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Journal of Milk and Food Technology

CALL FOR RESEARCH PAPERS FOR 1975 IAMFES MEETING

Contributed research papers will be an important part of the program at the 1975 Annual Meeting of IAMFES scheduled for August 10-13 at the Royal York Hotel, Toronto, Canada. Abstract forms and complete information about presenting papers can be found in the November, 1974 issue of this Journal.

NATIONAL MASTITIS COUNCIL ANNUAL MEETING

FEBRUARY 10-12, 1975

(SEE PAGE I)

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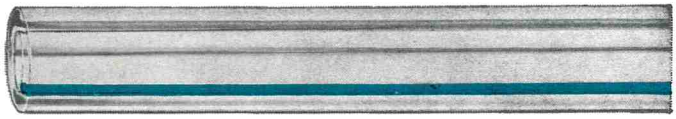

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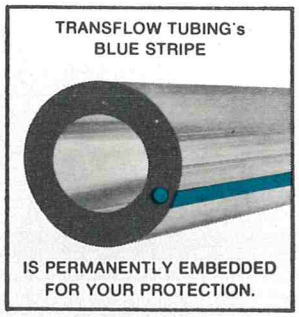

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NATIONAL MASTITIS COUNCIL ANNUAL MEETING

February 10-12, 1975

RADISSON HOTEL — MINNEAPOLIS, MINNESOTA

Everyone interested in prevention and control of bovine mastitis is cordially invited to attend the 14th Annual Meeting of the National Mastitis Council.

Vice President and Program Chairman Burdet Heinemann has planned an outstanding program for this meeting. Subject matter of interest to all segments will be presented.

International authorities from England, Dr. G. C. Brander, Beecham Research Laboratories, and Dr. James M. Booth, Milk Marketing Board, have been secured for the program. Dr. Brander will relate the Somerset mastitis control scheme and report on research results concerning the problem of gram-negative organisms in mastitis control. Dr. Booth will discuss mastitis control in the field and England's mastitis awareness scheme.

Dairy farmers will have an active part in this year's program. L. F. Viney, Arlen Schwinke, Melvin Leppo, and Elbridge Sullivan will present a panel discussion with John B. Adams as moderator. James R. Lefebvre will relate personal experience. John H. Nicolai Jr. will describe what the dairyman expects.

Subjects relating to recent research include: (1) teat dips by W. Nelson Philpot; (2) dry cow therapy by W. D. Schultze; (3) coliform mastitis by Louis Newman.

Reports dealing with somatic cell counts will include: (1) estimates of cells from WMT results by R. D. Mochrie; (2) electronic counting as a screening test by W. E. Ragsdale; (3) use of a mastitis test in DHIA programs by L. H. Schultz.

Dr. Curtis C. Miller, The Upjohn Company, will present considerations in the evaluation of mastitis treatment programs.

Make your plans to attend this excellent meeting. It will start at 8:45 a.m. on February 11 and will adjourn at noon on February 12. Request advance registration form from the National Mastitis Council, 910-17th Street, N. W., Washington, D. C. 20006.

Send request for room reservation directly to the Radisson Hotel, 45 South 7th Street, Minneapolis, Minnesota 55402. Ask for special NMC rates: Single — $19.00 per day; Twin — $27.00 per day.

W. Nelson Philpot, President
National Mastitis Council

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Journal of

MILK and FOOD TECHNOLOGY

INCLUDING MILK AND FOOD SANITATION

Official Publication

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

Reg. U. S. Pat. Off.

Vol. 37 October, 1974 No. 10

No one knows when he's coming

Public health officials who work with NSF as trustees, consultants or committee members are well aware that the awarding of the NSF seal to a manufacturer is only the *beginning*. Permission to place the seal on a product is granted only after it has passed evaluation tests confirming that the product does equal or exceed the requirements of an NSF standard.

The manufacturer's right to continue placing this seal on his product must be *continuously re-earned* at periodic intervals *each year*.

An NSF field manager appears at the factory, *and no one knows when he's coming*. He chooses samples of the product at random—for on-the-spot examination, or for testing in the NSF laboratory at Ann Arbor. Again the product must qualify for its NSF seal.

FOOD AND OTHER SOURCES OF PATHOGENIC MICROORGANISMS IN HOSPITALS. A REVIEW[1]

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(Received for publication June 12, 1974)

ABSTRACT

Persons entering a hospital are often more susceptible to infection than normal persons because of their debilitated condition. The hospital food supply is a potential source of infectious pathogenic microorganisms. Three infectious bacteria are of particular concern because of their ubiquitous nature and heartiness. They are *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli.*

Transfer of microorganisms to patients via hospital food supplies can occur in three ways. First, pathogenic bacteria can be brought in on food itself. If food is not cooked, as is true of many fruits and some vegetables, bacteria can be transferred from the food to the patient. Second, food service personnel may be carriers of pathogenic organisms that can contaminate food and then be transferred to the patient via the food supply. Third, mishandling of food by improper or insufficient heating, refrigeration, or storage can result in food-borne illness. Beside food, bacteria that cause nosocomial infections can come from such sources as hospital personnel (nurses, doctors, etc.), general hospital equipment, and burn victims. Proper food handling together with adequate employee education are probably the most practical means to control the problem of nosocomial infections resulting from hospital food.

When the physician recommends hospitalization of an individual, it is and should be considered as a serious undertaking by both doctor and patient. Such a decision is based on the premise that the hospital can provide benefits unavailable in the home environment. Among these benefits are facilities and equipment and a trained staff of doctors, nurses, nursing assistants, dietitians, and skilled technicians.

During a patient's stay, the hospital is responsible for maintaining the person's health and welfare. One of the factors involved in fulfilling this responsibility is maintenance of high sanitary standards.

A normal healthy individual is quite resistant to many bacterial infections. The human body has extraordinary defense mechanisms. An unbroken epidermal layer provides the initial line of defense against bacterial infection, and additional lines of defense include non-specific enzymes and phagocytes and specific antibodies. However, when someone enters the hospital it is because the patient either has a bacterial infection or has the potential to acquire one by way of wounds, surgery, or treatment with chemicals (antibiotics, immunosuppressive agents, etc.) that interfere with defensive responses. Occasionally the problem is compounded because the patient suffers from poor nutrition. Hence, it is evident that efforts to maintain adequate sanitation in a hospital must be greater than those in the home or in another public facility.

Problems encountered in maintaining this sanitary environment are complicated by the fact that a hospital is a public building. The population density creates a logistics problem for nurses, nursing assistants, sanitary engineers, and food preparation personnel. Contamination and cross-contamination from patient to nurse to patient and the chance that food may remain unprotected from bacterial invasion are very real possibilities. Additionally, new incoming patients or visitors may bring infections to an already susceptible population.

Evidence that problems of nosocomial infections exist can be obtained from recent literature. One of the major problems has been the invasion of hospitals by *Staphylococcus aureus,* giving rise to the phrase "hospital strains of staph." This refers to mutant strains of *S. aureus* that are immune to many common antibiotics. Such resistant staphylococci can be found in the nasal passages of hospital personnel and hence are available for transmission to patients who lack the necessary resistance. The magnitude of the problem with *S. aureus* has been reduced somewhat in recent years.

Currently another type of nosocomial infection has become of concern. *Pseudomonas aeruginosa* has been recognized as a potential human pathogen and recently research has been done to develop methods for identification of the bacterium and also to learn about the mode of transmission. Several other organisms also are of importance in nosocomial infections.

This paper discusses the role of food and some other materials in the transmission of microorganisms to patients in hospitals.

[1]A contribution from the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

ORGANISMS OFTEN ASSOCIATED WITH NOSOCOMIAL INFECTIONS

Several organisms are commonly associated with nosocomial infections. Among the most important are *P. aeruginosa, S. aureus,* and *E. coli.*

Pseudomonas aeruginosa

P. aeruginosa was originally known as *Pseudomonas pyocyanea* and occasionally this name still appears in the literature. The organism is a gram-negative motile rod with monotrichous flagellation *(14)* and occurs as single cells, pairs, or short chains. The term *aeruginosus* is derived from the Latin and means full of copper rust or verdigris hence, green. The common name of *P. aeruginosa* is the "blue pus organism." *P. aeruginosa* is a facultative aerobe with an optimum growth temperature of 37 C. Most strains produce pyocine, a phenazine that is blue when extracted with chloroform from alkaline solutions *(5).*

Pyocine can be used for epidemiological typing; this is advantageous because the procedure is simpler than serological or phage typing and results in a high percentage of typability (93%) *(54).*

P. aeruginosa is a primary cause of nosocomial infections and is replacing the staphylococcus problem of 10 to 15 years ago *(10, 25, 54).* The organism has been described as a bacterial hyena by Lowbury *(25)* and as a ubiquitous pathogen by Zabransky and Day *(54).* An extensive review of this organism was prepared by Brown *(8).*

P. aeruginosa is commonly found in patients with burns or with urinary or gastronintestinal infections *(9).* Darrel and Wahba *(10)* found it occurs most commonly in surgical ward infections. The bacterium is responsible for an increasing number of infections in patients being treated with broad spectrum antibiotics, immunosuppressive agents, and other metabolic inhibitors; patients debilitated by metabolic or oncogenic diseases; and increasingly in patients subjected to surgical manipulations *(54).*

This organism can establish itself so well because it is ubiquitous and is highly resistant to drugs. With leukemic patients, for example, 60% of the individuals who develop an infection are undergoing chemotherapeutic treatment. Use of immunosuppressive drugs to prevent organ rejection enhances the chance of infection. Antibiotics, while able to inhibit most organisms, will permit the selective colonization of *P. aeruginosa (15).* These authors also emphasized that, generally, defects in host resistance are important in establishing gram-negative infections. Buck and Cooke *(9)* reported that a person had to ingest 10^6 or more cells before *P. aeruginosa* could be recovered from feces. The proportion of the ingested dose present in the feces was usually small but it varied considerably. However, it should be noted that their experiments employed normal healthy volunteers and substantially fewer organisms may be needed to colonize the intestine of a debilitated person. The authors further noted that some institutionally prepared food may contain *P. aeruginosa.*

Bassett *(4)* found *P. aeruginosa* present in disinfectants like formalin and benzalkonium chloride. Goldschmidt and Bodey *(15)* tested four disinfectants, cytosine arabinoside, cyclophosphamide, methotrexate, and 6-mercaptopurine, and found that *P. aeruginosa* was most resistant of the bacteria that were studied. They found *P. aeruginosa* was not affected by 1000 μg methotrexate/ml. Some concentrations of cytosine arabinoside and 6-mercaptopurine actually may have enhanced its growth.

