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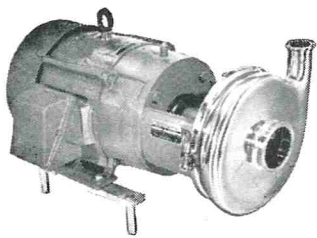
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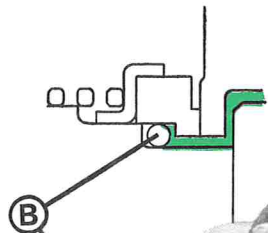
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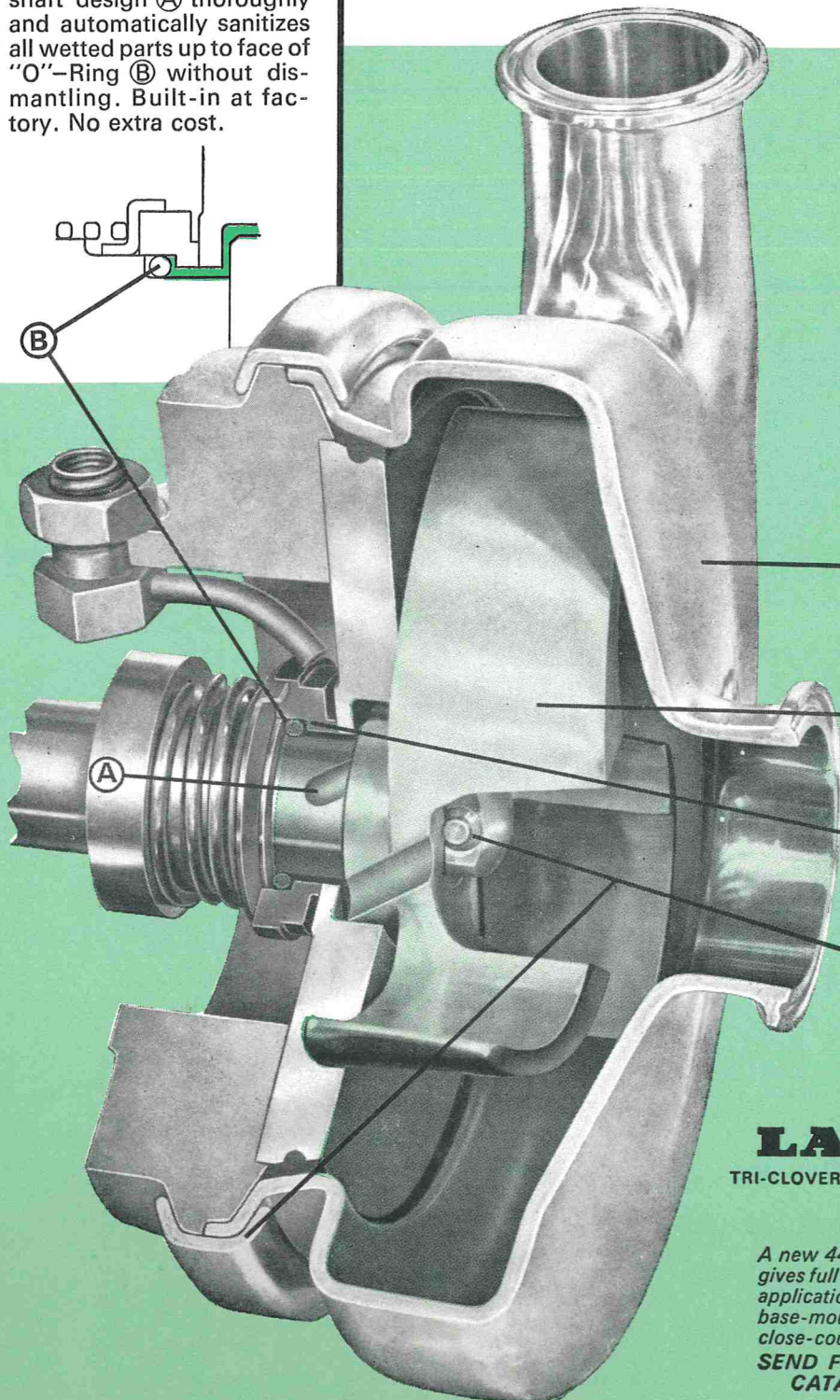
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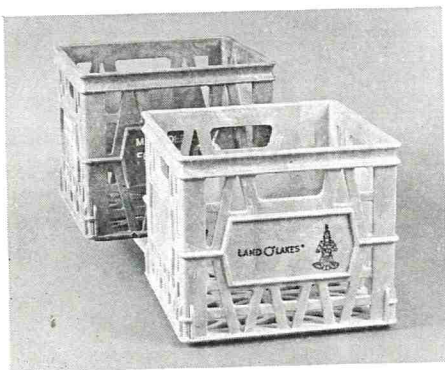
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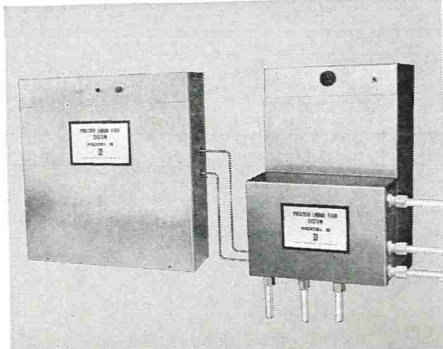
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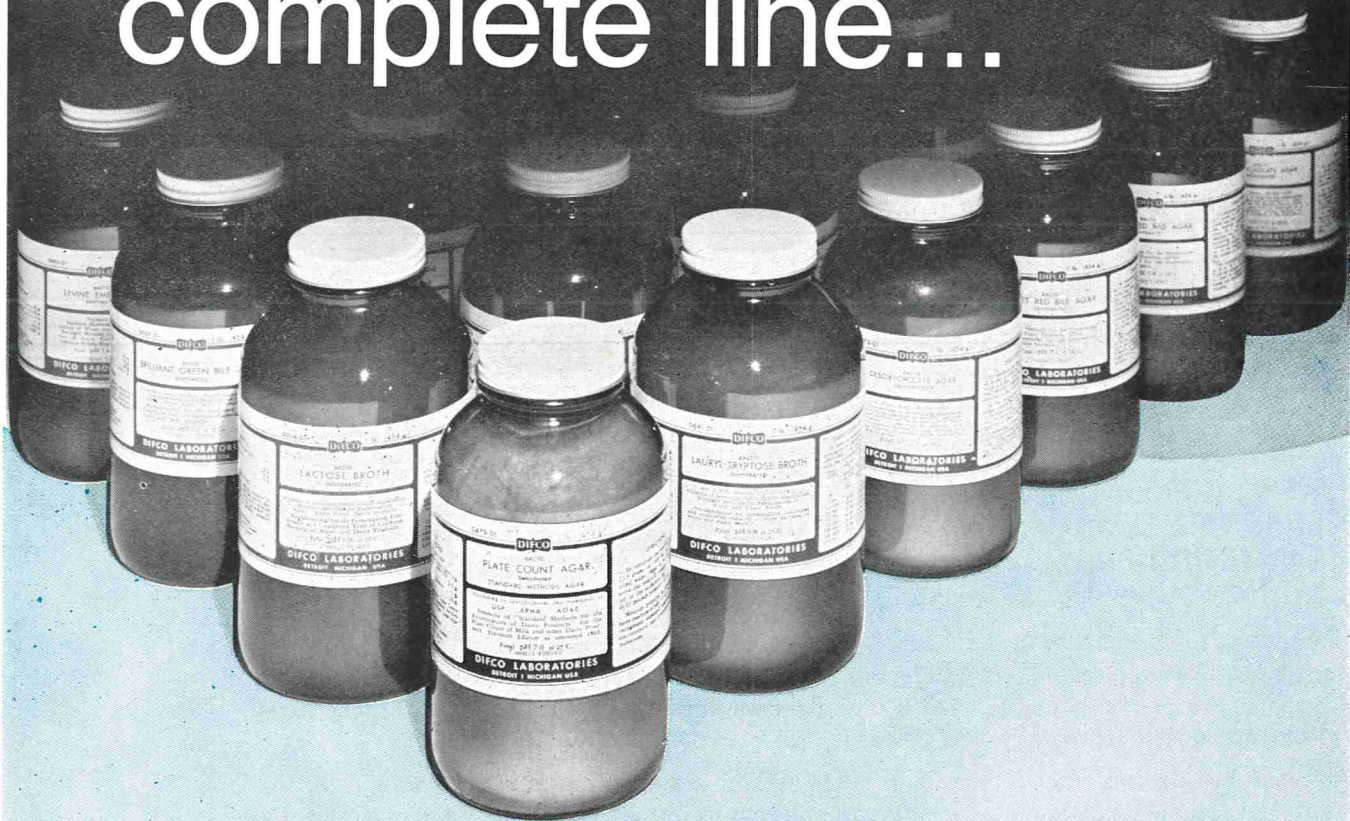


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# METHODS FOR PRODUCTION OF HIGH QUALITY RAW MILK

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the IAMFES Dairy Farm Methods Committee)

COMPILED AND EDITED BY

J. C. FLAKE, A. E. PARKER, J. B. SMATHERS, A. K. SAUNDERS AND E. H. MARTH

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# FLAVOR DEVELOPMENT AND MICROBIOLOGY OF SWISS CHEESE—A REVIEW

## I. MILK QUALITY AND TREATMENTS<sup>1, 2</sup>

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Department of Food Technology  
Iowa State University, Ames, Iowa 50010

(Received for publication January 31, 1973)

### ABSTRACT

This review, appearing in four parts, deals with relationships between flavor development and the microbiology of Swiss cheese. Body and texture characteristics of Swiss cheese also will be discussed, however, to a lesser degree and, wherever possible, in association with the flavor of Swiss cheese.

The first section, presented here, discusses use of raw milk and how the bacterial content of raw milk influences Swiss cheese quality. Thereafter, other milk treatments used in the cheese industry are discussed. They include clarification, heat-treatment, homogenization, and H<sub>2</sub>O<sub>2</sub>-catalase treatment of cheese milk. Changes in microbial flora and subsequent effects on Swiss cheese quality are included. Production of Swiss cheese with desirable body, flavor, and texture characteristics depends on use of low bacterial count, properly clarified milk. Mild [68 C (154.4 F) to 72 C (161.6 F) for 15 to 18 sec] heat treatment is recommended. Homogenization of cheese milk is not used and the H<sub>2</sub>O<sub>2</sub>-catalase treatment is not necessary.

Of the different varieties of ripened cheeses, Swiss cheese is important ranking third in total production in the United States after Cheddar and Mozzarella cheese. Swiss or Emmentaler cheese is characterized by having an elastic body with smooth texture; uniform, smooth eyes; and a unique, nut-like, sweet flavor. These factors are so closely interrelated that poor texture and irregular eye distribution can arise from a poor body. Flavor also may depend, to a certain degree, on this relationship.

The name Swiss cheese implies that it was first produced in Switzerland. It is called Emmentaler cheese in Europe and originated in the Emme Valley, Canton of Bern, in Switzerland about the 15th century (89). Other spellings of Emmentaler may be found in the literature. In the United States, production of Swiss cheese originated in the 1860's in Ohio. This cheese originally was made in wheels with firm rinds, individual wheels weighing up to about 100 kg (225 lb) each; large amounts now are

made in rindless blocks weighing about 36.4 to 40.9 kg (80 to 90 lb) (116). To facilitate cutting and packaging operations, some plants make larger blocks weighing about 91.0 kg (200 lb).

Traditionally, Swiss cheese was made from raw milk, but in 1938, a method to make Swiss-type cheese from pasteurized milk was developed in Iowa (136). This method was closely related to those used in Denmark, but has since been further modified (345). European countries other than Denmark also have developed Swiss-type cheeses. A variety closely related to Swiss cheese is Gruyère, originally produced in France since about 1288 (70). This cheese is made in smaller wheels and undergoes surface ripening, therefore having a stronger flavor arising from the proteolytic activity of the surface-grown microorganisms. There are other cheeses whose distinctive characteristics depend on growth of propionibacteria. They all possess eyes of various sizes and have the related nut-like, sweet flavor (345).

High-quality Swiss cheese is dependent on microbial fermentation of milk constituents. Propionic-acid bacteria are necessary to produce eyes and the typical flavor. *Streptococcus lactis* and *Streptococcus cremoris* are sometimes included for acid production, *Streptococcus thermophilus* to improve general quality, and *Lactobacillus* species to control abnormal fermentation (141). Propionic-acid bacteria are the special microorganisms of Swiss cheese; their presence in large numbers is necessary to develop the characteristic flavor and eyes that distinguish Swiss cheese and related varieties from all other cheeses.

Traditionally, Swiss cheese flavor has been evaluated organoleptically. Chemical methods were not sensitive enough, and only the influence of compounds such as propionic and acetic acid could be evaluated with some degree of accuracy. With the development of chromatographic methods (267) to analyze for amino acids and fatty acids (267), chemical analysis of Swiss cheese flavor compounds became possible (165). These compounds, however, do not completely account for the unique flavor of Swiss cheese. In 1952, gas-liquid chromatography

<sup>1</sup>Journal Paper No. J-7503 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1839.

<sup>2</sup>This review will appear in four parts: I. Milk Quality and Treatments; II. Starters, Manufacturing Processes and Procedures; III. Ripening and Flavor Production; and IV. Defects. Literature citations will follow part IV.

was introduced (177) and was first used in flavor chemistry in 1956 (85). Since then, development of flavor chemistry has been extremely rapid. Most flavor compounds are volatile, and careful methods of isolation are necessary (460). The concentration of these volatile compounds usually is very low, and sensitive analytical methods, such as mass spectrometry and nuclear magnetic resonance, are now successfully used for identification (427).

Identification of flavor components in Swiss cheese generally has been avoided by most investigators, but much work has been devoted to other cheese types, especially Cheddar cheese, and the literature concerning the flavor of Cheddar cheese is abundant (72, 115, 125, 147, 262, 266, 349, 373, 376). Some of the more significant work on Swiss cheese flavor has been done by Langler (241, 244).

This article will review how flavor of Swiss cheese is affected by milk quality, starter microorganisms, manufacturing processes, and ripening. Defects in Swiss cheese also will be discussed.

#### MILK QUALITY AND TREATMENTS

##### *Raw milk*

Traditionally, Swiss cheese was made from raw milk, and it was recognized early that the bacteriological condition of milk was an important factor in cheese quality. Studies in the United States in 1939 showed that, if kettle milk had a methylene-blue reduction time of <3 hr, only one-third of the cheeses made from that milk would be of high quality. When the reduction time exceeded 3 hr, two-thirds of the cheeses were of excellent quality (362). Therefore, raw milk used to manufacture Swiss cheese should have a reduction time between 3 and 6 hr (116).

Both the bacteriological condition of the milk and the types of bacteria present are important. Presence of coliforms in large numbers will severely reduce quality. About 30% of different strains of coliforms isolated from milk inhibited lactic-acid bacteria; *Enterobacter aerogenes* generally inhibited lactobacilli, and *Escherichia coli* inhibited streptococci (350). Presence of many coliforms generally led to failure of acid production and, therefore, production of cheese of inferior quality. Some micrococci promote, while others inhibit, growth of lactic-starter bacteria, with resultant diminished acid production. By reducing the numbers of these bacteria in cheese milk, defects in cheese related to inadequate acid production were eliminated (350). Anaerobic spore-formers in raw milk may lead to "blown" cheese. Milk from silage-fed cows often is implicated in these instances. There are different opinions about the importance of milk from silage-fed cows, but

most research workers believe that cheese made from milk of this sort is more prone to blowing. Constantinescu and Gondos (63) determined that feeding cows silage after instead of before milking resulted in a decrease in the number of anaerobic spores in the milk. In contrast, Kiermeier et al. (196) found no correlation between blown cheeses made of milk from silage-fed cows and of milk from cows not fed silage.

Because large quantities of milk are needed for cheese production, the manufacturing plant often stores milk overnight. Rapid cooling of milk to 10 to 12 C (50 to 53.5 F) instead of the usual overnight cooling usually improves cheese flavor with no effect on eye formation (410). Now, modern technology dictates use of lower storage temperatures and necessitates use of much longer storage periods.

In collection of milk from the farm, procedural changes have occurred; a primary example is the shift from can to bulk collection. Refrigerated farm-storage of milk for 2 or 3 days before collection may produce changes in the physical, chemical, enzymatic, and microbiological conditions of the milk. Multiplication of psychrotrophic microorganisms may sometimes be severe enough to partly destroy fat-globule membranes and promote lipolysis. Coagulation by rennin also is retarded; this problem, however, can be remedied by (a) adding soluble calcium salts, usually calcium chloride, (b) adding more rennet, (c) using a somewhat higher renneting temperature, (d) acidification, and (e) using a somewhat higher cooking temperature (357). Properly conducted refrigeration has advantages that outweigh the disadvantages, and Swiss cheeses made from refrigerated milk are whiter and have fewer but larger eyes, but otherwise show no difference in quality (425). To assure uniformity in make procedure and cheese quality, larger manufacturing plants prefer commingled storage in large silo tanks and use "holdover" milk.

In experiments in Switzerland, Emmentaler cheeses were made from milk with a normal somatic cell content (10,000-50,000/ml, CMT-negative) and from milk with increased cell content (310,000-650,000/ml, CMT-positive). Curd elasticity and firmness developed slower and to a lesser extent in cheeses made from CMT-positive milk. The cheeses needed a longer ripening period, and more flavor defects were noted. Yield also was lower (87).

##### *Clarification*

Clarification of raw milk leads to marked and consistent improvement in cheese quality. A decrease in cheese moisture content and yield may be noticed after clarification, and the percentage of fat



in the whey may increase (430). Clarification decreases the tendency of fat to form aggregates on standing. Most leucocytes, some bacterial cells, and about 98% of the clostridial spores are found in the slime. This treatment leads to an increase in multiplication rates of starter organisms and improvement in the methylene-blue reduction test. The oxygen content of the milk is increased, and carbon dioxide content is decreased. Clarification causes the oxidation-reduction potential to decrease more rapidly in milks held at 30 C (86 F). Clarified milk also has a slightly lower stability to alcohol (268, 423).

Removal of extraneous and cellular materials and more uniform distribution of bacteria are the most important contributions of clarification. Removal of these materials from milk leads to a decrease in eye number and an increase in eye size and uniformity. Swiss cheeses made from clarified milk also were found to contain fewer anaerobic spores, but formation of lactic and propionic acid and other aspects of the ripening process were slower in cheeses made from clarified milk (268, 408, 411, 423). Matheson et al. (268) mentioned that firmness of the cheese increased and the incidence of the "glæsler" (glass) defect also increased when cheese was made from clarified milk. Detrimental effects of clarification on cheese quality diminish through clarifying milk at a relatively low temperature [21 C (70 F) instead of 32 C (90 F)] and slow bowl speed (3500 rpm instead of 7000 rpm); but the quality of cheese improved by decreasing the flow rate of milk by one-half and by increasing the temperature from 21 to 32 C. Bactofugation, or bacterial centrifugation, lowers the number of eyes as well as the bacterial spore count in the cheese (423). It may reduce yield. This process has been studied in the United States but does not seem to have been adopted by Swiss cheesemakers to any extent (345).

#### *Heat-treatment of raw milk*

Pasteurization is defined by the U. S. Public Health Service as the heating of milk in approved apparatus to at least 62.83 C (145 F) for a minimum of 30 min (LTLH) or heating to at least 71.67 C (161 F) for a minimum of 15 sec (HTST) (430). Pasteurized milk is used to manufacture many cheese types, but its use for Swiss cheese production has caused difficulties. Most Swiss or Swiss-type cheeses made today are manufactured from heat-treated milk. In Switzerland, much of the Emmentaler cheese still is made from clarified or unclarified raw milk.

The first experimental Swiss cheese made from pasteurized milk was manufactured by von Freudenreich and Orla-Jensen in 1899 (122). They inoculated milk with various microorganisms and concluded

from their experiment that pasteurized milk was unsuitable to produce Swiss cheese. Their negative results may be explained by the use of low quality milk, and by too little knowledge of the beneficial bacterial flora of Swiss cheese. Later experiments, in the 1930's, showed that pasteurization had a beneficial effect through destruction of pathogens and other harmful bacteria, but made it necessary to add pure cultures to obtain good-quality Swiss cheese. Flash-pasteurization is suitable for treating good-quality milk, but earlier workers considered the holding method better for milk of lesser quality (124). It is extremely doubtful if any plants in the U. S. today use the holding method for milk heat-treatment for Swiss cheese manufacture.

One of the first successful methods to produce a Swiss-type cheese from LTLH pasteurized milk was introduced at Iowa State University in 1938 (136). A modified cooking procedure was introduced and involved removing a portion of the whey and raising the temperature of the curd to 38.8 C (102 F) by addition of hot water. This process produced cheese of good quality and has been used successfully in other countries (345, 469).

The most important advantage of pasteurization is the inactivation of much of the undesirable microbial flora, but it also has the adverse effect of reducing desirable flora if present. Milk, flash-pasteurized at 76 to 78 C (168.8 to 172.4 F) for 15 sec, was used by Demeter and Janoschek (78) to make Swiss cheese. They found that addition of a pure lactic-starter culture and a special *Lactobacillus casei* culture was necessary to produce quality cheese. From the viewpoint of flavor and aroma, the cheese was improved, although it was downgraded because of the development of splits. The effect on Swiss cheese quality of flash-pasteurization over a range of 64 to 94 C (147.2 to 201.2 F) was studied by Olšanský and Vychytová (307). Their results showed that cheese of high quality could be made with milk pasteurized in the range of 68 to 74 C (154.4 to 165.2 F) and that 72 C (161.6 F) with 15-sec holding time yielded the most desirable cheese. By reducing the temperature under 68 C (154.4 F), survival of deleterious bacteria was excessive, and their presence interfered with the ripening process. At temperatures above 74 C (165.2 F), reactions between the casein and whey proteins took place, resulting in deterioration of consistency of the cheese body and eye development. This deterioration was noted by the development of an "unclean sweet" taste and splits or cracks in the body of the cheese.

In Switzerland, it is thought that pasteurization is too severe a heat treatment for cheese milk. Therefore, a low-temperature heat treatment, "thermisa-

tion," of milk in the range of 56 to 65 C (132.8 to 149 F) for about 3 sec is utilized. Heat treatment at 56 C (132.8 F) caused fewer eyes to form, but yielded cheese of high-quality flavor and texture as compared with cheese made from raw milk (41). Even at 56 C (132.8 F), the numbers of bacteria were reduced. Coliforms showed strain differences in vulnerability, but at 61 C (141.8 F) for 5 min, all coliforms tested were greatly reduced in number. *Micrococcus* species were variably resistant, and some even survived pasteurization. *Streptococcus lactis* was not heat resistant, but *S. thermophilus* easily survived the treatment. The number of propionic-acid bacteria was appreciably reduced above 58 C (136.4 F) (293).

