13.11**
$B \times D$	3	22.41	7.51*
$C \times D$.	21	104.90**	37.96**
$A \times B \times C$	21	5.41	2.55
$\mathbf{A} \times \mathbf{B} \times \mathbf{D}$	9	15.68	4.56
$\mathbf{A} \times \mathbf{C} \times \mathbf{D}$	63	6.39	3.47
$\mathbf{B} \times \mathbf{C} \times \mathbf{D}$	21	29.57**	4.65**
$A \times B \times C \times D$	63	8.26	2.54
Experimental error	63	8.26	2.54

* Significant at 0.05 level.

**Significant at 0.01 level.

RESULTS AND DISCUSSION

Addition of liver concentrate

Data in Fig. 1 indicate that highest populations of L. *citrovorum* resulted when skimmilk was fortified with 0.1% liver concentrate and incubated at 22 C. The increase in population at the end of incubation was proportional to the amount of liver concentrate added to the skimmilk although the lowest level tested (0.001%) was essentially without effect. At 22 C, strains CAF-B and 9 exhibited the greatest response to the additive. Results similar to those at 22 C were obtained when cultures were incubated at 30 C

although the magnitude of the increase in population caused by 0.1% liver concentrate was somewhat less (results not shown). Generation times of all strains tested were reduced by 8.2 to 32.4% at 22 C when the highest level of liver concentrate was tested (Table 1). In contrast to this, at 30 C generation times of two strains were increased, whereas those of the other two strains were decreased. Both strains with increased generation times developed higher populations at 30 C in unfortified skimmilk and exhibited less response to the additive than did the other two strains.

Liver concentrate failed to significantly affect the

length of chains produced by the *L. citrovorum* strains under conditions of these experiments (Table 2). Instead, chain length was affected significantly by incubation time and temperature and strain of the organism. Goel and Marth (8) reported earlier that these factors affected chain formation by *L. citrovorum* during growth in unfortified skimmilk.

Addition of pancreas extract

Results reported in Fig. 2 indicate that highest populations at 22 C were attained by all strains of *L. citrovorum* when they grew in skimmilk fortified with the highest concentration (0.5%) of pancreas extract. Two of the strains, CAF-B and Da3, exhibited greater response to pancreas extract than did the other two cultures. In general, the response, in terms of population increase, was proportional to the amount of pancreas extract added to skimmilk. Behavior of the cultures at 30 C approximated that observed at 22 C except that populations of strain CAF-B in fortified skimmilks tended to be higher than at 22 C (data not shown). Generation times of all cultures were reduced by 0.5% pancreas extract when incubated at 30 but not at 22 C.

An analysis of variance of data on chain length of L. citrovorum demonstrated that pancreas extract significantly affected this characteristic of the organism (Table 3). In addition chain formation was affected by incubation time and temperature and strain of the bacterium. Data in Table 2 indicate that: (a) strain CAF-B tended to form longer chains during the 10-22 hr period of the incubation at 30 C when skimmilk contained any of the concentrations of pancreas extract, (b) strain Da3 produced longer chains in the presence of all levels of pancreas extract tested at 22 and 30 C with the effect being most pronounced during the 10-22 hr period of the incubation at 22 C and the 6-18 hr period at 30 C, and (c) strains 9 and 14 tended to form longer chains when skimmilk contained 0.1 or 0.5% pancreas extract and when incubation was at 30 C; differences were greatest during the first one-half of the incubation period.

Although no attempts were made to isolate the substances in these additives which stimulated the leuconostocs, other workers have suggested that peptides are responsible (2, 6, 18).

Acknowledgment

The authors thank Wilson Laboratories, Chicago, Illinois for supplying the liver concentrate and pancreas extract used in these experiments.

References

1. Agren, G. 1949. On the occurrence of bacterial growth factors in liver extract. Acta Physiol. Scand. 17:35-82.

2. Anderson, A. W., and P. R. Elliker. 1953. The nutritional requirements of lactic streptococci isolated from starter cultures. II. A stimulatory factor required for rapid growth of some strains in reconstituted nonfat milk solids. J. Dairy Sci. 36:608-613.

3. Berry, J. M., L. D. Witter, and J. F. Folinazzo. 1956. Growth characteristics of spoilage organisms in orange juice and concentrate. Food Technol. 10:553-556.

4. Elliker, P. R., A. W. Anderson, and G. Hannesson. 1956. An agar culture medium for lactic acid streptococci and lactobacilli. J. Dairy Sci. 39:1611-1612.

5. Foster, E. M., F. E. Nelson, M. L. Speck, R. N. Doetsch, and J. C. Olson, Jr. 1957. Dairy microbiology. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

6. Garvie, E. I., and L. A. Mabbitt. 1956. Acid production in milk by starter cultures. The effect of peptone and other stimulatory substances. J. Dairy Res. 23:305-314.

7. Goel, M. C., and E. H. Marth. 1969. Chain length of *Leuconostoc citrovorum* modified by dilution and shaking procedures of the plate count. J. Dairy Sci. 52:1941-1947.

8. Goel, M. C., and E. H. Marth. 1969. Growth of *Leuconostoc citrovorum* in skimmilk at 22 and 30 C. J. Dairy Sci. 52:1207-1213.

9. Goel, M. C., and E. H. Marth. 1969. Stimulation of the growth of *Leuconostoc citrovorum* by animal by-products. J. Dairy Sci. 52:893.

10. Kereszlesy, J. C., and M. Silverman. 1950. Partial purification of a factor essential for growth of *Leuconostoc citrovorum*. J. Biol. Chem. 183:473-479.

11. Kizer, D. E., L. Hankin, M. L. Speck, and L. W. Aurand. 1954. Stimulation of lactic bacteria by substances concomitant to certain proteolytic substances. J. Dairy Sci. 37:634.

Koburger, J. A., M. L. Speck, and L. W. Aurand. 1963.
 Identification of growth stimulants for *Streptococcus lactis*.
 J. Bacteriol. 85:1051-1055.

13. Marth, E. H., and R. V. Hussong. 1962. Effect of skimmilks cultured with different strains of *Leuconostoc citrovorum* on growth of some bacteria and yeasts. J. Dairy Sci. 46:1033-1037.

14. McAnelly, J. K., and M. L. Speck. 1956. The response of lactic acid bacteria to stimulants present in pancreas tissue. J. Dairy Sci. 39:914.

15. Sauberlich, H. E., and C. A. Baumann. 1948. A factor required for the growth of *Leuconostoc citrovorum*. J. Biol. Chem. 176:165-173.

16. Smyk. B. R. 1957. Studies of lactic acid bacteria. Part I. Effect of some growth factors. Nauk Rol. (series Zootechniczna) 71:301-312.

17. Speck, M. L., J. K. McAnelly, and J. Wilbur. 1958. Variability in response of lactic streptococci to stimulants in extracts of pancreas, liver and yeast. J. Dairy Sci. 41:502-508.

18. Sprince, H., and D. W. Wooley. 1945. The occurrence of the growth factor streptogenin in purified proteins. J. Amer. Chem. Soc. 67:1734-1736.

19. Walter, W. G. (ed.) 1967. Standard methods for the examination of dairy products (12th ed.) American Public Health Association, Inc., New York.

QUALITY CONTROL IN DAIRY MICROBIOLOGY

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Abstract

The principles of quality control are the same in any microbiology laboratory. Results are as good as the materials used and as the people who use them. Dairy microbiology could be called a narrower field than some areas of microbiology and fewer materials may be needed. However, because of the public health implications, development of standards and requirements began earlier and for this reason, it has been slow to use more scientifically constructed and more reliable media. Evaluation of materials, methods, and requirements are always in order in progressive quality control. Specific suggestions are included.

Quality control in dairy microbiology is essentially the same as for any area of microbiology. The organisms encountered are the same. Most of the materials used are the same. People do the work, with more or less skill and accuracy. In dairy work, limitations are greater than in some other areas. There are standard procedures required, which is not necessarily true in other areas, and there are limitations to the kinds of specimens tested, and possibly as to the variety of microorganisms sought.

Quality control is mostly common sense applied in the laboratory to obtain the most efficient results from test procedures. It begins, of course, with the manufacturer of materials and equipment employed and continues through their use in the dairy control laboratory.

Culture Media

Culture media are perhaps of primary importance. There has been much written and much work done on Standard Methods Agar used for plate counts. Two productivity tests have been developed, and they are both outlined in Appendix A (pp. 234-241) of the Twelfth Edition of the American Public Health Association (A. P. H. A.) Standard Methods for the Examination of Dairy Products (1). The first of these was developed by collaborative studies with Dr. Leon Buchbinder and his colleagues (4, 8). The test procedure was so complicated to interpret that it was neither understood nor widely used. The second one, developed by collaborative studies under the direction of Dr. Howard Bodily of the California State Department of Public Health, was more practical to perform. However, in May 1969, it was realized that it could not properly be used to obtain countable plates, having 30 to 300 colonies per plate, with many composites of pasteurized milks. The problem was that the diluted, pasteurized milks had too few organisms; the milk had to be first incubated, or aliquots of higher count milks added to get enough colonies. The test was, therefore, inapplicable and a uniform procedure could not be used. For this reason, it was agreed by the collaborators that studies with pasteurized milks would be discontinued.

It is a fact that the flora of milk and related products are numerous, diverse, and may vary seasonally and geographically. For this reason, it has been our belief that, to supply a test medium for this kind of work, it is inadequate to use tests such as those described, and it is necessary and more practical to use test organisms representative of those which may be encountered, without regard to local dairy products, seasons, etc. Our certificates, therefore, carry information on both types of testing (5). The organisms now used include *Bacillus subtilis*, *Corynebacterium diptheriae*, *Escherichia coli*, *Leuconostoc mesenteroides*, *Staphylococcus aureus*, *Streptococcus fecalis*, *Streptococcus pyogenes*, three lactobacilli, and two isolates that had been problems in dairy products.

The whole problem of productivity is complicated by the fact that Standard Methods Agar must not be too productive; allowable limits might have to be changed, affecting the grading of milk and various governmental regulations. This effect would be partially neutralized by the fact that collection and pasteurization methods have also improved in the many years which have elapsed since some of the limits were set. It is easy to make a more productive medium, one that will, in general, grow more of the flora present, and/or more of the microbes damaged, but not killed, by the heat of pasteurization. The requirements are that the medium must give approximately the same counts as media used decades ago. Relatively few people realize this situation, so different from a blood culture or sterility test in which an effort is made to detect all organisms present, and incorrectly believe that the function of the plate

¹Presented at the 47th Annual Meeting of the New York State Association of Milk and Food Sanitarians, Syracuse, New York, November 23-25, 1970.

count is for enumeration of the total aerobes in the product.

Coliform media and others required in the dairy laboratory should be thoroughly tested prior to use. Although not required by standard methods, selective media should be tested both for productivity of the desired organisms, and for inhibition of others, e.g. for growth of *E. coli* and for inhibition of streptococci.

Fortunately, laboratories seldom make their own media from the components. But if this is done, testing must begin with the ingredients, as it does with most commercial producers of media. Moreover, A. P. H. A. permits use of unidentified protein components, although it does specify the sugars and salts to be incorporated.

Whether selective or not, media should be tested for and have lot to lot equivalence and long term stability. Recent studies show, for example, that there are no significant differences between media (in *sealed* containers) made over a period of 5 years, but that changes may occur after that time, especially in selective media. A summary of stability studies on the following media, most of which are seldom used in the dairy laboratory, but which are representative of widely used formulas, yielded the following data (5, 6).

Media	No. lots	Satisfactory (Ages in month:		Unsatisfactory (Ages in months)
Selective				
Bismuth sulfite	5	2 - 67	9	72 - 114
agar				Poor reactions
Brilliant green	5	5 - 75	6	78 - 101
				Not selective
Nonselective				
C T A medium,	8	4 - 118	0	
Growth scores		79 - 83		
Lactose broth,	11	3 - 116	0	
Growth scores		71 - 72		
Trypticase soy				
broth,	11'	4 - 110	0	
Growth scores		71 - 72		

From such results, it is obvious that, properly stored in sealed containers, dehydrated media will keep for long periods of time, far longer than is necessary or desirable for good housekeeping. Recommended for control of dehydrated media, by monitoring monthly or biweekly (2, 7, 12, 13) are:

- 1. Purchase suitable package sizes, so that no one container will be entered over a period of more than 2 or 3 weeks. Deterioration of media in unsealed containers occurs at different rates, due to formulation, humidity, temperature, and other factors.
- 2. Date on receipt and rotate stock. Maintain a

supply adequate for 6 to 12 months. Discard media in sealed containers, if it is older than 2 (?) years, or retest thoroughly.

- 3. Discard materials in poor condition. Date when opened and then discard containers that have been opened for any considerable time, whether or not there is visible evidence of deterioration.
- 4. Store below 80 F in a cool, dry place. Store assay and other sensitive media in the freezer or refrigerator.

Generally it is convenient to maintain a log, showing receipt, testing, and remaining supply.

FINISHED MEDIA

Purchase of dehydrated media that have been thoroughly tested by a reputable producer does not by any means insure the use of properly functioning media in the using laboratory. Many precautions, some of them being covered by standard methods, must be taken at all times.

pH test. One test that can be done very quickly is a pH test, after the medium has been poured and solidified in a plate, without addition of a specimen. The reaction should be tested electrometrically, and the meter should be pretested with two fresh buffer solutions, one each of pH 4.0 and pH 7.0, and adjusted if necessary. Depending upon the type of meter available, the medium can be tested in the plate, or the solid agar can be removed by means of a clean knife, to the meter cup. If a medium has been over-heated at any time, the pH is likely to fall, so that this test indicates whether the medium has been made and handled properly, and whether or not it should be used.

The 1967 Standard Methods indicates permissible variation of the final reaction from zero to 0.3 for various media. Meters vary somewhat, and it would be more sensible and just as accurate to require that the final pH (of all media when used) should vary no more than \pm 0.2 from a stated figure. The U. S. Pharmacopeia (U. S. P.) formerly required, for a number of media, a variation of not more than \pm 0.1 from a stated pH, but this proved to be impossible to meet. The 1970 U. S. P. now requires variation to be within \pm 0.2, which then permits agreement from meter to meter, from year to year, and from one laboratory to another, and it also readily eliminates unsatisfactory media (10).

The *final* pH is specified for most media; this is the pH of the finished medium. An agar medium is not normally used in the liquid form at 45 C. A pH at room temperature (22 to 25 C) is about 0.5 unit less than at an incubator temperature of 35 C. At 45 C, results are likely to vary. Performance tests. It is desirable that performance tests (1, 2, 7, 11, 12, 13) be done routinely on all culture media. Perhaps a weekly check ought to be done, and wherever possible, at the same time as routine tests. The present A. P. H. A. productivity tests on Standard Methods Agar, discussed above, are exceedingly time consuming and complicated. They are probably rarely done in a practicing laboratory.

Most other media should be at least checked for the correct reactivity. For coliform media, minimal tests could be with a typical aerogenic *E. coli* and with *B. subtilis.* An overnight broth culture could be diluted 1:1000 with sterile water, buffer or broth, and a standard loopful of the dilution streaked on a plate, or added to a tube.

The test for inhibitory substances includes controls on the same plates. Penicillin control discs tend to deteriorate if kept in refrigerators that are frequently opened. If this is the case, store the bulk supply of discs in a freezer. Once a week remove sufficient discs to the refrigerator, for a week's supply in the laboratory.