When testing disinfectants on mixed populations of bacteria, these investigators reported that *P. aeruginosa* showned no sparing effect on *E. coli.* Thus it was concluded that *P. aeruginosa* can not only survive but can become dominant in a mixed population. Buck and Cooke *(9)* found that the organism is not affected by ampicillin. In contrast, some investigators noted that *P. aeruginosa* failed to survive normal cleaning measures *(10).*

P. aeruginosa has been recovered from a variety of sources. Zabransky and Day *(54)* isolated the organism from urine, stools, blood, sputa, ear infections, and fluids from wounds. Bassett *(4)* found the organism on suction devices, oxygen bags, catheters, eye irrigation fluids, endotracheal tubes, steroid creams, and ice from an ice machine. Goldschmidt and Bodey *(15)* reported isolation of the bacterium from skin, throat, and feces.

P. aeruginosa is a ubiquitous opportunistic pathogen often present in sufficient quantities to be of significant importance as a causative agent of infections.

Staphylococcus aureus

This gram-positive nonmotile coccus was, at one time, the major cause of nosocomial infections. The word *aureus* is derived from the Latin term golden, the characteristic color of colonies on media containing high levels of sodium chloride. The bacterium exists as single cells, in pairs, or in short chains; the cells being 0.8 to 1.0 μ in diameter. *S. aureus* is a facultative aerobe with an optimum growth temperature of 37 C, but it can grow at 10 and 44 C. It is extremely salt tolerant; some strains can grow in a solution of 10% or more sodium chloride *(7).* It is strongly catalase-positive and characteristically pro-

duces an enterotoxin described as a water soluble protein *(31)*. Presently six serologically different types of enterotoxins have been identified.

Several types of selective-differential media have been suggested to detect staphylococci; most are based on selective levels of an inhibitor such as sodium chloride. However, no single physiological characteristic or combination of characteristics possessed by staphylococci is an absolutely reliable indicator of enterotoxigenicity *(30)*.

S. *aureus* has been associated with many food poisoning outbreaks. Minor and Marth *(32, 33)* have extensively reviewed staphylococcal food intoxications caused by many contaminated foods including milk, cheese, meat and fish, and bakery products. Rather complete discussions of S. *aureus* and other gram-positive cocci have been prepared by Baird-Parker and Holbrook *(3)* and Minor and Marth *(30, 31)*.

Escherichia coli

E. coli, a normal inhabitant of the intestinal tract of man and other vertebrates, is a gram-negative nonspore forming rod about 0.5 × 1.0 μ in size. The word *coli* stems from the German term *colum* or colon, the large intestine. This aerobic or facultatively aerobic rod is either non-motile or motile, and if motile posses peritrichous flagella. The organism occurs singly, in pairs, and in short chains and is usually not encapsulated. It is catalase-positive and has an optimum growth temperature in the range of 30-37 C. It is generally not too heat resistant but some strains require 30 min at 60 C for inactivation *(6)*. *E. coli* was first isolated from feces of breastfed infants.

The species contains numerous serological types that differ in pathology. Some strains are termed enteropathogenic and cause either *Shigella* – like or *Salmonella* – like illness in humans.

Enteropathogenic *E. coli* have been found in market milk and market cheese *(17)* and have been associated with foodborne illness resulting from consumption of several other dairy products *(35)*. These strains of *E. coli* can cause infantile diarrhea and a form of food borne illness *(35, 38)*.

There are, of course, many other organisms that are occasionally associated with nosocomial infections. They are usually the result of an epidemic such as typhoid fever or specific mishandling of foods (salmonellosis). They generally originate from a local epidemic or a specific unique disease or source. However, the three organisms just considered are continuously present in the hospital environment and their source is often more difficult to pinpoint hence they are of primary concern in this discussion.

FOOD AS A SOURCE OF BACTERIA THAT CAUSE NOSOCOMIAL INFECTIONS

The means for transfer of microorganisms to patients via the hospital food supply can be divided into three categories. First is the food itself. Few foods entering the hospital are sterile. Potential human pathogens can be brought in on meat surfaces, with plants as clinging soil or as plant pathogens, and in or on other products. Second, transfer of microorganisms to patients can be directly attributed to food service personnel. Even if all basic rules of sanitation are followed, such as proper hand washing after handling any raw product or using the toilet, a small detail may easily be overlooked which could subsequently lead to a bacterial infection in a patient. The third means for transfer of infectious organisms from the food supply to patients is the mishandling of the products. This includes insufficient heating or refrigeration of the food as well as improper sanitation of equipment or storage of the product.

Food

Transfer of microorganisms to patients from hospital food supplies has been documented by several investigators. Kominos et al. *(22)*, extensively investigated the introduction of *P. aeruginosa* into hospitals via vegetables. These investigators isolated the organism from tomatoes, radishes, celery, carrots, endive, cabbage, cucumbers, onions, and lettuce found in hospital kitchens. They were further able to show through pyocine typing that *P. aeruginosa* cultures from clinical specimens were frequently identical to those recovered from the vegetables.

Tomatoes yielded *P. aeruginosa* most frequently and in greatest numbers. Samish et al. *(40)* investigated the tissue of normal healthy undamaged tomatoes and cucumbers and were able to isolate microorganisms despite the fact that their internal tissue is normally considered to be sterile. Varietal characteristics, climatic influences, and agrotechnical practices all seem to play a role in determining the bacterial content of a given vegetable. After more detailed experiments with tomatoes, Samish et al. *(39)* suggested that the stem depression area was the site of bacterial entry. Organisms can then penetrate into the inner pulp from this area. It is noteworthy that the central core of the tomato had a higher bacterial count than did any of the peripheral tissues except the stem depression.

It has been shown *(36)* that *P. aeruginosa* can cause disease in sugar cane, tobacco, and lettuce and that such strains of the bacterium are identical to those associated with animal infections.

Fresh meat, as one might expect, is commonly con-

taminated with microorganisms, particularly *E. coli*. It is a potential cause of infection. Shooter et al. *(42)* reported the absence of *E. coli* on meat after proper cooking. Other fresh meat, particularly poultry, can carry *Salmonella* spp. Kohler *(20)* described outbreaks of hospital salmonellosis caused by contaminated foods.

Shooter et al. *(42)* attempted to isolate *E. coli, P. aeruginosa,* and *Klebsiella* from food service foods and found various combinations of these organisms in salads, cold meat, cold sweets, cold food, pureed food, and, most significantly, in hot foods. Ross and Thatcher *(37)* investigated the presence of microorganisms in precooked market frozen foods and found coagulase-positive staphylococci and *E. coli* in a variety of frozen chicken, turkey, and beef liver dinners. Enteropathogenic *E. coli* was found in such frozen market meats as beef and pork *(16)*. These authors suggested that presence of the bacterium is more apt to result from contamination by a food handler than from being indigenous to the food. Eddy and Ingram *(12)* isolated *S. aureus* from bacon curing brines and stated that the bacterium can survive there almost indefinitely.

Milk is particularly important because it is often given to the most debilitated patients. Unfortunately milk is an optimal growth medium for many microorganisms and therefore if not handled properly it can be a potential source of infection. Jones et al. *(17)* found enteropathogenic *E. coli* in samples of pasteurized homogenized milk and other dairy products. Enteropathogenic strains of *E. coli* also have been identified as causing an outbreak of foodborne intoxication that included 105 episodes and 387 persons who ate imported soft cheeses *(28)*.

Raw milk or improperly pasteurized milk also can contain *S. aureus*. Olson et al. *(34)* showed that 15% of 157 *S. aureus* cultures identified as etiological agents of bovine mastitis produced enterotoxins.

The literature contains many other references to foodborne illness resulting from the ingestion of microorganisms or their toxins *(29, 51, 55)*.

Meats, always a carrier of organisms, can be freed from most of them by proper cooking; however many foods are not normally cooked before being eaten, and hence are of particular importance as potential vectors of infectious microorganisms.

Personnel

Food also can become contaminated during the preparation it must undergo before being served. Possible routes of microbial contamination are nearly unlimited. For example, almost all food is handled at one time or another by food service personnel. If one of these people has an infection it can be transmitted to the food. Eisenstein et al. *(13)* described a hospital epidemic of infectious hepatitis that was traced to breakfast orange juice. One of the kitchen workers had a subclinical infection that she probably acquired from her husband. She apparently transmitted her infection to the orange juice via some unknown route. Kominos et al. *(22)* isolated *P. aeruginosa* from 5 of 52 kitchen personnel. Hence, contamination of food with this organism is a real possibility. The currently fashionable longer hair and beards for men can be a source of pathogenic microorganisms *(1, 11)*. Transmission from workers is not the only route by which microorganisms can contaminate food. Kominos et al. *(22)* recovered *P. aeruginosa* from various cutting surfaces and knives used in salad preparation. Shooter et al. *(41)* were able to isolate *E. coli* from sinks, working surfaces, utensils, and floors of a hospital kitchen. They found concentrations as high as 2×10^3 organisms/ml on trays and bowls left to drain in the dishwashing room and concluded that some foods were free of infecting organisms but utensils to handle food were not.