The types of microorganisms present in raw milk before pasteurization severely affected the ripening process of Swiss cheeses. A "normal" milk flora slightly reduced the lactic-acid fermentation in cheese and enhanced defects in eye formation, such as splitting. Large numbers of coliforms stimulated lactic-acid production, but adversely affected eye formation and flavor production. Yeasts accelerated the lactic-acid fermentation, increased body elasticity, and augmented gas formation, but had a negative influence on flavor. Anaerobic spore-formers inhibited lactic-acid formation, caused production of blowholes and splits, and the cheese had an unpleasant flavor (309). Therefore, the initial microbial quality of the raw milk has an influence on cheese quality, even with pasteurization or other less severe heat treatments.

#### *Homogenization of cheese milk*

The first experiments with homogenized cheese milk resulted in cheeses of low quality because body and flavor defects developed. Later experiments using homogenized-pasteurized cheese milk in comparison with nonhomogenized milk showed that the milk lost less fat into the whey, cheese yields were increased, and the fat leakage from the cheese was reduced. The highest-quality cheese was obtained from milk homogenized at 500 psi, while higher homogenization pressures resulted in cheese of low-

er quality (328). With homogenization at 2000 psi and addition of propionic-acid bacteria and a *L. casei* culture, the quality was approximately the same as that of control cheese (329). Again, it is doubtful if any manufacturing plants in the U. S. use this treatment.

#### *H<sub>2</sub>O<sub>2</sub> - catalase treatment of cheese milk*

Another method to control the microflora of cheese milk is addition of hydrogen peroxide. Benefits of this treatment have been related to the supposed selective action of hydrogen peroxide on microorganisms commonly causing defects. Defect-forming types of microorganisms are claimed to be largely destroyed, while many of the desirable organisms are assumed to survive. Roundy (364) found that 0.02 to 0.05% hydrogen peroxide was sufficient to reduce the number of bacteria in cheese milk, while Demeter et al. (80) determined that a higher percentage of 0.2% was best for treatment of milk for cheese manufacture. This difference may have resulted because Roundy (364) heated the milk at 52 C (125.6 F) for 25 sec after addition of hydrogen peroxide, whereas Demeter et al. (80) heated the milk to 52 C (125.6 F) before hydrogen peroxide addition to destroy the natural catalase of milk. A hydrogen peroxide treatment of 30 min was used in both instances. Catalase was added to destroy the residual hydrogen peroxide.

A 0.2% concentration of hydrogen peroxide in the cheese milk reduced the numbers of acid-producing bacteria during the first 20 min, and the number then remained constant. Coliforms were completely destroyed in 10 min. The kill of propionic-acid bacteria was negligible according to Demeter et al. (80), but Roundy (364) determined that *Propionibacterium shermanii* was adversely affected. Bacteria were not appreciably reactivated during catalase treatment. This treatment of cheese milk is easily effected and can improve cheese quality (430). It is, however, expensive and should not be used as a substitute for farm sanitation and proper milk handling and cheese manufacturing procedures (345). Use of the H<sub>2</sub>O<sub>2</sub>-catalase treatment for milk also is believed to interfere with typical flavor development in resultant cheese.

# PROCEEDINGS OF THE FOURTEENTH NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS

DES MOINES, IOWA, MAY 20-24, 1973

J. C. McCaffrey

National Conference on Interstate Milk Shipments  
3306 Glouster-Kensington Park, Sarasota, Florida 33580

(Received for publication July 30, 1973)

## EXECUTIVE BOARD MEETING, MAY 21, 1973

Chairman Schilling convened the Executive Board meeting at 10:10 a.m. All Board members except McIntire of California, were present. Guests present: Newlin and Speer.

Chairman Schilling welcomed Shelby Johnson and introduced Harold Meister who replaced Floyd Fenton. Schilling explained duties of Jack Newlin as parliamentarian.

McCaffrey presented both the secretary's and the treasurer's report, indicating a balance of \$5,261.60 as of May 18, 1973. Both reports were accepted as read.

Chairman Schilling appointed a Credentials Committee consisting of Rowley, Van Devender, and Weckel as chairman; a Resolutions Committee of Cupps, Noles, and Luchterhand as chairman; a Nominating Committee of Osten, Jefferson, Van Buren, Brendle, Evans, and Vaux as chairman. Council changes made were: Council I—educational advisor, R. Mochrie, North Carolina State; Council II—replace Kenneth Feighner with Gene Dally; Council III—replace A. C. Fisher with Chris Sykes.

Earl Wright then read his "Conference Working Procedures" and asked for comments. The Board accepted the report as read and offered a "Resolution of Commendation" for a job well done.

There being no further business, meeting adjourned at 11:15 a.m.

## FIRST GENERAL SESSION

The first general session of the Conference was called to order at 1:30 p.m. on Monday, May 21, by Chairman Schilling. The invocation was by C. Fred Stout, Chief, Iowa Dairy Trade Practices, Iowa Department of Agriculture. The address of welcome was by Dr. Lee R. Komer, Dean, College of Agriculture, Iowa State University. The keynote address was by Donald E. Wilkinson, Secretary, Wisconsin Department of Agriculture, Madison, Wisconsin.

Chairman Schilling announced the members of the Credentials, Nominating, and Resolutions Committees, and gave them their respective charges. Earl Wright presented a report on "Conference Working Procedures" in which he explained the proposed workings of the Councils and how their reports would be presented. The first general session closed with assignment of rooms for Council meetings. Council chairmen were instructed to make their preliminary reports to the membership on Tuesday afternoon.

## SECOND GENERAL SESSION

The second general session was convened by Chairman Schilling at 1:35 p.m. on Tuesday, May 22, 1973. The first roll call of states and delegates authorized to vote on Conference agreements was taken by Secretary McCaffrey. The Chairman then called on each Council chairman to present the preliminary report of deliberations. Dudley Con-

ner reported for Council I, Jay Boosinger for Council II, and Milton Scherpf for Council III.

## THIRD GENERAL SESSION

Chairman Schilling convened the third general session at 1:40 p.m. on Wednesday, May 23, 1973. Each Council chairman presented his complete Council report, listing all problems and proposed solutions to be voted on at the final general session on Thursday, May 24, 1973. Each report included both changes in and additions to existing "Procedures".

## FINAL GENERAL SESSION

The final general session was called to order by Chairman Schilling at 8:35 a.m. on Thursday, May 24, 1973. The final roll call of states and delegates authorized to vote on Conference agreements was taken by Secretary McCaffrey. The roll call showed that 44 states were represented, 15 by both agriculture and health, 13 by agriculture only, and 16 by health only. The health department of the District of Columbia was also represented.

## COUNCIL I

Chairman Schilling called on Dudley Conner, Chairman, to give the Council report. The Council was assigned 44 problems, voted "no action" on 14, and transmitted 1 to Council II.

Problems 10, 11, 18, and 22 related to P.H.S. Publication No. 678, *Methods of Making Sanitation Ratings of Milksheds*. The Council recommended that the above mentioned publication be revised to more fully reflect current industry processes and procurement practices. The Council plans to undertake the responsibility of preparing such amendments at the earliest possible date.

Problem 13 referred to the alleged arbitrary application of changes in *Standard Methods* by a small group without participation of the majority affected. The Council recommended: (a) that the IMS Conference continue the standing Laboratory Committee to serve in an advisory capacity to the three Councils on laboratory problems, and (b) that the Food and Drug Administration forward to the IMS Conference Laboratory Committee any interpretations of the current *Standard Methods* and allow at least 15 days for comment before distribution of such interpretations.

The delegates concurred.

Problem 1 asked that the length of tygon hose for farm pickups and receiving stations be limited to an 8 ft. The Council recommended that the PHS/FDA reevaluate the problem and issue an official memorandum on the length of tygon hose. The delegates concurred.

Problem 4 asked for a discussion of the Grade "A" raw milk cooling requirements and enforcement by the 3 out of 5 method. The Council recommended that, if and when the PMO is revised, the 3 out of 5 temperatures for raw milk be removed from present enforcement procedures and that any product temperature exceeding that requirement be excluded from the market. The delegates turned down the recommendation by a vote of 23½ ayes and 21½ ayes.

Problem 7 stated that the results of WMT in millimeters is not understood by milk producers when the standards are in number of somatic cells. Results in millimeters must be interpreted in light of compliance with a maximum established cell count. The Council recommended (a) that the Laboratory Committee, in cooperation with and with approval of the Laboratory Development Section of the Food and Drug Administration, should develop a revised scale(s) or conversion chart(s) for reporting WMT screening test results. This direct reading cell count(s) should more realistically compare with and reflect (i) confirmed somatic cell counts, (ii) reported WMT screening test fluctuations in the test score where confirmation is necessary in different areas of the country, (iii) possible differences caused by everyday and every-other-day pickups and, (iv) any other conditions determined by the committee to produce a more accurate screening result. (b) WMT results (and possibly all screening tests) should be reported by the laboratory in terms of cell counts. (c) All screening tests should continue to be confirmed at the confirmatory level recommended in the Food and Drug Administration Guidelines, and punitive action should continue to be initiated only on confirmed somatic cell counts. The Laboratory Committee should study these confirmatory levels and, when appropriate, make recommendations to the Conference and to the Laboratory Development Committee of the Food and Drug Administration. The delegates approved the recommendations.

Problems 19 and 25 were concerned with Standards of Identity for certain dairy products and possible resultant label revisions. The Council recommended that IMS propose to the Food and Drug Administration that the PMO food standards be followed as adopted by states and recommended by the PMO except for all creams and half-and-half. The delegates concurred.

Problem 21 referred to present requirements as being too restrictive for reinstatement of producer permits after suspension for violation of bacteria standards. The Council recommended that, when the PMO is revised, the following be considered: Administrative procedures: (a) to avoid "clean slate" interpretations, and (b) to provide appropriate language to be incorporated in the PMO to provide a continuing history of violations of bacteria, coliform, and somatic cell counts limit after a temporary permit has been issued pursuant to Section 3. First paragraph—no change. Second paragraph, first sentence—delete "or cooling temperature standards" and substitute "somatic cell count standards." Second paragraph—Samples shall then be taken at the rate of not more than 2 per week on separate days within a 3-week period. Temporary permits shall be withdrawn if the first sample during this 3-week period is above the limit, including those taken before permit suspension, are above the limit. The health authority shall reinstate the permit upon compliance with the appropriate standards as determined in accordance with Section 6 of this ordinance. The delegates approved the recommendations by a roll call vote of 22½ ayes and 21½ naes.

Problems 23, 26, 27, 28, 29, 30, 31, 32, 34, 35, and 38 were submitted by the Milk Sanitation Branch, FDA/PHS, to get comments on the proposed revision of the PMO, and dealt with such problems as hauler evaluation, definitions, suspension of permit, standards for milk and milk products, bacterial examinations, etc. The Council recommended that IMS request the Food and Drug Administration to provide the Councils of the IMS with advance final draft copies of documents which specify what action the Food and Drug Administration intends to take regarding final publication of a revised PMO, statewide certification of interstate milk shippers, and other problems which have been considered previously and acted upon by one or more IMS Councils. The delegates approved.

Problem 37 stated that several states have state laws which do not place milk sanitation regulatory work in any of the classifications now listed in Section II. Supervision, subdivision 2. The Council recommended the deletion of a, b, and c of Section II, subdivision 2 and substituting: "The milk supply to be tested shall be under the full-time supervision of a state or local milk sanitation control agency. The delegates approved.

Problem 41 dealt with whether goat's milk should meet the same somatic cell count requirements as cow's milk, while problem 44-A dealt with recommended changes in bacteriological procedures for determination of bacterial counts in both Grade "A"

raw and pasteurized milk. The Council recommended that these problems be referred to the Laboratory Committee to gather information, collate and present a meaningful report to the 1975 conference. The delegates concurred.

Problem 9 was the report of the NCIMS Committee on Abnormal Milk, as well as the minority report of this committee, and the request to reduce the maximum acceptable somatic cell count to 1 million/ml, effective January 1, 1974. The essence of the report was "that the abnormal milk control program is progressing well and that the action level should remain at a somatic cell count in excess of 1,500,000/ml. The Committee recommended that laboratories be requested to report screening test results as estimated somatic cell counts and that the Committee on Abnormal Milk Control be continued. The delegates concurred.

The final action was the report of the NCIMS Committee on Single Service Containers and Closures. The main activity of the Committee was rewriting Public Health Service Publication No. 1465 and submission of it to the PHS/FDA for refinement. The delegates accepted the Committee report.

## COUNCIL II

Chairman Schilling called on Jay Boosinger, Chairman of Council II, to give the report. Boosinger reported that the Council had been assigned 30 problems, 5 of which were referred to other Councils, 3 of which were withdrawn at the request of the author; problems 1 and 9 were reported as no action. The delegates accepted the disposal of the problems.

Problems 2, 3, and 4 dealt with implementation of procedures without first having been voted on. The Council recommended that in Section VI. D a # 5 be added to read: "Enforcement ratings shall be made a part of check ratings." It recommended that Section VI. F. be amended to add the following: "Administrative procedures developed by PHS/FDA should be drafted and forwarded to the appropriate Council of IMS for review and comment prior to its adoption. The various councils of IMS should stand ready to deal with such problems on a continuing basis." The delegates concurred.

Problems 7 and 8 requested the Food and Drug Administration to rescind their interpretations set forth in the identical Memoranda IMS-a-6 and M-a-40 as it pertains to debiting of bacterial counts, coliform determinations, temperatures, and abnormal milk counts, when 2 of the last 4 samples exceed the limit. The Council recommended the above action; the delegates concurred.

Problems 10, 11, 12, 13, and 14 dealt with check

ratings. The Council combined these requests into one problem (13-a) and recommended that Section VI. D. 1. be deleted and the following be substituted: "The Food and Drug Administration shall conduct check ratings of the sanitation compliance status in each year of listed interstate milk shippers. Within a state, annual check ratings will be made of a representative sample of milk sheds. The selection of shippers for check rating in a given state will be made randomly from each of a series of categories defined by the number of pounds of raw milk produced and/or processed. This will assure that no conscious bias in selection is present and that small as well as large shippers are included in every sample.

To make effective use of Regional Office personnel, random selection of shippers to be check rated will be done in advance and assignments scheduled in each state. Selection of farms for rating will be made from records provided at the time of check rating.

The number of shippers selected for check rating will be based on consideration of the number of shippers in the state as well as the demonstrated validity of the state program. Validity will be measured by estimating the number of adverse actions (reinspections, resurveys, or withdrawal of certification) in the states based on results of previous check ratings. This approach will shift attention from states with demonstrated validity to problem states while still preserving an adequate level of monitoring.

In no instance can a check rating be made with more frequency than the official rating. The delegates concurred.

Problem 15 dealt with Section III. B. and Section VI. D. regarding ratings. The Council recommended that the Food and Drug Administration update the *Methods of Making Sanitation Ratings of Milksheds* keeping in mind the professional judgment aspect be kept to a minimum insofar as is practical. Proposed revised methods are not to be adopted before an opportunity for comment has been given. The delegates concurred by (roll call) 33 ayes.

Problems 18 and 19 dealt with penalizing shippers because of faulty laboratory procedures. The Council recommended (a) that Section V. C. 3 be changed to read: "If split sample results of the laboratories used by certified interstate milk shippers are not received by the appropriate FDA Regional Office within 12 months of the last split sample date, the regional office shall notify the State Laboratory rating agency to withdraw the certification of the laboratory," and (b) that Section V. C. 4 be changed to read: "If results of the most recent official laboratory and sampling survey are not received by the appropriate FDA Regional Office within 2 years and 6 months of the last survey date, the regional office shall notify the

State Laboratory rating agency to withdrawn certification of the laboratory." Delegates concurred.

Problems 20 and 21 dealt with standardizing procedures used by state sampling surveillance officers. The Council recommended that Section VI. A. 3. be changed to read: "The Food and Drug Administration shall standardize the evaluation procedures of state milk laboratory survey officers and state sampling surveillance officers." The delegates concurred. The Council recommended that Section V. C. 1. be changed to read: "Sample collectors who collect samples of milk and milk products of rated interstate milk shippers shall be approved by personnel outlined in Section III. D. in accordance with the Sample Collection Procedures specified in the *Public Health Service Evaluation of Milk Laboratories*. The delegates concurred.

Problem 25 dealt with the necessity for annual seminars. The Council recommended that Section VI. C. 2. be changed to read: "In order to coordinate ratings and evaluation procedures and interpretations, the Food and Drug Administration shall sponsor seminars at least biennially or annually at the request of the states, for the state milk sanitation rating and milk laboratory survey personnel in each of its regions. Food and Drug Regional personnel may invite others charged by law with the enforcement of Grade "A" milk regulations to attend such seminars. The delegates concurred (roll call) by 32 ayes.

Problem 27 questioned the procedure that under present requirements a listed rating must carry the oldest date of any portion of listed shippers survey. Council II recommended that the Food and Drug Administration take under consideration the rationale of this problem which reads: "It is recommended that the responsibility for seeing that a listed shipper utilizes only approved sources of raw milk in conformity with its published rating be placed on the rating agency which conducted the survey of the listed shipper. The listed shipper should then be listed with the date of its own survey without regard to the survey dates of its suppliers." The delegates approved.

Problem 28 dealt with the new state coding system and its adoption. The following amended Council recommendation was approved by the delegates. "Since the National Bureau of Standards, U.S. Department of Commerce, has developed a new state coding system which would change numbers assigned to each state under the plant identification coding systems used by nearly all fluid milk processors, Council II recommended to the Food and Drug Administration adoption of these new state code numbers for use in the quarterly bulletin simultaneously with old code numbers. The adoption date by state regulatory agencies should coincide with the proposed fluid

milk and cream standards, or by January 1, 1975 if Part 18 is not effective by that date. During the interim, states shall recognize both the old number and the new number as designated." The delegates concurred.

Problem 29 stated that no certification etc, of state survey officers should occur during a routine state survey or check rating. The Council recommended "that no certification of state survey officers should occur during a routine state survey of check rating by regular PHS/FDA representatives. The delegates concurred.

### COUNCIL III

Chairman Schilling called on Milton Scherpf, Chairman of Council III, to give the report. Council III received 9 problems, in addition to several proposed changes in the Constitution. The following changes in the Constitution were approved by the assembled delegates.

Amendments to Article IV. Section 2 and 4. Section 2: After the words "the immediate past chairman" in line 5, add "and the three Council Chairmen appointed by the Chairman and confirmed by Executive Board action, as non-voting members, along with the Program Chairman who also shall be a non-voting member." In the next sentences the word "thereafter" is to be deleted and the sentence will begin with the word "each." Section 4: To be changed to read: "The Board shall elect a chairman and a vice-chairman from its membership after each Conference and each may retain his position at the pleasure of the Board as long as he is officially a member of the Board. The Board shall also either elect or appoint an executive secretary-treasurer who shall be bonded. A monitor or special chairman may be appointed by the chairman for any special meeting."

Problem 1 dealt with official delegate status, since some states share the activities between agriculture and health and some states have other agencies entirely. The Council recommended that the report of the Credentials Committee be submitted to the Executive Board, to be studied and presented at the 1975 Conference for proposed constitutional changes. The delegates accepted the recommendation. (The Credentials Committee report submitted by K. G. Weckel, has since been given to the Board.)

Problem 3 requested that the IMS Conference reaffirm stipulations of Section III. paragraph D, page 3 of the 1971 Procedures. The Council so reported and delegates concurred in the report.

Problems 4, 5, and 8 dealt with the apparent lack of a provision for relisting a shipper whose certification has been withdrawn. This would involve chang-

ing Section VII. C. 1 and 2. The following amended recommendation was presented by the Council. Section VII. C. 1 to be amended by adding the following sentence after the words "in accordance with Section V. A. 4" on page 10, line 7 of the 1971 Procedures. In the case of withdrawal, a resurvey shall be made in not  $< 30$  days and not  $> 60$  days unless the state rating agency has reason to believe a new rating within a lesser time period, but in no event in  $< 15$  days, would result in an acceptable rating. Section VII. C. 2 to be amended to insert the following sentence after the words "in accordance with Section V, A. 4" on page 10, line 20 of the 1971 Procedures. In the case of withdrawal a resurvey shall be made in not  $< 30$  days and not  $> 60$  days, unless the state rating agency has reason to believe a new rating within a lesser time period, but in no event in  $< 15$  days, would result in an acceptable rating. The delegates concurred by a roll call vote of 31 ayes and 7 naes.

Problem 6 dealt with the proposed amending of Section III. F. 1. and G. The Council recommended that, in line 3, Section III. F. 1. the word "semiannually" be deleted and replaced by the phrase "90 days after the date of last rating." It recommended that, in Section III. G. the word "semiannually" be deleted and replaced by the phrase "90 days after the date of last rating." The delegates approved.

Problem 9 dealt with dry milk plants. The Council recommended that Section VIII be changed by adding after the words "milk products" the phrase "and Grade 'A' nonfat dry milk." The delegates approved.

Problem 2 dealt with reciprocity. The amended Council recommendations regarding reciprocity, which were approved by the delegates (roll call) by a vote of 33 ayes and 7 naes, follow.

Amend Section I. (Standards) A. (Milk Sanitation Standards) by inserting the number (1) in front of the single paragraph contained therein, and adding the following paragraphs, to be numbered (2) and (3).

(2) Milk and milk products from points beyond the limits of routine inspection shall be acceptable under the principles of reciprocity for sale in the state or local area concerned, provided they are produced and pasteurized under regulations which are substantially equivalent to the 1965 *Recommended Pasteurized Milk Ordinance* and have been awarded an acceptable milk sanitation compliance and enforcement rating made by a state milk sanitation rating officer certified by the PHS/FDA.

(3) Reciprocity for the purpose of NCIMS agreements shall mean that no action or requirements on the part of any regulatory agency shall cause or require any action in excess of the requirements of the

P.M.O. or conference agreements.

Amend Section VI. Responsibilities of the Public Health Service, B (Publication of Compliance Ratings) 2. by adding the following sentences. "Effective October, 1973, the quarterly issue of Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers shall identify those shippers located in states where complete reciprocity as defined in Section I. A. 2 and 3 is not recognized by the state and/or local regulatory agency. After July 1, 1975, no shippers located in those states which continue to disregard the principles of reciprocity as defined in Section I. A. 2 and 3 shall be listed.