Incidentally, inoculated spore plates make a good test method for toxic residues on plates. If toxic material remains on plates, clear areas after incubation indicate failure of growth.

Other control measures, which are nonspecific (3, 7, 12, 13), should include—

- 1. Dating and rotation of prepared media.
- 2. Discarding of plates showing any evidence of contamination, or drying, or other deterioration.
- 3. Preparation in sealed containers, if possible.
- 4. Storage under refrigeration.
- 5. Protection from light damage of all media containing indicators.

MONITORING

Equipment and other materials, as well as culture media should be monitored on a regular basis. One person should be responsible for the necessary inspections, records, and tests (2, 7, 9, 12, 13). Some examples are as follows.

CHECK LIST

Air filters (Monthly)

Autoclaves

(Constantly)

Periodically expose plates of nonselective medium for counts, e.g. for 3 hr exposure. See below.

Use of specified temperature and time for sterilization of media and for other loads. Automatic chart records should be kept; those with media can be attached to the test records on the media. Spore controls and tests on them.

Incubators (Daily)	Temperature during 24 hr. Temperature in various areas, using thermometers in tubes of water. Humidity.
Ovens and waterbaths	Record temperatures and adjust as necessary.
Refrigerators and freezers (Monthly)	Record and adjust temperatures. Discard outdated and deteriorat- ed materials. Rotate stock con- tents as necessary.
pH meters and buffers (Twice daily)	Proper adjustment of meters; cross check with two standards. Date buffers, solutions, tablets, etc.
Glass petri plates* and tubes	Discard any that are chipped or etched. Check cleanliness. Test sterility if sterilized "in- house." See that no detergent is used, or test that no detergent or other possibly toxic material remains.
Glass pipettes*	As for glass plates. In addition, check delivery.
Other glassware	Use glass or stainless steel ware. Check for cleanliness and for
Swabs*	freedom from detergent, etc. Test for sterility and for "tox- icity." Fibers, wood and adhe- sive may be toxic. One test method is to submerge for sev- eral days in a broth such as Trypticase Soy Broth. Then inoculate broth, with and with- out swabs, with small inocula of suitable test organisms, such as <i>S. pyogenes</i> .
*Alternatively, purchase pr do NOT use more than or	esterilized and pretested items and nee.

Milk and other dairy products may contain many organisms that can cause disease — e.g. *Brucella*, *Salmonella*, *Serratia*, *Pseudomonas*, etc. Pasteurized samples should be safer than raw ones, but ALL samples should be handled with care.

Dairy laboratories could well learn from other industries. General monitoring with a medium having a broad spectrum for microbial growth, such as plain U S P Soybean-Casein Digest Agar (BBL Trypticase Soy Agar) is recommended as being more convenient than the blood plates commonly used. Such monitoring could be applied not only to production areas, but also to the laboratory work bench. Settling plates may be used, but for surfaces "Rodac" plates are more convenient. In the voluntary federal control program, settling plates are required for plating work, with a maximum colony count of no more than fifteen colonies after fifteen minutes exposure to air (9).

For the future, the following should be considered for better quality control.

1. Standard Methods Agar. Development of a short practical test that could be run weekly or more

frequently.

- 2. Concurrent performance controls with all media, daily or weekly.
- 3. Improved selection of media.
 - a. Replacement of the Standard Methods Agar with a view to approximation of total microbial coverage.
 - b. Use of modern, well-defined, and more replicable media, which do not contain any crude or unspecified, undefined materials such as "peptone" or "extract".
 - (1) Violet Red Bile Agar contains crude bile salts rather than the sodium desoxycholate in M-F Endo Broth and in Desoxycholate Lactose Agar
 - (2) Nutrient Broth and Agar contain "beef extract" and could be replaced with U. S. P. Soybean-Casein Digest Agar and Broth (BBL Trypticase Soy Agar and Broth).
 - (3) Potato Dextrose Agar has been replaced in Europe by the International Dairy Federation medium, Dextrose Salt Agar. Incubation of plates is for 3 days at 20 C plus 2 days at 30 C.
- 4. Testing or pretesting of devices and pipettes, by lot (sterilization chamber load or "run"), prior to use. Tests should include sterility tests of the U. S. P. type and performance tests for freedom from toxicity and for delivery.
- 5. Pretesting of finished *media* and dilution buffer, by lot, for performance and for sterility. Thorough pretesting does not necessarily permit omission of concurrent control tests (2 above), unless new lots are made almost constantly. A year's supply of tubed or bottled media could be made or purchased. Concurrent controls really provide a systems check, not just of one or another item.

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- 6. Increased use of environmental checks, with more rigid requirements.
- 7. Increased purchase of quality control with satisfactory testing results included in the purchase, so that as much nonspecific work as possible can be eliminated from the dairy laboratory, and so that the laboratory can get on with its own important specific work.

References

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

2. Bartlett, R. C. 1967. Experience with a quality control program in a clinical laboratory. Warner-Lambert Pharmaceutical Co., Morris Plains, N. J.

3. BBL Manual of products and laboratory procedures. 5th ed. 1969. BBL, Division of BioQuest. Cockeysville, Md.

4. Buchbinder, L., Y. Baris, E. Alff, E. Reynolds, E. Dillon, V. Pessin, L. Pincus, and A. Strauss. Studies to formulate new media for the standard plate count of dairy products. Public Health Reports. 66:327-340.

5. Griffin, C. W. 1970. Personal communication. Excerpts from BBL quality control records. Cockeysville, Md.

6. Griffin, C. W., and H. D. Vera. 1969. Media standardization. Presented at the Amer. Public Health Ass. meeting. Philadelphia, Pa.

7. Jennings, E. R., J. W. Reynolds, R. L. Russell, and G. A. Schmidt. 1970. Quality control program for microbiology. Memorial Hospital, Long Beach, Calif.

8. Pessin, V., and L. A. Black. 1951. A comparative study of six agars proposed for bacterial plate counts of milk. J. Milk Food Technol. 14:98-102.

9. Survey form for milk laboratories. 1968. U. S. Dept. Health, Education, and Welfare. Public health service. Washington, D.C.

10. The United States Pharmacopeia. 18th ed. 1970. Mack Publ. Co. Easton, Pa.

11. Vera, H. D. 1967. Guidelines for control of routine microbiology. Warner-Lambert Pharmaceutical Co., Morris Plains, N. J.

12. Vera, H. D. 1970. Quality control in diagnostic microbiology. BioQuest. Cockeysville, Md.

13. Vera, H. D., and M. Dumoff. 1970. Culture media. pp. 633-674. *In* Manual of clinical microbiology. Amer. Soc. Microbiol. Bethesda, Md.

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SPORE DESTRUCTION WITH HOT CHEMICAL SOLUTIONS'

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(Received for publication February 26, 1971)

Abstract

Nine chemical solutions commonly utilized for sanitizing and/or cleaning were used in studies on destruction of *Bacillus subtilis* spores in laboratory trials and, subsequently, in trials with a pilot-plant size UHT tubular sterilizer. The strengths of the chemical solutions were varied in an effort to determine the most efficient usage. The temperature of these solutions was maintained at approximately 93 C, and the time was limited to 30 min.

In laboratory trials spores placed on strips of stainless steel were sterilized by nine chemicals. The concentration necessary for sterilization ranged from 50 ppm for an iodine compound to 12,150 ppm for an alkali compound. In pilot trials solutions of sodium dichloroisocyanurate and an acid sanitizer A, at 300 ppm and 1,500 ppm, respectively, were effective in sterilizing the UHT tubular sterilizer during 30 min.

Chrome-plated nickel resisted corrosion by each of the nine chemical solutions at 93 C during 8 hr of testing. Types 303, 304, 316, and 410 stainless steel showed the effect of corrosion from two or more of the nine hot chemical solutions. Sodium dichloroisocyanurate at 300 ppm had the most deleterious effects on the four stainless steels. The iodine compound, at 100 ppm, was second.

Of the 14 gasket and other materials tested, one showed no visible deterioration when exposed to the nine chemical solutions at 93 C for 8 hr, nine softened slightly in one of the nine solutions, and three materials showed slight softening in two of the solutions. Vespel disintegrated in more than half of the chemical solutions.

Sterilization of equipment before beginning ultrahigh temperature sterilization and aseptic packaging (UHT-AP) of fluid milk and other food products is essential. The common practice of sterilizing the equipment with steam has the disadvantage of requiring an excessive amount of time in many plants. This may be as long as 2 hr, depending upon various factors, such as steam pressure, kind and condition of equipment, etc.

UHT-AP processors desire to reduce the equipment sterilization time, but it must be by economical means. One possibility is to use a combination of heat and chemical solution, especially with the application of the full-flood method of cleaning. This paper presents the results of laboratory and pilot equipment sterilization trials using heated chemical solutions on bacterial spores.

Experimental

Bacillus subtilis A_1 which had been isolated from sterilized milk was grown and harvested according to the method of Angelotti et al. (1). These bacteria were held at 4 C. Tests indicated no significant change in viable numbers occurred while each supply was used. The spores inoculated into water were used for contamination of surfaces to be teststerilized.

In the laboratory trials 0.5- \times 2.0-inch strips of type 304 stainless steel, with a No. 4 finish, were manually washed with a detergent solution, rinsed, and dried. One-tenth milliliter of an aqueous spore suspension containing approximately 1,400 spores was pipetted onto the stainless steel strip and air dried at room temperature. The spores were adjusted to a water activity of 0.85 during a 24-hr hold in a chamber with reduced pressure and a saturated solution of potassium chloride at 25 C. The contaminated strips were inserted into test tubes (screw cap) with 20 ml of the chemical solution. The tube contents were heated rapidly to 93 C \pm 1 C in a waterbath. The strips were removed and drained aseptically at regular intervals between 20 to 60 min. Each strip was placed in a tube with 20 ml of cold, sterile water. After 5 min of shaking, a portion of the water was plated with dextrose tryptone starch agar and incubated at 35 C for 48 hr to determine survival number. Tests were made with known small inoculations for a possible growth inhibitory effect. No significant inhibition was observed.

Different sets of six stainless steel strips and 14 gasket materials were partially submerged in the nine different chemical solutions for 8 hr at 93 C. Each was examined visually for evidence of corrosion.

In the pilot plant trials the principal equipment, which included the tubular sterilizer³ and timing pump, were cleaned and rinsed by CIP. The product surfaces of this equipment were subjected to full-flood contact for cleaning, rinsing, and hot chemical sterilizing operations. Balance tank ahead of the timing pump was manually cleaned and rinsed.

The sterilizer was contaminated by circulating an aqueous suspension containing approximately 4,700 spores per ml for 5 min. Gaskets were dipped into the suspension of spores before replacement in the equipment system. Rough tests indicated the contamination of product contact surfaces of the equipment were very high compared to these surfaces without circulating the spore suspension.

After contamination, the water with the spores was drained from the system. The chemical solution was pumped into the system and heated to 93 C. It was maintained at that temperature during the 30 min recycling period for sterilizations. Recontamination can occur after sterilization if air is allowed into the system by draining the solution. Therefore, the solution temperature was increased to 135 C for an instant while immediately opening the water valve for indirect cooling after the sterilization step and also for introducing water into the system to thoroughly flush out the

³Spirotherm, Cherry-Burrell Corporation, Cedar Rapids, Iowa.

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TABLE 1	CONCENTRATION OF CHEMICAL SOLUTIONS AT 93	С
TO STER	UJZE STAINLESS STEEL STRIPS DURING 30 MINUTES	

Compound	Usage (%)	Active ingredient (ppm)
Quaternary A	0.37	400
Quaternary B	7.83	3,750
Acid sanitizer A	3.92	1,000
Acid sanitizer B	1.18	600
Iodine compound	0.31	50
Caustic soda	2.51	12,150
Sodium dichloroisocyanurate	0.12	200
Chloramine-T	1.86	3,000
Chlorine dioxide	18.00	3,600

TABLE 2. LETHAL EFFECT OF CIRCULATING CHEMICAL SOLUTIONS AT 93 C FOR 30 MINUTES ON SPORES IN SMALL TUBULAR STERILIZER

Compound	Usage (%)	Active ingredient (ppm)	Trial A samples 1, 2, 3	Trial B samples 1, 2, 3
Quaternary A	0.56	600	+1 + +	$-^{2} + +$
Ouaternary B	11.75	5,625	+ + +	_ + +
Acid sanitizer A	5.88	1,500	+ + +	+ + +
Acid sanitizer B	1.77	900	+ + +	_ + +
Iodine com pound	0.62	100	_ + +	+ + +
Caustic soda (48,3% NaOH)	3.77	18,225	+ + +	- + +
Sodium dichloro- isocyanurate	0.18	300	+ + +	+ + +
Chloramine-T	3.72	6,000	- + +	_ + +
Chlorine dioxide	27.00	5,400	+ + +	_ + +

 $^{1}+$ = no spoilage

 2 _ = spoilage of sample

chemical solution.

Nonfat dry milk reconstituted in water followed the rinse and was processed at a UHT of 135 C for 6 sec, before cooling to 21 C. Three samples, consisting of the first one gallon, the eighth quart, and the twelfth quart, were taken aseptically in glass bottles and incubated 2 weeks at 35 C. Each sample was checked by sensory means for spoilage and tested bacteriologically if questionable.

Nine different chemicals were used in the trials. They were commercial cleaning and/or sanitizing products, but most were commonly used as sanitizers: chlorine, iodine, and quaternary compounds. Also included in the study were acid sanitizing agents and an alkali compound.

The active chemicals in the various products were: Quaternary A, n-alkyl dimethyl benzyl ammonium chloride; Quaternary B, n-alkyl dimethyl benzyl ammonium chloride and tetrasodium ethylenediaminetetraacetate; Acid sanitizer A, ortho phosphoric acid and sulfonated oleic acid, Acid sanitizer B, ortho phosphoric acid and dodexyl benzene sulfonic acid; Iodine compounds, iodine and polyethoxy-polypropoxy alkanol iodine; Caustic soda. sodium hydroxide and organic sequestrant plus nonionic surfactant; Dichloroisocyanurate, sodium dichloroisocyanurate; Chloramine-T, chlorine of chloramine-T type and sodium carbonate plus complex phosphates; Oxine, chlorine dioxide.

RESULTS AND DISCUSSION

Results of extensive laboratory trials were used to

establish the approximate strength of each chemical solution at 93 C, necessary to destroy the B. subtilis spores on the stainless steel strips within 30 min. The recommended concentration of active ingredients for sanitizing or cleaning was used as a guide to initiate the trials. Thirty minutes was arbitrarily assumed to be the maximum time desirable for sterilization of UHT-AP equipment. Water definitely was not effective at 93 C for 30 min in destroying all the spores. The concentrations of nine different chemical solutions were increased until lethal for the spores within 30 min. Results are presented in Table 1. Sodium dichloroisocyanurate was effective at the low concentration of 200 ppm. Two and one-half per cent caustic soda compound was needed to destroy the spores. The minimum strength required of Quaternaries A and B varied substantially between these two in order to obtain sterility. The required concentrations were 400 ppm and 3,750 ppm, respectively.