Airborne bacteria also have been implicated as contaminants in some hospital dishwashing facilities. However, Jopke and Hass *(18)* found no gross contamination in facilities they examined but they were able to recover some gram-positive cocci and diphtheroids from air samples.

Generally hospitals that employ mechanical dishwashers have tableware with low microbial counts if the tableware is presorted to eliminate handling *(19)*. High counts that are found occasionally, can be correlated with low temperatures during washing. Hand washing of tableware, of course, results in very high counts because of the low water temperatures and excessive handling of the utensils. As a consequence, hand washing of tableware is not permitted in hospitals, at least in some areas.

The numerous ways for transportation of microorganisms in a hospital food service kitchen make a continuing conscientious effort to maintain a high degree of sanitation an absolute must.

Mishandling

Transfer of infection from mishandled hospital foods to the patient can be important. Hopefully it is infrequent. The old axiom, "keep it hot, keep it cold, or don't keep it long" applies in hospitals even more than it does elsewhere. Cooked foods must be kept hot while in transit from kitchen to patient. Foods must not remain at room temperature for long periods as some pathogenic organisms grow to potentially hazardous levels in a matter of hours *(23)*.

Additionally, if food is left in uncovered dishes or

uncovered carts the possibility exists that insects, known carriers of human pathogens, can contaminate the product *(1)*.

To properly refrigerate foods the rate of cooling must be satisfactory. A large tray or bowl of food (dressings, sandwich spread, etc.) may have an internal temperature sufficiently high to permit microbial growth even though the surface is at refrigerator temperature.

Of the three general means for bacterial transfer, contamination of food by the food service staff is probably the most important. Although microorganisms can enter the hospital via the food that is brought in, they may also enter with members of the food service staff. The human element continues to be the most unpredictable and difficult to control.

OTHER SOURCES OF INFECTION

Although this paper is primarily concerned with foodborne sources of bacteria that can cause nosocomial infections, it should be noted that there are numerous other sources of pathogenic organisms that can infect the patient. Brief mention will be made of some of these other sources.

Kominos et al. *(21)* studied the mode of transmission of *P. aeruginosa* in a burn unit and in an intensive care unit of a general hospital. These investigators found that direct handling of patients by nursing personnel was a major means of cross contamination. Hands are easily contaminated when bedding is changed and wounds of infected patients are dressed. Although the original sources were not identified, *P. aeruginosa* was recovered from such items as suction tubes, water containers, wash basins, hydrotherapy tanks, and saline solutions.

Shooter et al. *(43)* investigated sources of *P. aeruginosa* and noted that readmitted patients were highly likely to be carriers. He also found a higher incidence of infection among patients taking antibiotics or other chemotheraputic agents than among patients not taking these preparations. Wards devoted to burns and urological surgery were found to be the most important sources of infection.

According to Lowbury et al. *(27)* stringent regulation of sanitation including use of disposable catheters, steamed bedpens, boiled urinals, gloves for nurses, floors washed several times per day, and other measures failed to control the incidence of infection by *P. aeruginosa*. Lowbury and Fox *(26)* identified the nose, throat, nasopharynx, skin, and ears of patients as sources of organisms in burn units with hands of nurses often the likely vector. These investigators identified infected burns as the most important reservoirs of bacteria.

Original sources of infection vary widely and many possibilities have been identified. However, most researchers ignore the food supply as a source of organisms unless food was investigated exclusively.

PROPER FOOD HANDLING

Much space in periodicals devoted to hospital personnel and management has been allotted to descriptions of correct food handling procedures *(45-49)*.

Stauffer *(50)* outlined problems encountered by the food service manager with respect to sanitation and made the following five recommendations. *(a)* use of safe high quality food; *(b)* presence of healthy food service personnel having good personal hygiene, prompt reporting of communicable diseases, and proper education of workers; *(c)* use of proper, cleanable sanitary equipment; *(d)* proper preparation of food; and *(e)* protected service to the patient including adequate equipment for transportation of food and water.

Sperber et al. *(44)* made similar recommendations but gave greater emphasis to employee education in proper sanitation techniques. Jones et al. *(17)* suggested that greater care should be exercised by plant workers handling dairy products to eliminate enteropathogenic *E. coli* from such foods.

Food service managers seem particularly aware of *Salmonella* spp. in kitchens *(2, 24)*. Recommendations to prevent the problem include the complete absence of raw egg in any prepared food as well as careful clean up (cutting surfaces, hand washing, etc.) after handling poultry or breaking eggs.

Special procedures for communicable disease wards are necessary to prevent hospital epidemics *(53)*. This author urged the use of separate equipment in these wards and autoclaving everything that leaves the ward.

Isolation rooms for organ transplant patients pose an interesting problem for the food service manager because most commercial foods are pathogen-free but not sterile. Several procedures for supplying sterile food to these patients have been suggested by Watson and Bodey *(52)*. They used long-time low-temperature baking in a nylon bag to produce organoleptically satisfactory, sterile cakes, bread, etc. They also made extensive use of aseptic cooking techniques. Egg shells were carefully scrubbed with disinfectants before being opened in an aseptic atmosphere. Food trays were dipped in an iodinated antiseptic. They concluded that these methods were satisfactory for serving foods free of organisms and that such foods allowed patients to maintain their weight.

General Conclusion

This paper does not attempt to cover all aspects of the transfer of foodborne microbiological infections. However, sufficient literature sources have been reviewed so that some conclusions can be drawn.

Few microbiologists except those specifically interested in foods, seem to consider food as a potential source of infectious microorganisms. In several reports cited in this paper the investigators who searched for sources of infection did not consider food. Food microbiologists have considered this problem but most have failed to pursue it beyond the isolation and identification of microorganisms obtained from foods.

While food can be an important source of pathogenic microorganisms there are other sources equally or more important. Most authors consider other sources as more important than foods. Information appears to be lacking on the relative importance of different sources as causes of infection in hospitalized patients. Hence, this is an area that needs to be examined in some detail.

Another area of concern to many food service managers is the lack of appreciation by workers of bacteriologically sound procedures. The emphasis need not be on stricter rules for kitchen workers but rather on better education, i.e. an explanation of why certain procedures have been implemented. Kitchen rules need not, in fact should not, be elaborate and highly detailed but rather they should be a few simple easily understood but carefully followed procedures.

Foodborne pathogenic organisms need not be a hazard to hospital patients if the problems can be thoroughly researched, understood, and then controlled.

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BEHAVIOR OF *CLOSTRIDIUM PERFRINGENS* IN PRECOOKED CHILLED GROUND BEEF GRAVY DURING COOLING, HOLDING, AND REHEATING[1]

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Abstract

The suitability of handling practices used in school kitchens was evaluated using ground beef gravy that was contaminated with *Clostridium perfringens*. Cooked gravy was cooled to 110 F (43.5 C) and inoculated with a mixture of vegetative cells and spores of *C. perfringens* NCTC 8239 to provide approximately 10,000 organisms/g. After inoculation, gravy was packed in bags, refrigerated for 16 h, held for 5 h at 82 F (28 C) or 42 F (5.5 C), and then heated in a compartment steamer for 35 min or until the temperature of the gravy at the center of the pan reached 165 F (74 C). *C. perfringens* was enumerated at intervals during cooling, holding, and heating of the gravy. The number of viable cells after 16 h of refrigeration at 42 F (5.5 C) was influenced by the first 6 h of cooling when the temperature of the gravy was in the range that permitted growth of *C. perfringens* (65-122 F; 18.5-50 C). Plate counts of gravy held for 5 h at 82 F (28 C) or 42 F (5.5 C) indicated stablization of the *C. perfringens* population. When 165 F (74 C) was the final temperature to which the gravy was heated, no viable cells of *C. perfringens* were found.

In 1971 a food poisoning outbreak occurred in several Finnish schools that used precooked chilled food. A microbiological analysis of samples from food that was served indicated that brown gravy was heavily contaminated with vegetative cells of *Clostridium perfringens*. The place of mishandling and the reason for excessive multiplication of the organisms were not identified, but it was discovered that gravy had been inadequately heated in the school where the largest outbreak occurred. Of practical concern was the possibility that one or several of the stages in handling food in the chill food system could cause rapid multiplication of *C. perfringens*.

The optimal temperature for growth of *C. perfringens* is 109 to 115 F (43-46 C) *(2, 6)*. At these temperatures, in a favorable medium, the generation time averages 12 min and can be as short as 8.5 min *(3)*. At lower temperatures, multiplication is slower. The lowest temperature for growth has been reported as 65 F (18.5 C) *(1)*, although Duncan *(6)* gave the temperature range for growth of *C. perfringens* as 59 to 122 F (15-50 C), with growth below 18 C as very slow. Bryan *(2)* indicated that the range for rapid growth of the organism is between 88 and 122 F (31-50 C).

In foodservice operations cooked foods can contain *C. perfringens*, since spores often survive the heat process or the organism can be added as contamination after cooking. Results of several studies have shown that spores of heat-resistant strains of *C. perfringens* can survive routine cooking *(5, 10, 11, 14)*.

Although several investigations on the incidence of *C. perfringens* in raw and prepared foods have been reported in the United States, only eight studies *(1, 7, 8, 9, 10, 11, 12, 14)* were found that dealt with growth-death patterns of the organism during heating and holding of food. The literature contains no reports of growth-death studies of *C. perfringens* during heating and holding of food as practiced in mass feeding systems.