It is recommended that the Constitution of the NCIMS—Article VIII. Section 4, subd. 3 be amended at the 1975 biennial conference as follows: Only those delegates representing states recognizing complete reciprocity as defined in Section I. A. 2 and 3 of the Procedures are entitled to voting privileges and/or to serve as officers of the conference.

It is recommended that the Executive Board of the NCIMS designate Council III to act as the referee in determining whether complete reciprocity as defined in Section I. A. 2 and 3 is accepted by the state or local regulatory agency in question.

#### FINAL BUSINESS

H. H. Vaux, Chairman of the Nominating Committee, reported the selections from Region III for the Executive Board: Brace Rowley, Kansas Dept. of Agriculture; Keith Harvey, Idaho Dept. of Health; Harold E. Meister, U. S. Dept. of Agriculture; David E. Monk, Wichita-Sedgwick Kansas Health Dept.; and O. M. Russell, Foremost Foods. The slate presented was elected by a unanimous vote of the delegates.

#### RESOLUTIONS

The Resolutions Committee, under chairmanship of Clarence Luchterhand, presented 4 resolutions; 1 additional resolution was presented from the floor. All were accepted.

(1) Resolved, that the Chairman of NCIMS and the Executive Board shall make appropriate presentation to the Commissioner of the Food and Drug Administration that the official delegates to the 1973 Conference do not favor (a) publication of the *Pasteurized Milk Ordinance* in the *Federal Register* other than as a model regulation for adoption by states on a voluntary basis, and (b) development of Federal regulations covering fluid Grade "A" milk for publication in the *Federal Register* under the Federal Food, Drug, and Cosmetic Act.

(2) Resolved, that the National Conference on

Interstate Milk Shipments, through its Executive Board, convey to the proper FDA officials an expression of appreciation for clarification of their intentions to endorse the concept of the Conference and their continued participation in the attainment of the Conference goals.

(3) Resolved, that the 1973 National Conference on Interstate Milk Shipments urges all interested agencies and the industry involved to fully cooperate in the development of policies, laws, plans, educational efforts, and the administration to avoid conflicts and to bring about an orderly solution to problems which we now face in the area of environmental concerns.

(4) Resolved, that the Conference go on record to give a special vote of thanks and appreciation to (a) the Chairman and Co-chairman of the local arrangements committee, John Brockway and Hale Hansen, along with their members who made possible the fine facilities and whose outstanding hospitality will leave a fond memory of the State of Iowa with everyone in attendance; (b) John Speer and the program committee for setting up the challenges needed to stimulate and guide the Conference in its efforts to reach the goals established by the Conference; (c) the Chairmen and Co-chairmen, and members of the three governing Councils who, through their patience, understanding, and tolerance, guided us to the decisions which will be our guidepost for the next 2 years; (d) to John Schilling, our able Chairman, whose organizational abilities and keen understanding brought the Conference to a high plane of professional pride and accomplishment; (e) to Professor Earl Wright, whose organizational abilities and knowledge of conference structure made the ideas and dreams of those who pioneered the reorganization become a reality; (f) to the members of the Executive Board who have worked diligently in the past 2 years to establish policies which made the conference a strong, viable organization; (g) to hotel management for providing the fine facilities, the courtesies, and services which adds to the comfort of those in attendance; (h) to all those in attendance whose statesmanship and intelligent

deliberations make the goal of the best possible milk for all the people a reality.

(5) Resolved, that the Conference go on record as opposing any practices in any states which would permit the commingling of Grade "A" and manufacturing milk that could end up in Grade "A" milk supplies and be shipped to receiving states.

After the report of the Resolutions Committee, Chairman Schilling called for unfinished business. There being none, the Conference moved on to consideration of new business. There being none, the final general session of the 14th National Conference adjourned at 12:50 p.m., May 24, 1973.

#### EXECUTIVE BOARD MEETING, MAY 24, 1973

Secretary McCaffrey convened the meeting at 1:45 p.m. All voting Board members were present, except Johnson and Monk. Non-voting Board members present: Conner, Boosinger, Speer, and Smathers. McCaffrey called for nominations for the office of Chairman. John Schilling was nominated by acclamation.

Chairman Schilling called for nominations for the newly created office of Vice-chairman. Herb Vaux of Indiana was nominated and unanimously elected. Secretary McCaffrey, being no longer officially engaged in laboratory work, resigned from the Board. He was replaced as national representative of laboratories, by Berry E. Gay, Jr., the laboratory survey officer for the Illinois Dept. of Public Health. McCaffrey was hired to fill the newly created office of Executive secretary-treasurer, at a part-time salary of \$140 per month, plus expenses required for attendance at Executive Board meetings and the meetings of the Conference.

Cincinnati, Ohio, was selected as the site for the 1977 meeting of the Conference, with headquarters being the new Stouffer's Cincinnati Inn.

Chairman Schilling assigned Board members in advisory capacities to the Councils: Council I—Meister, Causey, Heine mann, Rich, Thompson, Van Patten; Council II—Rowley, Arledge, Harvey, Wright; Council III—Carr, Johnson, Russell, Monk, Vaux. Berry Gay was assigned as an advisor to the Laboratory Committee. Other appointments: Leland R. Lockhart, California Dept. of Food and Agriculture, Chairman of Single Service Containers committee; John C. Flake, Chairman of Abnormal Milk committee; John Speer, Chairman, Program Committee; Kenneth Whaley, Tenn. Dept. of Health, Chairman, Laboratory Committee.

There being no further new business, the meeting was adjourned at 3:10 p.m., May 24, 1973.



# LEBANON BOLOGNA

## I. MANUFACTURE AND PROCESSING

S. A. PALUMBO, J. L. SMITH, AND S. A. ACKERMAN

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(Received for publication June 22, 1973)

### ABSTRACT

A process devised in our pilot plant to manufacture Lebanon bologna consists of three steps: (a) aging salted beef at 5 C for 10 days; (b) smoking the stuffed bolognas at 35 C and high relative humidity for 4 days; and (c) mellowing the smoked bolognas at 5 C for 3 days. Aging the salted beef serves to enrich for a lactic microflora which will carry out the fermentation and for a micrococcal flora which will reduce nitrate to nitrite. Development of a firm cohesive structure which is characteristic of Lebanon bologna is related principally to acid production and only slightly to smoking. Fermentation occurs during the smoking period with the pH of the bolognas falling at least one pH unit during the first 2 to 3 days. Nitrate reduction and subsequent formation of nitrosylmyoglobin occur within the first 24 h. Flavor of Lebanon bologna is described as both acid and smoky. Both components develop during the lengthy incubation in the smoke house.

Lebanon bologna is a highly smoked, spiced, and fermented all-beef sausage originally made in the Pennsylvania Dutch area around Lebanon, Pa. Its manufacture probably represents an attempt to produce a sausage product similar to those of European origin. A sweet product, called sweet Lebanon bologna, is also produced. It is prepared in the usual fashion except that a larger quantity of sucrose (at least 10% instead of the usual 2%) is added along with the other ingredients just before the fermentation. Generally, the sweetness is great enough to mask the acid tang.

The traditional Lebanon bologna process may be summarized as follows: (a) beef is coarse chopped and salted (ca. 3%); (b) aged in wooden barrels in the cold (ca. 10 days at 5 C); (c) added KNO<sub>3</sub>, sugar, and spices; fine grind; stuff into casings; (d) given a lengthy smoke at relatively low temperature and high relative humidity in wooden smoke houses (smoked at least 4 days at 35 C and 90+% relative humidity); and (e) mellowed after smoking (held ca. 3 days at 5 C).

The process of Lebanon bologna manufacture appears to be similar to that of other fermented, semidry sausages (5, 11) though little is known about it. There are relatively few published processes for Lebanon bologna (2, 9, 10, 13), but, Federal specifications do exist for Lebanon-style bologna (6). Despite the apparent scarcity of knowledge of the microbiology

<sup>1</sup>Agricultural Research Service, U. S. Department of Agriculture.

and technology of Lebanon bologna, considerable quantities are made in the Pennsylvania Dutch area. One processor produces over 100,000 lb/week (2). Some manufacturers of Lebanon bologna claim that the sausage can not be made outside of the Lebanon area. Our purpose was to investigate the individual steps in Lebanon bologna manufacture and to define the technology of the process.

### MATERIALS AND METHODS

#### Meat

Freshly boned, whole, canner and cutter grade cow chuck was used throughout, except for one study in which cow knuckle was used. The meat was not trimmed before use.

#### Analyses

The moisture, fat, ash, and protein of the various sausages were determined by standard AOAC procedures (3). Samples of the different bolognas or other sausages were ground twice through a 3/16-inch plate and analyzed. Samples of the ground sausages were also used for water activity ( $a_w$ ) measurements using an Electric Hygrometer-Indicator (Model 15-3001, with gray sensor) (HygroDynamics, Inc., Silver Spring, Md.<sup>2</sup>). The pH was measured with a Radiometer Corporation pH meter (model 25) equipped with a single combination electrode. The electrode was inserted directly into the sausage or into the mass of coarse ground (3/4 inch) beef cubes. The acid content of the sausages was determined as follows: a 10-g sample of the fine ground (3/16 inch) sample was freeze-dried; the freeze-dried material was extracted 6 to 7 h with ethyl ether in a Soxhlet apparatus. The ether extract was then titrated with standard base to the phenol red end point and the percent acid calculated by assuming that all acid was lactic. Cured meat color was determined by the aqueous acetone extraction method of Hornsey (8).

<sup>2</sup>Reference to brand or firm name does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

#### Casings

Either fibrous or cellulose casings (Union Carbide) were used. They were presoaked at 130 F before use.

#### Starter culture

For most studies, fermentation was accomplished with the natural flora of the meat encouraged by aging the meat with salt. For certain studies, Merck's Lactacel MC starter culture was used for acid production.

#### Texture

Texture (firmness) of Lebanon bologna was measured in two ways: (a) with a Warner Bratzler-type shear device (J. Chatillon and Sons, N. Y.), and (b) with a subjective description of the fermented bolognas. For the Warner Bratzler shear values, measurements were made on core samples

formed by a #13 cork borer (I. D., 20 mm) and were cut parallel to the long axis of the bolognas. Warner Bratzler shear measurements were made in an attempt to assign a numerical value to firmness of the bolognas. However, because of the non-homogenous nature of the bologna cores (it contained pieces of tough connective tissue along with the fine ground muscle), Warner Bratzler shear values did not agree completely with our subjective evaluation of texture, i.e., bolognas with similar numerical Warner Bratzler shear values were given different subjective descriptions of texture. In general, with most of the Warner Bratzler values, there was agreement between them and the subjective descriptions. In some experiments (c.f., Table 4), there was a progression of Warner Bratzler shear values; these data suggested that the bolognas became firmer with longer incubation.

#### *Salt, spice mixture, and curing agents*

Except where indicated, all bolognas contained 3% added salt (NaCl). Either potassium nitrate (1.85 g/kg meat) or sodium nitrite (0.078 g/kg meat) was used as the curing agent. Sodium nitrite was used in only a few experiments when Lactacel MC was employed. The following sugar-spice mixture was formulated based on published spice mixtures (9, 10, 13) and sugars used in Lebanon bologna:

sugar or spice	g/kg meat
glucose	20.0
sucrose	20.0
black pepper	2.50
nutmeg	1.25
allspice	1.25
red pepper	0.62
cloves	0.62
cinnamon	0.62
ginger	0.62
mustard	0.62
mace	0.02

The sugars and spices were premixed in a large quantity and weighed out as a single addition when the bolognas were prepared.

#### *General procedure*

The general procedure for Lebanon bologna preparation was as follows: Beef chuck was coarse ground through a 3/4-inch plate, 3% salt added and mixed with the meat; salted meat was then aged for 10 days at 5 C; after aging, the spice mixture and KNO<sub>3</sub> were added to the aged meat and mixed; this mixture was then fine ground through a 3/32-inch plate, stuffed into casings, and incubated in either (a) a Mepaco smoke house for 4 days at 35 C and 93% relative humidity (wet oak sawdust was used to generate the smoke); or (b) a constant temperature-constant humidity cabinet for 3 days at 35 C and 80% relative humidity. Eighty percent relative humidity was used to discourage mold growth on bolognas incubated in the cabinet; however, in the smoke house even at 93% RH, no mold growth occurred. After smoking, the bolognas were mellowed for three days at 5 C to allow desirable flavor changes to occur.

In the traditional Lebanon bologna process, meat is aged in a wooden barrel. We aged salted meat in a wooden barrel or in plastic bags and both methods were equally successful.

The bolognas were stuffed with either an E-Z Pak hydraulic stuffer (Minneapolis, Minn.) or a small laboratory hand stuffer. Using a small laboratory grinder and the hand stuffer, we were able to prepare bolognas with characteristics similar to those prepared with large-scale equipment from as little as 1 kg of meat.

## RESULTS

To determine the composition of Lebanon bologna as well as other fermented sausages, several commercial samples were obtained and analyzed. These data and those from our Lebanon bolognas are in Table 1. Our sweet Lebanon bologna, though it contained 10% sucrose, was not as sweet (judged by tasting) as commercial sweet Lebanon bologna. Percentages of acid in our sweet Lebanon bologna were at least double those in the commercial bolognas, indicating that high levels of sugar might have limited the fermentation in the commercial products.

The procedure for Lebanon bologna manufacture was derived empirically based on the few published formulae (2, 9, 10, 13). The process appeared to be a lactic fermentation along with reduction of the nitrate to nitrite to yield cured meat color. In addition to its low pH, Lebanon bologna also has a characteristic firmness and cohesive texture. It was therefore decided to investigate the effect of the three main steps of Lebanon bologna manufacture, aging, smoking, and mellowing on flavor, as well as the factors responsible for texture of Lebanon bologna.

#### *Texture study*

Inside portions of cow knuckle were handled in such a fashion as to minimize contamination and keep the bacteriological count low. These inside portions were then coarse ground through a sterile grinder and held in sterile trays. The coarse ground meat was then divided into 1-kg batches and aged at 5 C, 4 batches with and 8 batches without salt. When this aged meat was made into bolognas, salt was added to half of the non-salted batches. Bolognas were made from meat aged 0, 5, and 10 days, with and without the addition of Lactacel MC starter culture and/or the gram-negative rod culture isolated from unsalted aged beef cubes (see Table 2). After incubation in the cabinet, the bolognas were evaluated bacteriologically (Smith and Palumbo, in preparation) and for texture (firmness) and pH. The data from bolognas made from meat aged 10 days are in Table 2. Similar data were obtained from bolognas made from meat aged 5 days. Of bolognas prepared without aging, the only bologna that was firm and had the typical structure was the one made with both salt and starter culture.

Salt added to meat before aging inhibits the gram-negative rod microflora (Smith and Palumbo, in preparation). When gram-negative rods were absent, the characteristic texture of Lebanon bologna was obtained by adding salt to the aged meat before preparation of the bolognas.

#### *Salt concentrations*

TABLE 1. COMPOSITIONAL ANALYSES AND CHEMICAL MEASUREMENTS OF LEBANON BOLOGNA AND OTHER SAUSAGES

Sausage/Company/Description	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	a <sub>w</sub>	pH	Percent acid as lactic
<i>Regular Lebanon bologna</i>							
Our product — Expt. #3	61.13	3.96	10.76	20.19	0.963	4.50	1.27
Our product — Expt. #2	62.53	5.04	12.15	—	—	4.50	—
Our product — made with Lactacel MC	—	—	—	—	0.963	4.60	1.31
Company A	59.60	4.90	13.30	—	0.958	4.60	0.86
Company B	50.95	4.52	22.34	—	0.977	4.60	0.86
Company C	47.39	4.91	22.25	—	0.934	4.70	0.77
Company D	49.80	5.0	24.4	17.6	0.98	4.80	0.46
Company F	59.75	4.67	12.15	22.38	0.965	4.60	1.22
Company G	57.50	5.14	16.09	19.46	0.955	4.90	0.77
<i>Sweet Lebanon bologna</i>							
Our product	58.65	3.69	7.70	18.37	0.960	4.40	1.12
Company A	51.90	5.90	11.90	—	0.982	4.90	0.34
Company B	49.14	3.98	13.64	—	0.98	4.80	0.39
Company D	47.82	4.76	17.39	16.31	0.937	4.90	0.33
Company G	54.77	4.38	16.20	17.18	0.965	4.90	0.65
<i>Sweet bologna (non-emulsion, non-fermented)</i>							
Company C	57.01	4.62	16.13	—	0.99	5.6	0.19
<i>Lebanon bologna (non-smoked)</i>							
Our product	53.34	4.74	13.67	24.80	0.95	4.60	1.09
<i>Italian salami</i>	24.25	7.14	39.77	20.32	0.793	5.20	0.47
<i>Cervelat</i>	29.40	4.60	44.4	—	0.930	4.80	0.40
<i>Thuringer</i>	36.58	5.05	37.15	17.28	0.923	4.95	0.70

TABLE 2. EFFECT OF SALT AND STARTER CULTURE ON TEXTURE DEVELOPMENT (FIRMNESS) IN LEBANON BOLOGNA<sup>1</sup>

Addition of 3% salt		Addition of culture		Firmness		
Before aging	Before bologna preparation	Gr — rod	Lactacel MC	pH	Warner-Bratzler shear value, lb.	Description
+	—	+	+	4.60	3.3	Firm
+	—	+	—	5.25	2.2	Soft
+	—	—	+	4.50	4.5	Very firm
+	—	—	—	5.30	0.8	Very soft
—	—	+	+	4.75	1.9	Soft & grainy
—	—	+	—	4.60	1.6	Soft & grainy
—	—	—	+	4.75	1.4	Soft & grainy
—	—	—	—	4.70	2.0	Soft & grainy
—	+	+	+	4.60	3.8	Very firm
—	+	+	—	4.70	2.3	Soft & grainy
—	+	—	+	4.60	3.9	Very firm
—	+	—	—	5.20	0.3	Soft

<sup>1</sup>Meat aged 10 days at 5 C.

Meat was aged for 10 days with salt concentrations varying from 0 to 4%. At the time of preparation of bolognas, additional salt was added to portions of aged meat having < 3% salt to bring the final salt content to 3% total added salt. The stuffed bolognas were incubated in the cabinet for 3 days and evaluated for pH, color, and texture. These data are in Table 3.

Amount of drip and odor of the meat during aging appeared to be related to the concentration of salt. Meat aged with 2 to 4% salt did not drip at any time during the 10-day aging period or develop off-odor.

In contrast, meat aged with none or 1% salt showed considerable drip and had pronounced off odor that seemed to be related to the development of the gram-negative rod microflora (Smith and Palumbo, in preparation).

Four percent salt present during the aging process appeared to interfere with development of the desired lactic microflora since the pH did not drop (Table 3). However, meat aged with 3% salt that had an additional 1% added during processing yielded bolognas similar in texture, color, and pH to those made from meat aged with 3% salt.

### Smoke study

To determine the exact sequence of changes occurring in the meat during normal Lebanon bologna manufacture, salted meat and the resulting bolognas were evaluated sequentially using the criteria of pH, color, and firmness. During the 10-day aging period, the pH of the salted meat remained at the starting pH of 5.6. During incubation in the smoke house or the cabinet, the pH fell (Table 4). The meat was completely cured within the first 24 h and no further cured meat color developed during the last 3 days of incubation. During incubation, the bolognas became firmer (Table 4); smoked bolognas appeared to be firmer than non-smoked ones. Based on the aforementioned studies, firmness and texture of Lebanon bologna were shown to be dependent principally on acid production, whether by the natural flora or added starter culture, and only to a limited degree on smoking. Salt is also necessary for firmness and good texture. One commercial firm smokes their Lebanon bologna until it firms up and has the proper color and texture.

### Casings

Among the three casings tested for Lebanon bologna, the cellulose casing allowed less than half as much moisture loss as the fibrous casing (Table 5). Bolognas in cellulose casing, however, had to be hung in stockinettes during smoking; bolognas in fibrous casings were hung without additional support.

### Length of aging

Meat for preparation of Lebanon bologna normally is aged for 10 days. In one study, salted meat was made into bolognas after different aging times, and after incubation in the cabinet, bolognas were evaluated for texture and pH. In this particular study, it took 14 days instead of the usual 10 to give the desired pH drop and subsequent texture development (Table 6). The additional aging time required for this batch of meat to reach the proper acidity was due to the slow development of lactic acid-producing bacteria (Smith and Palumbo, in preparation).

In a separate experiment, fresh meat was salted and made into Lebanon bologna without aging. These bolognas were evaluated daily for pH and texture. During 12 days' incubation in the cabinet, the pH did not change and firm texture did not develop; cured meat color was observed after 2 days' incubation. A separate portion of this fresh meat was salted, aged at 5 C for 12 days, and then made into bolognas. The pH of these bolognas fell to 4.7 after 3 days' incubation in the cabinet. The microbiology of this experiment is considered elsewhere (Smith and Palumbo, in preparation), but the lack of fermentation in the bolognas made from fresh meat

appeared to be related to the starting concentration of lactic acid bacteria in the meat. The fermentation in the aged-meat bolognas demonstrated that the meat would support a fermentation if aged at 5 C to develop the desired bacterial flora.

TABLE 3. INFLUENCE OF DIFFERENT SALT CONCENTRATIONS DURING AGING ON pH, COLOR, AND FIRMNESS OF LEBANON BOLOGNA<sup>1</sup>

% Salt	Characteristics of finished bolognas				Description
	pH	Hornsey cured meat color value	Firmness		
			Warner-Bratzler shear value, lb.		
0	4.71	2.90	0		Soft
1	4.78	2.70	1.5		Soft
2	4.60	2.20	2.1		Firm
3	4.85	3.50	2.4		Firm
4	5.55	0.50	0		Soft
3 + 1 <sup>2</sup>	4.60	3.0	3.0		Firm

<sup>1</sup>Meat aged 10 days at 5 C.

<sup>2</sup>Meat was aged with 3% salt; an additional 1% salt was added when the bologna was prepared.

TABLE 4. CHANGES IN pH, COLOR, AND FIRMNESS OCCURRING DURING SMOKING OR INCUBATION OF LEBANON BOLOGNA<sup>1</sup>

Days	pH	Hornsey cured meat color value	Firmness		Description
			Warner-Bratzler shear value, lb.		
<i>Smoked</i>					
1	5.05	1.7	2.9		Firm
2	4.40	1.7	4.6		Very firm
3	4.60	1.7	4.7		Very firm
4	4.60	1.7	4.4		Very firm
<i>Incubated</i>					
1	5.1	1.7	1.6		Slightly firm
2	4.50	1.7	2.1		Firm
3	4.80	1.7	3.0		Firm
4	4.75	1.7	2.9		Firm

<sup>1</sup>pH of meat before smoking or incubation was 5.6.