Sterilization of the tubular UHT equipment was not accomplished with the same strength of chemical solutions used for the submerged stainless steel strips when the same temperature and time were maintained. The results of a 50% increase in concentration of chemicals, but for the same time (30 min) and temperature (93 C), are given in Table 2. The two chemicals with the lowest concentration were increased 100%. However, the viable spores were not completely eliminated with seven of the nine different chemical solutions at the concentrations used. Sodium dichloroisocyanurate effectively sterilized the tubular sterilizer at 300 ppm. Acid sanitizer A was effectively sterilized the tubular sterilizer at 300 ppm. Acid sanitizer A was effective at 1,500 ppm.

The use of Quaternary B and chlorine dioxide seems to be less practical because an even higher concentration will be needed to obtain sterility than shown in Table 2. Under conditions of the trials, the sampling and incubation of the first gallon of product given the UHT treatment appeared to be a more critical test for equipment sterility than subsequent samplings. The higher concentrations required to sterilize the UHT sterilizer compared to the stainless steel strips was attributed to the greater difficulty in destroying the spore contamination on the valve components and the tubing unions with gaskets.

Table 3 presents the influence of the chemical solutions on common dairy equipment metals. The concentration of the chemical solutions used for these corrosion studies was the same as used for spore destruction in the tubular sterilizer (Table 2). Corrosion was considered to be a visible or physical change in the material such as roughing of the sur-

TABLE 3. THE EFFECT OF CHEMICAL SOLUTIONS AT 93 C ON VARIOUS METAL SHEETS

- Chemical	Concentration	303 SS		304 SS	316 SS	410 SS	Nickel	Chrome- plated nickel
Quaternary A	600 ppm	MED ⁴ EX ⁷ COR ³	MED	SU COR	NE	ME EX COR	NE	NE
Quaternary B	5,625 ppm	NE ¹	SL	EX COR	NE	NE	NE	NE
Acid sanitizer A	1,500 ppm	NE	SL	EX COR	NE	SL EX COR SL SU COR	NE	NE
Acid sanitizer B	900 ppm	NE	SL	EX COR	NE	SL SU COR SL EX COR	NE	NE NE
Iodofor	100 ppm	NE		SU COR EX COR	NE	DEF EX COR MED SU COR	DEF EX COR	NE
Caustic soda	18,225 ppm	SL ² EX COR	SL	EX COR	NE	NE	NE	NE
Sodium dichloroiso- cyanurate		DEF ⁵ EX COR SL SU ⁶ COR	DEF SL	EX COR SU COR	DEF EX COR SL SU COR	DEF EX COR SL SU COR	NE	NE
Chloromine-T	6,000 ppm	SL EX COR		NE	NE	NE	NE	NE
Chlorine Dioxide	5,400 ppm	NE	SL	EX COR	SL EX COR DEF SU COR	NE	NE	

 $^{1}NE = no effect$

⁴MED = medium ⁵DEF = definite

face, wearing away, pitting, softening, or disintegration.

Chrome-plated steel showed no visual evidence of corrosion by the nine chemical solutions. The other metals exhibited various amounts. Type 304 stainless steel, one of the most commonly used types in dairy equipment, was corroded by eight of the nine chemical solutions. Type 410 stainless steel was next most susceptible. It was affected by five of the chemical solutions.

In commercial operations corrosion of equipment But with surfaces is an important consideration. reasonable care a hot chemical solution for sterilization is not anticipated to present too serious a problem. First, the full-flood system, necessary for sterilization, would necessitate that the surface be completely covered with the solution during the steriliz-Secondly, chemically accelerated ing procedure. corrosion has been a problem with faulty sanitizing procedures for many years. Consequently, the common practice is to sanitize just before use. The same practice must be applied to a hot chemical solution sterilization of the equipment. By sterilizing immediately before processing and a start-up with water to adjust controls, the chemical residues are rinsed out of the system before the product processing begins.

A few of the solutions can be adjusted in pH for greater effectiveness. Trials are warranted to determine the adjusted pH at which optimum sterilization occurs with minimum corrosion. Since a difference in corrosive effect was observed among the chemical solutions when the material was completely submerged (Table 3), this fact, as well as cost of the chemical usage, should be considered in selection of the chemical. $^{6}SU = portion submerged in solution$

 $^7\mathrm{EX}$ = portion exposed above solution

Presumably, the spray method would be difficult to use for hot chemical sterilization of equipment within a reasonable length of time where complete destruction of all microbes is essential.

Sodium dichloroisocyanurate, which was effective in spore destruction at the low solution strength of 300 ppm, was corrosive for all four types of stainless steel used in the trials. The other effective chemical, acid sanitizer A, was much less corrosive at 1,500 ppm than sodium dichloroisocyanurate at 300 ppm. The corrosive effect of the other chemical solutions can be expected to increase as the concentration is increased sufficiently to obtain complete destruction of the spores within 30 min in the tubular sterilizer.

Laboratory trials were conducted with 14 gasket and other materials to determine their resistance to chemical solutions at 93 C. The same chemicals and concentrations shown in Table 3 were used. Glassepoxy plastic showed no effect in any of the nine solutions after exposure for 8 hr. Ethylene propylene rubber, nitrile rubber-182, nitrile rubber-733, hycar, teflon, silicone ruber, nylon, glass-filled teflon, and graphitar showed only a slight softening in one of the nine solutions and no effect in the other eight. Neoprene, nitrile rubber-ST, and vitron softened slightly in two of nine solutions. Vespel disintegrated in five solutions and definitely softened in a sixth. It was not tried in the remaining three solutions. Selection of resistant material for gaskets and tubing is necessary in order to avoid a serious deterioration problem.

The possibility was considered of the breakdown of the chemicals while in solution and maintained at temperatures up to the boiling point for extended periods. An official (2) of the company supplying

 $^{^{2}}SL = slight$ $^{3}COR = corrosion$

all but one of the chemicals stated that there was no danger of toxic products being formed under the temperature and other conditions of the trials.

When an equipment sterilization procedure is changed, economics as well as effectiveness must be considered. The cost of sodium dichloroisocyanurate, at 65 cents per pound, would amount to approximately 1 cent per gallon of solution with 0.18% (300 ppm of active ingredient). Acid sanitizer A, at \$2 per gallon, and used at the rate of 5.88% (1,500 ppm active ingredient) would cost 11.76 cents per gallon of solution. Since UHT sterilization equipment is cleaned in place, the use of hot chemical solution for sterilization should not increase the labor costs. Some initial cost could be involved in CIP programming for the use of the chemical.

In conclusion, hot chemical solution sterilization

can be effective with certain chemicals and conditions. The practicality under commercial conditions for full-flood equipment systems warrants investigation by plant officials.

Acknowledgment

The assistance of Mr. Gail Smith and the partial support of the Wyardotte Chemicals Corporation are appreciated. The excellent cooperation of V. R. Carlson, of Cherry-Burrell Corporation, and F. J. Misfeldt, of CP Division, St. Regis, also is appreciated.

References

1. Angelotti, R., J. H. Maryanski, T. F. Butler, J. T. Peeler, and J. E. Campbell. 1968. Influence of spore moisture content on dry heat resistance of *Bacillus subtilis* var *niger* spores. Appl. Microbiol. 16: 735-745.

2. Smith, Gail A. 1970. Private correspondence, Jan. 3.

BOOK REVIEW

ULTRA-HIGH-TEMPERATURE PROCESSING OF DAIRY PRODUCTS. Ey T. R. Ashton, E. L. Crossley, F. O'Conner, and A. C. O'Sullivan (ed.) Society of Dairy Technology, 17 Devonshire Street, London, W1N 2BQ, England, 1969.

This pamphlet is the result of a seminar presented in May, 1969, in conjuction with the opening of a new research laboratory by the Agricultural Institute, Moorepark, Ireland. It is significant that a seminar on UHT processing should be held in association with dedication of a research laboratory since, as the participants point out, there are still several problems in UHT processing to be solved. This publication will be of interest to all those presently processing UHT products and to scientists engaged in research on UHT processing.

"Engineering Aspects of UHT Processing" was presented by D. T. Stone. This included a review of the engineering design problems for both direct and indirect UHT systems. Problems with scaling, residence time, and construction materials (especially gaskets) were described and commercial plant designs discussed. The only major inadequacy of the presentation is in the discussion on scaling and the factors that influence it (other than flow velocity and surface roughness).

H. Burton presented the second paper, "Aseptic Packaging," and offered an enlightening review of the bacteriological principles in aseptic packaging. Some participants questioned the values for spore counts and potential spoilage rate that he used but the principle was still valid. Available aseptic systems were discussed from the product contamination standpoint.

The problems encountered in planning a large UHT installation were discussed by T. R. Ashton in "UHT Milk-Practical Application and Associated Problems." Possible plant arrangements with the Tetra-Pak unit, actual start-up conditions, and flavor and oxidative changes of milk were reviewed.

W. K. Downey, A. C. O'Sullivan, and M. K. Keogh presented the fourth paper entitled, "Physical and Chemical Characteristics of UHT Creams." They described the effects of milk composition (as a function of seasonal variation) and storage conditions on the flavor, physical, and whipping characteristics of some UHT creams. As participants in the seminar pointed out, this was not a complete study since the possible influence of seasonal variation on specific milk constituents was not investigated.

"Nutritional Aspects of UHT Processed Products" was the title of a paper by S. Y. Thompson. The nutritional quality of milk and a comparison of nutritive changes occurring in HTST and UHT milk were reviewed. This paper contains an excellent literature review and was one of the best papers in the seminar.

The seminar was closed with a paper by R. S. Renwick entitled, "Market Potential for UHT Products." After the scientific and technological papers it is only fitting that the market potential be explored and exposed. Mr. Renwick showed some imagination in describing possible UHT procesed products.

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MICROFLORA OF COCOA BEANS BEFORE AND AFTER ROASTING AT 150 C

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(Received for publication February 12, 1971)

Abstract

Cocoa beans representative of different fermentation practices, flavor types, and geographic origins were collected and analyzed microbiologically before and after roasting under laboratory conditions which simulated commercial practices. The detectable number of microorganisms per gram of bean before roasting ranged from 3×10^5 to 4.7×10^7 . After roasting the beans for 40 min at 150 C the counts ranged from 10^3 to 1.6×10^5 per gram. The genus *Bacillus* accounted for 91% of the 277 isolates. Only bacilli were found in roasted beans and *Bacillus stearothermophilus* and *Bacillus coagulans* were the predominant species. Microorganisms identified in unroasted beans were nineteen species of *Bacillus*, two of *Streptococcus*, two of *Micrococcus*, two of *Escherichia*, and single species of *Aerobacter*, *Flavobacterium*, and *Microbacterium*.

Cocoa beans are exported primarily from West central Africa and Brazil with smaller, but still significant, quantities being harvested in Ecuador north through the southern part of Mexico, the islands of the Carribean Sea, and the Southwest Pacific Ocean. The variability in quality of this agricultural commodity is of great concern to chocolate manufacturers. Much is known about the chemistry of roasted cocoa beans. However, published information on microbiological aspects is quite limited. The objective of the present study was to more fully characterize the microflora of raw and roasted cocoa beans.

After harvesting, cocoa beans usually undergo a fermentation which facilitates development of flavor precursors and helps to remove mucilage from the beans. At the start of fermentation, the predominant microflora are yeasts which are then replaced by lactic acid bacteria, acetic acid bacteria, and finally spore-forming bacilli as the fermentation is completed (3). In Jamaican beans, *Bacillus sub-tilis* was identified as the predominant organism after fermentation (7). During this process, the internal temperatures in the bean heap may reach 50 C (3).⁴ Moisture in beans is then reduced to 6% or less, most commonly by sun drying, but mechanical dry-

ing is finding increasing acceptance. Dried beans are exported in bags and are usually stored in silos at the processing plant until roasted.

One of the most important processes affecting quality involves the roasting of cocoa beans in the chocolate factory. During roasting, flavor is developed, moisture is removed, the microbial population is reduced, and the shell is loosened from the nib. Roasting time and temperature combinations may vary from 15 min to 2 hr and from 105 to 150 C (2).

MATERIALS AND METHODS

Bean samples

Four chocolate manufacturers supplied the following beans representing different fermentation patterns, flavor types, and geographic origins: Arriba (Ecuador), Bahia (Brazil), Ghana (Africa), New Guinea, Samoa, Sanchez (Dominican Republic), Trinidad, and a mixture of Bahia and Arriba.

Temperature measurement and roasting procedure

Raw beans were laboratory-roasted in a forced air STABIL-THERM constant temperature cabinet (Blue M Electric Co.). The mixture of Bahia and Arriba beans was used in a preliminary study to determine the temperature gradient in beans at various air temperatures. Thermocouples inserted in the geometric center of the beans were attached to a Honeywell-Brown Electronik potentiometer and temperature recordings were taken periodically for 40 min. At 135 150, 160, and 180 C oven air temperature equilibrium was reached after exposure periods of approximately 10, 15, 15, and 20 min, respectively.

The effect of roasting variables on the reduction in microbial populations was assessed by roasting beans for 30 min at the above mentioned air temperatures and by varying the exposure time (20, 30, and 40 min) at constant roasting temperature (150 C). All identification work involved beans roasted at 156 C. Organoleptic evaluations and chemical comparisons between samples roasted in the laboratory and roasted cocoa beans supplied by chocolate manufacturers indicated that time-temperature combinations employed in this study were representative of commercial practices.

Microbiological analysis

Immediately after roasting, 30 g of beans were blended for 2 min with 270 ml 0.1% sterile peptone water in a sterile Waring Blendor. Decimal dilutions were prepared using 0.1% sterile peptone water and were plated in duplicate on plate count agar (Difco) with 0.1% K₂HPO₄ added. After 48 hr incubation at 37 C the total number of organisms per gram was determined. Unroasted beans were tested for coliforms using the three-tube MPN method and lauryl tryptose broth (Difco). Samples from tubes showing gas

¹Authorized for publication on January 6, 1971, as paper No. 3905 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

TABLE 1 TOTAL NUMBER OF MICROORGANISMS IN COCOA BEFORE AND AFTER ROASTING FOR 30 MIN AT VARIOUS TEMPERATURES.

Roast temperature (C)	Number of organisms/g
Unroasted	1.5×10^7
135	2.6×10^6
150	2.7×10^{5}
165	2.8×10^3
180	0

¹Average of three trials.

TABLE 2. NUMBER OF MICROORGANISMS PER GRAM OF COCOA BEAN BEFORE AND AFTER ROASTING AT 150 C FOR VARIOUS PERIODS OF TIME.

		Roast time	(min)	
Bean type	0	20	30	40
	(Num	ber of micro	oorganisms/g	g)1
Trinidad	$4.7 imes 10^7$	$2.2 imes 10^6$	$4.2 imes 10^5$	$6 imes 10^4$
Mixed Bahia				
and Arriba	$1.5 imes 10^7$	$1.3 imes10^{6}$	$5.8 imes10^5$	$1.6 imes10^5$
Sanchez	$5.0 imes10^6$	$2.6 imes10^4$	$3.8 imes 10^4$	$2.7 imes 10^3$
Samoan	$2.8 imes 10^6$	$1.1 imes10^{5}$	$1.5 imes10^4$	$3.1 imes 10^3$
New Guinea	$2.0 imes 10^6$	$8.1 imes10^4$	$5.9 imes10^4$	$1.4 imes 10^4$
Ghana	$1.7 imes 10^{6}$	$1.7 imes 10^5$	$4.4 imes 10^4$	$6.4 imes10^3$
Arriba	$8.9 imes10^5$	$6.3 imes10^4$	3.9×10^4	$6.0 imes10^3$
Bahia	$3.0 imes10^5$	$1.1 imes 10^5$	$2.7 imes 10^3$	$8.4 imes10^2$

¹Average of three trials.