Studies of the growth-death patterns of *C. perfringens* in food held above 120 F (49 C) indicate that vegetative cells can survive the usual hot-holding temperatures of food, 140-160 F (60-71 C), in foodservice operations *(7, 9, 11, 14)*. Several reports showed as variable the length of time for rapid multiplication of *C. perfringens* in food held at temperatures between 65 and 122 F (18.5-50 C) *(1, 7, 8, 10, 11, 14)*.

Survival of *C. perfringens* during storage at low temperatures appears to vary with strain, vegetative cells versus spores, and substrate. Although the organism is not very stable at low temperatures, studies show that the bacterium could survive at refrigeration temperatures overnight or even several days *(4, 11, 12 14)*.

The purpose of this study was to observe the effect of the cooling, holding, and heating practices on the rate of heat transfer in precooked chilled ground beef gravy and to evaluate how these practices affect the behavior of *C. perfringens*.

Methods and Materials

The ingredients, formula preparation, stages of handling,

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TABLE 1. TIME IN THE REFRIGERATOR REQUIRED TO REDUCE THE TEMPERATURE OF COOLED GROUND BEEF GRAVY FROM 102 F (39 C) TO 65 F (18.5 C)

Trial	Time[a] (h) required to cool gravy in bags			
	1	2	3	4
1	4.5	6.5	3.5	7.0
2	4.0	5.5	7.0	7.5

[a]Time was recorded by a potentiometer with a thermocouple inserted in the approximate center of each bag.

TABLE 2. TIME REQUIRED TO RAISE THE TEMPERATURE OF CHILLED GROUND BEEF GRAVY TO 65 F (18.5 C) DURING HOLDING AT ROOM TEMPERATURE

Trial	Time[a] (h) required to warm gravy in bags			
	1	2	3	4
1	>5	>5	3.0	3.0
2	5.0	>5	>5	4.5

[a]Time was recorded by a potentiometer with a thermocouple inserted in the approximate center of each bag.

TABLE 3. CHANGE IN TEMPERATURE OF GROUND BEEF GRAVY AFTER HEATING IN A COMPARTMENT STEAMER

Preceding holding treatment	Pan	Temperature of gravy (F)[a] Heating time in min		Additional time in min required to reach 165 F
		0	35	
82 F	1[b]	64	172	0
	2[c]	64	165	0
	3[d]	64	150	7
	4[e]	64	148	10
	Mean	64	159	4
42 F	1[b]	44	100	30
	2[c]	44	116	23
	3[d]	44	128	17
	4[e]	44	148	30
	Mean	44	123	25

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

sampling methods, and analyses for *C. perfringens* were the same as those reported previously *(13)*. Identical conditions were maintained in the two trials of this experiment. After cooking, gravy was cooled to 110 F (43.5 C), inoculated with *C. perfringens*, and packed in plastic bags. Bags were refrigerated for 16 h, then held at 82 F (28 C) or 42 F (5.5 C) for 5 h. All gravy was then heated in a compartment steamer for 35 min. At this time, if the temperature of the gravy was not 165 F (74 C), heating was continued until this temperature was reached.

The temperature of gravy inside the bags during cooling and holding was recorded continuously by a potentiometer. A thermometer was used to measure the temperature of gravy after heating in the compartment steamer. Samples were taken at six stages: *(a)* before inoculation, *(b)* immediately after inoculation, *(c)* after cooling in the refrigerator for 16 h, *(d)* after holding for 5 h at 82 F (28 C), and 42 F (5.5 C), *(e)* after heating in the steamer for 35 min, and *(f)* after heating in the steamer until the temperature of the gravy at the center of the pan reached 165 F (74 C).

Inoculation procedure

The stock culture of *C. perfringens*, strain NCTC 8239, Hobb's serological type 3, which is a food poisoning strain and produces heat resistant spores, was obtained from the Food Research Institute, University of Wisconsin-Madison. The stock culture was kept frozen in veal broth in the laboratory between the experiments.

Before conducting an experiment, preliminary tests were needed to determine the procedure for inoculation of gravy. Such tests revealed that *C. perfringens* was in the logarithmic growth phase after 5 h in Fluid Thioglycollate Medium at 37 C. Hence, subsequently, incubations of 6-7 h were used.

The second preliminary test was made to establish the temperature of the gravy at the time of inoculation. In the study previously reported *(13)* gravy was cooled to 158 F (70 C), packed in plastic bags, and held in bags in chilled water for 1 h. However, most of the added cells of *C. perfringens* were inactivated when the gravy was inoculated at 158 F (70 C). If the inoculation were done after gravy in plastic bags was cooled in chilled water, uniform distribution of cells in gravy was not possible. Hence, cooling in chilled water was omitted in this experiment. The method developed for use was to cool gravy to 110 F (43.5 C) in the steam kettle. Forty-four pounds of the cooled gravy were weighed into two pans; each pan contained 22 lb. The inoculum was divided equally between the two quantities of gravy. The 44 lb. of gravy were then distributed among eight bags. Each bag was placed in one corner of two cartons. Gravy for the two center bags in each carton was measured from the steam kettle and was not inoculated with *C. perfringens*.

The amount of inoculum to be added to the gravy was determined in another preliminary test. A small amount of gravy was inoculated with different amounts of a broth culture of *C. perfringens*. If the inoculum provided only 1000 cells or less of *C. perfringens* per gram of gravy, no growth of the organisms occurred when the gravy was held in the refrigerator for 16 h followed by holding at room temperature for 5 h. Therefore, to insure growth when conditions were right, an inoculum yielding approximately 10,000 cells per gram of gravy was chosen.

Methods thus established in preliminary tests were used in later trials. A 0.1-ml quantity of stock culture in veal broth was transferred to 10 ml of Fluid Thioglycollate Medium in

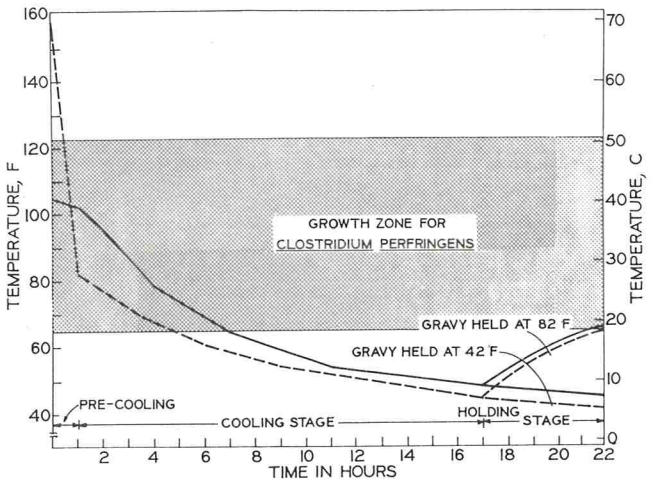

Figure 1. Experiments one and two: Time-temperature relationships of ground beef gravy during cooling and holding stages (Experiment One, broken line) (Experiment Two, solid line).

TABLE 4. NUMBERS OF *Clostridium perfringens* IN GROUND BEEF GRAVY DURING COOLING IN REFRIGERATOR

Sample[a]	Plate count of *C. perfringens* (cells/g × 10^4) Before inoculation	After inoculation	After refrigeration	Subsequent holding treatment
1	0	1.0	4.0	82 F
2	0	1.0	4.2	
3	0	2.4	7.8	
4			5.1	
5	0	2.1	6.7	
6	0	1.0	6.2	
7	0	1.0	5.8	
8			4.1	
Mean	0	1.4	5.5	
1	0	1.0	3.6	42 F
2	0	1.0	3.6	
3	0	2.4	1.8	
4			1.1	
5	0	2.1	4.4	
6	0	1.0	6.4	
7	0	1.0	5.4	
8			4.6	
Mean	0	1.4	3.9	

[a]Samples 1 to 4 are counts from trial 1, samples 5 to 8 are counts from trial 2.

test tubes. Tubes were incubated at 98.6 F (37 C) for 6 to 7 h and sufficient of this broth culture was added to provide approximately 10,000 organisms/g of gravy. Brown coloring, an ingredient of the gravy, was added and mixed in together with the inoculum. The adequacy of mixing could be ascertained from the uniformity of color.

RESULTS AND DISCUSSION

Cooling of gravy

Figure 1 shows the cooling rate of gravy with and without initial cooling in chilled water. In the work previously reported *(13)*, cooked gravy was cooled to 158 F (70 C) in the steam kettle, packed in bags, and then cooled in chilled water for 1 h. Thus at the beginning of the 16-h cooling stage in the refrigerator, the mean temperature of eight bags of gravy was 82 F (28 C). In this experiment cooling in chilled water was omitted. Cooked gravy was cooled to 110 F (43.5 C) in the steam kettle, inoculated, and packed in bags. Thus at the beginning of the 16-h cooling state in the refrigerator, the mean temperature of eight bags of gravy was 102 F (39 C). This temperature was 20 F higher than was found when chilled water was used. Hence, during cooling in the refrigerator, gravy remained in the zone for growth of *C. perfringens* for 4 h when the gravy was precooled in chilled water and for 6 h when this was not done. When precooling in chilled water was omitted, gravy cooled by refrigeration was in the temperature range for rapid growth of *C. perfringens* (88 to 122 F; 31 to 50 C) for 2 h. As can be seen from data in Fig. 1, the time-temperature relationships of gravy during the cooling stage in this experiment were more favorable for growth of *C. perfringens* than those in the experiment previously reported *(13)*.