TABLE 5. EFFECT OF TYPE AND SIZE OF CASING ON SHRINK DURING FOUR DAYS OF SMOKING AT 35 C AND 93% RH

Code	Casing type	Diameter	Percent shrink (moisture loss)
1A	cellulose	85 mm	2.44
1B	fibrous	85 mm	6.26
1C	fibrous	55 mm	7.01

TABLE 6. INFLUENCE OF LENGTH OF AGING WITH 3% SALT ON pH AND TEXTURE OF LEBANON BOLOGNA

Days of aging	pH	Firmness		Description
		Warner-Bratzler shear value, lb.		
0	5.55	0		Soft
1	5.65	0		Soft
3	5.75	0		Soft
6	5.35	2.1		Firm
8	5.25	2.1		Firm
10	5.15	3.3		Very firm
14	4.50	3.3		Very firm

TABLE 7. INFLUENCE OF DIFFERENT FREEZING TREATMENTS ON pH, COLOR, AND FIRMNESS OF LEBANON BOLOGNA

Meat and treatment	pH	Color	Bologna evaluation	
			Warner-Bratzler shear value, lb.	Description
Frozen, thawed, salted, and aged 10 days	4.55	+	4.8	Very firm
Salted and aged 10 days; frozen, thawed, and made into bolognas	4.55	+	4.9	Very firm
Salted and frozen with no aging; thawed and made directly into bolognas	5.8	+	0	Soft
Frozen with no salt or aging; thawed and salt added and made into bolognas directly	5.72	+	0	Soft
Frozen, thawed, and salted; no aging (lactacel MC added) (incubated only 24 hr in smokehouse)	4.60	+	4.5	Very firm
Fresh; salted; no aging	5.70	+	0	Soft

#### *Fresh versus frozen meat*

Throughout most of these studies fresh meat was used. However, under certain circumstances, it might be desirable to prepare Lebanon bologna from frozen beef. Data on bolognas prepared from beef frozen at different stages of Lebanon bologna manufacture are presented in Table 7. These data indicate the following: (a) frozen meat could be made into Lebanon bologna of good texture, color, and pH if the meat was aged with salt after thawing or if starter culture was added; and (b) meat could be frozen after aging, thawed, and made into Lebanon bologna (the bacterial flora remains viable during the freezing and thawing). All bolognas described in Table 7 showed the typical cured meat color of Lebanon bologna. Thus, freezing did not seem to interfere with the nitrate-reducing flora or with the meat's enzymatic systems for producing cured meat color.

#### *Flavor*

A trained taste panel evaluated Lebanon bologna produced in our laboratory. Various bolognas made by the above described process were judged by the panel using the triangle test. The panel was best able to distinguish differences when one of the samples was smoked. Smoke was important in picking out differences between samples and in preferences; smoked samples were preferred over non-smoked in almost all instances. Some samples had higher than usual pH values (4.9 versus 4.5) and in general, panelists could distinguish and preferred the more acid bolognas.

The mellowing process was also evaluated by the taste panel by employing a hedonic scale. Lebanon

bolognas just removed from the smoke house and cooled were compared with Lebanon bolognas mellowed 3 days at 5 C. The panel rated the mellowed bolognas just slightly higher than those freshly removed from the smoke house. However, the difference was not statistically significant. Our own observation indicated that during mellowing, the flavor of the spices became less pronounced and the acid tang less sharp, but, the taste panel was unable to pick out these subtle differences. Most commercially fermented sausages are probably mellowed during the period between production and consumption and this period is generally at least the 3 days suggested for these products (9).

Several commercial Lebanon bolognas were compared to our own Lebanon bolognas using the triangle test. Based on flavor, the panel could distinguish ours from the commercial samples and preferred ours over the commercial samples in all instances. The commercial Lebanon bolognas used included representative samples of the major Lebanon bolognas produced and available in this area.

#### DISCUSSION

In the preparation of Lebanon bologna, the most critical step appears to be aging of salted beef. An optimum concentration of salt is needed to produce a bologna with good texture (Table 3). Too much salt appears to inhibit development of proper microflora as evidenced by limited pH drop and inadequate development of cured color. Too little salt permitted extensive development of gram-negative rods, producing bolognas with good color and pH, but soft,

grainy texture. The time salted meat is aged is also related to development of the necessary lactic microflora to produce the pH drop. In most experiments, a 10 day aging period was adequate.

Pederson and Albury (12) considered the influence of salt concentration in another naturally fermented food, sauerkraut. They found that salt concentration was the single most important factor which governed the course of the fermentation. Too high a salt concentration (3.5%) allowed formation of sauerkraut with lower acidity (as lactic acid) and lower pH; this sauerkraut was poor in color, flavor, and texture. Too low a concentration of salt (1%) yielded sauerkraut with soft texture, but good color. At 1% salt, the acidity (% acid as lactic) was not affected, but the pH did not fall as low as it did when 2.25 and 3.5% salt were added.

Salt is necessary for proper aging of chopped beef for bolognas since it discourages development of gram-negative spoilage bacteria. While inhibiting almost all bacteria, salt is especially valuable because it encourages growth of micrococci (Smith and Palumbo, in preparation). Salt is also necessary for development of texture (cohesive structure) of the Lebanon bologna. When not needed to suppress growth of gram-negative rods during aging, salt can be added at the time bolognas are prepared (Table 2).

Data in Table 3 suggest that the presence of high concentrations of salt (4%) during aging inhibited both cured meat color formation and production of acid during fermentation. The lack of acid production (high pH) was due to the inhibition of the growth of the lactic acid bacteria by the 4% salt (Smith and Palumbo, in preparation). However, the number of micrococci which reduce nitrate are stimulated by increasing concentrations of salt. Development of cured meat color also may be influenced by the high pH. Fox and Thomson (7) observed that formation of nitrosylmyoglobin was very pH-dependent; the reaction was 20 to 30 times as fast at pH 4.5 as at pH 5.5. Since the pH of the bolognas made from meat aged with 4% salt remained high, formation of nitrosylmyoglobin apparently was inhibited. Analyses of the nitrate and nitrite content of the different bolognas might have clarified this point. Despite the presence of a micrococcal flora capable of reducing nitrate to nitrite and conditions not favorable for nitrosylmyoglobin formation, "nitrite burn" (4) was not observed.

Considerable variation in Hornsey color values was observed between the different experiments (Tables 3 and 4), and reflects differences in the amounts of nitrosylmyoglobin formed during the respective experiments. Since the amount of nitrosylmyoglobin is dependent upon factors such as pH and amount of

nitrite (formed by bacterial reduction of nitrate) as well as other factors including the meat pigment itself, variation can be expected. There is good agreement within the respective experiments.

The compositional and chemical analyses (both of our own and of commercial samples of Lebanon bologna) represent the only data of this type that are available in the literature (Table 1). Examination of the compositional analysis data indicate that Lebanon bologna produced in our pilot plant was similar to the commercial product. Generally, our Lebanon bologna had less fat than the commercial ones. These compositional data for all sausages except the Italian salami, Cervelat, and Thuringer (Table 1) show that these sausages are high in protein and low in fat and would provide good nutrition in the diet. The last three sausages in Table 1 are considered to be of the dry type and their low moisture content reflects this. They are also characterized by a much higher fat content.

In general, the percent acid of Lebanon bolognas prepared in our laboratory was higher than that found in commercial Lebanon bolognas. Our Lebanon bologna was prepared under more carefully controlled conditions of salt concentration, and of time and temperature of aging. This rigid control may be reflected in better acid production. Furthermore, we used freshly boned chuck with no trimmings added. The meat was ground and salted either the same day or the next day after boning.

The  $a_w$  values observed for most of these sausages were relatively high and thus  $a_w$  probably did not form the sole basis for the long shelf life of Lebanon bologna (Table 1). One sample of commercial Lebanon bologna sliced in our laboratory showed no sign of visible spoilage after 12 months' storage at 20 C. This long shelf life was probably related to the extensive smoking of the bolognas along with their lactic acid content and low pH.

The shelf lives of several smoked and non-smoked Lebanon bolognas produced in our pilot plant were studied. The variables included smoked and non-smoked bolognas, with and without spices which were fermented with natural lactic flora or with Merck's Lactacel MC. The bolognas were taken to a local market where a small quantity of each was sliced; these slices were wrapped in Saran, stored at 20 C, and observed daily. The slices from the various non-smoked bolognas showed mold growth within 1 week. Those from the various smoked bolognas showed no mold after 4 weeks. This observation further supports the above statement that the extensive smoking contributes a major portion to the shelf life of Lebanon bologna.

Throughout these studies, we employed an aging

temperature of 5 C, except for one study in which we used 11 and 16 C. These two elevated temperatures appeared satisfactory for development of the necessary microflora, as evidenced by the pH drop and color of the bolognas. However, 5 C is the recommended aging temperature because growth of food-borne pathogens is inhibited at this temperature (1).

The special wooden smoke houses used by most commercial firms to smoke Lebanon bologna allow them to achieve, without difficulty, the long low-temperature incubation required to produce this sausage product. We employed a modern air conditioned smoke house and had difficulty maintaining 93% relative humidity at 35 C. After modification of the smoke house to function at low temperature and high relative humidity, we were able to maintain these conditions for the regular 96-h smoke given our product.

Our Lebanon bologna did have one defect which we were unable to correct. The bolognas had a somewhat dry, dark outer layer just beneath the casing. The layer was about 1/2-inch thick and dark brown in color. The layer may represent a heavy accumulation of smoke along with some dehydration of this outer portion of the bologna. This dried layer was observed with both fibrous and cellulose casings with as little as one day of smoking. One explanation may be that the wooden smoke houses used to make this product commercially have no means of circulating the smoke, while in our smoke house, the smoke was continuously circulated and forced through the house. This continual circulation of slightly less than water-saturated smoke (93% relative humidity) may provide a drying effect and produce this dry, dark layer.

The acid production and nitrate reduction pattern observed during smoking of Lebanon bologna was similar to that reported for summer sausage by Deibel et al. (5). Nitrate reduction and subsequent curing occurred very early in the smoking, while acid production was somewhat slower. Deibel et al. (5) also reported that soft texture of summer sausage was associated with high pH values in the sausage. This is essentially our observation with Lebanon bologna; firm, cohesive characteristic texture was produced only with a good fermentation (pH drop) and in the presence of salt.

In defining Lebanon bologna flavor, two criteria seem to describe it: smoke and acid tang. Bolognas possessing both these characteristics were preferred

by the taste panel and judged "typical." Certain commercial Lebanon bolognas are only smoked for relatively short periods and in general these lack typical Lebanon bologna flavor. These same bolognas are prepared with starter cultures. We have found that with starter culture, too long an incubation (smoking) tends to yield undesirable flavors. Starter cultures allow a faster pH drop, and make the bolognas safer from a public health point of view, but they do not permit the long smoking necessary to give the typical Lebanon bologna flavor.

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## ACCEPTABILITY OF MAIN DISHES (ENTREES) BASED ON MIXTURES OF GROUND BEEF WITH GROUND FISH OBTAINED FROM UNDER-USED SOURCES

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### ABSTRACT

Technological, economic, and nutritional considerations suggest that ground fish can be better utilized if a greater variety of acceptable food items are developed. Using well-known main dish recipes, the feasibility of mixing ground beef and ground fish was tested. Several types of main dishes received favorable acceptance ratings. Some of these evaluations were based on ground fish that had been stored in a freezer for up to a year.

### INTRODUCTION

#### *Seafood resources*

Nutritionists estimate that food production is becoming less adequate to meet our requirements for good quality protein at moderate prices. Seafoods, in general, are well known as excellent sources to meet these requirements. However, there is a growing awareness that we may be reaching the limit of the oceans' resources for seafood in traditional forms.

During the last decade, several groups in this country and overseas have sought new ways to increase the yield of food from species presently landed or to utilize other species which are not now landed for food uses. Japanese groups have pioneered in this effort (16, 17). Although several popular products for Japanese diets have been developed, they have found a limited acceptance in America. In contrast, the relevant technology and engineering is being adapted in many American and European countries to suit local sources of fish and local food preferences.

In the United States, at least, the emerging technology of flesh separation and utilization considers two general categories of raw material sources. One source is filleting leftovers. Present day filleting operations could provide a source of machine-separable meat. By using these leftovers, as well as fillets, for food, an increase in edible yield is possible. Since these species are already landed for food uses, this increased yield is attainable without any extra harvesting effort. The second source includes a larger number of species. Some are presently caught incidentally to other species and thrown back into the ocean because their anatomy or size is not

amenable to present filleting methods. Some are not harvested at all for these reasons. Included in this category are species which, if harvested, could improve the ecological balance among the oceans' resources. In general, these species can be headed and gutted and either used in this form or put through a meat-bone separator to recover their edible flesh.

#### *Sources of ground flesh and their suitability for making fish sticks or portions*

In recent years, some products have emerged in U. S. markets which are based on ground fish recovered by machine. These products are generally patterned after fish sticks or fish portions which have become increasingly popular since their introduction in the 1950's. The typical appearance, taste, and texture of sticks or portions made from fillet (regular) blocks have become well established. The resemblance of products made from ground fish to these "regular" sticks and portions depends strongly on the type of raw material used.

Ground fish obtained from V-cuts (fillet trimmings) is the most suitable material to prepare a product which resembles a regular fish stick. Headed and gutted fish are sometimes used to make stick or portion-like products. In comparison, fish frames (filleting left-overs) represent a source of ground meat that has a good potential if problems of color and texture can be solved. The color problem originates from the concentration of blood tissues near the spinal column while the texture problem results from using a strainer to improve its appearance.

Thus, a series of grades (and prices) can be visualized for ground blocks from different sources of machine-separable flesh if one considers suitability for making fish sticks and related items. On the basis of appearance (whiteness) and texture, at least, it can be assumed that blocks made from V-cuts would be near the top and blocks from frames would be near the bottom of this series. Suggestions to improve the suitability of blocks from pigmented sources do have merit. However, they are vulnerable to criticism for reasons of increasing cost or reducing



TABLE 1. RECIPE FOR BEEFISH LOAF

---

1/2 lb. Ground fish
1/2 lb. Ground beef
2 tsp. Chopped onion
3/4 tsp. Salt
1/8 tsp. Black pepper
1/4 Cup bread crumbs, "Italian Style," flavored with cheese, spices, parsley, herbs, monosodium glutamate, Progresso Foods Corp., Jersey City, N. J. No endorsement implied compared to competing trade names.
1/4 Cup milk
1 Egg, slightly beaten
Mix ingredients together and place in baking dish. Bake at 350 F for 25 min or until done. Place under broiler for 5 min to brown top surface.

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yield to unacceptable levels compared to the present sources of supply for the fish stick trade.

*Mixing ground beef and fish: A different approach to using colored fish flesh*

Instead of altering ground fish derived from frames or headed and gutted fish to suit requirements for fish sticks, we have suggested developing food products which take advantage of this material's natural characteristics (8, 9). For example, its color (due to blood pigments) suggests a resemblance to mammalian meats, especially beef. This resemblance led us to consider economic and nutritional factors in this product development work.

Economic considerations are definitely in favor of mixing beef and blood-colored fish. One estimate of processing costs for preparing minced fish blocks resulted in a value of 17 to 18 cents a pound for frozen ground fish delivered to final user (8). This estimate was based on utilization of fish frames or headed and gutted fish. It was compared to 1970 selling prices of 15 cents per pound for cod blocks (using ground meat obtained from V-cuts) and 30-33 cents per pound for regular cod blocks or fillets. Since 1970, average prices for all three items have increased. Seasonal fluctuations in prices have occurred, but the price of ground cod has remained at about half of the prices for regular cod blocks or fillets. It is still reasonable to propose that ground fish derived from frames or headed and gutted fish would be priced similarly. Prices for ground beef have risen more noticeably during these last 3 years. At current prices, it appears that ground beef is still roughly four times as expensive as ground fish.

Nutritionally, there are positive advantages in mixing ground fish with ground beef (1). The protein value of fish is comparable to beef both in quantity and in quality as well as being more easily digestible (4). The fat content of fish is typically lower than beef and contains less cholesterol. Fish fats also contain a greater proportion of unsaturated fatty acids in comparison to the "hard" saturated fatty

acids of beef. For adults, there are positive health benefits in lowering fat intake and decreasing the proportion of saturates in this fat (3, 12). Such dietary choices can be more significant to the continued health of younger people compared to older people who have already experienced coronary heart disease (1). Nutritional studies have shown that these health benefits can start in the diets of teenagers (7, 11).

Despite the importance of nutritional and economic considerations, acceptance is the major factor in the development of such new products. In a survey of food preferences among people in military service, Peryam et al. (14) found that main dishes using ground beef had an average preference rating considerably above averages for other main dishes which involved a complex recipe or preparation. We are unaware of similar studies for the School Lunch Program or other mass feeding groups. However, the presence of several main dish items based on ground beef in their recipes attests to the popularity of these dishes in American diets.

Thus, the purpose of this investigation is to test the acceptability of mixing ground beef and ground fish using well-known main dish recipes. We call these mixtures "beefish." This investigation does not include beefish patties, which are described elsewhere (10), or sausage and frankfurter type products (which are being developed in NMFS laboratories in Gloucester, Massachusetts, and Seattle, Washington.)

#### RESULTS AND DISCUSSION

Results of sensory evaluations for a wide variety of beefish main dishes are given in Table 2. In most instances, the averages were between 6 and 8 on a 9-point hedonic scale. Since these averages were well above the mid-point of this scale, it is suggested that these main dish items, at least, are acceptable products. Preferences for particular items were not evident statistically from this data. In contrast to this small in-house taste panel, Peryam et al. (14) used a large consumer-type panel to determine preference ratings for several ground beef main dishes. Their results led us to consider that an average acceptance rating of 7 to 8 might be the practical maximum for a beefish main dish item.

Our decision to use equal weights of ground beef and fish in the recipes of Table 2 was based on preliminary evaluations. We tried different proportions of beef and fish using a meat loaf recipe. The results in Table 3 suggest that the ratio of beef to fish can vary from whitish (obtained from V-cuts) to the blood-pigmented meat obtained from frames without altering acceptability of the final product.

The results in Tables 2 and 3 do not reveal how

TABLE 2. SENSORY EVALUATIONS OF MAIN DISH ITEMS USING 1:1 MIXTURES OF GROUND BEEF AND FISH IN PLACE OF GROUND BEEF (HAMBURG)

Recipe	Mean of taste panel ratings (9-point hedonic scale)				
	App.	Odor	Flavor	Text.	Overall <sup>1</sup>
Lasagna <sup>2</sup>	8.0	7.7	7.5	7.8	
Meat/potato burgers <sup>2</sup>	7.4	6.4	6.0	6.3	
Spanish rice <sup>2</sup>	7.7	7.8	7.3	7.5	
Ground beef and spaghetti <sup>2</sup>	8.1	8.1	7.2	7.6	
Chili con carne <sup>3</sup>	7.7	7.8	7.0	7.3	
American chop suey <sup>4</sup>	8.0	8.0	7.8	7.8	
Beef stroganoff <sup>3</sup> or Hamburg stroganoff <sup>4</sup>	5.7	7.3	7.4	7.4	
Beef porcupines <sup>3</sup> or Beef a la Lindstrom <sup>4</sup>	5.6	6.4	5.8	5.7	
Beef porcupines <sup>3</sup> or Beef a la Lindstrom <sup>4</sup> with 4% NM139-88	5.7	6.9	6.8	6.2	
<i>Meat balls</i>					
"Plain" style <sup>3</sup>	7.1	7.0	6.4	6.6	
All-beef <sup>3</sup> (served simultaneously)	7.1	7.5	7.2	7.1	
Swedish style <sup>4</sup>	7.2	7.2	6.1	6.4	
All-beef <sup>4</sup> (served simultaneously)	6.5	7.0	6.9	6.0	
Italian style <sup>3, 4</sup>	6.9	6.8	6.7	7.4	
All-beef <sup>3, 4</sup> (served simultaneously)	6.9	7.1	7.0	7.5	
Barbecued beef <sup>3</sup> (Sloppy Joe)	—	—	—	—	7.9
Barbecued beef <sup>5</sup>	—	—	—	—	8.0
Chinese chop suey <sup>5</sup>	7.4	7.3	7.1	7.4	
Chinese chop suey <sup>5</sup> with 4% NM139-88	7.5	7.5	7.1	7.7	
Foo young <sup>3</sup> (with fish) <sup>6</sup>	—	—	—	—	7.9
Foo young <sup>5</sup> (with fish and egg) <sup>6</sup>	—	—	—	—	7.7
<i>Beefish</i>					
cheeseburger <sup>5</sup>	7.9	7.9	7.8	7.7	
Chili con carne <sup>5</sup>	7.8	8.0	8.0	7.8	
Salisbury steak <sup>3</sup>	6.5	6.8	5.9	6.7	
Salisbury steak <sup>3</sup> with 4% NM139-88	6.5	7.1	7.2	7.2	
<i>Meat loaf</i> <sup>3, 4</sup>					
Beefish with cheese	7.0	7.0	6.8	7.1	
With cheese cover	—	—	—	—	7.2
Porkfish (1:1) with cheese <sup>6</sup>	6.7	7.6	7.3	7.0	
Beef-pork-fish (1:1:1) with tomato sauce <sup>6</sup>	7.2	7.1	6.5	7.5	

<sup>1</sup>For the five recipes in which no figures are given for appearance, odor, flavor, and texture, the overall means were based on "overall" sensory ratings.

<sup>2</sup>Adapted from recipe in the Type A School Lunch Program (19).

<sup>3</sup>Adapted from recipe in the Armed Forces Recipe Service (6).

<sup>4</sup>Based on recipe in a domestic cookbook (13).

<sup>5</sup>Based on recipe furnished on package of commercial seasoning mixture. No endorsement implied compared to competing trade names.

<sup>6</sup>In these recipes, the stated ingredients were used in place of a 1:1 beefish mixture.

long ground fish can be frozen stored and retain its acceptability as an ingredient of a beefish entree. This may be an important consideration in mixing fish and beef under practical conditions. Blood pigments are known to catalyze lipid oxidation (5, 18). The practical consequences of this reaction and other storage-induced changes were measured by using frozen-stored ground cod as an ingredient of a beefish loaf recipe.

The results of this storage study indicate that ground cod can be used as a meat loaf ingredient even after it has been stored for a year at 0 F. Unfortunately, we were unable to determine how much longer it could have been stored because we ran out of samples. Extrapolating the results actually obtained, led us to suggest that this period of useful storage might be significantly greater than 1 year.