TABLE 3. THE MOST COMMONLY ISOLATED MICROORGANISMS IN COCOA BEANS BEFORE AND AFTER ROASTING FOR VARYING TIMES AT 150 C.

		Time Roas	sted (min)	
Organisms	0	20	30	40
		(% of is	solates)—–	. <u> </u>
B. stearothermophilus	18.4	23.2	18.6	20.5
B. licheniformis	14.9	5.4	14.0	9.1
B. circulans	11.5	21.4	11.6	15.9
B. coagulans	8.0	10.7	3.6	22.7
B. megaterium	3.4	5.4	7.0	4.5
B. brevis	10.3	5.4	3.6	4.5

production after 48 hr incubation at 37 C were transferred to 2% brilliant green bile broth (Difco) and incubated at 37 C for 48 hr. Positive tubes were then streaked on eosin methylene blue agar for confirmation of coliforms. To determine the number of yeasts and molds on unroasted beans, dilutions of 10^{-3} and 10^{-4} were plated on potato dextrose agar [(Difco), pH adjusted to 3.5 with 10% tartaric acid], and incubated for 72 hr at 25 C.

Individual colonies were isolated from plates having 30-300 colonies. Each isolate was cultured on tryptic soy agar slants (Difco) and classified according to Bergey's Manual (1) using procedures described in Laboratory Methods in Microbiology (6) and Manual of Microbiological Methods (10), except for sugar fermentation tests in which the microtiter method described by Fung and Miller (4) was employed.

RESULTS AND DISCUSSION

The numbers of microorganisms per gram of mixed beans before and after roasting for 30 min at 135, 150, 165, and 180 C are shown in Table 1. As evidenced, roasting at 180 C and 165 C for 30 min had an extreme bactericidal effect, but these temperatures also imparted scorched flavor characteristics.

The number of organisms found before and after roasting for various periods of time at 150 C are shown in Table 2. Microorganisms in unroasted beans ranged from approximately $3 \times 10^5/\text{g}$ for Bahia beans to $4.7 \times 10^7/\text{g}$ for Trinidad beans. Yeast and mold counts were not significant, being less than $10^3/\text{g}$ in unroasted beans. Only one sample, unroasted Trinidad, was positive for coliforms (110-MPN/g). After roasting for 40 min, microbial counts varied from $8.4 \times 10^2/\text{g}$ for Bahia beans to $1.6 \times 10^5/\text{g}$ for the mixed bean sample. As might \checkmark be expected, the number of microorganisms which survived roasting was highest in the most heavily contaminated samples.

A total of 277 isolates from unroasted and roasted beans were identified. The majority of these microorganisms belonged to the genus Bacillus (91%). Bacilli commonly found in unroasted beans but not in roasted beans were Bacillus cereus var mycoides, Bacillus firmus, Bacillus laterosporus, Bacillus macerans, Bacillus polymyxa, and Bacillus pumilus. Microorganisms other than bacilli detected in unroasted beans were Enterobacter (Aerobacter) aerogenes, Escherichia coli, Escherichia freundii, Flavobacterium lactis, Microbacterium flavum, Micrococcus candidus, Micrococcus conglomeratus, Streptococcus lactis, and Streptococcus thermophilus. With the exception of M. conglomeratus, these organisms were isolated from 1:10 dilutions and were not considered a part of the predominant microflora. They were not detected in roasted beans and presumably were destroyed during roasting.

Bacillus stearothermophilus was the most frequently identified isolate in both roasted and unroasted beans. Other commonly occurring species were Bacillus brevis, Bacillus circulans, Bacillus coagulans, Bacillus licheniformis, and Bacillus megaterium.

All microorganisms isolated from roasted cocoa beans belonged to the genus *Bacillus*, of which nineteen species were identified. As shown in Table 3, *B. stearothermophilus*, which is extremely heat resistant, and *B. coagulans* accounted for nearly onehalf of the isolates from beans roasted for 40 min at 150 C. *Bacillus stearothermophilus*, *B. coagulans*, and *B. circulans* showed more resistance to roasting than other species.

Cocoa beans analyzed in this investigation represented different fermentation practices, flavor types, and geographic areas. Despite these differences, the microflora of the samples were remarkably similar. Results are in general agreement with those of Ostovar who studied the fermentation patterns in Trinidad beans (9). He found that the genus Bacillus appeared at the fifth day of fermentation and predominated thereafter. The fact that all of the isolates from roasted beans were Bacillus is consistent with the report of Mundt (8) who found B. cereus and its variants as the principal microorganism in finished cocoa powders. Results from this investigation are also congruent with the isolation of bacilli and micrococci from 36 samples of cocoa powder reported by Gabis et al. (5).

Acknowledgements

This research was supported in part by the Chocolate Manufacturers' Association of the USA.

References

1. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957.

Bergey's manual of determinative bacteriology. 7th ed. The Williams and Wilkins Company, Baltimore.

2. Cook, L. R. 1963. Chocolate production and use. Catalog and Book Division Magazines for Industry, Inc. New York.

3. Forsyth, W. G. C., and V. C. Quesnel. 1963. The mechanism of cocoa curing. Advances Enzymol. 25:457-492.

4. Fung, D. Y. C., and R. D. Miller. 1970. Rapid procedure for the detection of acid and gas production by bacterial cultures. Appl. Microbiol. 20:527-528.

5. Gabis, D. A., B. E. Langlois, and A. W. Rudnick. 1970. Microbiological examination of cocoa powder. Appl. Microbiol. 20:644-645.

6. Harrigon, W. F., and M. E. McCance. 1966. Laboratory methods in microbiology. Academic Press. London and New York.

7. Kempf, N. W. 1964. The technology of chocolate. The Manufacturing Confectioner Publishing Company. Oak Park, Illinois.

8. Mundt, J. O. 1948. Aerobic bacteria in cocoa. J. Tenn. Acad. Sci. 23:201-203.

9. Ostovar, K. Unpublished observations.

10. Society of American Bacteriologists. 1957. Manual of microbiological methods. McGraw-Hill Company. New York.

BOOK REVIEW

BYPRODUCTS FROM MILK, Second Edition, *Edited by B. H. Webb and E. O. Whittier*, Published by the AVI Publishing Company, Inc., Westport, Connecticut, 1970, 428 p., Price \$18.00.

The detailed addition of progress in byproduct research and development during the 20 years between editions of this text has made it very current and worthwhile. The theme, "Gather up the fragments that remain that nothing (John 6:12), has been the apparent goal of be lost." the contributing authors; both to assemble available fragmentary references and to deal with a subject of special concern in our ecology-conscious society. The quality of the work is reflected in the selection of contributors. The majority represent the Eastern Utilization Research and Development Division, Agricultural Research Service, USDA, most responsible for the utilization and development of new products from milk. In addition, W. S. Arbuckle, M. F. Brink, E. H. Marth, and T. A. Nickerson strengthen coverage of some of these complex subjects.

New chapters are included describing utilization of milk fat, not generally considered a 'byproduct in the past. There also are new contributions reviewing nutritional characteristics and waste disposal.

The text is very readable and adequately reflects the purpose of the editors to assemble the various methods into one volume. Authors have combined bulletins, patents, and journal contributions making it easy to review the state of the art.

My criticisms of the text are minor. The title of chapter two, "Fermentation Products from Skimmilk," doesn't adequately cover inclusion of some whole and partially defatted milk products. Centigrade and Fahrenheit temperatures were used without consistent policy. Summaries of current practices did not always reflect their relative merits. Preparation of some of the lesser-known products such as milk crumb, casein glue, and lipolyzed milkfat products were inadequately described; reflecting the lack of published reference material.

In general, this text represents an excellent compilation by competent experts. It is certainly an indispensable reference work for anyone involved in the research and development of dairy products. It also is a valuable source work for those in practical plant operations and for students of dairy technology.

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SANITATION IN THE FOOD INDUSTRY

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Abstract

Plant sanitation and housekeeping go hand in hand. Sanitary standards are continually changing in direct relation to our standards of living. There is an unconscious resistance to change; the desire to retain old methods, some of which may have been extremely insanitary. Some food industries have established sanitary codes for the design, construction, and installation of machinery and equipment. Properly drained floors and adequate cleaning facilities are important features of plant design and construction. Certain aspects of equipment design are mentioned including holding vessels, product pipelines, valves, and conveyor belts. An effective cleaning program is necessary to maintain a plant in a sanitary condition. The necessary tools must be provided. Check lists can be used as an index of sanitation in a plant. Bacteriological line checks, the diacetyl test in the citrus industry, and swabs and contact plates for equipment contamination are also important tools. Good housekeeping is an important adjunct to sanitation. One company employs a Good Housekeeping/Safety contest as a method of maintaining a safe and neat appearing plant.

Sanitation in the food industry is an extremely broad subject. It touches upon plant design and construction, equipment, cleaning, etc. Therefore, this paper touches briefly on certain aspects of these topics. Much of the material was obtained from the citrus industry but would also be applicable to other food operations.

When one thinks of plant sanitation or the sanitation of an operation, one usually associates cleanliness with plant appearance. In other words: Is the plant clean, is it neat, well painted and is it being kept in good repair?

Being a sanitarian is a thankless job. It is like hitting one's head against a stone wall. The wall usually is the "red light" put up by management who usually states, "What's wrong with the place? It looks alright to me. Why the continual harp about keeping a plant clean?" One of the most timely reasons for keeping a plant clean appeared in the *Oakite News* (3). "Henry Ford, one of the most successful industrialists this country has ever produced, was asked what he would do if he suddenly found himself the head of a business that was failing because of excessive production costs. He quickly answered: "The first thing would be to see if the plant was clean. It is a hundred to one that I would find it dirty. I would clean it up. There is nothing so demoralizing to personnel as a dirty shop. Such a place drives away good men and attracts bad ones."

This statement was made a generation ago but it's just as timely today. Given a choice, no one would elect to work in a dirty plant. Every plant manager and foreman wants a man who will do his job well, but if that man is forced to work in filthy, unkept surroundings the chances are his worst traits, rather than his best will come to the surface. No one can be expected to give his most for a company that seemingly doesn't care about him or his personal working conditions. I repeat, no one can be expected to give his most for a company that seemingly doesn't care about him or his personal working conditions. This was true 60 years ago and it's true today and we venture to say will be true 60 years from now. Contented personnel produce more. There are fewer rejects. Quality is higher and costs are lower. Again: in a clean, well managed plant there are fewer rejects, quality is higher and costs are lower. Most readers are concerned with costs; directly or indirectly management is continually looking for ways to cut costs, but let me say this: costs cannot be reduced in a dirty, sloppy plant operated by discontented personnel. In other words, you can't have your cake and eat it too.

Have you ever been to Disneyland, Anaheim, California? If so, maybe you obtained the same impression I did the first time I paid this fabulous place a visit. What struck me more than anything else was the cleanliness of the whole operation. The grounds were so clean that one felt as if he committed a minor offense by dropping a cigarette butt or a chewing gum and/or candy wrapper on the ground. In other words, cleanliness promotes cleanliness.

SANITARY INDEX

The first topic I would really like to discuss is what I call the sanitary index. Our sanitary index, or standards for sanitation are continually changing like a stream of water which during a spring flood has just overflowed its banks. It spreads out with great force and fans out over the countryside. People who were never concerned with flood waters are now suddenly faced with them. As the water spreads,

¹Presented at the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., Cedar Rapids, Iowa, August 17-20, 1970.

more and more people suddenly find their feet getting wet, so-to-speak. This analogy also can be applied to the food industry that is now suddenly faced with FDA's GMPs. Those that are most concerned might be looked on as the food manufacturers closest to the break in the dike; or the bank where the force is the greatest. That would be, for example, the shrimp industry. As we spread out, others are finding that they now are suddenly getting their feet wet in regard to sanitation. People who heretofore never thought much about the importance of sanitation are now faced with it.

As I stated, our sanitary standards are fluid and continually changing in direct relation to our standards of living. We now demand better sanitation in our food processing operations. What was good yesterday is not acceptable today. As an example, 75 to 100 years ago, the outhouse was considered modern. It was the ultimate in design and convenience. Wouldn't it be a catastrophe if we had resisted change, and when people take their coffee break they have to look forward to this type of facility. Fortunately, we now have come a long way and now accept our modern restrooms as commonplace. Food processing plants also have changed and modernized their methods of operation from wooden equipment and hand operations to highly mechanized processes of today.

RESISTANCE TO CHANGE

We have seen how our living standards are continually changing and applying this to the food industry, one is faced with a number of obstacles. This, I call resistance to change-the desire to maintain old standards in food processing. Every one has a certain amount of built-in resistance to change. Unfortunately, this resistance to change is still with us in the food industry. Many readers perhaps have heard the remark: "I've done it this way for 30 years. Why should I now suddenly change?" I, personally, have been confronted with this statement on many occasions in the pea and corn industry where the processor was resisting changing from wooden to metal equipment. In this particular case wood was responsible for spoilage which was occurring in his product after it had been processed and stored in the warehouse. Unfortunately, this resistance to change is something that we are going to be faced with continually. Some industries, who were once very prosperous, have now gone out of existence because of their adherence to staid old methods and their lack of foresight to change with the times.

SANITARY CODES

Sanitary codes have been developed not only to

overcome resistance to change, but primarily to have a uniform standard of design. The primary purpose was to establish criteria for manufacturers in the design, construction, and installation of machinery and equipment which can readily be maintained in a clean and sanitary condition. For example, the dairy industry has the 3-A Standards covering equipment, design, fabrication, installation, etc.

PLANT DESIGN AND CONSTRUCTION

In order to have a sanitary operation, one must first have a plant that has sanitation designed into it. Along this line, I would like to mention several items which have caused me a certain amount of anguish throughout the years.

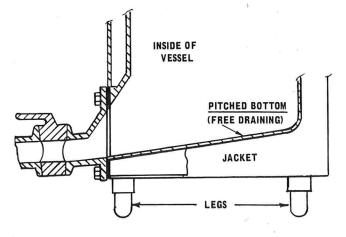
- The first is: Floors which should be selfdraining, readily cleanable, kept clean, and in good repair.
- The other: Cleaning facilities which should be adequate for the operation to be performed.

This might sound facetious but if we could place floor drains in the lower part of the floor, in my opinion, we would come a long way in sanitary design of a plant. I have seen floor drains not only in the citrus industry, but in many other food plants as well, where the drain is placed in the high part of the floor and the employees are required to squeegee the water to the drain. I just recently visited a new food processing plant. Floors in this case were perfectly level with drains spotted here and there. Water had to be squeegeed to the drains; a time-consuming operation. In connection with the floors themselves, there is great need for a surface material that will stand up under acid conditions. Epoxies have been used but there is still room for improvement.

The other item, many times overlooked, is the detergent system. Plants are frequently designed without any thought given as to how the equipment is to be cleaned. The ability to keep equipment clean and sanitary is just as important as the processing operation itself. Equipment might be of sanitary design and construction but unless proper tools are provided for cleaning, it cannot be kept in this condition. This item is so important but is frequently overlooked in a large number of our food processing plants.

Equipment Design and Plant Layout

Likewise, I would like to touch upon equipment design. Equipment should be so designed and of such material and workmanship as to be adequately cleanable.