Table 1 shows that the maximal time the temperature of gravy remained in the range for growth of *C. perfringens* was 7 h for the first trial of this experiment. In trial 2, the time that gravy remained at a temperature suitable for growth of *C. perfringens* ranged from 4 h to 7.5 h. Under optimal conditions *C. perfringens* can multiply very rapidly, therefore large numbers of cells may be produced in 2 to 3 h *(6)*. Thus, in this experiment during the 2 h that

TABLE 5. NUMBERS OF *Clostridium perfringens* IN GROUND BEEF GRAVY DURING HOLDING (42 AND 82 F) AND HEATING IN A COMPARTMENT STEAMER AT 7 LB/IN^2 PRESSURE

Holding temperature	Sample[a]	Plate count of *C. perfringens* (cells/g × 10^8) Holding time (h) 0	Holding time (h) 5	Heating time (min) 0	Heating time (min) 35	Additional time
82 F	1	4.0	4.1	4.1	0	–
	2	4.2	3.8	3.8	0	–
	3	7.8	0.7	0.7	0	–
	4	5.1	0.6	0.6	0	–
	5	6.7	0.6	0.6	0.007	0
	6	6.2	0.9	0.9	0.008	0
	7	5.8	7.2	7.2	–	0
	8	4.1	5.6	5.6	–	0
	Mean	5.5	2.9	2.9	–	–
42 F	1	3.6	3.4	3.4	0.01	0
	2	3.6	2.3	2.3	0.007	0
	3	1.8	1.7	1.7	–	0
	4	1.1	1.5	1.5	–	0
	5	4.4	6.6	6.6	0.008	0
	6	6.4	1.9	1.9	0.03	0
	7	5.4	5.7	5.7	–	0
	8	4.6	7.0	7.0	–	0
	Mean	3.9	3.8	3.8	–	0

[a]Samples 1 to 4 are counts from trial 1, samples 5 to 8 are from trial 2.

gravy remained at a temperature in the optimum growth zone, multiplication at a high rate could start, continue for several hours and decrease as the temperature of gravy decreased. Hall and Angelotti *(7)* found that multiplication of *C. perfringens* inoculated into meat started without any lag phase at 113 F (45 C), but at lower temperatures (58 to 99 F; 14.5 to 37 C), a lag phase of 4 h occurred. If there were no lag phase in the beginning of the cooling stage of the gravy, the necessity to pass through the optimal growth zone before refrigeration is evident.

Holding of gravy

Data in Fig. 1 show that the rate of heat transfer of gravy during holding for 5 h at 82 F (28 C) or 42 F (5.5 C) was similar in this experiment to the one reported previously *(13)*. The temperature of gravy in this experiment differed only 2 F from that of the first test. Thus, the mean temperature in the center of eight bags of gravy did not reach the minimum needed for appreciable growth of *C. perfringens*. The temperature of gravy in the areas close to the edges of the case may reach the minimum for growth of *C. perfringens*, but temperature measurements and sampling from several points in the bags were not possible in this study.

As can be seen from data in Table 2, 65 F (18.5 C) was reached in four bags during the holding stage. Results of trial 1 suggest that growth of *C. perfringens* was possible in bags 3 and 4, because the gravy was warmed to 65 F (18.5 C) in 3 h. Growth of *C. perfringens* in any bag of gravy from the second trial was doubtful.

The heat transfer of gravy held under refrigeration in the second experiment was similar to that in the first experiment *(13)*. The mean temperature of gravy decreased 3 F, from 47 F (8.5 C) to 44 F (6.5 C), during the 5-h period.

Heating of gravy

Results obtained when gravy was reheated are in Table 3. Gravy initially at 64 F (18 C) was at 148 to 172 F (64.5 to 78 C) after 35 min of heating in the compartment steamer at 7 lb/in^2 pressure. The maximum time required to heat gravy to 165 F (74 C) in the center of pan was 45 min. The temperature of gravy held in the refrigerator increased from 44 F (6.5 C) to a maximum of 148 F (64. 5 C) in 35 min. Heating for 30 additional minutes was required before the temperature of the gravy at the center of the pan reached 165 F (74 C). The minimum temperature of gravy after 35 min of heating was 100 F (38 C). The maximum heating time to reach 165 F (74 C) was 65 min.

The rate of heat transfer was more rapid in this experiment than the one reported previously *(13)*. In the first experiment *(13)*, the mean temperature after 35 min of heating in the compartment steamer was 119 F (48.5 C) for gravy initially at 64 F (18 C) and 109 F (43 C) for gravy initially at 44 F (6.5 C). In contrast, data in Table 3 show that the mean temperature after 35 min of heating was 159 F (70.5 C) for gravy initially at 64 F (18 C) and 123 F (50.5 C) for gravy initially at 44 F (6.5 C). Since the temperature inside the compartments of the steamer could not be recorded, it is unknown if these temperatures were different in the two experiments. The physical nature of the gravy in the second experiment differed from that in the first in that the liquid and solids fractions separated during cooling and storage. This may have contributed to differences in temperature of the heated gravy.

Growth of C. perfringens

The gravy was tested before inoculation, and no samples yielded *C. perfringens*. After inoculation there were approximately 1.4×10^4 cells of *C. perfringens* per gram of gravy. Data in Table 4 indicate that rapid multiplication of the organism occurred during the cooling period, and resulted in a 30,000- to 40,000-fold increase in population of *C. perfringens*.

The rapid increase of *C. perfringens* during cooling was expected since the temperature of the gravy remained in the growth range for the organism for 6 h. During the first 2 h of cooling the gravy was in the temperature range for rapid growth. The substantial increase in population that occurred when the cooked gravy was refrigerated emphasizes the danger when a large quantity of food is cooled slowly. The most common reason reported in the literature for outbreaks of perfringens food poisoning is refrigeration of cooked food in quantities too large to permit rapid cooling.

When gravy was held at 42 or 82 F, samples were taken from bags after the contents were thoroughly mixed. As can be seen from data in Table 5, plate counts of *C. perfringens* in samples taken after holding gravy for 5 h at 82 F (28 C) and 42 F (5.5 C) indicated that no multiplication or inactivation of organisms occurred during this treatment.

The lack of growth by *C. perfringens* even with a large initial inoculum indicates that conditions which prevailed during holding of gravy were not conducive to growth of the organism. Since the temperature of chilled gravy in pouches remained in the range for growth of *C. perfringens* only during the last 2 h of holding, high initial numbers of the organism would be required to achieve a potentially hazardous high final population. The temperature of the chilled gravy at the center of the bags did not reach the

minimum for growth of *C. perfringens* during the holding period, thus it is doubtful if any growth of the organism could occur.

Data in Tables 3 and 5 show that no viable cells of *C. perfringens* were found after heating when the temperature of gravy at the center of the pan reached 165 F (74 C) or more. This temperature was reached in 35 min in gravy that initially was held at room temperature. In contrast, approximately a 100-fold decrease in population occurred when gravy held at 42 F (5.5 C) was heated for 35 min.

Findings from this trial show that the way to assure the adequacy of the heating treatment is to establish a standard terminal temperature of 165 F (74 C). Heating for 35 min was sufficient to raise the temperature of gravy held at room temperature to 165 F (74 C) in one trial; but this time was not sufficient in the second trial. For gravy held at 42 F, heating for 35 min was not sufficient to raise the temperature of gravy at the center of the pan to 165 F (74 C).

Most important in controlling *C. perfringens* is what happens when the organism is in the logarithmic growth phase. When gravy was cooled, multiplication of *C. perfringens* was most rapid.

Rapid growth of the organism during this time identifies the cooling stage as the most critical period in maintaining the safety of such foods as gravy. Time-temperature measurements indicated that the temperature of gravy at the center of the bags during the cooling period remained in the range for growth of *C. perfringens* for 6 to 7 h. No multiplication of *C. perfringens* occurred during the 5-h holding period at 82 F (28 C) when the temperature of gravy at the center of the bags was in the range for growth of the organism for 2 h or less.

Implications for foodservice operations

To prevent foodborne disease outbreaks in foodservice operations, time-temperature relations and sanitation must be controlled throughout the total chill food system. The safety of precooked chilled food has to receive primary consideration in food processing plants, during food transport, and in foodservice operations.

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LEAD IN PAPER: A POTENTIAL SOURCE OF FOOD CONTAMINATION

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Abstract

Many paper products, including newspapers and paper goods used in packaging foods, contain lead. The lead content of newspapers, an important constituent of recycled paper, varies with color of ink, type of printing process, and source of newsprint. Recycled newsprint is richer in lead than virgin newsprint. Lead on or in food packages ranged from 2 to 10,000 ppm, and varied with the quantity of printing and color of ink. Printed packages contained more lead than unprinted material, although some unprinted ones contained up to 58 ppm. The potential for introducing lead into meat and milk by feeding waste paper to ruminants is explored. Negligible amounts of lead are introduced into soils and plants from newspaper mulch.

Paper products such as newsprint and other printed material may be contaminated with lead, or other potentially hazardous substances *(25)*. The possible inadvertant contamination of food by lead from new or used, printed or unprinted, paper is not well understood, although the potential toxicity of ingesting paper printed with lead inks has been reported *(9, 10)*. Contamination of food by lead assumes increasing importance, because current environmental levels of lead are reportedly detrimental to animals *(12, 13, 23, 24)*. Therefore, we have examined how lead on or in paper may become associated with food.

Possible routes of lead transfer to food through new or recycled, printed or unprinted, paper are shown in Fig. 1. Because of the present paper shortage, reclaiming waste paper by recycling, and the development of alternative uses for waste paper are being considered. Paper for food wrappers may come from either virgin or reclaimed paper stock, and we show that lead in newspapers is retained during recycling. Further, lead on food packages is associated with printing. Alternative uses of printed paper that might also associate lead with food, such as the feeding of paper to ruminants or the mulching of plants are also examined. In the ensuing sections, we present new evidence and review published observations to illustrate these points.