Averages of the sensory ratings for flavor are illustrated in Table 4. As a group, ratings for the two samples which contained a minimum of blood pigment (meat from V-cuts) or which contained visible blood pigmentation (meat from frames) and added antioxidant appear to be higher than ratings given to the other two samples. This apparent difference was not statistically significant ( $p = 0.05$ ).

In addition to flavor, sensory ratings for appearance, odor, and texture were obtained for these samples. Throughout the study, the averages for appearance and texture remained between 7 and 8. The ratings for odor followed the trends already noted for flavor, but they were much less pronounced.

#### MATERIALS AND METHODS

##### *Preparation of materials*

Material was obtained from local, commercial cod, had-dock, pollock, or cusk filleting lines. These fish had been eviscerated at sea. After the fillets had been removed, we obtained the frames (filleting leftovers). After beheading the frames, we used a Bibun<sup>1</sup> meat-bone separator (drum perforations 7.0 mm diameter) and strainer (screen perforations 1.0 mm diameter) or the separator alone (drum perforations 5.0 mm diameter) to obtain deboned, ground flesh (8, 9). No attempt was made to remove blood pigments or other water soluble components by washing this ground flesh. The ground fish was packaged in wax board boxes, plate frozen (plates at -40 F), and stored at 0 F until used. All of the other ingredients in the recipes were purchased locally and used within 24 h. The ground beef ("hamburg") contained an average of 26% fat.

If the ground fish had been frozen-stored, it was first slow-thawed in a package held 10 h at 36 F or fast-thawed in a package immersed in a 68 F water bath until it was slack-thawed (some ice crystals remaining). If brown areas (due to surface oxidation) were present on the fish or the beef, they were trimmed off. Only ground fish or beef having a red coloration typical of unoxidized blood was used.

For some of these recipes, hydrolyzed plant protein seasoning NM 139-88 was added to the ground beef and fish mixture before proceeding with the rest of the recipe instructions.

TABLE 3. SENSORY EVALUATION OF MEAT LOAVES CONTAINING DIFFERENT PROPORTIONS AND SOURCES OF GROUND FISH WITH GROUND BEEF

Source of ground fish	Ratio ground fish/ground beef w:w	Mean of taste panel rating $\pm$ 2 standard deviations (9-point hedonic scale)			
		Appearance	Odor	Flavor	Texture
V-cuts from haddock <sup>1</sup>	1:1	7.8 $\pm$ 2.6	7.2 $\pm$ 1.8	6.7 $\pm$ 1.7	7.2 $\pm$ 1.6
Frames from haddock <sup>1</sup>	1:1	7.8 $\pm$ 0.74	7.6 $\pm$ 1.7	7.5 $\pm$ 1.2	7.8 $\pm$ 1.3
V-cuts from cod <sup>2</sup>	1:1	7.2 $\pm$ 2.7	7.4 $\pm$ 1.9	7.6 $\pm$ 1.9	7.4 $\pm$ 2.5
Frames from cod <sup>2</sup>	1:1	7.5 $\pm$ 1.7	7.5 $\pm$ 1.2	7.6 $\pm$ 2.1	7.5 $\pm$ 1.6
V-cuts from cod <sup>2</sup>	2:1	7.5 $\pm$ 2.0	7.2 $\pm$ 2.0	7.2 $\pm$ 2.9	7.6 $\pm$ 2.1
Frames from cod <sup>2</sup>	2:1	7.3 $\pm$ 2.6	7.1 $\pm$ 2.2	7.4 $\pm$ 2.5	7.3 $\pm$ 1.5
Frames from cod <sup>3</sup>	1:2	7.3 $\pm$ 1.7	7.4 $\pm$ 2.0	7.6 $\pm$ 1.3	7.2 $\pm$ 2.7
All beef <sup>3</sup>	0:1	7.8 $\pm$ 1.6	7.2 $\pm$ 2.4	7.6 $\pm$ 2.1	7.2 $\pm$ 2.1
Frames from cod <sup>3</sup>	1:1	7.2 $\pm$ 1.9	7.1 $\pm$ 3.0	6.4 $\pm$ 3.0	7.2 $\pm$ 2.8
Frames from cod <sup>3</sup>	2:1	6.8 $\pm$ 2.9	6.8 $\pm$ 2.8	6.3 $\pm$ 2.7	6.9 $\pm$ 3.2

<sup>1, 2, 3</sup>Served at same time as other loaves with same number.

TABLE 4. SENSORY EVALUATIONS OF BEEFISH MEAT LOAVES USING FRESH INGREDIENTS EXCEPT FOR THE GROUND COD (SEE TABLE 1 FOR RECIPE USED)

Day of storage at 0 F for the ground cod ingredient	Mean of ratings for flavor (9-point hedonic scale) $\pm$ 2 standard deviations			
	Ground fish obtained from cod frames			
	Control (Ground fish not mixed)	Antioxidant mixed with the ground fish	Ground fish mixed but no antioxidant added	Ground fish obtained from V-cuts
1	7.8 $\pm$ 1.6	7.8 $\pm$ 0.75	7.2 $\pm$ 1.4	8.1 $\pm$ 1.3
2	7.6 $\pm$ 1.5	7.5 $\pm$ 1.9	7.6 $\pm$ 1.5	7.8 $\pm$ 1.4
3	7.4 $\pm$ 1.5	7.2 $\pm$ 1.8	7.2 $\pm$ 2.0	7.4 $\pm$ 1.5
9	7.3 $\pm$ 1.5	7.4 $\pm$ 2.1	6.0 $\pm$ 2.9	7.7 $\pm$ 1.5
16	6.9 $\pm$ 2.2	7.7 $\pm$ 2.1	7.2 $\pm$ 2.0	7.0 $\pm$ 2.6
23	7.2 $\pm$ 1.4	7.2 $\pm$ 2.0	7.2 $\pm$ 1.4	7.5 $\pm$ 1.9
30	7.1 $\pm$ 2.3	7.8 $\pm$ 1.4	6.7 $\pm$ 3.4	7.5 $\pm$ 1.5
36	6.8 $\pm$ 2.3	7.4 $\pm$ 1.5	6.7 $\pm$ 2.1	7.2 $\pm$ 1.2
51	5.8 $\pm$ 4.0	7.3 $\pm$ 1.6	6.9 $\pm$ 3.6	7.4 $\pm$ 1.6
62	6.5 $\pm$ 2.1	7.6 $\pm$ 2.1	6.8 $\pm$ 1.8	7.7 $\pm$ 1.7
86	6.5 $\pm$ 2.2	7.3 $\pm$ 2.1	6.3 $\pm$ 2.3	8.0 $\pm$ 0.85
112	6.4 $\pm$ 1.3	7.6 $\pm$ 1.0	5.8 $\pm$ 2.3	7.6 $\pm$ 1.0
158	6.3 $\pm$ 1.6	7.5 $\pm$ 1.7	6.6 $\pm$ 1.0	7.1 $\pm$ 1.7
164	7.4 $\pm$ 1.0	7.6 $\pm$ 1.0	6.8 $\pm$ 1.8	7.8 $\pm$ 0.80
218	7.5 $\pm$ 1.6	7.4 $\pm$ 1.0	7.5 $\pm$ 1.8	7.7 $\pm$ 1.0
228	5.6 $\pm$ 1.5	6.9 $\pm$ 1.1	5.8 $\pm$ 3.2	7.6 $\pm$ 1.0
280	6.5 $\pm$ 2.2	6.9 $\pm$ 1.8	6.6 $\pm$ 2.6	7.5 $\pm$ 2.2
294	5.8 $\pm$ 3.2	7.5 $\pm$ 1.0	7.1 $\pm$ 1.0	7.8 $\pm$ 1.4
309	5.5 $\pm$ 3.2	7.0 $\pm$ 1.8	7.0 $\pm$ 1.6	6.7 $\pm$ 1.7
329	---	7.1 $\pm$ 1.7	---	7.6 $\pm$ 1.4

Quantities are expressed as a percentage of the final weight of the mixture. This seasoning was developed by Nestle Company, Inc.<sup>1</sup>, Food Ingredients Division, White Plains, New York 10605, to improve the acceptability of ground fish.

#### Recipe sources

The recipes used were based on main dish items (entrees) in a School Lunch recipe set (19) and in the Armed Forces Recipe Service (6). Both of these institutional sets of recipes are based on feeding 100 persons. Since our taste panel consisted of 12-15 people, we scaled down these recipes. A household set of recipes (13) was very useful for this scaling down. For some recipes, we purchased seasoning mixtures at local grocery stores and used the recipe on the package. These seasoning packages include:<sup>1</sup> (a) "Sloppy Joe Seasoning Mix", Whyler Foods, Borden, Inc., Chicago, Illinois 60618; (b) "Durkee Chop Suey Sauce Mix", Durkee Famous Foods, SCM Corporation, Cleveland, Ohio 44115; (c) "Egg Foo Young Dinner", Chun King Division, RJR Foods, New York, N.Y. 10017 (when used without egg, called "Fish Foo

Young"); (d) "Durkee Ground Beef Seasoning with Onions," Durkee Famous Foods, SCM Corporation, Cleveland, Ohio 44115 (used to prepare "cheeseburger"); (e) "Maggi Chili Mix," The Nestle Co., Inc., White Plains, N. Y. 10605; and (f) "Brookwood Chili Con Carne Dry Mix", Gentry Co., Inc., Gilroy, California 95020.

#### Use of Recipes

For each recipe used, we followed instructions except for substituting a mixture (usually 1:1, w/w) of ground beef and fish for the hamburger ingredient.

#### Sensory evaluations

Sensory evaluations were based on a 9-point hedonic scale (2). For most tests, the 12-15 evaluators were asked to rate appearance, odor, flavor, and texture separately using this scale. In a few tests, the evaluators were asked to give a single overall rating of the item.

#### Storage study of ground fish ingredient

For a storage study, cod frames and the meat-bone separa-

tor without strainer were used to obtain three lots of ground fish. One lot was packaged in 5-lb. wax board boxes, plate frozen (-40 F plates) and stored at 0 F until used. The second lot was mixed with enough Sustane-3 to give a final antioxidant (BHA + PG) concentration of 0.03% based on a 1.0% estimate for the fat content of the fish flesh. Sustane-3 is a solution obtained from Universal Oil Products Company, Chemical Division, East Rutherford, New Jersey<sup>1</sup>. Its composition is based on a proposal called AMIF-72 from the American Meat Institute Foundation (15). A Hobart Meat Mixer<sup>1</sup> was used to mix the Sustane-3 with the ground fish. The third lot was put through the Hobart Meat Mixer in the same manner as the second lot except that no Sustane was added to the third lot. Both the second and third lots were packaged and frozen in the same manner as the first lot.

A fourth lot was purchased from a commercial manufacturer of fish sticks. It was made from V-cuts (a mixture of white, skinless cod fillet meat and "pin" bones). This lot was also stored at 0 F until used.

At intervals during the storage period, samples were removed from the lots for sensory evaluation. After thawing the fish, it was mixed with the other ingredients of a meat loaf recipe (Table 1). Portions of each loaf were coded and served in random sequence to the evaluators who used the 9-point hedonic scale.

#### CONCLUSIONS

Acceptable main dish items can be made from mixtures of ground beef and ground fish. The sensory results of this study and of a study on beefish patties (10) suggest a wide variety of acceptable recipes with their variations of seasonings and proportions of ground beef to fish. Even the limited storage data support the concept of substituting mixtures of ground beef and fish in these main dish recipes. These product applications appear to be especially suitable for ground fish that is visibly colored by blood pigments.

It is hoped that the results of this investigation will stimulate further development of food items based on mixtures of ground beef and ground fish. There is a definite need for foods which meet our nutritional requirements, both qualitatively and quantitatively, which decrease health risks, which are reasonably priced, and which are readily available to consumers. Beefish food items appear to meet these criteria—with the exception of commercial availability. Acceptance testing under typical consumer mass feeding or domestic conditions should be done. In such testing, there is no reason to impose legal or regulatory barriers if these foods are easily identified by appropriate labeling (1).

#### ACKNOWLEDGMENTS

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<sup>1</sup>The use of trade names is merely to facilitate descriptions; no endorsement is implied.

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## REMOVAL OF GLUCOSE FROM EGGS: A REVIEW<sup>1</sup>

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### ABSTRACT

Glucose affects adversely the storage stability of dried eggs and causes undesirable changes in physico-chemical and functional properties of the powder. Therefore, microbial or enzymic techniques are used to desugar eggs before drying. A comprehensive review of the microbial and enzymic techniques to remove glucose from eggs is presented. Factors affecting the efficiency of the two methods are discussed as well as their effect on the physico-chemical and functional properties of the final product. Regardless of the techniques used in desaccharification, egg powders prepared after removal of glucose exhibit better functional properties than untreated samples.

Eggs contain approximately 1.0% carbohydrate, of which nearly 0.6% occurs in a free form (20). Glucose constitutes the major carbohydrate in eggs and has been shown to react readily with egg proteins. Fee-ney et al. (6) observed a reaction between glucose and egg white proteins which was delineated in starch-gel electrophoretic patterns. They reported that the changes in the electrophoretic patterns could be prevented by dialyzing the egg white, indicating that glucose reacted with most proteins. Also, these changes could be simulated by adding back glucose to the dialyzed egg white.

Glucose in eggs has been known to affect the storage properties of egg powder in three ways: (a) change in solubility of proteins; (b) discoloration of the product because of the development of a brown color; and (c) development of fluorescing substances. Also, it has been known to exert a detrimental effect upon certain functional properties of egg solids. Therefore, several methods have been considered and developed to remove glucose from eggs before drying. In general, the methods used to remove glucose from eggs fall into two main categories, viz. microbial and enzymic. Since Lightbody and Fevold (16) have discussed at some length the work done on desaccharification of eggs before 1948, this review article deals mainly with research carried out in the post '48 period. Also, the numerous patents on this subject will not be dealt with in this article.

### MICROBIAL METHODS FOR DESACCHARIFICATION

The principle in such techniques is based on the fact that microorganisms require carbohydrates as a source of energy. Therefore, both bacteria and yeast have been used to deplete the sugar from eggs.

Removal of sugar from raw egg white by *Saccharomyces cerevisiae* was described by Ayres and Stewart (2). They observed that *cerevisiae* was able to remove glucose from egg white, provided 0.1% yeast extract was added as a growth stimulant. When large masses of yeast cells were used, the need for yeast extract was obviated. Yeasts were found to be more suitable than such bacteria as streptococci, *Aerobacter*, and *Escherichia* since the fermentation could be more readily controlled. They reported that the initial pH of egg white had a marked effect on the speed of glucose removal; pH levels below 8.5 greatly accelerated the rate of fermentation. Since oxygen transfer is a function of surface area and volume, the pH of the glucose-free stage of fermentation could be kept above pH 6.4 (surface to volume ratio of 1:3.3 or greater) by suitable selection of an appropriate surface area. This permitted fermentation to be accomplished without altering the physical properties of the mucin, and thus permitted retention of good angel cake-making properties of the albumen.

Hawthorne (8) prepared dry albumen with the removal of sugar before drying. The yeast used was *S. cerevisiae*. His preliminary experiments indicated that the rate of fermentation was proportional to the amount of yeast added, at least up to a concentration of 4 g yeast (ca. 25% dry weight) per 100 ml albumen. However, high levels of yeast produced yeasty flavor in the product. He observed that 1 g of yeast per 100 ml albumen gave optimal rates of fermentation at 37 C, without producing any undesirable flavor defect. Initial pH of egg white did not affect fermentation rates and, thus, was not in agreement with earlier data reported by Ayres and Stewart (2). When fermentation was complete, the bulk of the yeast was removed from the white by centrifugation, and the egg white was pan dried. The desugared albumen exhibited good solubility (95-98%) and could be stored up to 6 weeks at 50 C. The product was also free from objectionable odors and flavors.

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Carlin and Ayres (4) carried out storage studies on yeast fermented dried egg white. They used commercial dry yeast pellets which had the advantages of convenience, supply, and speed of action. Yeast fermented dried egg white thus obtained was soluble in water, rehydrated readily, and could be used to make "high" quality angel cakes. The egg white did not have an objectionable odor and retained all the properties of fresh albumen, particularly whipability. After 16 weeks of storage at 22 C, there were no detectable changes in color and angel cake-making properties of yeast fermented dried egg white. However, storage at 40 C caused some deterioration in this product. For example, there was a decrease of 5% in the volume of angel cake made from a product stored at 40 C for 2 weeks. After this initial loss, the material was quite stable until after 12 weeks of storage by which time there was a slight additional decrease of 5%. In contrast, on storage of the control, unfermented dried egg white, at 40 C, its functional properties deteriorated rapidly. A decrease of 26% in angel cake volume was noted after 2 weeks storage of the egg white, and angel cakes made with this product after storage of 4 weeks were definitely not satisfactory. Also, solubility of the egg white had decreased to an extent where it was impossible to rehydrate all of the albumen. When stored at 22 C for 16 weeks, the product gave cakes with 7% less volume than that observed with unstored or fresh egg white.

Removal of glucose from whole egg melange by yeast fermentation before drying was also attempted by Kline and Sonoda (13). They observed that the fermentation proceeded in an essentially linear manner, after a slight initial lag phase. Although the yeast could ferment glucose at pH 7.4, the normal pH of whole egg melange, its fermentation rate increased significantly as the pH was lowered to 6.0. They investigated fermentation rates over the pH range of 5.4 to 7.4, and over a temperature range of 10 to 38 C, with optima being at pH 6.0 and 30 C. At 10 C approximately 4 to 5 times as much time was required to remove the glucose as at 30 C. Also, the time required to remove glucose varied with the yeast concentration but not in a linear fashion. Use of 0.07 to 0.15% (dry wt. of yeast per 100 ml egg melange) of yeast at 30 C was found to provide optimal conditions for rapid fermentation within 2 to 3 hr, with minimum residual yeastiness in the resulting lyophilized glucose-free powders. Six different yeast strains grown on identical media were compared as to fermentation rates and as to the residual foreign flavor imparted to the resulting glucose-free powders. *Torulopsis utilis*, although slightly superior as to the fermentative rate, imparted a distinct foreign flavor.

The two strains of *S. cerevisiae* and *Saccharomyces carlsbergensis* appeared to be equal choices, combining rapid fermentation rate with minimum foreign flavor. Commercially available *S. cerevisiae* (baker's yeast) was found to be nearly as satisfactory as laboratory grown strains. Three pounds of fresh baker's yeast were used to ferment 1200 lb. of whole egg melange in about 3 h at 25 to 30 C. The fermented liquid was pasteurized at 61 C for 3 to 4 min and spray dried to 3.5% moisture. The flavor of the powder thus produced was as satisfactory as that of the control powder prepared from the same batch of egg without desugaring. They further reported that the laboratory process could be used successfully on a commercial scale, and when used on that scale, the process removed the yeast flavor and odor much more effectively than the laboratory process.

In a subsequent study, Kline et al. (14) reported that upon drying whole eggs a browning reaction occurred, involving glucose and cephalin. They observed a visible browning of the total lipid or phospholipid fraction with development of fluorescence, increased ultraviolet absorption, and loss in amino nitrogen content. The amount of browning in the phospholipid fraction was estimated by both visible inspection and spectrophotometric measurements of ether extracts at 400 to 500 nm. These changes in the normal glucose-containing powders were not significantly influenced by the presence or absence of oxygen during storage; nor were they induced by storing glucose-free powders in air. In fact, the browning observed in the phospholipid fraction from stored glucose-containing powders was found to be invariably greater when powders were stored in a nitrogen atmosphere. They indicated that the most rapid reaction contributing to deterioration of phospholipid during storage of egg powders was a lipid amine-aldehyde reaction, with glucose being the reactive aldehyde involved.

Further work of Kline et al. (15) revealed that flavor stability of whole egg powder stored at 37.5 C was primarily dependent on elimination of glucose-induced reactions. Non-glucose type deterioration, both oxidative and non-oxidative, was readily demonstrated by use of glucose-free powders. These reactions were neither as rapid nor as marked as those involving glucose. A combination of glucose removal, low moisture content, and inert-gas packing yielded a product which showed little significant flavor deterioration even after storage for 2 to 3 months at 37.5 C. Glucose removal from egg powders eliminated or greatly reduced physical and chemical changes that occurred during storage, which included: visible browning, pH decrease, increase in lipid fluorescence, increase in ultraviolet absorption by lipid

constituents, and decrease in protein solubility. Additionally, oxygen uptake by air-packed powders was reduced 2 to 3 fold by glucose removal. These changes were largely manifestations of the glucose-protein and glucose-cephalin interactions.

In addition to use of yeast for glucose fermentation, bacterial cultures, such as streptococci, appear to hold promise as a pure culture method for fermentation of glucose in egg white before drying (11). Kaplan et al. (11), using resting cell concentrates of *Streptococcus liquefaciens* NRRL 453, *Streptococcus lactis* NRRL L-21, and *S. lactis* ATCC 7963, observed that glucose in egg white was rapidly fermented without any proteolysis or production of any off-flavors and -odors. The temperature range of 26 to 37 C, over which the process could be carried out, was fairly wide, and desugared egg white could be produced with any desired pH value between 5.5 and 6.5. Since fermentation of glucose in egg white at its natural pH results in a powder with a pH of 6.5, the necessity, inconvenience, and cost of neutralizing acid egg white could be obviated if near-neutral egg white powders were produced. The dried albumen prepared from egg white fermented in this manner had the same pH after reconstitution as before drying.

Further, these workers (11) suggested that use of bacterial cell concentrates for fermentation of glucose eliminated contamination of egg white by broth constituents and did away with preparation of mother cultures with all the accompanying hazards of contamination. Bacterial cell concentrates could be held for relatively long periods without loss of viability and could be held in stock for future use. Also, they reported that use of resting cells resulted in extremely rapid fermentation (at 37 C the fermentation was completed in 3 h) and microorganisms responsible for "off" fermentations were suppressed.

Also, Ayres (1) compared methods of depleting glucose from egg albumen before drying, using *S. cerevisiae*, *S. lactis*, and *Aerobacter aerogenes*. There was little multiplication of cells of *S. cerevisiae* or *S. lactis* when these organisms were introduced into egg white. Unless large inocula were introduced or yeast extract was added to the cells before they were placed with egg white, there occurred a slight decrease in the microbial numbers during the first 24 h. On the other hand, cell numbers of *A. aerogenes* did increase in egg white, and by adding yeast extract their rate of growth was further accelerated. The fermentation by *S. cerevisiae*, *S. lactis*, and *A. aerogenes* could be successfully completed in 4.5 to 5.5 h by incorporation of 0.1% yeast extract. He noted that by use of yeast extract, it was possible to eliminate the need for unnecessarily large inocula. Also, if inoculum levels were wisely selected, only the number of cells neces-

sary to remove the glucose needed to be introduced. The minimum inoculum procedure eliminated much of the yeasty or bready aroma in *Saccharomyces* fermentation, the high acidity developed in *Streptococcus* fermentation, and growth of excessive numbers of cells and formation of large amounts of acetyl methyl carbinol in the *Aerobacter* fermentation.