CROSS SECTION OF PROCESSING VESSEL

Figure 1. Cross section of processing vessel for self draining (Courtesy of Cherry-Burrell Corp.).

Holding vessels; that is, storage tanks of all sizes, should be self-draining (Fig. 1). Unfortunately, this is not usually true. I have seen tanks as large as 50,000 gal capacity which could not be completely drained. Such a holding vessel is almost impossible to properly clean and, as you can imagine, creates sanitation problems.

Manholes used on many large holding vessels also should be of sanitary design, as illustrated in Fig. 2. Tanks are sometimes designed with manholes protruding from the side wall (Fig. 3). This is an extremely insanitary type of construction. It not only creates a dead pocket but the cover is also difficult to remove. I have observed tanks as large as 50,000 gal capacity with this type of manhole. The gasket, in this instance, was a serious source of contamination. Some vessels have covers bolted in place making them difficult to remove when cleaning the tank. A hinged cover with over-lapping lip would be more sanitary.

It is highly important that the engineer and sanitarian work together closely to make sure that sanitation is designed into the equipment and/or the plant itself.

PRODUCT PIPELINES

Product pipelines should be self-draining or otherwise designed so that the food product will not remain in them after they have been flushed with water. For sanitary reasons, pipes should be heli-arc welded instead of resistance welded. The first type results in a smooth surface inside the pipe in direct contrast to the latter, which produces a very rough condition.

A sanitary fitting should have the ferrule properly attached to the pipe; otherwise a crack or crevice will result. Welded connections and fittings provide areas for microbial growth which may be serious if a large number exist in a plant.

Flange connections should not be used, if possible. Instead, pipes should be either welded or a sanitary fitting used. These types of connections,

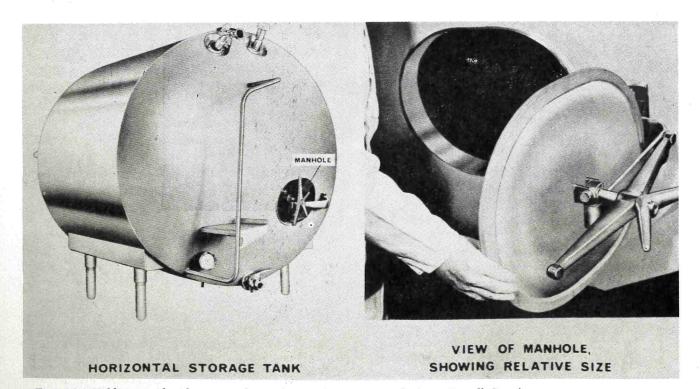


Figure 2. Holding vessel with sanitary designed manhole (Courtesy of Cherry Burrell Corp.).

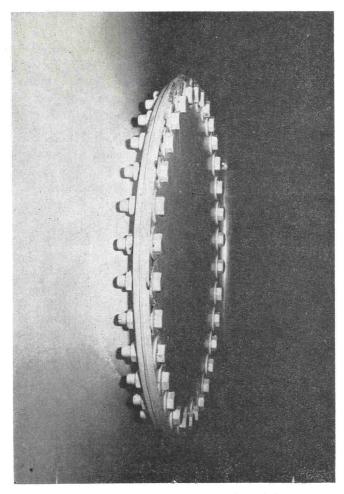


Figure 3. Tank with insanitary designed manhole.

when used in food product pipelines, are extremely insanitary. I noted 30 of these flanges on a 2-inch line in one plant over a distance of approximately 60 ft, and 29 on a 6-inch line. A swab made from one of the gaskets on the 6-inch line showed a total of 16,000 organisms per 3 inch² area when plated. In this instance, product entering this piping system contained less than 10 organisms per milliliter and was being recontaminated through use of numerous pipe flanges.

VALVES

Product valves should be of sanitary design. There are a number that fall in this category such as Keystone, Mills-McCanna, and the dairy sanitary stainless steel valve. However, there are still a number of insanitary valves used in the food industry. The gate valve (Fig. 4), for example, is extremely insanitary. In order to clean this valve properly, it must be completely dismantled. Valves of this type cannot be cleaned-in-place. Several years ago I visited a dog food canning plant which was having trouble with heat-resistant organisms of the "putrefactive anaerobe" type which caused spoilage in their finished warehoused product. In this installation gate valves were used throughout. An inspection showed the equipment to be cleaned properly, except for the gate valves, which when dismantled were found to contain maggots. When these valves were changed to a sanitary type, the processor experienced no more spoilage.

DEAD ENDS

A dead end is a space without an outlet. It is an area where product, cleaning and sanitizing agents, or extraneous matter may be trapped, retained, or not completely displaced in normal operation or cleaning procedures. A dead end can take various forms. Dead ends usually result when the processor endeavors to make his plant extremely versatile. In so doing, he has pipelines available which permit him to use various processing arrangements. Consequently, the possibility exists that certain lines may not be cleaned and subsequently when they are re-used, they become a serious source of contamination. Another source of dead ends is where the old product lines or unused lines are left in place, resulting in a

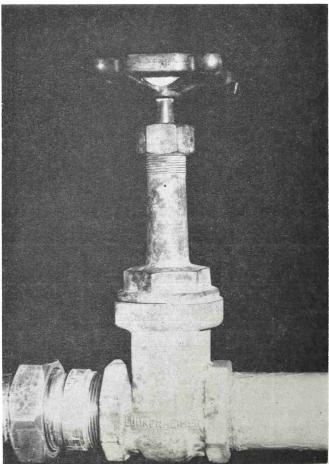


Figure 4. Gate valve-not designed to be used on food product pipelines.

portion of the line creating a dead pocket.

CONVEYOR BELTS

Conveyor belts in food processing plants should be kept clean and sanitary at all times. The design of some belts leaves a lot to be desired. For example, some have wooden sides, others have an open space between the edge of the belt and the sides, allowing food particles to become trapped permitting microorganisms to grow; thus contaminating product as it passes by. On the other hand, one might have a sanitary designed belt but neglect the roller bed on which it travels. If rollers are not operative, they will collect organic matter; thus, contaminate the belt. Where practical, belts should be continually sanitized by means of chlorinated water sprays.

PREVENTIVE SANITATION

One can have the most modern food processing plant but unless the equipment is maintained in a sanitary condition through an effective cleaning program, a modern installation can become an extremely insanitary operation. In order for the program to be effective, the necessary tools must be provided to accomplish the clean-up in a minimum of time with a minimum of effort. The ultimate in any cleaning is to have the operation programmed. In the not too distant future, I predict that most of our cleaning operations will be programmed. Industry will be forced to take this route not only because of the human factor one is faced with in a clean-up operation where every clean-up is different, but the difficulty in obtaining competent help and the cost of such help.

SANITATION AUDIT

How does one determine the condition of his plant? One method is by making frequent inspections of the operation. I like to use check lists for this purpose. It can be made a combination of sanitation and housekeeping since both go hand in hand. Various types of lists can be used. The usual practice is to develop a form for the plant involved. However, a universal form can be used if you are to cover plants producing the same type of product such as frozen citrus concentrates.

In making checks of a plant, it is advisable to have the foreman of each department accompany the inspector. In our operation, results of the inspection are kept at the plant level. It is circulated to the plant manager and Quality Assurance supervisor and the sanitarian, if one is attached to the plant. However, if no action is obtained, a summation report is published monthly listing all insanitary items that still exist after two or more inspections. This is given a wider circulation. In other words, top management is notified where the insanitary or poor housekeeping practices exist.

BIOLOGICAL APPROACH

I have discussed the use of plant inspections as a means of determining the sanitary and/or housekeeping condition of a plant. Now I would like to discuss with you what I call the biological approach.

In the citrus industry the diacetyl test, which requires less than an hour to perform, has been developed as a rapid procedure for detection of microbial activity in processing orange and grapefruit concentrates (1, 2). The diacetyl test is a colorimetric method for detection of diacetyl and acetylmethylcarbinol, end products of bacterial growth produced in orange juice principally by the organisms in the genera *Lactobacillus* and *Leuconostoc*. It is a very effective quality control tool.

Another method is the use of line checks which involves collecting samples of product from various processing operations and then plating the samples. However, line checks require 48 hr or more before the results are obtainable.

Another tool is the use of swabs. This is an effective method that can be used to determine equipment contamination. Various other methods have been developed to determine contamination on surfaces—such as the Rodac and contact plates.

HOUSEKEEPING

We have discussed the various methods that can be used to point out and/or detect poor sanitary practices. How does one maintain a neat and clean operation? Of course, management's attitude in this regard plays an important role. One method to create the incentive to keep the plant clean is a housekeeping contest. Each year, our Citrus Division operates a contest which is a combination of both safety and good housekeeping. This is the 12th year we have carried on this program. Posters using a catchy slogan announcing the contest are placed in each plant prior to the start of the program. Some examples are: "STOP ACCIDENTS," "CAUTION-THINK FIRST," "GO CLEANER AND SAFER," "WANTED-CLEANER AND SAFER PLANTS FOR '65," and currently, "STAY SAFE-KEEP IT CLEAN IN 1970." A booklet describing the program is prepared. One item which has aroused considerable interest is our Safety Sam slogans. During the day of the plant inspection, or twice each month, a Safety

Sam slogan is posted on the bulletin boards at each plant. At the end of each review the area personnel and safety manager draw from a hat at least 10 plant employees names. The personnel manager then contacts the eligible hourly employees in the order in which their names were drawn from the hat. The first one to repeat the slogan word for word, is awarded trading stamps redeemable at the local redemption store. If the employee is working in an inspection area receiving a perfect score (no points lost) during the current review, the award will be 5,000 trading stamps; otherwise, 3,000. Of course, the purpose of this phase of the program is to make our employees more safety minded.

Prizes in the form of stamps are drawn at the end of each contest period. The winning plant receives \$2.00, in the equivalent of stamps, per eligible employee and the second plant \$1.00 per eligible employee. The annual cost of this plant contest is from \$2,300 to \$2,600—this is for two of our plants. As a result, the morale of the employees, overall plant sanitation, and efficiency have been improved.

I hope that I have been able to give you some worthwhile information concerning sanitation and housekeeping as it applies to the food industry. Before closing, however, I would like to include a short poem which I believe is appropriate.

He walked up to the heavenly gates,
His face was scarred and old.
He stood before the man of fate
For admission to the fold.
"What have you done," Saint Peter asked,
"to gain admission here?"
"I was a sanitarian
Sir, for many, many a year."
The pearly gates swung open wide
When Saint Peter touched the bell.
"Come in and choose your harp," he said—
"you've had your share of hell."

References

1. Murdock, D. I. 1967. Methods employed by the citrus concentrate industry for detecting diacetyl and acetylmethyl-carbinol. Food Technol. 21:643.

2. Murdock, D. I. 1967. Diacetyl test as a quality control tool in processing frozen concentrated orange juice. Food Technol. 22:90.

3. Oakite News Service. 1969. Why clean. First quarter, 60th anniversary edition. Vol. LIII.

LETTER TO EDITOR

Perishable and potentially hazardous foods defined

Dear Sir:

At a meeting of the Board of Health of the Department of Health held April 13, 1971, the following resolution was adopted:

- RESOLVED, that the section heading of section 81.07 appearing in the schedule of section headings of Article 81 of the New York City Health Code, as amended by resolution adopted on the sixteenth day of November, nineteen hundred sixty-seven and filed with the city clerk on the fourth day of December, nineteen hundred sixty-seven, be and the same hereby is amended, to read as follows:
- 81.07 Food; refrigeration required if perishable; refrigeration or heat treatment required if potentially hazardous; microbiological examination authorized and conformance to standards required if designated potentially hazardous by Commissioner

Resolved further, that section 81.07 of the New York City Health Code, as enacted by resolution adopted on the twenty-third day of March, nineteen hundred fifty-nine and filed with the city clerk on the twenty-fourth day of March, nineteen hundred fifty-nine, be and the same hereby is amended, to be printed together with explanatory notes, to read as follows:

§81.07 Food; refrigeration required if perishable; refrigera-

tion or heat treatment required if potentially hazardous; microbiological examination authorized and conformance to standards required if designated potentially hazardous by Commissioner

(a) Perishable foods, unless otherwise provided and except those specified in subsection (b) of this section, shall be kept at all times under appropriate refrigeration at a

temperature no higher than 45 F in order to prevent spoilage.

(b) Perishable foods consisting, in whole or in part, of milk or milk products, eggs, meat, poultry, fish, shellfish or other ingredients capable of supporting rapid and progressive growth of pathogenic microorganisms shall be deemed to be potentially hazardous foods. Such potentially hazardous foods shall be kept at all times under appropriate refrigeration at a temperature no higher than 45 F or under appropriate heat treatment at a temperature no lower than 140 F.

(c) The Commissioner may designate, by administrative order, the names or types of potentially hazardous foods which shall be subject to microbiological examination by the Department. Such administrative order or orders shall be published forthwith in the City Record.

(d) No person shall manufacture, produce, pack, possess, sell, offer for sale, deliver or give away any potentially hazardous food of the name or type designated pursuant to subsection (c) of this section which contains bacteria in excess of the following standards:

(1) More than 100 per gram of hemolytic Staph-

ylococcus aureus; or, (2) More than 100 per gram of coliform organisms: or,

(3) More than 100,000 per gram in the total bacteria plate count; or,

(4) No fecal coliform and no Salmonella, Shigella or other pathogenic microorganisms.

(Continued on Page 384)

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GERMINATION OF SPORES OF CLOSTRIDIUM PERFRINGENS

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(Received for publication February 5, 1971)

Abstract

Germination of spores of *Clostridium perfringens* S-45 was optimal at pH 6.0 at a temperature of 30 C in a medium freed of dissolved oxygen. Heat activation was necessary, and maximal germination occurred when spores were heated for 20 min at 75 C. Of the various materials examined, a combination of cystine and sodium chloride was most effective for inducing rapid and complete germination.

Clostridium perfringens is an anaerobic spore-forming bacterium known to cause food poisoning, particularly as the result of the consumption of certain meat dishes (11) containing this organism. Dische and Elek (5) suggested that spores of this organism present on meat may survive ordinary cooking and subsequently germinate if conditions are suitable. Although a number of agents can induce germination in bacterial spores (9), the specific conditions for and stimulants of germination of spores of this organism have not been well defined. The purpose of the present work is to further determine some of the environmental conditions and nutritional requirements for rapid germination of spores of a strain of C. perfringens.

MATERIALS AND METHODS

Clostridium perfringens strain S-45 was used in these studies. The original culture was obtained from Dr. K. Weiss, University of Wisconsin, Madison. The organism was maintained on cooked meat medium stored at room temperature.

Spores were produced on the following medium (AW medium) consisting of yeast extract, 1.5%; Bacto tryptone, 1.0% soluble starch, 0.5%; K_2 HPO₄, 1.75%; urea, 0.5%; barbituric acid, 0.1%; bovine serum albumin (BSA, crystallized and lyophilized) (Sigma Chemical Co., St. Louis, Mo.), 0.4%; and mineral mixture, 1.0% (by volume). The mineral mixture contained MgSO₄ · 7H₂O, 2.0 g; MnSO₄ · H₂O, 0.46 g; NaCl, 1.17 g; CaCl₂ · 2H₂O, 6 g; ZnSO₄ · 7H₂O, 1.34 g; and FeSO₄ · 7H₂O, 1.11 g. Each salt was separately dissolved in 0.1 N HCl and combined to a final volume of 1 1. BSA was sterilized by Seitz filtration and added with the mineral mixture at the time of inoculation.