Methods

Newspapers, newsprint, and ink were obtained from the printer to preclude contamination during distribution. Packages were purchased at local markets.

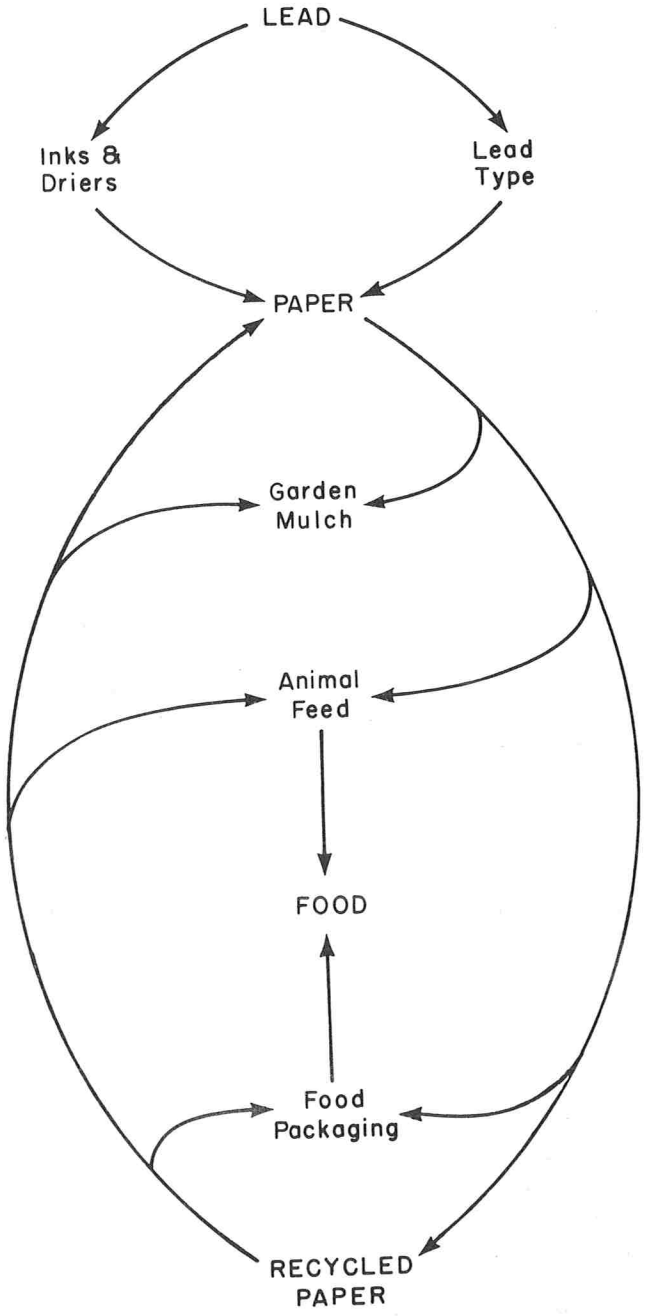

Figure 1. The possible circulation of lead to food through paper.

TABLE 1. LEAD CONTENT OF NEWSPAPERS[a]

Sample	Printing method	Lead (ppm)
1a	Modified letterpress	1
1b	Modified letterpress	69
2	Offset	1
3	Offset	1
4	Offset	2
5	Offset	3
6	Offset	4
7	Offset	12
8	Lead plate	4
9	Lead plate	5
10	Lead plate	6
11	Lead plate	8
12	Lead plate	8
13	Lead plate	9
14	Lead plate	11
15	Lead plate	23

[a]All papers were printed in black and white except 1b, which was a comic section printed in color.

TABLE 2. LEAD CONTENT OF A NEWSPAPER PRINTED BY OFFSET ON VIRGIN AND RECYCLED STOCK

Sample	Lead (ppm)
1. Printed on virgin paper	3
2. Printed on 100% recycled paper	13
3. Black ink	1
4. Unprinted virgin paper	2
5. Unprinted 100% recycled paper	12

Samples for analysis weighed at least 3 g. All the samples were ashed at 500 C for not more than 1 h. The ashed material was digested in 4 ml of a mixture of one part concentrated HNO_3 to three parts distilled water and concentrated to about half the volume. Finally, the concentrate was diluted in distilled water and either centrifuged or filtered *(20)*. Filtrates were analyzed for lead with a Perkins Elmer Model 303 atomic absorption spectrophotometer. The limit of detection for the method of assay was 1 part of lead per million parts of the original sample, e.g. paper, ink, or dried plant material.

RESULTS AND DISCUSSION

Newspaper

The lead content of newspapers varies with printing methods and color of ink (Table 1). A newspaper printed with black ink by modified letterpress (lead-free plates) contained 1 ppm lead (sample 1a), but the comic section of the same newspaper printed with colored inks contained 69 ppm lead (sample 1b). Ink color clearly mediates the lead content of newspaper.

Lead in newspapers printed with black ink by offset (lead-free process) ranged from 1 to 12 ppm (samples 2 to 7). Newspapers printed with lead plates and black ink ranged from 4 to 23 ppm (samples 8 to 15). Clearly, newspapers printed with black ink and lead plates were often richer in lead than those printed with black ink by offset or modified letterpress. However, the ranges of lead content among newspapers printed with black ink by the three methods contained either common or similar values, so factors other than printing method probably contributed to the lead content of the newspaper.

During this investigation, two newspapers changed from lead plates to offset or modified letterpress without changing inks. For one, the lead content decreased from 8 to 1 ppm (samples 12 and 1a; Table 1), and the other decreased from 23 to 12 ppm (samples 15 and 7; Table 1). Again factors other than the printing method or ink clearly contributed to the lead content of the newspaper because the decrease upon changing from lead plates to offset printing or modified letterpress was different between the two newspapers.

Samples of newsprint and ink were obtained from a newspaper that changed its printing method from lead plate to offset (Table 2). The same ink was used in both methods. A four-fold increase from 3 to 13 ppm lead was observed between an issue printed on virgin and one printed on recycled paper (samples 1 and 2). The ink contained little lead (sample 3), and a comparison of unprinted virgin and recycled paper confirmed that the recycled paper was indeed 6-fold richer in lead than virgin paper (samples 4 and 5). The lead in recycled newsprint or other paper would, of course, vary with the lead in the wastes. Clearly, the type of newsprint, the color of ink, and the printing process all contribute to the lead content of newspapers.

Paper products other than newspapers, such as magazines or other paper printed with leaded inks may also be recycled. The inks used in printing colored inserts in magazines contained up to 29,000 ppm lead *(9)*, hence magazines may raise the lead content of the recycled stock. Thus, we must conclude that recycled paper made primarily from paper printed by lead type or with colored inks might contain more lead than that made from paper printed by offset, modified letterpress, or with black ink. However, the lead on or in paper must also be considered in subsequent applications of printed or unprinted, virgin or recycled, paper stock (Fig. 1).

Food packaging

Because printed and unprinted containers are widely used in food packaging and vending, the lead content of paper used in food packages (Table 3) was determined. The lead content of the packages ranged from 2 to more than 10,000 ppm (Table 3). Some

TABLE 3. LEAD CONTENT OF PAPER PACKAGES

Sample	Color[a]	Printing[b]	Lead (ppm)
1. Kraft grocery bag	tan	−	2
2. Interior of bread crumb container	tan	−	2
3. Towel	white/tan	+	2
4. Lollypop stick	white	−	3
5. Plate	white	−	3
6. Tray	white	−	6
7. Spaghetti dinner box	blue/white	+	7
8. Straws	mixed	+	8
9. Rice dinner box	blue/yellow/red	+	10
10. Frankfurter tray	white/orange/green	+	13
11. Candy wrapper	red/yellow	+	14
12. Flour bag	white/orange/blue/red	+	20
13. Candy wrapper	yellow/white/red	+	29
14. Candy tray	brown	−	42
15. Spaghetti box	yellow/red/black/green	+	50
16. Candy divider	brown	−	58
17. Bubble gum trading card (from sample 22)	yellow/red/blue	+	88
18. Frozen confection wrapper	white/red	+	481
19. Straws (confection filled)	mixed	+	488
20. Bread wrapper (waxed)	white/red/black	+	1062
21. Sugar bag (outer wrapper)	yellow/red/black	+	1450
22. Bubble gum wrapper	blue/red/white	+	1650
23. Bread inner liner (waxed)	yellow/red/brown	+	6125
24. Bread wrapper (waxed)	white/red/green/black	+	6500
25. Candy bar wrapper	yellow/white/red/brown	+	7125
26. Bakery confection wrapper	yellow/blue/red/brown	+	10,125

[a]Color listed in order of predominance.
[b]+ Indicates printing, − indicates absence of printing.

of the items often touch the mouths of children, e.g. straws filled with confection, lollypop sticks, and candy bar wrappers. For many of the printed samples, e.g. candy wrappers, frozen confection wrappers, inner liners of bread packages, and bakery confection wrappers, the food product touched the unprinted inner surface of the paper. Unprinted materials like paper trays, plates, candy trays, and candy dividers directly touch the food and are not separated from the product by an intervening layer of paper or by a protective barrier like foil or a waxed liner. Although several items contained less than 20 ppm, samples 18 through 26 (Table 3) were substantially richer in lead and included wrappers from a frozen confection, bread, candy, gum, a bakery confection, a bread inner liner, confection-filled straws, and the outer layer of a sugar bag.