Iyengar et al. (9) have developed a desugaring process of eggs, which could be applied successfully in the laboratory as well as the pilot plant. The process involves desugaring whole eggs with 0.5% yeast for 90 min at 36 C, liquid egg is then pasteurized at 61 C for 30 min, cooled to 8 C, and stabilized by the addition of 1N HCl, to pH 5.5 prior to drying. They (10) further studied the keeping quality of whole egg powder prepared by different methods. The samples used in the study were: (a) untreated (control), (b) only acid stabilized (not desugared), (c) yeast desugared, and (d) yeast desugared and acid stabilized. After packing under nitrogen, samples were stored for 1 year at 4.4, 21, or 37.7 C for assessment of chemical and organoleptic properties and at 8 to 10, 30, or 37 C for assessment of changes in bacterial load. Samples were tested at 3-month intervals for solubility in 10% KCl, glucose content, salt water soluble fluorescence, sponge cake volume, scrambled egg palatability, total bacterial count, and coliform count. Their results revealed that the acid stabilized powder could be stored up to 9 months at room temperature as compared to the yeast desugared powder which kept up to 1 year at 37 C without loss of any quality. The acid stabilized, yeast desugared powder retained its original properties for more than 1 year at 37 C, whereas the control or untreated sample had a shelf life of only 3 months.

Niewiarowicz et al. (17) desugared egg whites at 5 C with the aid of cell-free yeast extract mixed with 5% NaCl. Glucose removal could be accomplished in 4 to 5 h. The prepared dry egg white contained low levels of microbiological impurities and had good physico-chemical and organoleptic properties immediately after drying and even after 42 days storage at 37 C.

More recently, another commercial culture for desugaring of egg white has been produced by Micro-life Technics (Personal communication). Reportedly, the frozen concentrated culture consists of  $>10^{12}$  viable *Aerobacter* Group A cells/ml. They report the fermentation time to be 16 h at 32.2 C (90 F). Approximately 4 oz. of this culture concentrate can desugar 1000 lb. of egg white. The culture has been claimed to be essentially free from *Salmonella*, *Escherichia coli*, *Staphylococcus*, pathogenic streptococci, *Clostridium botulinum*, *Pseudomonas aeruginosa*, yeast, and molds.

## ENZYMIC METHODS FOR DESACCHARIFICATION

A considerable amount of work has been done to desugar eggs with glucose-oxidase-catalase systems. This process has been applied successfully to egg white, yolk, or whole egg. The use of this double enzyme system has been recommended for: (a) exhaustive removal of oxygen in the presence of an excess of glucose, and (b) exhaustive removal of glucose in the presence of an excess of molecular oxygen.

One of the first group of researchers to recommend use of glucose-oxidase in the processing of foods, with special emphasis on the desugaring of egg white, was Baldwin et al. (3). Their deoxygenase enzyme system was supplied by Vita-Zyme Laboratories and contained both glucose-oxidase and catalase. Thus, in the presence of molecular oxygen, it brought about oxidation of glucose to gluconic acid as shown in Fig. 1, and hydrogen peroxide thus produced during the reaction was depleted by the enzyme catalase. They observed that the enzyme-treated, pan-dried albumen rehydrated readily and was free of any objectionable odor or flavor. Also, Carlin and Ayres (5) corroborated the results of Baldwin et al. and further observed that angel cakes made with rehydrated enzyme-treated egg white compared favorably in volume, texture, and flavor with angel cakes made with frozen egg white.

Scott (21) has developed empirical relationships concerning the glucose level, reaction time, enzyme level, and hydrogen peroxide demand for conversion of glucose to gluconic acid, in whole eggs or its components by the glucose-oxidase-catalase system. For removing glucose from yolks, the yolks were warmed to 35 to 38 C and two "standard" pounds<sup>1</sup> of glucose oxidase were added per 1000 lb. of yolk. To accelerate the process, hydrogen peroxide was added at an overall level of 0.3%. This process reduced the glucose level of yolks from the initial 1% down to 0.1% (dry basis) in 3.5 h. Further increase in the enzyme level to 3 lb. per 1000 lb. reduced the process time to 2 h.

Whole eggs contain about 1.2% glucose on a dry basis with a pH of about 7.5. For desugaring of whole eggs, Scott (21) has recommended use of three "standard" pounds of the enzyme mixture for each 1000 lb. whole eggs. He further suggested the incorporation of about 10% sucrose in the liquid whole egg

<sup>1</sup>One "standard" pound of glucose-oxidase-catalase contained 75,000 "Savette" units of glucose oxidase. A Savette unit of glucose oxidase is defined as that quantity of enzyme that causes an oxygen uptake of 10 mm<sup>3</sup> per minute at pH 5.9 and 30 C in the presence of an excess of catalase. The substrate being 3.3% glucose monohydrate in a dibasic sodium phosphate-dehydroacetic acid buffer (21).

before drying. The sucrose being non-reducing did not exert any deleterious effect on the resultant egg solids.

Paul et al. (18) compared the storage stability of egg yolk solids, which had been enzyme-treated to remove glucose, with that of the untreated solids. Samples of each lot were stored at 95 F for 7, 14, 25, 40, and 90 days, and at 65 F for 3, 6, 9, and 12 months. Additional control samples were stored under refrigeration. Doughnuts were selected as the product for testing the quality of yolk solids. Performance was evaluated on the basis of fat content, specific volume, and eating quality of doughnuts. The enzyme treatments materially improved the storage stability of the egg solids at 95 F. The untreated samples gave doughnuts of lower fat content and decreased palatability as the storage time of the egg yolk solids increased. Results obtained when storage was at 65 F showed that differences in functional properties of treated and untreated samples were much smaller than in those stored at 95 F.

Kiss (12) also investigated the acceptability of the glucose oxidase treatment for increasing the storage stability of pulverized eggs. Of all temperatures investigated, optimum reaction rates were observed at

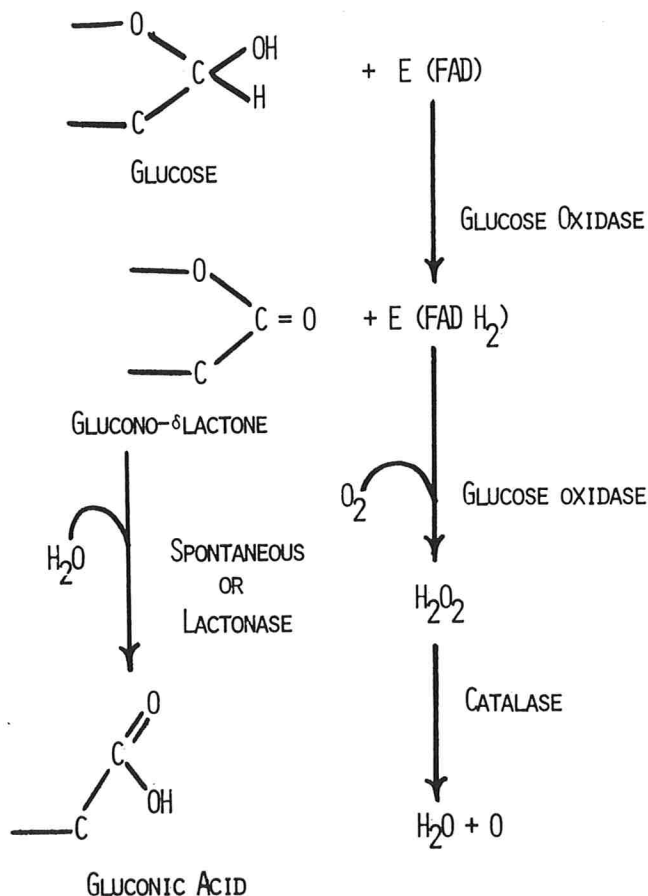


Figure 1. Mechanism of the glucose oxidase-catalase system. Source: Reed (19).



32 C, with enzyme level of 150 Savette units per 100 ml egg white. During the reaction period, it appeared practical to add 1.7 ml of a 1:3 mixture of 30% H<sub>2</sub>O<sub>2</sub>: H<sub>2</sub>O to 100 ml of egg. In the case of egg white, it was advisable to adjust the pH to 7.0 with HCl. No pH adjustments were necessary for yolks or whole eggs.

Sigmund (22) studied the effect of oxygen on enzymatic desaccharification of liquid whole eggs. He used hydrogen peroxide at the concentrations of 0.25, 0.5, and 1.0% by weight of egg as a source of oxygen, and glucose oxidase was used for enzymic desaccharification. The lowest level, 0.25% of added hydrogen peroxide, was found to be adequate for complete removal of sugars. He further observed that use of hydrogen peroxide in the process helped in maintaining the bacteriological quality of the product and exerted no undesirable effect upon its functional properties.

Ayres (1) compared the properties of egg albumen desugared by microbial fermentation and enzymic treatment. He observed that the microbiologically fermented egg white produced foams of lighter weight than did the enzyme treated albumen. However, in practical applications, the desugared albumen manufactured by both methods produced angel cakes of satisfactory volume.

In his article on the manufacture of egg products in the U.S.A., Forsythe (7) has discussed a cold desugaring process developed by Fermco Laboratories. He indicated the optimum temperature of the process to be 27 C, although it could be carried out at 7 to 10 C in which case the process took approximately 1.5 times as long. At the lower temperature the process took more time presumably because of the greater solubility of oxygen under those conditions. He further suggested that although the desugaring process at the lower temperature took longer, it permitted use of less enzyme.

#### CONCLUSIONS

Glucose, the major carbohydrate in eggs, affects the storage stability of egg powders in that it causes undesirable changes in physico-chemical as well as functional properties of the powder. Thus, desaccharification of liquid eggs, before drying, is an important step in the manufacture of egg powders. Essentially, methods used to remove glucose from egg melange, before drying, fall into two main categories: microbial and enzymic. In the microbial methods both bacteria and yeast have been investigated extensively for their ability to metabolize glucose. The microorganisms commonly used include *S. lactis*, *S. liquefaciens*, *A. aerogenes*, *T. utilis*, *S. cerevisiae*, and *S. carlsbergensis*.

In the enzymic methods glucose oxidase is employed to desugar eggs. Normally, catalase is used in conjunction with glucose oxidase to eliminate H<sub>2</sub>O<sub>2</sub> produced in the process.

In general, egg powders prepared after removal of glucose exhibit superior storage and functional properties than untreated samples, regardless of the technique used in desaccharification.

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## PROPOSED ADDITION TO THE THIRTEENTH EDITION OF STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS<sup>1</sup>

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### CHAPTER 3. SAMPLING

#### 3.1 Fluid Milk and Cream Samples

##### 3.11 Equipment for collecting samples

c - Sample containers: (4) Single-service vials for samples of *raw* milk and cream provided that: (a) maximum viable bacterial counts in rinse tests of containers do not exceed 1/ml of capacity; (b) containers made according to each different formulation are nontoxic and are not bacteriostatic or bac-

teriocidal; and (c) closure is designed so the container can be opened and closed easily without contamination of the lip of the vial or inner surface of the closure.

#### Discussion

This addition to SMEDP will permit use of non-sterile containers with very low numbers of viable bacteria as receptacles for samples of *raw* milk and cream. Members of the Intersociety Council believe as negligible the few viable organisms added to *raw* milk or cream from vials which conform with the standard outlined in the proposed addition to SMEDP.

The Council also recognizes that: (a) the plant(s) in which containers are manufactured and prepared for shipment will be inspected and certified by State Milk Sanitation Rating Authorities as being in compliance with requirements found in *Fabrication of Single-Service Containers and Closures for Milk and Milk Products*, PHS Publication No. 1465, and will be listed in the quarterly publication *Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers*; (b) laboratories and procedures will be approved by the Food and Drug Administration Laboratory Development Section or the State Laboratory Certifying Agency, where applicable; and (c) a sampling plan will include the testing of 60 randomly selected vials from each lot and the lot to be accepted only if all vials tested are in compliance with the standard of 1 viable organism or less/ml of capacity; each lot will be tested and certified; results of tests will be included with the lot of containers when it is purchased.

<sup>1</sup>Approved on April 13, 1973 by the Intersociety Council on Standard Methods for the Examination of Dairy Products.

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The Intersociety Council approved for publication the following proposed addition to the 13th edition of *Standard Methods for the Examination of Dairy Products* (SMEDP).

(Continued on Page 525)

## CHANGES IN RESIDUAL NITRITE IN SAUSAGE AND LUNCHEON MEAT PRODUCTS DURING STORAGE<sup>1</sup>

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### ABSTRACT

Changes in residual nitrite content of 18 sausage products during storage for various lengths of time are reported. Most pre-packaged processed meats (storage temperature  $5 \pm 2$  C) showed decreasing residual nitrite levels during storage. All dried meat products (storage temperature  $24 \pm 1$  C) showed increasing residual nitrite levels during storage. Residual nitrite decreased with cooking for the three products evaluated.

Retention of cured meat color is presently dependent on use of nitrite in the curing process. It has been reported that approximately 20 ppm residual nitrite are sufficient to produce acceptable color and flavor in pork but sausage products were not included in this study (8). The residual nitrite level in cured meat products has been the basis for determining the desirable level of nitrite added for curing meat products. However, the desirable level of residual nitrite during refrigerated storage of cured sausage products has not been satisfactorily resolved. In addition, factors which influence the degree of retention of residual nitrite in cured sausage products have not been fully explained. Among factors which have been identified as influencing the level of residual nitrite in cured meat products are time and temperature of processing, pH, reducing activity of the system, storage time and temperature, and meat to water ratio (9, 11).

Kolari and Auman (9) reported that 20-25% of the nitrite has been estimated to disappear during mixing of raw meat mixtures. Nordin (10) noted that depletion of nitrite during storage of commercially canned hams has been observed in the industry but has not been reported. In a study of fresh ground ham, canned by a commercial technique, Nordin (10) found that nitrite was depleted at a rate independent

of the initial nitrite concentration. Unsterilized samples, held at room temperature showed a rate of depletion of nitrite that increased rapidly with time. For samples held at other temperatures (28 - 225 F) and for sterilized samples held at room temperature, the higher temperatures and lower pH resulted in a greater rate of depletion. Nordin (10) concluded that bacterial utilization of nitrite accounted for the more rapid depletion of nitrite in unsterilized samples held at room temperature. Kolari and Auman (9) reported that franks and bologna decreased in residual nitrite levels after 3 and 8 weeks storage, respectively, whereas meat loaves increased in residual nitrite after 5 weeks storage, then decreased. Liver sausage showed no appreciable change after 3 weeks storage. The presence or absence of ascorbate did not affect residual nitrite level during storage. Oldsman and Krol (11) concluded that the level of nitrite added, sodium chloride content, and vacuum chopping and canning had no effect on the rate of nitrite depletion.

Nitrate can be converted to nitrite by bacteria (4) and the nitrite is, in turn, converted to nitric oxide, which reacts with myoglobin and hemoglobin (2, 15). Nitrite was found to have significant bactericidal properties if the pH was in the range of 4.5 to 5.5, provided the bacterial population was not abnormally high (5, 12). Shank et al. (12) proposed that oxidation-reduction reactions of nitrite resulted in production of nitrite, nitric oxide, and nitrogen dioxide. Nitrite can be in equilibrium with nitrous acid in the presence of water. The equilibrium condition favors nitrous acid at pH 4.5 - 5.5, with nitrous acid having bactericidal properties. Nitrite has been shown to prevent growth of germinated spores of *Clostridium botulinum*. (5, 7). Castellani and Niven (3) and Tarr (13) showed that sodium nitrite had a bactericidal effect on selected bacteria. Thus, the level of residual nitrite in processed meats has importance in maintaining the shelf-life of processed meats.

This study was made to determine the changes in residual nitrite during the shelf-life storage and the effect of cooking on selected processed sausage products.

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<sup>2</sup>Present address: GoodMark, Inc., Raleigh, North Carolina.

TABLE 1. TYPE OF PACKAGING, INITIAL NITRITE AND NITRATE ADDED, FINISHED PRODUCT pH LEVELS, AND RESIDUAL NITRITE OF FRANKFURTERS STORED FOR VARIOUS TIMES AT 5 C

Product	Type packaging <sup>1</sup>	NO <sub>2</sub> <sup>2</sup> added (ppm)	NO <sub>3</sub> <sup>2</sup> added (ppm)	Storage time (days)	Initial pH	Residual <sup>3</sup> NO <sub>2</sub> (ppm)
Frankfurters-10/lb all beef	Vacuum film	110	119	0	6.24 ± .14	27 ± 4a
				10		16 ± 2b
				20		13 ± 1bc
				31		11 ± 1c
Frankfurters-8/lb all meat	Vacuum film	116	124	0	6.07 ± .05	33 ± 1a
				10		12 ± 1b
				20		10 ± 0c
				31		9 ± 2c
Frankfurters-10/lb all meat	Vacuum film	116	124	0	5.91 ± .01	39 ± 2a
				9		20 ± 5b
				20		15 ± 3bc
				31		11 ± 1c
Frankfurters-12/lb all meat	Vacuum film	116	124	0	5.92 ± .03	42 ± 2a
				9		37 ± 2b
				20		31 ± 2c
				31		15 ± 2d
Frankfurters-14/lb all meat	Vacuum film	116	124	0	6.03 ± .03	25 ± 1a
				10		21 ± 1b
				20		17 ± 2c
				31		9 ± 1d

<sup>1</sup>Vacuum film (21 in. Hg) consisted of a non-forming web (mylar, laminated to polyethylene) and a forming web (Saran coated mylar).

<sup>2</sup>Initial NO<sub>2</sub> and NO<sub>3</sub> values were calculated from the weighed amounts of NaNO<sub>2</sub> and NaNO<sub>3</sub> added to the emulsions during processing.

<sup>3</sup>Mean and standard deviation of 4 determinations. Any two means for a specific product were significantly different at the 0.05 level, when not having the same letter suffix.

#### MATERIALS AND METHODS

In the shelf-life storage experiments, various commercially processed meat products were selected from specific production lots and placed in a controlled temperature unit 48 h after processing. The beginning of the storage period was designated as day 0. All products were processed by standard commercial methods of manufacturing. The quantities of sodium nitrite and sodium nitrate initially added to each product were below the maximum allowed by federal regulations (14). The amounts of nitrate and nitrite added at the time of processing are shown in Tables 1, 2 and 3. These were determined by calculation of the quantities added during formulation. Sodium erythorbate was added to all products, except souse loaf, at a level of 0.05% based upon initial meat weight.

The products used in the shelf-life storage study, with days on which nitrite analysis were done in parenthesis, were: frankfurters (0, 9, 10, 20, 31); Polish and smoked sausage (0, 5, 8, 15); salami, spiced luncheon meat, and souse loaf (0, 10, 21, 30); pickle and pimento loaf (0, 7, 14, 20); bologna (0, 6, 12, 17); pickled hot sausage (0, 30, 64); pizza beef stick, chili beef stick, salami beef stick, spice beef jerky (0, 30, 60, 88). The prepackaged processed meats which are normally refrigerated were stored 5 ± 2 C and the dried and pickled sausage products were held at 24 ± 1 C. The storage times were selected to coincide with the estimated shelf-life of each product. Storage times and temperatures are listed for each product in Tables 1, 2, and 3. The pH measurements were made on samples from packages of products with similar treatments to those used for nitrite analysis.

The effect of cooking was determined on day 0 for smoked sausage, Polish sausage, and five frankfurter samples. Cooking refers to heating for serving and was accomplished 48 h

after processing. Frankfurters were cooked by heating tap water (1 pt.) to boiling (100 C), introducing 2 frankfurters, bringing the water to a second boil, turning off the heat source, covering and holding for 10 min. The frankfurters were removed, blotted of excess water, and prepared for analysis. Polish sausage was cooked by placing 5 sausages in cold tap water (1 pt.), heating to the boiling point (100 C), and maintaining the temperature for 10 min. The sausages were removed, blotted free of excess water, and prepared for analysis. Smoked sausages were split lengthwise, placed in a preheated (149 C) fry pan, cooked 2 min on each side, removed, and prepared for analysis.

Two packages of each product were sampled in duplicate for each nitrite determination. All shelf-life products were taken directly from the package and prepared for analysis. Each sample was homogenized for 1 min in a pint jar using a Sunbeam blender. The residual nitrite content of the samples was determined according to the A.O.A.C. method (1) which is not influenced by the presence of ascorbate.

#### RESULTS AND DISCUSSION

The range of residual nitrite at 0 days storage for frankfurters was 25-42 ppm (Table 1). This variation did not appear to be related to type or size of frankfurter. The residual nitrite content decreased significantly and steadily during storage but varied among types of frankfurters. In all instances there was a significant decrease in residual nitrite by storage day 9 or 10. The rate of decrease in nitrite was slightly different for each type of frankfurter but after 31 days storage the final range in residual ni-

trite levels (9-15 ppm) was relatively close.

Data in Table 2 indicate that Polish and smoked sausages, salami, and spiced luncheon meat showed similar initial residual nitrite levels to that of the frankfurters. However, there was a significant decrease by storage day 8 or 10 for these products. Also, the rates of decrease during storage for Polish and smoked sausages were similar to those for frankfurters.

Salami and spiced luncheon meat had rapid rates of decrease in residual nitrite, especially during the first 10 days of storage, but these products had relatively low levels of residual nitrite after 30 days storage. During the first 12 days of storage, the residual nitrite decrease for bologna was not significant but by storage day 17 the value showed a significant increase above the levels previously observed. The low variation (standard deviations) within samples suggested that the observed increase in residual nitrite in bologna was actual but there is no evident explanation for the difference in the rate of change in bologna as compared to salami and spiced luncheon meat.