The inoculum for production of spores was prepared by inoculating 15 ml of fluid thioglycollate medium (Difco) with 1 ml of stock culture and incubating at 37 C for 20 hr. One ml of this culture was transferred to 15 ml of broth containing yeast extract, 1.5%; tryptone (Difco), 1.0%; soluble starch, 0.5%; and K₂HPO₄, 1.74%. After 16 hr at 37 C, the cultures contained vegetative cells and endospores; these cultures were aerated by shaking for 4 hr at room temperature to enhance maturation of the spores. If spores were not observed at this stage, production of large quantities of spores was not likely to occur in later steps; this medium was not satisfactory for sporulation of all strains of *C. perfringens*. After pasteurization at 75 C for 20 min, the resultant suspension was subcultured in fluid thioglycolate medium at 37 C for 8 hr. These subcultures were used as inocula (10% by volume) for production of spores in AW medium.

The medium containing spores was stored overnight at 5 C before harvesting. Spores were collected by centrifugation and washed several times in cold phosphate buffer (0.067 M, pH 6.0). Aeration at room temperature followed by storage overnight at 5 C permitted lysis of vegetative cells. After several additional washings, the final preparation contained essentially refractile spores. Spores were kept frozen, and were thawed and suspended in distilled water when needed. These suspensions contained approximately 1.5×10^8 spores/ml and were used within a week because, after storage for 1 or 2 months at refrigeration, microscopic examination revealed some germination.

Medium 1 was used to correlate optical density (O.D.) with loss of heat resistance of spore suspensions, to determine optimal temperature for heat activation, and to observe effects of pH, temperature, and oxygen on germination. The medium consisted of veast extract, 0.5%; casitone, 0.75%; glucose, 0.5%; NaCl, 0.25%; sodium thioglycollate, 0.05%; and cystine, 0.5%. Cystine was dissolved first in water adjusted to a highly alkaline pH. The final pH of Medium 1 was adjusted to pH 7.0. Before inoculation the medium was steamed to drive off dissolved oxygen. Germination approached 90% within approximately 1 hr; little or no outgrowth occurred during this time. Medium 2, which was Medium 1 diluted by one-half, was used in experiments in which a slower rate of germination was desired. Extent of germination was estimated on a Spectronic 20 Colorimeter (Bausch and Lomb) by the decrease in O.D. at 610 nm. The percentage of germinated spores was calculated by using the equation of Hachisuka et al (10). Decrease in O.D. and survival at 60 C for 15 min were compared since loss in O.D. and loss of heat resistance are related to germination (12, 23, 31). Spores were incubated at 37 C in Medium 1; samples were withdrawn at various time intervals, and O.D. and number of spores surviving the heat treatment were determined. Surviving spores were enumerated on SPS agar without antibiotics (1) using the pouch technique (4). The percentages of germination calculated from the two techniques differed by less than 5%.

For heat activation, spore preparations were heated for 20 min at 75 C. All germination studies were done after removal of dissolved oxygen by steaming the medium before inoculation.

RESULTS AND DISCUSSION

Heat activation of spores of C. perfringens S-45

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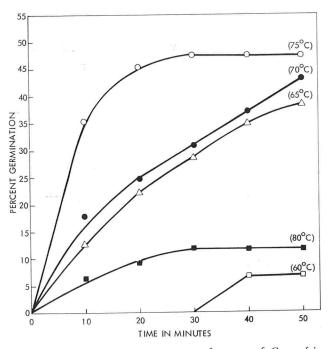


Figure 1. Percentage germination of spores of *C. perfringens* upon incubation for various time intervals at 37 C in Medium 2 after heat activation for 20 min at the indicated temperatures.

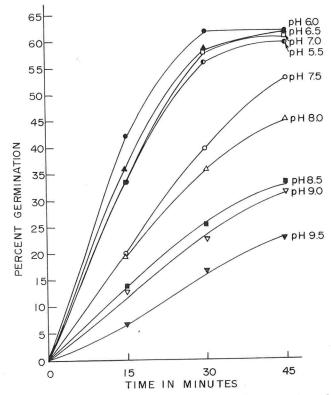


Figure 2. Effect of pH on the extent of germination of spores of *C. perfringens* incubated at 37 C in Medium 2 for various time intervals.

increased with an increase in temperature from 60 C to 75 C (Fig. 1). Germination decreased when the temperature was raised to 80 C. A temperature

of 75 C for 20 min was considered optimal for the activation of spores of this strain of C. perfringens. This treatment was used in subsequent experiments. The optimal heat-activation requirements may vary for other strains of this organism and for spores produced under other conditions. Barnes et al. (2) observed that, in blocks of fresh meat, spores of heat-resistant strains germinated poorly unless the spores were activated at 70 C for 30 min either in the meat or in suspension before inoculation. Roberts (25) reported that spores from food poisoning strains defined as producing exceptionally heat resistant spores showed a high degree of heat activation; whereas spores of "classical" strains showed little heat activation. He also observed differences in degree of heat activation between spore crops of the same strain obtained from two different sporulation media. Duncan and Strong (7) found that heating at 60 C to 70 C for 20 min yielded optimal activation of heat-sensitive spores; whereas heating at 80 C for 10 or 20 min was optimal for spores showing heat resistance. The spores in our work were not considered to be exceedingly heat resistant and had an average D₉₀c of 2.85 min.

The influence of pH on germination was observed over a range of 5.5 to 9.5 (Fig. 2). Values between pH 5.5 and 7.0 favored germination, but the greatest percentage of germinated spores consistantly occurred at pH 6.0. Rapid germination of PA 3679h occurs also at pH 6.0 in the presence of sodium nitrate (6); but in the presence of L-alanine and so-

TABLE 1. INFLUENCE OF COMBINATIONS OF CYSTINE, CYSTEINE, TRYPTOPHAN, AND GLUCOSE ON GERMINATION OF SPORES OF *C. perfringens* incubated at 30 C, pH 6.0, for 40 min.

Media*	Per cent germination		
L-Cystine plus glucose	53		
L-Tryptophan plus glucose	25		
L-Cysteine plus glucose	17		
L-Tyrosine plus glucose	0		
L-Cystine plus L-tryptophan plus L-cysteine plus L-tyrosine plus glucose	49		
L-Cystine plus L-tryptophan plus L-cysteine plus	50		
glucose L-Cystine plus L-cysteine plus L-tyrosine plus	0		
glucose L-Cystine plus L-tyrosine plus glucose	0		
L-Cystine plus glucose plus sodium nitrite	51		
L-Cystine plus sodium bicarbonate plus glucose	52		
L-Cystine plus sodium bicarbonate plus gateobe	60		
L-Cystine plus sodium bicarbonate plus glucose	65		
L-Cystine plus sodium blearbonate plus glucose	82		
L-Cystine plus sodium chloride	82		

^aThe following levels of constituents were used: cystine, 0.5 mg/ml; tryptophan, 1.0 mg/ml; cysteine, 1.0 mg/ml; tyrosine, 0.4 mg/ml; glucose, 0.5%; sodium bicarbonate, 0.25%; sodium chloride, 0.5%.

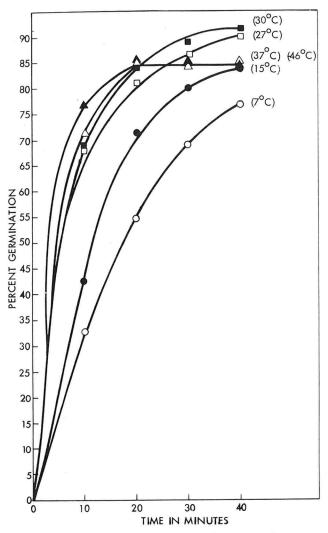


Figure 3. Effect of temperature on the extent of germination of C. *perfringens* spores incubated in Medium 1, pH 6.0, for various time intervals.

dium pyrophosphate, germination is most rapid at pH 8.5 (29).

Germination occurred at temperatures ranging from 7 C to 46 C (Fig. 3). The extent of germination increased with increasing temperatures; a broad range of favorable temperatures was observed between 27 C and 46 C, but the most favorable temperature consistently appeared to be 30 C. Germination occurred readily at 7 C and 15 C, but outgrowth did not occur at these temperatures.

Germination was rapid in medium 1 after removal of oxygen by bubbling nitrogen through the medium or by steaming the medium. Germination was minimal when oxygen was not removed from the medium. These observations are in agreement with those made on *C. perfringens* by Wynne and Harrell (33) and Wynne et al. (4). Some clostridial spores do germinate, however, under aerobic conditions (10, 29, 33, 34).

Subsequent observations on germination of spores

of this organism were made at pH 6.0 and at a temperature of 30 C in media freed of dissolved oxygen and inoculated with heat-activated spores.

Several complex nitrogenous substances enhanced germination (Fig. 4). Germination was most extensive in the presence of vitamin-free casamino acids (Difco) and next most extensive with casamino acids with vitamins (Difco). Yeast extract (Difco) and trypticase (B.B.L.) were the least effective of the substances examined. Differences in germination may be attributed to variability in the constituents of these products. Vitamin-free casamino acids contained 38% sodium chloride, casamino acids with vitamins contained 14% sodium chloride. In addition, the latter preparation contained 2% phosphate. Yeast extract and trypticase contain lower concentrations of sodium chloride than do casamino acids. Vitamin-free casamino acids enhance germination of C. bifermentans (8), but evidently affect spores of

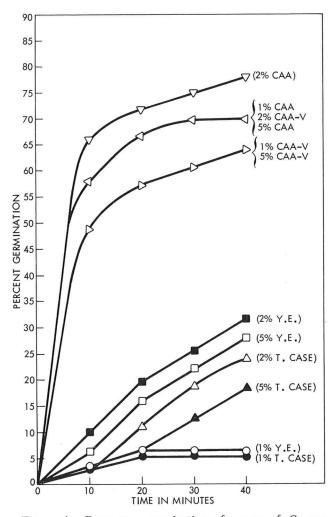


Figure 4. Percentage germination of spores of *C. perfringens* in the presence of various concentrations of yeast extract (Y.E.), trypticase (T. case), vitamin-free casamino acids (CAA), and casamino acids (CAA-V) after incubation for various time intervals. A pH of 6.0 and incubation temperature of 30 C were used.



C. botulinum very little (15, 28). Yeast extract (5%) causes rapid germination of spores of C. botulinum (15, 28). These variations probably reflect species differences as well as compositional differences in various batches of these nitrogenous materials.

Testing of 24 amino acids in the presence of yeast extract and glucose showed that only L-cystine, Lcysteine, L-tryptophan and L-tyrosine enhanced germination. Absence of glucose resulted in decreased germination (Fig. 5). Stimulation of germination by L-cysteine, L-cysteine, and L-tyrosine has been dem-

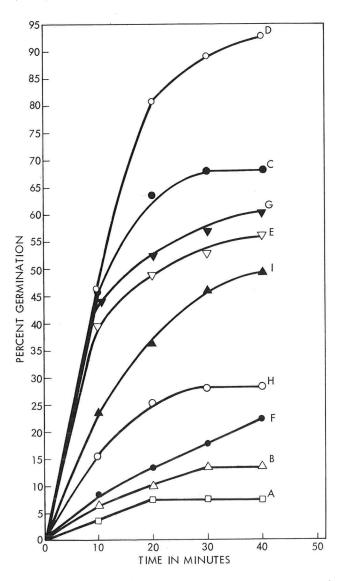


Figure 5. Effect of amino acids on the germination of suspensions of spores of *C. perfringens* in the presence of yeast extract and yeast extract plus glucose after incubation for various time intervals. A pH of 6.0 and incubation temperature of 30 C were used. All observations were in the presence of 1.0% yeast extract plus the following: A. control (1.0% yeast extract) and cysteine, B. 0.5% glucose, C. 0.5 mg/ml cysteine plus 0.5% glucose, E. 1 mg/ml cysteine plus 0.5% glucose, H. 0.4 mg/ml tyrosine, and I. 0.4 mg/ml tyrosine plus 0.5% glucose.

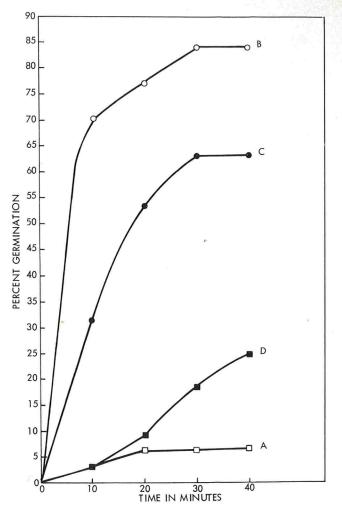


Figure 6. Percentage germination of spores of *C. perfringens* incubated for various time intervals at 30 C, pH 6.0, in 1.0% yeast extract and in 1.0% yeast extract plus the following: A. 1.0% extract only, B. 0.3% sodium bicarbonate, C. carbon dioxide (bubbled into medium for 5 min), D. 0.1% sodium lactate.

onstrated for certain *Bacillus* spp. and for other clostridial species (8, 13, 14, 18, 20, 21, 22, 30). None of these amino acids produced stimulatory effects in the presence of vitamin-free casamino acids.

Addition of 0.3% sodium bicarbonate or bubbling carbon dioxide into a 1% yeast extract medium stimulated germination (Fig. 6). The pH of the medium fell below 6.0 when carbon dioxide was bubbled into it; adjustment of the initial pH to 6.5 produced a pH of 6.0 ± 0.1 after bubbling carbon dioxide into the medium for 5 min. Bicarbonate ions and carbon dioxide have been shown to increase germination in spores of other clostridia (26, 28, 32). Sodium lactate stimulated germination in the presence of yeast extract (Fig. 6). Increasing concentrations of lactate did not produce additional germination, and addition of glucose to the medium had no effect. Lactate (8) and thioglycollate (16, 24) can increase

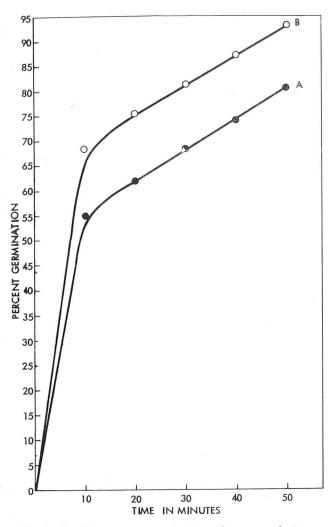


Figure 7. Percentage germination of spores of *C. per-fringens* in 2.0% vitamin-free casamino acids incubated for various time intervals at 30 C, pH 6.0. Curve A shows percent germination in vitamin-free casamino acids alone; curve B represents germination upon addition of 0.1% sodium bicarbonate, carbon dioxide, 0.1% sodium lactate or 0.05% sodium thioglycollate.

germination in spores of other clostridial species. Addition of sodium bicarbonate, carbon dioxide, sodium lactate, and sodium thioglycollate to vitaminfree casamino acids enhanced both rate and extent of germination (Fig. 7). The extent of germination was essentially the same for all combinations under these conditions. Little or no germination occurred in the absence of yeast extract or casamino acids. Evidently, an additional unidentified factor or factors in these materials was necessary for germination.