Although lead on or in the packaging materials generally increased with the number and diversity of colors (Table 3), some highly colored packages (e.g. samples 9 and 13) contained less lead than others (e.g. sample 23). Packages printed with yellow were substantially higher in lead than most other samples. One yellow box (sample 15) had little lead (50 ppm), but this might be dilution of the lead in the ink by the thick cardboard. Some white packages with red printing were also rich in lead (samples 18, 20, 24). The wrapper from a frozen confection, sample 18, was especially revealing because the white, or unprinted, part of the wrapper contained only 10 ppm lead compared with 481 ppm in the total wraper. Only about 5% of the wrapper was printed in red, the only color used. Thus, relatively little ink can increase the lead content of packages. Some unprinted packaging materials contained up to 58 ppm lead (samples 14, 16) but unprinted samples numbered 1, 2, 5, and 6 did not exceed 6 ppm. The paper was probably the principal determinant of lead concentration in unprinted packaging material. Clearly, the lead on or in packaging materials, like colored magazine pages *(9)*, is primarily associated with ink. Letterpress magazine inks, especially yellow with 29,000 ppm and red with 4100 ppm lead, were especially rich in lead *(9)*. Similar inks may contribute to the relatively rich lead content of the yellow and red samples listed in Table 3, and to the lead in other samples having yellow and red incorporated with other colors. Moreover, lead on food packages is not restricted to paper. For example, printed polyethylene food bags contained up to 24,000 ppm of lead *(11)*.

Most foods are separated from the printing by an

intervening barrier, but some candies contact unprinted paper having about 50 ppm lead (samples 14 and 16; Table 3). Additionally, printed frozen confection wrappers become torn or unglued because of moisture, and we have observed the product in direct contact with the printing. Furthermore, a printed trading card with 88 ppm lead (Table 3) was found in direct contact with chewing gum. Although the abrasion of printed surfaces by food or machines during packaging and marketing might possibly contaminate food, we found less than 1 ppm lead on a candy and a bread sample in a companion experiment. Nevertheless, because the source of the low levels, i.e. <1 ppm, of lead in food *(2)* is unclear, the possibility remains that food might acquire lead from packaging (Fig. 1).

Animal feed

Because ruminants can degrade cellulose, several investigators have tested the feeding of waste paper (Fig. 1) to cattle and sheep *(5, 6, 7, 17, 19)*. Cattle apparently tolerate about 24% paper in the ration before gain is substantially reduced. Additionally, colored magazine paper inhibited dry matter disappearance attributable to isolated rumen bacteria *(16)*. Although the decreased digestibility of colored, compared with brown, paper was attributed to organic constituents in the inks, it is possible that lead, e.g. from letterpress inks *(9)*, suppressed the rumen bacteria because 0.1 ppm lead in the growth medium prevents multiplication of *Escherichia coli (21)*.

The movement of lead to meat from a test ration supplemented with waste paper was explored with beef cattle *(5)*. When cattle were fed a ration with 15 ppm lead that was formulated by substituting a total of 24% paper with 60 ppm lead into the feed no significant (>0.2 ppm) accumulations of lead were observed in the edible muscle.

Although the lead in printing inks might inhibit rumen bacteria, and the feeding of waste paper slowed the weight gain of beef cattle, the present rates of feeding waste paper with 60 ppm lead to cattle do not contribute significant amounts of lead to meat used as human food. However, we get about 30% of our lead from meat *(2, 8)*, so even seemingly insignificant increases in the amount of lead in meat from feeding paper might raise the percentage intake of lead from this source, if other sources of dietary lead remained unchanged.

About 8.2% of human dietary lead comes from milk *(2, 8)* that normally averages 0.04 ppm lead *(18)*. To our knowledge, only Mertens et al. *(17)* have published on the feeding of newspapers to dairy cattle. Although newspapers contributed up to 20% of the diet in a test ration, neither the lead content of the feed nor of the milk was reported. However, we estimate from other published data *(5)* that feeding a ration with 24% waste paper at 60 ppm lead to a 450-kg dairy cow would cause a lead intake of about 0.04 mg lead/kg^{-1} bodyweight/day^{-1}. Interestingly, milk from dairy cattle fed up to 0.28 mg lead/kg^{-1} bodyweight/day^{-1} from lead arsenate, or 7-fold more lead than that ingested from a ration with waste paper *(5)*, contained less than 0.05 ppm lead *(15)*. Therefore, the possibility of enrichment of milk with lead by feeding cows a ration contaminated with lead from waste paper seems small.

Garden mulch

Paper has been used as mulch by vegetable growers. Gardeners often use discarded newspapers and magazines around plants to inhibit weeds, and conserve moisture *(14)*. Since lead salts are more soluble in acid than in alkaline and neutral solutions, acid soils when moistened with acid rainfall should leach the maximum lead. In the Northeast during the growing season, the rain has pH of 4.4 to 5.0 *(26)*.

To test whether lead could migrate from a newsprint mulch to plants, six 7-cm squares of newsprint with either 2 ppm lead (control) or 12 ppm lead were placed on soil in pint pots in which onion and lettuce seedlings, and radish and corn seeds were planted. The edges of a 30-mm hole through the center of the paper squares were sealed to a plastic collar to allow emergence of the plants and ensure that all water reached the soil through six layers of paper mulch. During four weeks commencing either at the planting of seed or transplanting of seedlings, 6 inches of water adjusted with HCl to 10^{-3}N and pH 4 to 4.3 were applied to the surface of the paper mulch layer in daily increments of 100 to 150 ml. The soil was sufficiently dry so that all water was retained.

At the end all plants were analyzed, but no uptake of lead was observed. The paper mulch that remained after treatment was tested, and it was found that negligible amounts of lead had been leached from the paper. Since the water added was similar to eight weeks of rainfall, we conclude that negligible lead will reach soil and plants from newspaper mulch.

Implications and conclusions

Although more attention has been given to lead ingestion from paint *(1)*, some lead is ingested from food *(2)*, and subclinical levels of lead are detrimental to animals. For example, rats fed 40 ppm lead in milk and feed displayed hyperactivity *(23)*, and increased lead in the blood (below the clinical level) was associated with hyperactivity in children *(4)*.

Further, a single injection of 1 mg of lead acetate elicited increased sensitivity of mice to bacterial endotoxin *(24)*, and 0.1 mg lead nitrate per day for 30 days increased susceptibility of rats to *Salmonella typhimurium (12)*. The present levels of environmental lead might also be deterimental to mental health *(3)*. Further, Hernberg and Nikkannen *(13)* showed a close negative correlation between lead in blood and the activity of delta-aminolevulinic dehydrogenase in 26 individuals never occupationally exposed to lead, and they reasoned that current levels of environmental contamination by lead can produce a measurable biochemical alteration in man.

Although about two-thirds of the daily intake of lead of humans is attributable to food *(22)*, many of the sources of lead in food are not yet understood. The use and reuse of printed and unprinted paper may inadvertently contribute lead to the food supply. We have examined paper because it is widely used as food containers and wrappers, because paper is being tested as feed for ruminants and because paper is used to mulch plants.

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ELECTRONIC COUNTING OF SOMATIC CELLS IN FARM BULK TANK MILK

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ABSTRACT

Electronic counting of somatic cells in farm bulk tank milk, using chemical fat dispersion has been studied. The method used was the Pearson et al. modification of the original Tolle et al. procedure. It was found that fixation of the original sample with formalin was critical, and that with bulk tank milk this was best accomplished by the use of buffered formalin at 30-32 C for 24 h. No significant differences could be found, in samples kept up to 48 h at 4 C, between adding milk to buffered formalin and buffered formalin to milk. Good agreement was found between the DMSCC made on the day samples were taken, and electronic counts obtained following 24 and 48 h refrigeration storage before fixation, and 24 and 48 h following fixation after 24 h refrigeration. Correlation coefficients were + 0.876, + 0.924, and + 0.853, + 0.870, respectively. Replicate counts on both fresh bucket and bulk tank, and single counts on replicate samples from bulk tanks yielded coefficients of variation between 2.98% and 9.69%. The procedure shows promise of providing rapid, accurate somatic cell counts on large numbers of bulk tank milk samples.

Somatic cells in milk may be counted electronically by several published procedures, which can be divided into *(a)* those employing a centrifuge to remove the fat globules *(2, 8, 10)*, and *(b)* those in which chemical dispersion of fat is utilized *(12)*. Because it is more rapid, more economical in cost, equipment and labor, and is adaptable to automation, chemical dispersion of fat is to be preferred *(3, 5)*. While the method of Tolle et al. *(12)* is widely used in Europe, there is but one report of its use in North America *(7)*.

Tolle et al. *(12)* obtained a high degree of correlation between electronic and microscopic counts, and this was confirmed by Philpot and Pankey *(7)*, although the latter investigators encountered variations ascribed to relatively minor procedural changes.

We have used the method of Pearson et al. *(6)* for several years to estimate somatic cell counts in freshly drawn quarter and bucket milk from cows on a number of experiments, with results that have not been different from those obtained by the centrifuge technique *(8)* used previously. In attempting to apply the procedure to farm bulk tank samples we have examined the relationships of the DMSCC *(4)* on the day the samples were taken to the electronic counts made after various storage times. The original publication stated that samples might be stored for 3 days in the refrigerator following addition of formalin fixative *(12)* and both refrigeration storage for up to 2 weeks, and 5 to 7 days at 22 C have also been said to be satisfactory, although no data were given to support these *(6)*.

The cost of the equipment for doing electronic counts is such that centralized laboratories are preferable, in which large numbers of samples can be handled, samples which may have come sometimes from long distances and consequently are of different ages. Depending on the sampling system in a given area it may or may not be possible to add formalin to the samples at the time they are taken. We have therefore, investigated not only the stability of counts with storage after addition of formalin, but also the effect on counts of storage before formalin is added.

The work reported here was done over a period of approximately 2 years. At the beginning the method was as outlined by Pearson et al. *(6)*, with certain details recommended by Dr. Phipps (personal communication). In the later or second part procedures were modified, based on our earlier experience. In both parts we have attempted to compare electronic counts with the DMSCC's *(4)*, and to examine the relationship between the two following storage times both before and after formalin fixation of the milk samples.