No significant change was found in residual nitrite content of pickle and pimento loaf during the storage period (20 days) but the initial level was only 9 ppm. The reason(s) for the differences in rate of change for residual nitrite among those four luncheon loaves is not evident on the basis of data obtained in this study. Souse loaf increased from no detectable residual nitrite initially to 13 ppm after 10 days storage but the nitrite content was highly variable during the 30-day storage period. The only significant increase was between the initial and 10th day of storage. The reason for this result is not evident since a level of 83 ppm was added at the time of curing. It was postulated that all of the available nitrite was depleted during curing and processing with a subsequent conversion of the sodium nitrate to nitrite during storage. It can be noted that pickled hot sausage showed an increase in residual nitrite from 4 to 10 ppm during the 64 days of storage (Table 3). These three products (pickle and pimento loaf, souse loaf, and pickled hot sausage) were relatively low in initial nitrite levels and the high variability in all products indicates that the trends in residual

TABLE 2. INITIAL NITRITE AND NITRATE, RESIDUAL NITRITE, AND pH LEVELS OF SELECTED SAUSAGE PRODUCTS STORED FOR VARIOUS TIMES AT 5 C

Product	Type packaging <sup>1</sup>	NO <sub>2</sub> <sup>2</sup> added (ppm)	NO <sub>3</sub> <sup>2</sup> added (ppm)	Storage time (days)	Initial pH	Residual <sup>3</sup> NO <sub>2</sub> (ppm)
Polish sausage	Overwrap film	128	139	0	5.90 ± .05	24 ± 2a
				5		22 ± 1a
				8		18 ± 0b
				15		16 ± 1c
Smoked sausage	Overwrap film	123	132	0	6.23 ± .02	30 ± 2a
				5		30 ± 2a
				8		26 ± 1b
				15		19 ± 2c
Salami, sliced	Vacuum film	126	136	0	6.81 ± .02	42 ± 10a
				10		12 ± 1b
				21		6 ± 1c
				30		6 ± 1c
Pickle and pimento loaf, sliced	Vacuum film	81	87	0	5.99 ± .01	9 ± 2a
				7		7 ± 1a
				14		8 ± 1a
				20		8 ± 1a
Bologna, sliced	Vacuum film	126	135	0	6.50 ± .02	20 ± 2a
				6		18 ± 1a
				12		17 ± 0a
				17		25 ± 3b
Spiced luncheon meat, sliced	Vacuum film	140	150	0	6.44 ± .18	39 ± 3a
				10		12 ± 1b
				21		11 ± 1b
				30		10 ± 2b
Souse loaf, unsliced	Overwrap film	83	89	0	4.24 ± .02	0
				10		13 ± 8b
				21		4 ± 2b
				30		7 ± 1b

<sup>1</sup>Vacuum film (21 in. Hg) consisted of a non-forming web (saran coated mylar laminated to polyethylene and a forming web (saran coated polyvinyl chloride laminated to polyethylene). Overwrap film consisted of polyethylene.

<sup>2</sup>Initial NO<sub>2</sub> and NO<sub>3</sub> values were calculated from the weighed amounts of NaNO<sub>2</sub> and NaNO<sub>3</sub> added to the emulsions during processing.

<sup>3</sup>Mean and standard deviation of 4 determinations. Any two means for a specific product were significantly different at the 0.05 level, when not having the same letter suffix.

TABLE 3. INITIAL NITRITE AND NITRATE, RESIDUAL NITRITE, AND pH LEVELS OF BEEF STICK SAUSAGE STORED FOR VARIOUS TIMES AT 24 C

Product	Type packaging <sup>1</sup>	NO <sub>2</sub> <sup>2</sup> added (ppm)	NO <sub>3</sub> <sup>2</sup> added (ppm)	Storage time (days)	Initial pH	Residual <sup>3</sup> NO <sub>2</sub> (ppm)
Pizza flavored beef stick (1 cm. dia.)	Non-vacuum film	125	135	0	5.00 ± .01	2 ± 1a
				30		10 ± 2b
				60		8 ± 0b
				88		15 ± 3c
Spice flavored beef stick (1 cm. dia.)	Non-vacuum film	101	109	0	4.54 ± .07	3 ± 1a
				30		5 ± 2ab
				60		7 ± 1b
				88		12 ± 1c
Chili flavored beef stick (1 cm. dia.)	Non-vacuum film	126	135	0	4.66 ± .11	3 ± 0a
				30		9 ± 2b
				60		10 ± 1b
				88		18 ± 1c
Salami flavored beef stick (1 cm. dia.)	Non-vacuum film	101	109	0	4.86 ± .01	4 ± 1a
				30		8 ± 1b
				60		10 ± 1b
				88		17 ± 3c
Beef jerky (1 cm. dia.)	Non-vacuum film	—	—	0	5.59 ± .01	4 ± 0a
				30		4 ± 1a
				60		11 ± 1b
				88		14 ± 1c
Pickled hot sausage	Bottled, glass jar	123	132	0	4.06 ± .04	5 ± 0a
				30		5 ± 1a
				64		10 ± 1b

<sup>1</sup>Non-vacuum film was saran coated cellophane.

<sup>2</sup>Initial NO<sub>2</sub> and NO<sub>3</sub> values were calculated from the weighed amount of NaNO<sub>2</sub> and NaNO<sub>3</sub> added to the emulsions during processing.

<sup>3</sup>Mean and standard deviation of 4 determinations. Any two means for a specific product were significantly different at the 0.05 level, when not having the same letter suffix.

TABLE 4. EFFECT OF COOKING ON THE RESIDUAL NITRITE LEVEL OF SMOKED SAUSAGE, POLISH SAUSAGE, AND FRANKFURTERS AT 0 DAYS STORAGE (48 H AFTER PROCESSING)

Product	Before cooking, residual nitrite, (ppm) <sup>1</sup>	After cooking, residual nitrite, (ppm) <sup>1</sup>	Residual nitrite loss (%)
Smoked sausage	29 ± 2a	28 ± 2a	3.4
Polish sausage	24 ± 2a	16 ± 1b	25.0
Frankfurters			
All beef, 10/lb. (13.2 x 2.17 cm.)	27 ± 4a	22 ± 3a	18.5
All meat, 8/lb. (13.2 x 1.76 cm.)	33 ± 1a	27 ± 1b	15.0
All meat, 10/lb. (13.2 x 2.17 cm.)	39 ± 2a	23 ± 3b	41.0
All meat, 12/lb. (13.2 x 1.92 cm.)	42 ± 2a	30 ± 2b	28.6
All meat, 14/lb. (13.2 x 1.77 cm.)	25 ± 1a	19 ± 1b	24.0

<sup>1</sup>Mean and standard deviation of 4 determinations. Any two means for a specific product were significantly different at the 0.05 level, when not having the same letter suffixes.

nitrite changes during storage may be of little consequence at low residual nitrite levels.

Without exception, all varieties of dried sausage products increased in residual nitrite content during the 88-day storage period (Table 3). The initial values for dried sausage and jerky were extremely low (2-4 ppm) and reached a final residual nitrite content of 12-18 ppm. Although the moisture level was not measured during storage, the moisture-proof film was sealed and it was not possible that these relatively dry (22% moisture) products could have lost sufficient moisture to cause the rate of increase observed. Olsman et al (11) reported that the rate constant for nitrite depletion was directly propor-

tional to the meat:water ratio based on studies of model systems; the greater the water content, the faster the depletion. It is possible that the meat:water ratio was the basis for an increase in residual nitrite in the dried products. However, further research is needed to establish the cause of the increase.

The highest residual nitrite value observed for any pre-packaged processed meat product was 42 ppm. All products (except bologna on storage day 17), that showed significant decreases in residual nitrite, had initial levels of 20 ppm or higher and were of relatively high pH (>5.9). With the exception of bologna and beef jerky samples, all samples which showed an increase in residual nitrite during storage

had initial nitrite levels of  $< 4$  ppm and were relatively low in pH ( $< 5.0$ ). The beef jerky had a pH of 5.59 but an initial residual nitrite level of 4 ppm. Even with these exceptions, the results suggest that low initial levels of nitrite and/or relatively low pH values and/or moisture levels are associated with an increase in residual nitrite during storage. Also, those products which increased in residual nitrite level during storage apparently developed conditions which favored conversion of nitrates to nitrites. The observed decreases in residual nitrite suggest that oxidation-reduction reactions occurred to convert the nitrite to nitrous oxide. The mechanism for these changes was not investigated in this study, and this indicates the need for further research on this problem.

Residual nitrite levels obtained by chemical analysis of the cooked frankfurters are in Table 4. The decrease in nitrite caused by cooking ranged from 3.4 to 41.0% and did not appear to be related to a common parameter such as emulsion type, product size, or quantity of nitrite added initially or present at the onset of the storage study.

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## INJURY AND REPAIR OF SEVERAL SALMONELLA SEROTYPES AFTER FREEZING AND THAWING IN MILK SOLIDS<sup>1</sup>

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### ABSTRACT

Freezing and thawing of *Salmonella anatum* cells induced increased sensitivity to selective agents in the plating medium. Selective plating media containing the bile salt sodium desoxycholate were most inhibitory. Injury was demonstrated in 11 *Salmonella* serotypes. The percent injury ranged from 20% (*Salmonella heidelberg*) to about 60% (*Salmonella pullorum*, *Salmonella anatum*, *Salmonella infantis*, and *Salmonella cubana*). The percent death ranged from 10% (*Salmonella meleagridis* and *Salmonella infantis*) to 27% (*Salmonella pullorum* and *Salmonella oranienburg*). The data indicated that 2,4-dinitrophenol prevented repair of injury in all serotypes that exhibited at least 25% injury. This suggested the importance of energy synthesis in the repair process.

Several sublethal environmental stresses have been reported to produce bacterial injury. Since the early work of Straka and Stokes (21), most investigators have found injured cells to be capable of forming colonies on a nutritionally complete medium but not on a minimal medium. Metabolic injury was reported in bacteria subjected to freezing (3, 10, 21) and freeze drying (19, 20). Recently, several reports have indicated that injured cells were sensitive to selective agents in the plating medium that were normally not inhibitory. This selective agent injury was demonstrated in bacteria that were irradiated (2, 8, 22), heated (11, 12), freeze dried (14, 15), frozen (9, 13, 16), and exposed to sanitizers (9, 17, 18).

This study was conducted to determine if the effects of stress resulting in damage of cells would be observed after freezing and thawing of several *Salmonella* serotypes in milk.

### MATERIALS AND METHODS

The *Salmonella* serotypes used were isolated in our laboratory from naturally contaminated nonfat dry milk. The test organisms were propagated and maintained in reconstituted nonfat dry milk (10% solids-not-fat) as described previously (14). A 1-ml portion of this culture was added to 100 ml of sterile reconstituted nonfat dry milk (NDM) with 10% solids-not-fat (SNF) and incubated at 35 C for 16 to 20 h. This was then diluted by transferring 1-ml culture to 100 ml milk. A 10-ml portion of this diluted culture was added to a sterile screw-cap tube (150 × 25 mm). It was then frozen by static placement in a dry ice-acetone bath for 10 min. The contents were thawed in a water bath at 4 C for about 45

min and tested. Treatment with antimicrobial materials was described previously (13).

The following plating media were used: bismuth sulfite agar (Difco), desoxycholate agar (Difco), *Salmonella*-*Shigella* agar (Difco), brilliant green agar (Difco), eosin methylene blue agar (Difco), xylose lysine agar base (Difco) supplemented with 0.5% peptone (XLP), and XLP supplemented with 0.2% sodium desoxycholate (XLDP). These media were prepared according to directions supplied by Difco.

The following formulas were used to calculate the percentage of injury and death: percent injury =  $1 - (\text{counts on XLDP} / \text{counts on XLP}) \times 100$ ; percent death =  $1 - (\text{counts on XLP of treated sample} / \text{counts on XLP of untreated control}) \times 100$ .

### RESULTS AND DISCUSSION

Previous reports have indicated that freezing and thawing *S. anatum* cells in water (13) or milk (Janssen and Busta, submitted for publication) resulted in injury as manifested by an inability to form colonies on a selective medium containing sodium desoxycholate. This present study evaluated (a) the recovery of *S. anatum* cells, frozen and thawed in milk, on several plating media selective for gram-negative bacteria, and (b) the effects of freezing and thawing on the injury and death of different *Salmonella* serotypes.

Data on the recovery of frozen and unfrozen *S. anatum* cells on several plating media selective for gram-negative bacteria are presented in Fig. 1. Unfrozen *S. anatum* cells were recovered equally well on the selective media tested, except for *Salmonella*-*Shigella* agar (SS) which inhibited about 50% of the untreated population. However, after freezing and thawing, reductions in the number of colony forming units were apparent on xylose-lysine-desoxycholate-peptone agar (XLDP), desoxycholate agar (DA), and SS agar. The greatest number of injured cells was demonstrated with the plating media containing sodium desoxycholate (XLDP and DA media). Several reports have indicated that the presence of bile salts, especially sodium desoxycholate, in the plating media was inhibitory to gram-negative bacteria injured by heating (9), freezing (9, 13, 16), freeze drying (14, 15), and exposure to sanitizing compounds (17, 18). Hill (5) found that bile salts dissolve part of the cell wall material from disrupted *E. coli* cells. Also, Woldringh (23) showed that ionic detergents such as sodium dodecyl sulfate, sodium lauryl sarcosinate,

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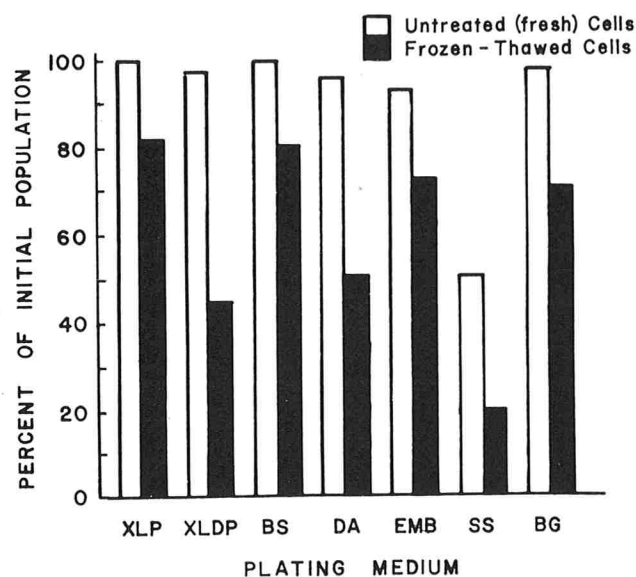


Figure 1. Effect of several plating media on the recovery of frozen and unfrozen *Salmonella anatum* NF3 cells in nonfat milk solids (10% solids-not-fat). The plating media used were: xylose-lysine-peptone agar, XLP; XLP with 0.2% sodium desoxycholate added, XLDP; bismuth sulfate agar, BS; desoxycholate agar, DA; eosin methylene blue agar, EMB; Salmonella-Shigella agar, SS; and brilliant green agar, BG.

and sodium desoxycholate dissolve the plasma membrane of *E. coli* resulting in changes of the organization of nucleoplasm and cytoplasm. Ray et al. (15) reported that freeze-dried *S. anatum* cells failed to repair when desoxycholate was added to the recovery medium. They suggested that the bile salt might interfere with the rehydration process. Ray and Speck (16) indicated that workers using minimal agar for measurement of damage generally obtained a far lower percentage of injury than those using selective plating media containing desoxycholate. They suggested that the former method measured only metabolic injury, whereas the latter method measured injury in a structure which normally protected cells from the bactericidal effect of surface active agents.

Freezing injury in salmonellae was not limited to *S. anatum* and was demonstrated for 11 different serotypes (Table 1). The amount of injury ranged from 20% (*S. heidelberg*) to about 60% (*S. pullorum*, *S. anatum*, *S. infantis*, and *S. cubana*). All of the serotypes exhibited an ability to repair the damage within 2 h of incubation at 25 C in the presence of 10% milk solids. The amount of death ranged from less than 10% (*S. infantis* and *S. meleagridis*) to 27% (*S. pullorum* and *S. oranienburg*) after freezing and thawing in milk.

Data on the effect of 2,4-dinitrophenol (DNP) on repair of several serotypes after freezing and thawing in milk are presented in Table 2. DNP is an uncoupler of oxidative phosphorylation that apparently

hydrolyzes an intermediate necessary for formation of ATP (1). DNP prevented the repair of injury in all serotypes that exhibited at least 25% injury. This was indicated by the high percentages of injured cells after 2 h incubation at 25 C. Energy synthesis thus appeared to be necessary for the repair of injured cells. The repair of injury induced by freeze drying, sublethal heating, aerosolization, and ethylenediaminetetraacetic acid (EDTA) also involved energy metabolism (4, 6, 7, 11, 12, 19). These data indicate that the injury and repair of salmonellae frozen and thawed in milk are not limited to one or two laboratory strains and must be considered in food or analytical systems.

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TABLE 1. EFFECT OF FREEZING AND THAWING OF SEVERAL *Salmonella* SEROTYPES IN RECONSTITUTED NONFAT DRY MILK (10% SOLIDS-NOT-FAT)

Serotype	Percent injury			Percent death
	0 h	1 h	2 h*	
<i>S. anatum</i>	58	15	10	16
<i>S. pullorum</i>	61	10	10	27
<i>S. cubana</i>	62	25	12	15
<i>S. infantis</i>	55	16	10	10
<i>S. derby</i>	34	13	10	24
<i>S. senftenberg</i>	44	17	12	20
<i>S. meleagridis</i>	35	15	11	10
<i>S. oranienburg</i>	32	10	10	27
<i>S. typhimurium</i>	26	10	10	17
<i>S. enteritidis</i>	27	10	10	17
<i>S. heidelberg</i>	20	10	10	18

\*Time thawed cells were held in milk (10% solids-not-fat) at 25 C.

TABLE 2. EFFECT OF 2,4-DINITROPHENOL (100  $\mu$ G/ML) ON THE REPAIR OF INJURY IN SEVERAL *Salmonella* SEROTYPES AFTER FREEZING AND THAWING IN RECONSTITUTED NONFAT DRY MILK (10% SOLIDS-NOT-FAT)

Serotype	Percent injury		Percent injury with 2,4-dinitrophenol	
	0 h	2 h*	0 h	2 h*
<i>S. anatum</i>	56	10	56	37
<i>S. enteritidis</i>	25	10	25	10
<i>S. meleagridis</i>	35	12	35	21
<i>S. typhimurium</i>	26	10	25	27
<i>S. heidelberg</i>	20	10	20	10
<i>S. senftenberg</i>	44	12	44	27
<i>S. infantis</i>	55	10	55	50
<i>S. oranienburg</i>	32	10	32	41
<i>S. cubana</i>	67	14	67	43
<i>S. derby</i>	43	10	43	23

\*Time thawed cells were held in diluent (0.1% solids-not-fat milk) at 25 C. Diluent consisted of water with or without antimicrobial and residual milk in 1:100 dilution.

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## NATIONAL CONFERENCE ON SOLID WASTE

The U. S. Environmental Protection Agency, Office of Solid Waste Management Programs, and the National Solid Wastes Management Association are cosponsoring a national conference on major solid waste management issues and policy alternatives on November 15 and 16, 1973, at the O'Hare Inn, O'Hare Airport, Chicago, Illinois. The program for this intensive two-day workshop will span the most current legislative and technological activities affecting this field in the areas of resource recovery, processing technology, and sanitary landfill. The format for the conference is intended to provide an opportunity for government and industry officials to discuss issues and alternatives in an informal atmosphere with several hundred leading Federal, State and local government officials and industry representatives.

The OSWMP has arranged for representatives of State solid waste agencies, EPA regional offices, and members of the OSWMP headquarters staff to participate in this meeting. We expect several hundred public officials and industry representatives to at-

tend the conference and we believe it is an excellent opportunity for public officials to obtain, first hand, the latest information on key Federal, State, and local programs as well as industry activities in these areas of the field. Some of the major topics that will be included in this conference are the following:

- Legislative Background and Planning for Connecticut's Resource Recovery Program
- New Orleans Waste Disposal/Resource Recovery Program
- Federal Solid Waste Legislative Priorities and Congressional Activities
- State of the Art—Resource Recovery Technology
- Atlanta Rail Haul—Regional Processing and Disposal Program
- St. Louis Waste Energy Recovery Project
- Sanitary Landfilling in Flood Plain Areas
- Landfill Equipment Selection and Application
- Role and Application of Shredding and Baling Technology

# A COMPARISON OF THE BABCOCK, MOJONNIER, AND MILKO-TESTER MARK III METHODS IN THE ANALYSIS OF MILKFAT IN CREAM<sup>1</sup>

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## ABSTRACT

The Babcock, Mojonnier, and Milko-Tester Mark III methods of milkfat analyses were compared on creams ranging in milkfat from 6.6 to 50.0%. Variances ( $s^2$ ) of the three methods on 243, 246, and 336 observations, respectively, were 0.062, 0.048, and 0.017. Variability tended to increase with an increase in milkfat content of the creams. This was true for all three methods. Regression formulae for estimating Babcock and Mojonnier values from Milko-Tester values were, respectively,  $\hat{y} = -0.94795 + 1.06699(X)$ , and  $\hat{y} = -0.60222 + 1.04850(X)$ . Correlation coefficients for the Milko-Tester Mark III with the Babcock and Mojonnier methods were 0.9987 and 0.9983, respectively. These relationships were noted with the Milko-Tester unit calibrated on whole milk, and indicate the feasibility of Milko-Tester use on cream samples whether calibrated at lower milkfat levels and calculated from regression formulae, or calibrated at the approximate range of milkfat test expected and read directly.

With high-volume continuous buttermaking operations demanding interplant shipments of tanker loads of cream, the need for improved methods and a better understanding of traditional methods of cream testing has become critical. The Babcock method has been used extensively and the Mojonnier procedure occasionally. Both methods are slow and tedious. Moreover, their within-test repeatability on high percentage milkfat products has not been well established. This latter condition could account in part for the difficulty often encountered between buyer and seller in reconciling milkfat tests on cream.

Beyond the obvious concern for accuracy and precision, there is also a great need for faster methods. The Milko-Tester Mark III offers an excellent prospect in this regard. However, the relationship between the Milko-Tester method and the Babcock and Mojonnier procedures has not been documented for cream analyses, and it was with this in mind that

the work reported herein was done.

Several investigators have compared the Babcock and ether extraction methods for milkfat in milk and cream (2, 4, 5). In general, higher results have been noted for the Babcock than for ether extraction methods, the disparity widening with increasing milkfat test of the product.

Hileman et al. (2) reported differences of 0.059 to 0.193% for milk samples ranging in milkfat levels from 3.00 to 5.00%, the Babcock results being higher in all instances. In creams of 20, 40, and 50% milkfat, this difference averaged 0.158, 0.527, and 0.717%, respectively.

O'Dell (5) also found the Babcock test to yield higher results than the Mojonnier, and stated that the difference was greater as the milkfat level went up. Similar findings have been reported by Randolph et al. (5). In the latter work, difference between the two tests was found to range from 0.27 to 0.65% on creams averaging, by the Babcock test, 26.0 through 45.93% milkfat.