Considerable data are available on the role of ions in different germinative systems (6, 17, 19, 27). Therefore, other ionizable substances, in addition to those mentioned previously, were examined. The influence of sodium nitrate, sodium nitrite, sodium chloride, and ammonium chloride is shown in Fig. 8. Sodium nitrate exhibited no stimulatory effect toward germination; germination, however, was accelerated by increasing concentrations of sodium nitrite from 0.01% to 0.2%; at concentrations higher than 0.2%, germination decreased. Labbe and Duncan (19) also have shown a decrease in germination of spores of *C. perfringens* with the addition of sodium nitrite in excess of 0.2%. Nitrite-induced germination has been observed with *C. butyricum* and *C. tyrobutyricum* (3) and PA-3679h (6).

The rate and extent of germination increased as the concentration of sodium chloride was increased between the levels of 0.1% to 0.5% (Fig. 8); no further stimulation was observed when levels of 0.6% to 3.0% were added to the medium. Beyond this point, the stimulatory effect of the salt was reduced with increasing concentrations until the effect was absent at 8.0% sodium chloride. Ammonium chloride also enhanced germination in the presence of yeast exact; maximal effect was observed at 0.2%. No further enhancement occurred with addition of levels from 0.3% to 0.5%. Optimal concentrations of sodium chloride (0.5%) and sodium nitrite (0.2%) in combination produced no additive effect when added to yeast extract. Salts alone or in combination in the absence of yeast extract caused litle or no germination. Seemingly, some factor or factors in the yeast extract were necessary for the activity of these salts.

Since cystine, cysteine, tryptophan, and tyrosine produced germination in spores of C. perfringens when added individually to yeast extract-glucose medium and since certain inorganic ions enhanced germination in the presence of yeast extract or vitaminfree casamino acids, attempts were made to determine specific amino acid and (or) ionic requirements for induction of complete germination. All four amino acids were tested individually and in combination in the presence or absence of glucose. None of the amino acids individually or in any combination induced germination in the absence of glucose. Different degrees of stimulation of germination were observed when these amino acids were tested individually or in combination in the presence of glucose (Table 1). Cystine produced the greatest amount of germination of any of the amino acids in the presence of glucose. Tyrosine inhibited germination when added to the cystine-glucose or cystine-cysteineglucose mixture.

The ability of the cystine-glucose mixture to induce germination was improved by addition of carbonate, whereas nitrite had little effect (Table 1). Sodium chloride was effective in enhancing germination in the presence of cystine-glucose or cystine alone; the same level of germination occurred under these conditions as occurred in 2.0% vitamin-free casamino acids or in broth Medium 1. Of the various materials

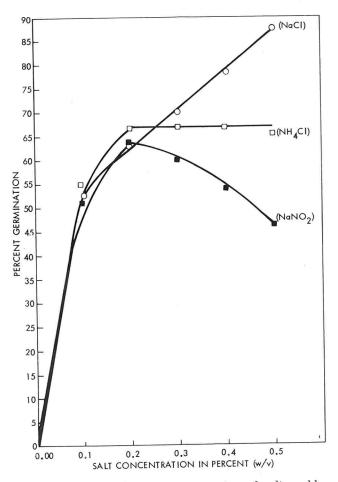


Figure 8. Effect of varying concentrations of sodium chloride, ammonium chloride, and sodium nitrite on germination of spores of *C. perfringens* when added to 1.0% yeast extract medium and incubated at 30 C, pH 6.0. Measurements were taken after 40 min. Germination induced by yeast extract alone was approximately 7.0%.

examined, cystine and sodium chloride were the most effective for inducing rapid and complete germination of spores of *C. perfringens* S-45.

Acknowledgements

Journal Paper No. J-6802 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1393, Center for Agricultural and Economic Development cooperating. This investigation was supported in part by PHS Grant No. FD-00106.

References

1. Angelotti, R., H. E. Hall, M. J. Foter, and K. H. Lewis. 1962. Quantitation of *Clostridium perfringens* in foods. Appl. Microbiol. 10:193-199.

2. 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.

3. Bester, B. H., J. W. Claassen, and P. M. Lategan. 1968. Nitrite induced germination of *Clostridium butyricum* and *Clostridium tyrobutyricum* spores. South Afr. J. Agr. Sci. 10:1055-1058.

4. Bladel, B. O., and R. A. Greenberg. 1965. Pouch method for the isolation and enumeration of clostridia. Appl. Microbiol. 13:281-285.

5. Dische, F. E., and S. D. Elek. 1957. Experimental food-poisoning by *Clostridium welchii*. The Lancet 2:71-74.

6. Duncan, C. L., and E. M. Foster. 1968. Nitrite induced germination of putrefactive anaerobe 3679h spores. Appl. Microbiol. 16:412-416.

7. Duncan, C. L., and D. H. Strong. 1968. Improved medium for sporulation of *Clostridium perfringens*. Appl. Microbiol. 16:82-89.

8. Gibbs, P. A. 1964. Factors affecting the germination of spores of *Clostridium bifermentans*. J. Gen. Microbiol. 37: 41-48.

9. Gould, G. W. 1969. Germination. pp. 397-444. In G. W. Gould and A. Hurst (eds.) The bacterial spore. Academic Press, New York.

10. Hachisuka, Y., N. Asano, M. Kato, M. Okajima, M. Kitaori, and T. Kuno. 1955. Studies on spore germination. Effect of nitrogen sources on spore germination. J. Bacteriol. 69:399-406.

11. Hauschild, A. H. W. 1970. Enumeration of *Clostridium perfringens* in foods—a review. Can. Inst. Food Technol. J. 3:82-84.

12. Hills, G. M. 1949. Chemical factors in the germination of spore-bearing aerobes. The effect of yeast extract on the germination of *Bacillus anthracis* and its replacement by adenosine. Biochem. J. 45:353-362.

13. Hills, G. M. 1949. Chemical factors in the germination of spore-bearing aerobes. The effect of amino acids on the germination of *Bacillus anthracis*. With some observations of the optical forms to biological entities. Biochem. J. 45:363-370.

14. Hills, G. M. 1950. Chemical factors in the germination of spore-bearing aerobes: observations on influence of species, strain and condition of growth. J. Gen. Microbiol. 4:38-47.

15. Hitzman, D. O., H. O. Halvorson, and T. Ukita. 1957. Requirements for production and germination of spores of anaerobic bacteria. J. Bacteriol. 74:1-7.

16. Holland, D., A. N. Barker, and J. Wolf. 1969. Factors affecting germination of clostridia, pp. 317-323. *In* L. L. Campbell (ed.) Spores IV. American Society for Microbiology, Bethesda, Maryland.

17. Hyatt, M. T., and H. S. Levinson. 1961. Interaction of heat, glucose, L-alanine and potassium nitrate in spore germination of *Bacillus megaterium*. J. Bacteriol. 81:204-211.

18. Krask, B. J. 1961. Discussion on L-alanine dehydrogenase in spores. pp. 89-100. In H. O. Halvorson (ed.) Spores II. Burgess Publishing Co., Minneapolis, Minn.

19. Labbe, R. C., and C. L. Duncan. 1970. Growth from spores of *Clostridium perfringens* in the presence of sodium nitrite. Appl. Microbiol. 19:353-359.

20. Levinson, H. S., and M. T. Hyatt. 1962. Nitrogenous compounds in germination and postgerminative development of *Bacillus megaterium* spores. J. Bacteriol. 83:1224-1230.

21. Lund, A. J. 1956. Investigation of factors influencing food sterilization and preservation: Sporulation in genus *Clostridium*. Hormel Inst. Univ. of Minnesota, Ann. Rept. 1955-56:88-97.

22. O'Connor, R. J., and H. O. Halvorson. 1961. L-Alanine dehydrogenase: A mechanism controlling the specificity of amino acid induced germination of *Bacillus cereus* spores. J. Bacteriol. 82:706-713.

23. Powell, J. F. 1951. The sporulation and germination of a strain of *Bacillus megaterium*. J. Gen. Microbiol. 5:993-1000.

24. Riemann, H. 1963. Germination of bacterial spores with chelators. With special references to the calcium dipicolinic acid germination system. Aarhuss Stiftsbogtrykkerie, Copenhagen.

25. Roberts, T. A. 1968. Heat and radiation resistance and activation of spores of *Clostridium welchii*. J. Appl. Bacteriol. 31:133-144.

26. Roberts, T. A., and G. Hobbs. 1968. Low temperature growth characteristics of clostridia. J. Appl. Bacteriol. 31:75-88.

27. Rode, L. J., and J. W. Foster. 1962. Ionic germination of spores of *Bacillus megaterium* QM B1551. Arch. Mikrobiol. 43:183-200.

28. Treadwell, P. E., G. J. Jann, and A. J. Salle. 1958. Studies on the factors affecting the rapid germination of spores of *Clostridium botulinum*. J. Bacteriol. 76:549-556.

29. Uehara, M., and H. A. Frank. 1965. Factors affecting alanine induced germination of clostridial spores, pp. 38-46. *In* L. L. Campbell and H. O. Halvorson (eds.). Spores III.

LETTER TO EDITOR (Continued from Page 377)

NOTES: Subsection (a) is derived from S.C. §148 Regs. 13 and 26 (part), 149 Reg. 12, 150 Regs. 19, 38, 60 and 61, and 156 Reg. 145(part). It is to be noted that the required temperature is to be maintained also during transportation of perishable foods. Former section 81.07 was amended and renumbered as subsection (a) by resolution adopted on April 13, 1971.

Subsection (b) is new. It was added by resolution adopted on April 13, 1971 to require potentially hazardous foods as defined therein to be kept refrigerated or under heat treatment within specified temperature ranges which are known to control the growth of pathogenic microorganisms.

Subsection (c) is new. It was added by resolution adopted on April 13, 1971 to mandate the microbiological examination of certain potentially hazardous foods designated by the Commissioner.

Subsection (d) is new. It was added by resolution adopted on April 13, 1971 to prohibit the preparation, sale or distribution of designated potentially hazardous foods which have not been prepared or held in such manner that the product conforms to the specified bacteriological standards.

Resolved further, that section 103.05 of the New York City Health Code, as enacted by resolution adopted on the twenty-third day of March, nineteen hundred fifty-nine and filed with the city clerk on the twenty-fourth day of March, nineteen hundred fifty-nine, be and the same hereby is amended, to be printed together with explanatory notes, to read as follows:

§103.05 Brining of fish

Only clean refined salt, or filtered brine of a temperature not exceeding 38 F, shall be used in the brining of fish.

NOTES: This section is derived without substantive change from S.C. §148 Reg. 92. This section was amended by resolution adopted on April 13, 1971 to conform its refrigerated temperature standard for the brining of fish with Title 21 — Food and Drugs, Part 121, Code of Federal Regulations.

Resolved further, that section 113.19 of the New York City Health Code, as enacted by resolution adopted on the twenty-third day of March, nineteen hundred fifty-nine and filed with the city clerk on the twenty-fourth day of March, nineteen hundred fifty-nine, be and the same hereby is American Society for Microbiology, Ann Arbor, Mich.

30. Woese, C. R., H. J. Morowitz, and C. H. Hutchinson. 1958. Analysis of action of L-alanine analogues in spore germination. J. Bacteriol. 76:578-588.

31. Wynn, E. S., and J. W. Foster. 1948. Physiological studies on spore germination with special reference to *Clostridium botulinum*. 1. Development of a quantitative method. J. Bacteriol. 55:61-68.

32. Wynne, E. S., and J. W. Foster. 1948. Physiological studies on spore germination with special reference to *Clostridium botulinum*. III. Carbon dioxide and germination. J. Bacteriol. 55:331-339.

33. Wynne, E. S., and K. Harrell. 1951. Germination of spores of certain *Clostridium* species in the presence of penicillin. Antibiotics and Chemotherapy 1:198-202.

34. Wynne, E. S., D. A. Mehl, and W. R. Schmieding. 1954. Germination of clostridium spores in buffered glucose. J. Bacteriol. 67:435-437.

amended, to be printed together with expanatory notes, to read as follows:

§113.19 Standards; pasteurization of frozen desserts mix No Class A. D or E permittee operating a wholesale plant shall offer for sale, sell, give away or distribute frozen desserts mix containing milk or a milk product or use such frozen desserts mix for manufacture of frozen desserts unless it is pasteurized by heating (1) to a temperature of 155 F and held at such temperature for at least 30 minutes, or (2) to a temperature of 175 F and held at such temperature for at least 25 sec, or (3) to a temperature of 200 F and held at such temperature for at least 3 sec, or (4) to such temperature and for such holding period as the Department may approve. Immediately after heating the mix shall be cooled to a temperature no higher than 45 F.

NOTES: This section was amended by resolution adopted on April 13, 1971 to conform its required refrigeration standard for pasteurized frozen desserts mix to the related standard contained in §81.07 as amended by the same resolution.

Resolved further, that subsection (4) of section 115.17 of the New York City Health Code, as enacted by resolution adopted on the twenty-third day of March, nineteen hundred fifty-nine and filed with the city clerk on the twenty-fourth day of March, nineteen hundred fifty-nine, be and the same hereby is amended, to be printed together with explanatory notes, to read as follows:

§115.17 Standards; labeling of containers

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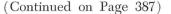
(4) The date of preparation and a statement that the formula milk must be kept under refrigeration at a temperature no higher than 45 F.

\$

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NOTES: Subsection (4) was amended by resolution adopted on April 13, 1971 to conform its required refrigeration standard for the labeling of formula milk to the related standard contained in §81.07 as amended by the same resolution.

Resolved further, that subsection (a) of section 115.27 of the New York City Health Code, as enacted by resolution adopted on the twenty-third day of March, ninteen hundred fifty-nine and filed with the city clerk on the twenty-fourth day of March, nineteen hundred fifty-nine, be and the same hereby is amended, to be printed together with explanatory notes, to read as follows:





MILK FLAVOR: THE TRUE TEST OF QUALITY

D. K. BANDLER

Department of Food Science Cornell University Ithaca, New York 14850

Abstract

Since the consumer's appraisal of a glass of milk is based solely on taste, flavor and keeping quality are of paramount importance to maintaining consumption of fluid milk and byproducts.

For the past 5 years, the New York State Milk Flavor Program has identified off-flavor problem areas to the industry, particularly with regard to the farm supply. There has been a marked improvement where companies and the college have cooperated. Emphasis now is being placed on getting the finished product protected to the highest degree in an effort to improve shelf life. The program is carried out primarily by the Quality Control staffs currently employed by industry. This force is assisted by the extension staff in the Department of Food Science at Cornell in the following ways: (a) individual plant seminars for each plant or company participating in the program; (b) follow-up sessions to help solve special problems, to evaluate progress, and to maintain interest and enthusiasm; and (c) direct contact with top management to evaluate shelf life performance, route returns, relationships with non-owned outlets, and promotion of extra high quality dairy products.

The quality control procedures for milk in the past few decades have been in a rut. Reliance on bacteria counts, sediment tests, and farm inspection scores as a measure of quality has led to a false sense of security. The consumer (satisfied that milk is completely safe) is concerned about only two things: "How does it taste?" and "How long will it keep?" It therefore would seem reasonable that milk also should be evaluated by these two criteria.

We found 5 years ago, that milk in our area of the U. S. was not being checked for flavor. The industry simply did not have the personnel who were trained to judge milk. Many plant people claimed that milk was sour when actually it was rancid. They could not detect oxidized flavor and generally, when the milk did not taste right, they said it had "turned." With such a vague understanding of flavor defects, it was virtually impossible to correct a problem.

TRI-STATE FLAVOR PROGRAM

In 1965, I got together with Sid Barnard of Penn State and Dick Kleyn of Rutgers and formed the

Tri-State Milk Flavor Program. In a short period of time we produced a Milk Flavor Handbook, Producer and Consumer bulletins, two charts, two slide sets, and we were off to tell the producers and processors of the problem they had and how we could help them solve it. For example, by picking up samples throughout the state we found that only 23% of the milk was free from flavor defects, 60% had slight off flavors, and over 17% was downright awful. The most serious off-flavors were oxidized, rancid, and unclean—the flavors that leave a lingering after taste (Tables 1 and 2).

We found that at least 10% and as many as 20% of the producers shipping milk to plants were sending milk that scored 36 or lower (consumer complaint level). The first step was to correct the really bad supply. To do this we had to get the plant personnel to taste the supply. The first step was to train them to judge milk.

FLAVOR EVALUATION AT THE PLANT

A major problem in training flavor judges has been the lack of convenient off-flavor samples in quantity for judging. We solved this problem by the use of 1 oz Tetra-pak containers, color coded for all the common off-flavors found in milk. Off-flavor samples, procured from can receiving stations, or prepared in the laboratory were packaged and frozen in these color coded Tetra-paks. These were used in workshops throughout the state and kept on hand in plants for comparison and refresher training. Flavors were stable for 3-6 months. These were also used to demonstrate to producers the problem of flavor defects that originate at the farm.

Plants were encouraged to flavor check each producer's supply at least once a month. Samples were taken by haulers in 4 oz brown glass bottles on days requested by the plant. Many haulers resisted this extra work and all too often samples were improperly taken or not taken at all. To remedy this we recommended a Universal Sample program where a fresh 4 oz sample is taken every time a farm tank is pumped. This provides an opportunity to screen the milk at any time since there is a sample available for testing in the plant at all times. (The sample

¹Presented at the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Cedar Rapids, Iowa, August 17-20, 1970.

TABLE	1.	FLAV	OR S	SCORES	OF	501	SAM	PLES	OF	BOT	FLED	MILK	
IN	NE	w Ye	ORK	STATE	, J	ANUA	ARY.	1968	-Ar	RIL,	196	9	

Classification of milk sample ¹	Number	Per cent
Good	113	22.6
Fair	302	60.3
Poor	86	17.1

Off-flavor criticism of 501 samples

Flavor defect	Number	Per cent
No criticism	64	12.8
Cooked (Feedy)	65	12.9
Feedy	57	11.4
Feedy-Unclean	85	17.0
Unclean	68	13.6
Rancid	61	12.2
Oxidized	92	18.3
Foreign	9	1.8

¹GOOD: 39-40 score, consumer would have complete satisfaction and use milk at maximum level.

FAIR: 37-38 score, consumer would tolerate milk and consume at a level dictated by habit and promotion.

POOR: 36 score and below-consumer would reject in some manner-(a) complain, (b) consume less, (c) change brands, (d) switch to substitute product.

TABLE 2. FLAVOR SCORES OF 3484 HERD SAMPLES OF PRODUCER MILK RECEIVED AT NEW YORK STATE PLANTS, JANUARY, 1968-April, 1969

Classification of milk sample ¹	Number	Per cent
Good	1,079	30.9
Fair	1,631	46.9
Poor	1,631 774	22,2

Off-flavor criticism of the 3484 samples

Flavor defect	Number	Per cent
No Criticism	904	25.9
Feedy	725	20.8
Feedy-Unclean ¹	527	15.2
Unclean ¹	542	15.6
Rancid	318	9.1
Oxidized-Flat	405	11.6
Foreign	63	1.8

¹Selective testing for Acid Degree Value (ADV) shows elevated reading on some unclean and feedy-unclean samples (an indication of rancidity).

also is used for regular bacteriological work, other adulteration work, for our modified 4 oz sediment test and will be used later for random fat testing with the Milko Tester when that becomes legal.) Producers with the serious off-flavors were visited by the fieldmen to help in the correction. Plants that have cooperated soon found a marked improvement in the farm supply.

Phase Two

In 1970, an industry advisory group, pleased with our farm progress recommended that we begin "phase two." They suggested that the N. Y. State Milk Flavor Program become the N. Y. State Dairy Industry Quality Assurance Program. Under the new program there is continued work on producer supply, but greater emphasis is placed on plant processing and distribution, particularly in the area of improved shelf life. Here plant procedures are closely checked, especially for temperature control. Several plants have added after coolers where the short-time unit did not have sufficient capacity to cool the milk to 35 F, to assure temperatures of 40 F or less in the final package.

Of greatest help in this project is an electronic temperature sensing device. This unit allows us to monitor package, filler bowl, and pipeline temperatures from the outside. We have learned for example, exactly how much temperature increase to expect in filling various size packages on a variety of filling equipment. On the average there will be no detectable increase in temperature between the filler bowl and a sealed gallon paper container. On the other hand, we normally expect an average of 5° increase in the filling of 1/2 pints. Quarts, on the average, will be heated 2-4°, whereas 1/2 gallons may increase from 0.75° to 1.5° . With this information we can lower the incoming milk temperature to achieve our 40 F package goal.

Likewise the machine can be used to monitor activity in the cooler, on the trucks, and in stores. Our bacteriological study has told us a basic fact. Increasing the storage temperature of milk 5° cuts the shelf life in half. Milk that will keep 10 days at 40 F will spoil in 5 days at 45 F or in 2.5 days at 50 F.

Quality assurance also demands protection from



Figure 1. "Tele-Thermometer" equipped with surface probe accurately indicates internal product temperature from the outside without disturbing product.





light. The work at Pennsylvania State University by Sidney Barnard clearly supports the well known fact that milk flavor can be damaged by direct sunlight or strong fluorescent light. In a recent survey of 1,057 samples of milk from retail stores, 165 were judged as oxidized. Of 104 samples in blow molded plastic containers, 79% were oxidized compared to 49% of the milk in glass and only 7% in plastic coated paper. If transparent or highly translucent containers are to be used, they must be protected from overdoses of light. This may mean partially darkening the milk display cases.

Milk flavor control is the key to survival of the fluid milk industry. If the milk does not taste good,

LETTER TO EDITOR (Continued from Page 384)

§115.27 Operations; sterilization of formula milk

(a) No formula milk permittee shall offer for sale, sell, give away or distribute formula milk otherwise than in individual containers for a single feeding. Immediately after filling of a container of formula milk, it shall be closed with a cover or cap which effectively seals and protects the mouth of the container. Container may be closed with suitable, incised nipples which shall be protected with suitable outside fitted caps. When closed, the container shall be heated in an autoclave for at least 10 min at 230 F so as to render the formula milk and its container sterile. After sterilization, containers of formula milk shall be immediately cooled to and kept at a temperature no higher than 45 F until delivery to the consumer.

why drink it? Therefore, the taste test is the only test that is going to provide the assurance of good flavor in the finished product.

To official agencies, bacteria counts, sediment tests, and barn and plant inspection scores are present day standards for quality. But none of these tests will assure that milk is free of rancid, oxidized, feedy, barny, or other unnatural flavors. Only a working quality assurance program which includes the taste test will assure a satisfactory product to the consumer.

(Sample copies of all materials used in the Tri-State Milk Flavor Program are available by writing the author.)

NOTES: Subsection (a) was amended by resolution adopted on April 13, 1971 to conform its required refrigeration standard for the cooling of sterlized formula milk to the related standard contained in §81.07 as amended by the same resolution.

Resolved further, that this resolution shall take effect immediately.

It is hoped that the information contained in these amendments may be of interest to readers of the *Journal of Milk* and Food Technology.

> Sincerely yours, ABRAHAM E. ABRAHAMSON Environmental Health Services Department of Health 125 Worth Street New York, N.Y. 10013

NEWS & EVENTS

NEW DAIRY COMPUTER SURVEY PINPOINTS CLEANING COSTS AND PROCEDURES

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A breakthrough concept in analyzing and evaluating dairy cleaning and sanitizing procedures has been announced by Klenzade Products of St. Paul, Minnesota. Klenzade, a division of Economics Laboratory, Inc., calls the new service a Computer Cost Survey Analysis.

The net result of the new Computer Survey provides a dairy with the exact $\cos t$ – to the penny – of each and every cleaning function within the plant. The computer analyzes soil conditions, detergent concentrations, water volume, water hardness, and even the iron content in the water. By simply scanning the computer printout, cleaning costs per cycle can be determined. In addition, total costs by day and month are included.

Bob Barrett, Technical Director for Klenzade,

points out that while the Computer Cost Survey Analysis is a new concept, the system has been proven in a number of Klenzade customers' plants, and is now available on a select basis to the dairy industry.

According to Barrett, the Computer Survey can provide the major dairy processor with a method of comparing cleaning variables. An example might be a dairy plant with two available sources of water supply. Municipal water or the dairy's own well. Given the two water samples and the cleaning variables by function within the plant, Klenzade can deliver a printout comparing exact costs of total plant cleaning and sanitizing procedures using the two water sources.

This direct comparison will establish the exact cost differences created by differing degrees of water hardness, mineral content, temperature and volume.

Prior to the introduction of the Computer Survey,

a cost analysis of a dairy plant would require personnel up to three days to complete. Now, this same data can be analyzed and evaluated electronically in a matter of minutes. A further advantage is that once a basic Cost Survey is finished, future plant changes in procedures or functions can be instantly analyzed.

For more information contact Robert Barrett, Klenzade Products Division, Economics Laboratory, Inc., Osborn Building, Dept. 103, St. Paul Minnesota 55102.

USDA OFFERS EXCLUSIVE LICENSES TO MAKE DEHYDRATED FOODS, WASHABLE LEATHERS

Prospective manufacturers can now obtain exclusive licenses to operate under U. S. Department of Agriculture patents for making such products as fruit juices, dried honey, dry whole milk, and washable and drycleanable leather. In a recent change in its historic patent policy, USDA has announced that licenses to operate under some specific patents are offered on an exclusive basis. Under the new policy, manufacturers can gain exclusive rights to use these patents for periods up to 5 years.

The *Federal Register* and the *Official Gazette* of the U. S. Patent Office have listed the first USDA patents for which exclusive licenses are being offered. Five of them cover developments of the Eastern marketing and nutrition research laboratory in Philadelphia. These include three basic patents on the manufacture of full-flavor fruit juice powders and dried honey, one on the drying of whole milk, and one on a process for making leather so it retains its softness when washed or drycleaned.

All of these are developments of considerable commercial promise. They have been available on a nonexclusive basis for some years. It is felt that the added inducement of providing a competitionfree period for market development will stimulate interest in the patents on the part of prospective manufacturers. The patents for making fruit juice powders and dried honey (No. 2,816,039, No. 2,816,-840, and No. 2,906,630) cover a basic process developed at the Philadelphia laboratory for stripping the essential aroma or "essence" from natural fruit juices or from foods such as honey, dehydrating the juice or food, then restoring the essence to it under superatmospheric pressure in such a highly concentrated form that the product can be quickly chilled to convert it to a brittle solid.

The juice powders, which reconstitute instantly in water, would provide for the first time a natural juice with all of its original flavor in the form of a convenient powder that can be stored without refrigeration. The dried honey would make this widely used sweetener available to bakers, confectioners, and other food processors in a free-flowing, granular form with none of the inconvenience and difficult cleanup connected with the use of liquid honey.

The dry whole milk patent (No. 2,964,407) covers a process for the vacuum dehydration of whole milk to a powder that dissolves instantly in cold water to make a beverage virtually indistinguishable from fresh milk. The product, which would be sold from the dairy case, has a refrigerated storage life of at least 9 months.

For a complete listing of these and other USDA patents available for exclusive licensing, see the *Federal Register*, February 3, 1971, or the Patent Office Official Gazette, February 23, 1971. The rules and regulations governing exclusive licensing were spelled out in the *Federal Register*, May 14, 1970. Prospective manufacturers can apply for these licenses to the Administrator, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C. 20250. For further technical information on any of the particular patents described above, write to the Director, Eastern Marketing and Nutrition Research Division, 600 E. Mermaid Lane, Philadelphia, Pa. 19118.

ASSOCIATED ILLINOIS MILK SANITARIANS SPRING CONFERENCE MAY 3 ELGIN, ILLINOIS



The highlight of the program was the presentation of the "Outstanding Sanitarians' Award" to Wilbur McLean. Mr. McLean, now retired from the Public Health Service, has contributed much to the advancement of environmental health in Illinois during his many years as Region V Milk and Food Consultant.

The Association was fortunate to have Darold Taylor and Harold Thompson of the Public Health Service on the program and to add to our surprise to W. R. McLean. W. R. McLean was the 1969 recipient of the International Sanitarians' Award and is well known throughout the nation for his contributions.

WASHINGTON MILK SANITARIANS MEETING EVERETT WASH., JUNE 8, 1971



Dr. Frank W. Crews has announced that he is retiring from his position of Laboratory Director for the Washington State Department of Agriculture and is leaving the United States to seek a new life for himself and family in Australia.

Dr. Crews received his B.S. in Dairy Science at Washington State University, his M.S. in Dairy Science at Ohio State and his Ph. D. in Dairy Bacteriology under Dr. Hammer at Iowa State University. He has spent several years as a Milk Sanitarian and has been an instructor at the college level.

8

Dr. Crews has been continually employed by the Department of Agriculture since 1948, being involved in both animal disease diagnosis and dairy products examinations. Dr. Crews is a Past President of the Washington Milk Sanitarians Association and pioneered the Laboratory Certification program in Washington State. He has been Chairman of the Laboratory Methods Committee of the Washington Milk Sanitarians Association since its inception.

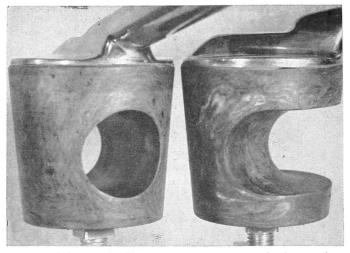
Dr. Crews has been kept too busy with his multiple responsibilities in the State to participate in National meetings or complete articles for publication as we would have liked, but professional microbiologists that are familiar with his work recognize Dr. Crews as an outstanding, thorough microbiologist and a leader in the field of Direct Microscopic examination of milk.

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Recommended By INTERNATIONAL ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC.

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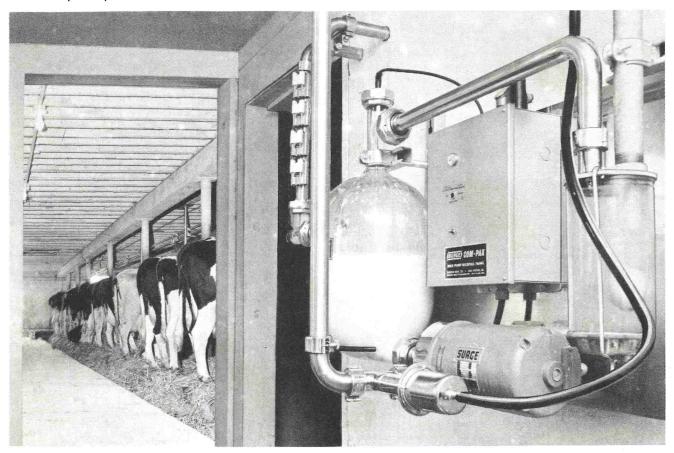
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