## METHODS

in a laboratory model disc separator. Samples were immediately diluted with warm skimmilk to provide a range of milkfat levels. Samples thus obtained were split into three lots for Babcock, Mojonnier, and Milko-Tester analyses. They were held warm (90 F) until representative portions were taken for milkfat analyses. No more than 4 hr elapsed between sample preparation and completion of testing. Babcock and Mojonnier tests were done in triplicate, Milko-Tester analyses in quadruplicate.

Initially, to be certain that hydrolytic rancidity would not be a problem, the Acid Degree Value (ADV) method of Thomas et al. (6) was applied to a portion of the cream held for that length of time required to finish sampling. The ADV results, after several trials, indicated normal levels (0.60 to 0.70) and further testing was discontinued.

The procedure followed for the Babcock method was as outlined in the *Official Methods of Analysis of the Association of Official Agricultural Chemists (1)*, Method II, the only modification being the use of a Sartorius Balance, Type 1105, with a sensitivity of  $\pm 0.01g$ , rather than the cream scales indicated. The Mojonnier analyses were made accord-

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TABLE 1. AVERAGE MILKFAT PERCENTAGES<sup>1</sup> FOR THE BABCOCK MOJONNIER AND MILKO-TESTER METHODS OVER A RANGE OF MILKFAT LEVELS IN CREAM

Range of tests	Number of samples	Average Babcock test (%)	Average Mojonnier test (%)	Difference from Babcock	Average Milko-Tester test (%)	Difference from Babcock
25-35%	28	30.03	29.82	-0.21	29.10	-0.93
35.1-45%	17	39.52	39.11	-0.41	37.90	-1.62
45.1-50%	8	47.41	46.76	-0.65	45.14	-2.27

<sup>1</sup>Babcock and Mojonnier analyses were made in triplicate, Milko-Tester analyses in quadruplicate.

TABLE 2. AVERAGE VARIANCE<sup>1</sup> OF BABCOCK, MOJONNIER AND MILKO-TESTER METHODS OVER A RANGE OF MILKFAT LEVELS

Range of milkfat	Babcock		Mojonnier		Milko-Tester	
	No. of samples	Variance (s <sup>2</sup> )	No. of samples	Variance (s <sup>2</sup> )	No. of samples	Variance (s <sup>2</sup> )
5.0-10.0	11	0.023	11	0.0096	10	0.0054
10.1-15.0	4	0.030	4	0.0346	6	0.0092
15.1-20.0	7	0.023	8	0.0763	6	0.0072
20.1-25.0	8	0.044	8	0.0329	12	0.0193
25.1-30.0	16	0.045	15	0.0247	14	0.0201
30.1-35.0	12	0.118	13	0.0538	15	0.0223
35.1-40.0	8	0.069	8	0.0753	10	0.0265
40.1-45.0	8	0.071	8	0.0568	7	0.0207
45.1-50.0	8	0.125	10	0.0635	5	0.0107

<sup>1</sup>Variance is based upon triplicate Babcock and Mojonnier analyses, and quadruplicate Milko-Tester analyses. A variance (s<sup>2</sup>) value was calculated for each set of replicate analyses and these individual variances were averaged for the range of milkfat levels indicated.

ing to the procedure in *Laboratory Manual*, Milk Industry Foundation (3). Milko-Tester analyses were made on a Mark III Model (Foss Electric, Hilleroed, Denmark) operated under procedures recommended by the manufacturer.

Between replicate analyses on the Milko-Tester, cream samples in Erlenmeyer flasks were agitated by slowly rocking them back and forth about 5 times. It should be noted that the Milko-Tester had been previously calibrated and standardized on whole milk by the method prescribed in A.O.A.C., using the Babcock test as reference standard.

While the Mark III unit is automatic for sampling and testing milk, it is semiautomatic for cream analyses. A sample of mixed cream is drawn into the sampling tube (or pipette), the sample is diluted and discharged from the equipment. It is the diluted sample, then, that passes through the testing cycle of the device. Following initial uptake of cream, a drop remains hanging on the intake pipette. Since this drop of cream would alter the dilution rate, and might not be consistent in quantity from sample to sample, the procedure was standardized by allowing 5 to 10 sec for the drop to form, then wiping it off with a brown paper towel. This type toweling is not too absorbent and serves essentially to remove only the excess cream protruding below the pipette. For the replicate analyses, four separate samples were tested, all taken in this manner.

## RESULTS AND DISCUSSION

Table 1 shows the average milkfat percentage over a range of milkfat levels by the three methods, and the difference between the Babcock and the Mojonnier and Milko-Tester results. The Mojonnier analyses averaged slightly lower than the Babcock. This disparity widened with increasing milkfat level

as has been observed by other workers (2, 5). The Milko-Tester values at these milkfat levels averaged lower than both Babcock and Mojonnier values, the difference becoming progressively wider as milkfat levels went up. There appeared to be a good general relationship, however, and one that could provide close agreement by formulation of appropriate regression equations.

In Table 2 may be seen the variance of the three procedures over a wide range of milkfat levels. Although there were some inconsistencies, perhaps caused by the small number of samples, the variability in all three instances tended to increase as the milkfat level increased. The Mojonnier variability at any level of milkfat appeared to be only slightly better than the Babcock, while the Milko-Tester in general was the least variable—the most precise—of the three methods. On a total of 243, 246, and 336 observations respectively for the Babcock, Mojonnier, and Milko-Tester methods, the variance (s<sup>2</sup>) averaged 0.062, 0.048, and 0.017%. Correlation coefficients for the Milko-Tester versus Babcock and Mojonnier methods were 0.9987 and 0.9983, respectively.

Regression formulae for estimating Babcock and Mojonnier values from Milko-Tester values were derived from these data. Using the relationship  $\hat{y} = a + bX$ , the formulae were calculated to be:  $\hat{y} = -0.94795 + 1.06699(X)$ , and  $\hat{y} = 0.60222 +$

1.04850 (X) for the two methods, respectively.

Considering both precision and relative accuracy, it would appear that Milko-Tester milkfat analyses could be made on cream with the equipment calibrated and standardized on whole milk. Because the Milko-Tester procedure is fast, duplicate, triplicate, or even quadruplicate analyses could be made in less time than it takes to do either a Babcock or Mojonnier analysis, with the resultant improvement in accuracy and precision so necessary in today's high volume processing.

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#### PROPOSED ADDITION

(Continued from Page 514)

##### *Sampling and testing of vials*

One sampling plan which can be followed in testing vials is outlined below. Publication of this plan does not preclude use of other equally valid procedures.

*Objective.* This sampling plan will allow only one lot in 20 to pass inspection when 5% of the vials are defective (e.g. >1 organism/ml of capacity). No defective vials will be allowed ( $c = 0$ ) in the plan.

*Defective vial.* A residual bacterial count of 50/50-ml vial has been approved by the Intersociety Council. One laboratory method currently employed requires that 2 ml of rinse water be placed in the container as per *Standard Methods* procedure (16.223) and 1 ml plated. Plates are incubated at 32 C for 48 h and colonies are counted. A defective vial is one having a count of 26 or more colonies when tested as just described.

*Lot.* A lot of vials may be defined as one day's production or in another suitable manner. It is assumed that the lot is large (>5000 vials) for derivation of the sampling plan.

*Sampling plan.* The attribute sampling plan does

not depend on the specific definition of a defective vial. It will yield the same size of sample ( $n$ ) for a probability of 0.05 that a lot contains only 5% defective vials for any definition of a defective vial. Dodge and Romig (*Sampling Inspection Tables*, 2nd ed., John Wiley and Sons, New York, 1959) have given the statistical details for deriving sampling inspection plans. For the condition stated above and  $c = 0$ , the value of  $n$  will be 60.

*Application of sampling plan.* The sampling plan as described requires that 60 vials be randomly selected from a lot and tested by the recommended procedure. The lot will be accepted if no defective vials are observed and rejected otherwise. All lots must be examined.

##### *Comments requested*

Readers desiring to comment on the proposed addition to the 13th edition of SMEDP are requested to do so within 60 days after publication of this announcement. Comments should be sent to Dr. E. H. Marth, Chairman, Intersociety Council on Standard Methods for the Examination of Dairy Products, Department of Food Science, University of Wisconsin, Madison, Wis. 53706.

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(All affiliate secretaries are request-  
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 up-to-date)

**NOTICE  
 IAMFES AWARDS 1974**

Each year IAMFES recognizes outstanding contri-  
 butions and performance by it's members.

The success of this program is dependent not only  
 on those organizations who so generously support  
 the monetary aspects of these awards, but it is equal-  
 ly dependent on your individual help in furnishing  
 the Awards Committee with appropriate information  
 and names of potential award winners.

Will you please give serious thought to the follow-  
 ing Awards, which will be considered for presenta-  
 tion at our 1974 IAMFES Annual Meeting.

1. *The Sanitarian's Award* of \$1000 to a county or  
 municipal sanitarian, who, during the past seven  
 years has made outstanding contributions to the  
 health and welfare of his community.
2. *Educator-Industry Award* of \$1000 to a Uni-

versity or Industry employee who has made out-  
 standing contributions to food safety and sani-  
 tation.

3. *The Citation Award* to a member who has given  
 outstanding service to IAMFES in fulfilling it's  
 objectives.
4. *The Shogren Award* to the affiliate organization  
 that has the best statewide or regional program.
5. *Honorary Life Membership* to those members  
 who have given long and outstanding service to  
 IAMFES.

Please contact Orlowe M. Osten, Chairman of the  
 IAMFES Recognition and Awards Committee, Minne-  
 sota Department of Agriculture, 530 State Office  
 Building, St. Paul, Minnesota 55155 for details.

## ANNOUNCEMENT CONCERNING THE SANITARIAN AWARDS FOR 1974

Announcement is made that nominations will be accepted for the annual Sanitarians Award and the Educator-Industry Award until June 1, 1974, and the members of the International Association of Milk, Food and Environmental Sanitarians, Inc. are requested to give consideration to the nomination of individuals whose professional work in the field of milk, food, or environmental sanitation has been outstanding.

The Sanitarians Award consists of a Certificate of Citation and \$1,000 in cash, and is sponsored jointly by the Diversey Chemical Corporation, Klenszade Products, Inc., and Pennwalt Corporation. It is administered by the International Association of Milk, Food and Environmental Sanitarians, Inc., and is presented annually. The next presentation of the Sanitarians Award will be made at the 61st annual meeting of the Association which is to be held at St. Petersburg, Florida in August 1974.

The Educator-Industry Award consists of a Certificate of Citation and \$1,000 in cash and is sponsored by the National Milking Machine Council. It is administered by the International Association of Milk, Food and Environmental Sanitarians, Inc. and is presented annually. The next presentation will be made at the 61st annual meeting at St. Petersburg, Florida.

The Executive Board of the Association has established the following rules and procedures governing the Sanitarian Awards.

### *Eligibility:*

#### 1. *General Criteria*

To be eligible for nomination of these Sanitarian Awards offered annually by the International Association of Milk, Food and Environmental Sanitarians, candidates must:

- a. Have been a member of IAMFES in good standing for a period of five years prior to the date when the Award is to be presented;
- b. Be a living citizen of the United States or Canada who, at the time of nomination, is employed as a professional sanitarian in the field of milk, food, and/or environmental sanitation by a county, municipality, state or federal government provided that in the odd years beginning with 1969 the Sanitarians Award will be limited to state and federal employees and the even years to county and municipal employees.

The Educator-Industry Award is limited solely to members who are employed in educational or industrial field.

Members of the Executive Board, members of

the Committee on Recognition and Awards of the International Association of Milk, Food, and Environmental Sanitarians, shall not be eligible for the Award. Race, sex or age shall not enter into the selection of the Award recipient.

- c. Have made a meritorious contribution in the field of milk, food or environmental sanitation, to the public health and welfare of a county, counties, district, state or federal government with the United States or Canada.
- d. Have completed the achievements and contributions on which the nomination is based during the seven-year period immediately preceding January 1, of the year in which the Award is to be made.

#### 2. *Additional Criteria*

- a. Co-workers are eligible for nominations if both have contributed equally to the work on which the nomination is based and each independently meets the other qualifications for nomination.
- b. Where co-workers are selected to receive the Award, each shall receive a certificate and share equally in the cash accompanying the Award.
- c. No person who has received, or shared in receipt of the Award, shall be eligible for re-nomination for this Award.

#### *Nominations*

Nominations of candidates for the Sanitarian Awards may be submitted by the Affiliate Associations of the IAMFES, or by any member of the Association in good standing except members of the Executive Board, members of the Committee on Recognition and Awards, and employees of the sponsoring companies. Nominations from persons who are not members of the Association cannot be accepted. No member or Affiliate may nominate more than one candidate in any given year.

Each nomination must be accompanied by factual information concerning the candidate, a resume of his work and achievements, evidence supporting his achievements and if available, reprints of publications. A form for the submission of nominations may be obtained upon request from H. L. Thomasson, Executive Secretary, International Association of Milk, Food and Environmental Sanitarians, Inc., P. O. Box 437, Shelbyville, Indiana 46176.

#### *Submission of Nominations*

The deadline for submission of nominations is set annually, and all nominations and supporting evidence must be postmarked prior to midnight of that date. The deadline this year is June 1, 1974. Nom-



inations should be submitted to Orlowe M. Osten, Chairman, Committee on Recognition and Awards.

#### *Selection of the Recipient*

The Committee on Recognition and Awards of the International Association of Milk, Food and Environmental Sanitarians, Inc., has full responsibility for selecting from among the candidates nominated the recipient of the Sanitarian Awards. In judging the contributions of each candidate, the Committee will give special consideration to (a) originality of thought, mode of planning, and techniques employed, (b) the comprehensive nature of the candidate's achievements, and (c) their relative value as they affect the health and welfare of the area served by the candidate. The Committee will give consideration also to the efforts of the candidate to establish professional recognition in the area in which he serves, as well as to his research, administrative development, program operation and educational achievements. Additional information or verification of submitted information will be requested when considered necessary by the Committee. Testimonial letters in behalf of a candidate are not desired.

If after reviewing the nominations and supporting evidence, the Committee decides that the work and achievements of none of the candidates have been significantly outstanding, the Award shall not be made. In this connection, it is fundamental that if meritorious professional achievement cannot be discerned the Award shall be omitted for a year rather than to lower the standards for selections of a recipient.

Orlowe M. Osten, Chairman,  
Committee on Recognition and Awards,  
Minnesota Dept. of Agric.,  
530 State Office Bldg.,  
St. Paul, Minn. 5155

#### **FOOD SCIENTIST RETIRES**

Robert F. Holland, an authority on New York's dairy industry who headed the Department of Food Science at Cornell University for more than 17 years, has been named Professor Emeritus by the University Board of Trustees, effective October 1.

During Holland's tenure as department head the department's name was changed from the Department of Dairy Industry to the Department of Dairy and Food Sciences and finally, in 1966, to the Department of Food Science.

To reflect this change, the programs of teaching, research and extension were modified. The curriculum shifted from dairy only, to one that includes all foods. Also, the department's research base was broadened and extension activities became concerned

with environmental preservation.

At this time, Stocking Hall, the base of the Food Science Department, was almost completely refurbished and re-equipped.



Before joining the faculty of the N. Y. State College of Agriculture and Life Sciences in 1945, Holland served as director of chemical research for GLF, now known as Agway.

He has also had experience as a bacteriologist for Inlet Valley Farms, Inc., as a dairy sales engineer for Cherry-Burrell Corp., and he was a research associate at the N. Y. State Agricultural Experiment Station at Geneva.

At Cornell he has been in charge of the extension work in his field and has taught specialized courses on the dairy industry, dairy chemistry, and bacteriology. Holland's research has focused on the microorganisms of importance in milk, food products, and industrial fermentations.

A native of Holley, he received the B.S., M.S., and Ph.D. degrees from Cornell. In addition to his membership in Phi Kappa Phi, Sigma Xi, and Alpha Gamma Rho, Prof. Holland is affiliated with the American Dairy Science Association, the Society of American Bacteriologists, and the International Association of Milk, Food and Environmental Sanitarians.

#### **DR. J. C. FLAKE NAMED EXECUTIVE VICE PRESIDENT**

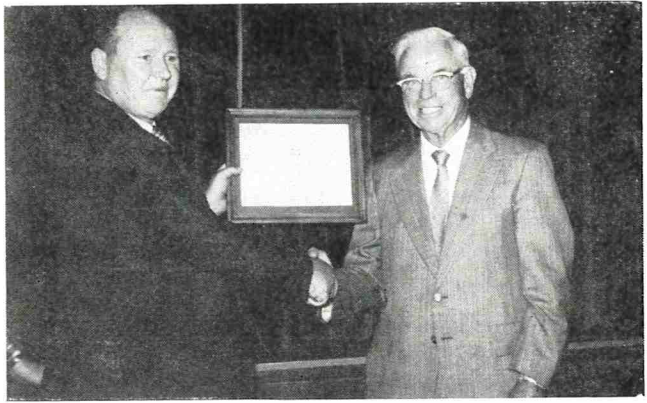
Dr. J. C. Flake has been named executive vice president of the Evaporated Milk Association and will assume his new duties October 1, the Association's president Ray Morris, president of the Grocery Products Division, Pet Incorporated, announced. In this post he succeeds Fred J. Greiner, who recently accepted the executive vice presidency of the Dairy and

Food Industries Supply Association, also based in Washington.

Dr. Flake joined the Sanitary Standards staff of the Evaporated Milk Association in 1940. During World War II, Dr. Flake served as a commissioned officer in the U. S. Public Health Service with responsibilities in milk sanitation. He returned to the Association in 1946 as assistant director of the Sanitary Standards program, becoming director in 1954, a position held to date.

Dr. Flake received his BS degree from the University of Tennessee, his MS from Purdue University and was awarded his PhD in dairy science by the University of Wisconsin.

Dr. Flake is the administrative secretary and treasurer of the National Mastitis Council, headquartered in the Association's offices. He currently chairs the Committee on Abnormal Milk Control of the National Conference on Interstate Milk Shipments. Dr. Flake is a fellow of the American Public Health Association, an associate member of the Association of Food and Drug Officials of the U. S., and an active member of the American Dairy Science Association, the International Association of Milk, Food and Environmental Sanitarians, and the Institute of Food Technologists.



Dr. Samuel T. Coulter (right) receiving an Honorary Life Membership from Dr. Walley Lawton at annual meeting of Minnesota Sanitarians Association, Sept. 13, 1973.



Russell Lerfald (right) recipient of the Association's Certificate of Achievement receiving award from Harold Johnson, Minn. Dept. Agric. at Minnesota Association of Sanitarians Annual Meeting Sept. 13, 1973.

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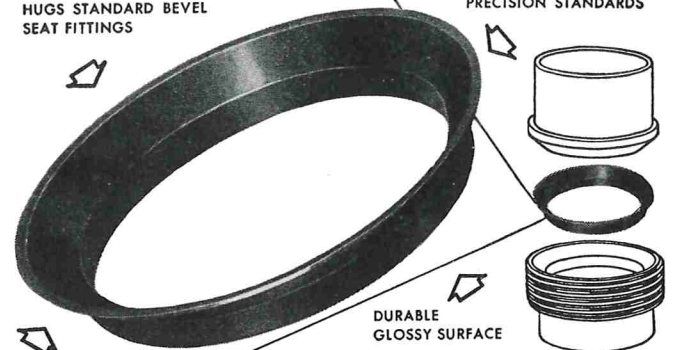


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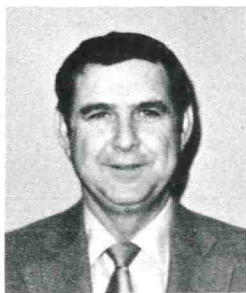
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## Dairy authorities speak out on better cow milking



Walter F. Wilson/President/  
International Association of Milk,  
Food and Environmental Sanitarians

### Welded lines: Great help in producing a quality product.

Reviewing changes in California's dairy milking practices over the past ten years brings two important changes to mind:

One—nearly 100 percent of all milking operations use a pipeline system.

Secondly—well over 50 percent of those pipeline milking systems use welded joints completely throughout except where joining to other equipment, such as receiver jars, pumps, tanks, filters, etc.

Neither of these changes happened overnight but once they were tried, their use by California dairymen continued to grow steadily. Why? A summary of the superior features of a welded pipeline, whether the summary was made by an inspector, a milk producer, or a milk distributor, would certainly include the following:

1. Better and easier cleaning and sanitizing
2. Less possibility for loss of vacuum control
3. Less foaming of milk in the pipeline
4. Lower initial cost of installation and lower maintenance costs.

Observations on dairies and milk plants in California during the last 25 years have resulted in the following conclusions relating to welded joint pipeline milking systems.

In-place circulation cleaners cannot accommodate excessive foaming because the foaming interferes with cleaning action. Pockets of foam prevent cleaning solutions from reaching the surface of the pipeline. Air leaks which so often occur at unions in jointed pipelines can cause foaming and thus interfere with good cleaning action. Sanitizing solutions cannot work on improperly cleaned surfaces. Properly welded pipeline joints clean as completely as the entire length of the pipeline. By eliminating air leaks, welded joints therefore help provide superior cleaning and sanitizing

of pipelines. Welds in pipelines must be of good quality without pits in the welded areas.

*Vacuum stability is an essential part of all successful pipeline milking systems.* Proper size of lines, vacuum pump, regulator, and vacuum reserve are all necessary parts but all of these good features can be ruined by air leaks at joints in the pipeline milkers. The best vacuum system ever designed cannot compensate for continual air leaks. Without good control of vacuum in a milking system, milking-rest ratios are not clearly separated. This poor milking procedure may result in mastitis aggravation. Welded lines prevent this possible cause of vacuum loss.

*Mixing of air and milk at body temperature* can cause two of milk's most severe flavor defects—oxidized and rancid milk. Both of these flavors have other factors involved but it has been demonstrated that air mixed with milk through air leaks in pipeline milking does contribute to these bad flavors. They both reach a point at which the average consumer can detect them and will refuse to drink the milk. Obviously, no milk distributor can have these flavors in his milk and hope to retain customers. There is no practical way that either of the flavors can be removed, or even masked in milk once the defect has developed. Welded pipeline joints reduce the possibility of air leaks to a minimum.

*The final but not the least important consideration is cost.* The cost of each welded pipe union is less than the cost of a conventional sanitary pipe union. Add to this the continuing cost of opening the joints for cleaning when needed, the use of gaskets when leaks occur, and you will agree that welded pipe joints start cheaper and maintenance remains cheaper.

These four benefits: better sanitation; reliable vacuum control; control of flavor; and lowered costs are all important to quality-minded milk producers.

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