MATERIALS AND METHODS

Samples

With the exception of a few samples noted later, all samples were provided to us by fieldmen of the Milk Industries Branch of the Ontario Ministry of Agriculture and Food. They were taken either by drivers of milk transports picking up bulk tank milk from farms, or by fieldmen riding with the drivers. They were from tanks containing four milkings at the time of pick up, and in all instances were delivered to our laboratory in the afternoon of the day on which they were taken. Samples were taken in 12-dram or 16-dram clear plastic vials with plastic snap caps. Samples were kept in ice-water until delivered to our laboratory.

On receipt of the samples in the laboratory, slides were prepared for DMSCC's and the samples placed in the rerigerator at 4 C until divided and fixed. The samples were

subdivided into a number of subsamples of approximately 10 ml, the number dependent on the number of procedural variations being investigated. A single subsample was prepared for each count to be made, as our experience suggests that multiple shaking and sampling of a single bottle or vial results in erratic counts.

Fixation of samples

The original methods of Tolle et al. *(12)*, and Pearson et al. *(6)* called for fixation of milk by addition of unbuffered 10% formalin solution, containing 0.02% eosin, in the ratio of 0.2 ml of fixative to 10 ml of sample. Tolle et al. completed fixation by maintaining the formolized samples at room temperature. In the first part of our work ambient room temperature for 21-24 h was used. In the last parts of our work, because of erratic counts, and a suggestion from Dr. Phipps (personal communication) that buffering the fixative might be useful, the fixative was buffered by the addition of 2.7 ml of molar phosphate buffer, pH 7.0, to 500 ml of eosin-formalin solution. In addition, fixation was carried out at 32 C for 21 to 24 h (for brevity, hereafter, any time within this period is referred to as 24 h)

In most instances the formalin was dispensed into screw-capped glass sample vials before addition of milk. In one trial the formalin was added to milk in the vials, by means of a Cornwall Repipetting syringe[4] with a 19-gauge needle, the tip of which was held below the surface of the milk to ensure rapid dispersion. All vials containing milk and formalin were inverted two or three times to mix.

Fat dispersing solution

This was composed of 0.9% (W/V) sodium chloride solution, 84.5 parts; Triton X-100[1], 2 parts; 95% ethanol, 12.5 parts; 37% (W/V) formaldehyde solution[2], 1 part. These were mixed and buffered at pH 7.0 with 0.5 molar Tris solution (THAM, primary standard)[2]. The complete solution was filtered through Millipore filters, 0.45 Mμ M.P.S., and stored in 5-liter plastic containers at room temperature.

Procedure for fat dispersion

At the appropriate time, fixed samples were shaken on a Vortex Jr. mixer for approximately 10 sec and left for 2 to 3 min to allow the foam to disperse. Then, using a Fisher Automatic Diluter[2], O.1 ml of milk was dispensed with 9.9 ml of the fat dispersing solution into suitable capped containers, which in the beginning were 1 oz glass vials with plastic screw-caps, washed between uses, but later Falcon disposable clear plastic test tubes[4], 17 × 100 mm, with caps, were used. These were held in stainless steel racks, and heated in a water bath at 80 ± 1 C for 10 min. They were then cooled to room temperature by standing in cold tap water for 5 min.

Electronic counters

The first part of the work was done using a Coulter Model B counter, equipped with a 70 mμ orifice tube accurately calibrated and adjusted to count all particles 54.5 mμ^3 and larger in 50 μl. In later work a Coulter Model TA was used equipped with a 100 mμ orifice tube which was adjusted to count all particles 44.6 mμ^3 and larger in 0.5 ml. Both instruments were used with the same measuring manometer on an external sample stand, equipped with a Unopette Flow-through Cell[4] (Bull, 1967). No corrections were made in the counts for coincidence.

DMSCC

These counts were made by the National Mastitis Council Method *(4)*. A total of 177 counts were analysed by the method outlined by Smith *(11)*. The average error mean square/mean and film mean square/mean were + 0.8397 and + 0.1128, respectively.

Plan of experiments

1. On receipt at the laboratory, after slides were prepared for DMSCC's, 78 samples, in groups of 16-25 were divided into four subsamples. One of these was mixed with unbuffered formalin immediately (0 h), while three were placed in the refrigerator at 4 C. One of these was removed from the refrigerator at each of 24, 48, and 72 h and fixed as described. All formolized samples were held at ambient room temperature for 24 h, at which time they were treated for dispersion of the fat, and electronic counts made.

2. On receipt at the laboratory, after slides were prepared for DMSCC's, 100 samples were each divided into three subsamples, in vials containing unbuffered formalin. These were maintained at ambient room temperature, and one subsample treated and electronic counts made at each of 24, 48, and 72 h.

These experiments constituted what has been referred to as early work. At this point the results were analysed, and the technique modified as already described, following some further unreported trials. The modifications were *(a)* use of buffered formalin and *(b)* fixation in the incubator at 32 C.

3. To determine the accuracy and reproducibility of the methods adopted, electronic counts were made as follows: *(a)* 36 counts on a single bucket milk sample from an infected cow, *(b)* 36 counts from a single sample of low-cell count farm bulk tank, *(c)* one count on each of 16 samples taken from a single low cell count bulk tank by each of three sampling methods, and *(d)* one count on each of 15 samples taken by straws from a single farm bulk tank containing high cell count milk. All samples were fixed on the day they were taken and after 24 h at 32 C fat was dispersed and counts made.

Up to this point cell counts were determined using the model B Coulter counter. All work after this was done using the model TA Coulter counter.

4. 186 samples, in groups of 16-25, were obtained. After slides were prepared for the DMSCC's, each was divided into two subsamples, one of which was formolized after 24 h at 4 C, the other after 48 h at 4 C. These were treated and counted after 24 h at 32 C. From 125 of these samples an additional subsample was made, this being formolized after 24 h at 4 C, and counted after fixation at 32 C for 24 h followed by storage at ambient room temperature for 24 h. Thus, two storage times before fixing were compared and two storage times after fixing.

5. Following suggestions that addition of formalin to milk, rather than milk to formalin, might cause protein coagulation and precipitation, and hence erratic counts, 5 groups of samples totalling 76 were each divided into two subsamples. One of each was added to buffered formalin in a vial, while milk in the other received buffered formalin by means of a syringe as described earlier. 38 pairs of subsamples were

[1]British Drug Houses, Toronto, Canada
[2]Fisher Scientific Co., Toronto, Canada
[3]Falcon Plastics, Oxnard, California, U.S.A.
[4]Becton, Dickinson and Co., Rutherford, N.J., U.S.A.

TABLE 1. REPRODUCTIBILITY OF COUNTS BY ELECTRONIC METHOD

Type of milk	No. of samples	No. of tests per sample	Mean Count ± S.D. (× 10^3)	Co. of var. (%)
Fresh bucket milk from infected cow	1	36	1032.5 ± 30.7	2.98
Bulk tank, low count	1	36	191.0 ± 10.96	5.74
As above, taken with straws[1]	16	1	235.7 ± 14.1	5.99
As above, taken with dipper[1]	16	1	226.4 ± 21.9	9.69
As above, large sample subdivided[1]	16	1	237.7 ± 13.25	5.57
Bulk tank, high count	15	1	1138.8 ± 93.5	8.21

[1]All 48 samples from a single tank.

TABLE 2. THE EFFECT OF METHOD OF ADDITION OF FORMALIN FIXATIVE TO MILK SAMPLES ON THE ELECTRONIC COUNT AND ITS RELATIONSHIP TO THE DMSCC

	38 samples fixed after 24 h at 4 C		38 samples fixed after 48 h at 4 C	
	Milk added to formalin	Formalin added to milk	Milk added to formalin	Formalin added to milk
r	+0.973	+0.970	+0.973	+0.966
Regression equation	Y = 1.199 X −51.44	Y = 1.248 X −53.52	Y = 1.254 X −94.56	Y = 1.201 X −47.75
S.E. of Reg. coefficient	±0.048	±0.052	±0.049	±0.054
S.D. about Reg. line (× 10^3)	±63.02	±65.50	±67.26	±75.75
Mean count ± S.D. (× 10^3)	506.39 ± 216.8	488.05 ± 207.8	547.47 ± 224.1	532.65 ± 232.2

stored for 24 h at 4 C before formolizing and 38 pairs for 48 h at 4 C. Counts were made after fixation for 24 h at 32 C.

Statistical analysis

Correlation coefficients and regression equations were determined as described by Quenouille *(9)*. Significance of differences between mean counts were as described by Arkin and Colton *(1)*.

RESULTS

Comparison of DMSCC on fresh milk with electronic counts following sample storage at 4 C for 0, 24, 48, and 72 hours before fixing

The correlation coefficients (r), and regression equations of microscopic counts (Y) on electronic counts (X) for the 4 storage periods are shown in Fig. 1. The mean counts ± S.D. for DMSCC at 0 h, and electronic counts at 0, 24, 48, and 72 h were 10.25 ± 5.0 × 10^5, 10.27 ± 4.5 × 10^5, 9.59 ± 5.0 × 10^5, 9.82 ± 5.8 × 10^5, and 16.51 ± 20.44 × 10^5, respectively. While there was no significant change in the mean counts up to 48 h storage before fixing, there was a steady reduction in the correlation coefficients and the regression coefficients. After 72 h storage before fixing no prediction of the DMSCC on fresh milk from the electronic count was possible.

Comparison of DMSCC on fresh milk with electronic counts following sample storage at ambient room temperature for 24, 48, and 72 hours after fixing

The correlation coefficients (r), and regression

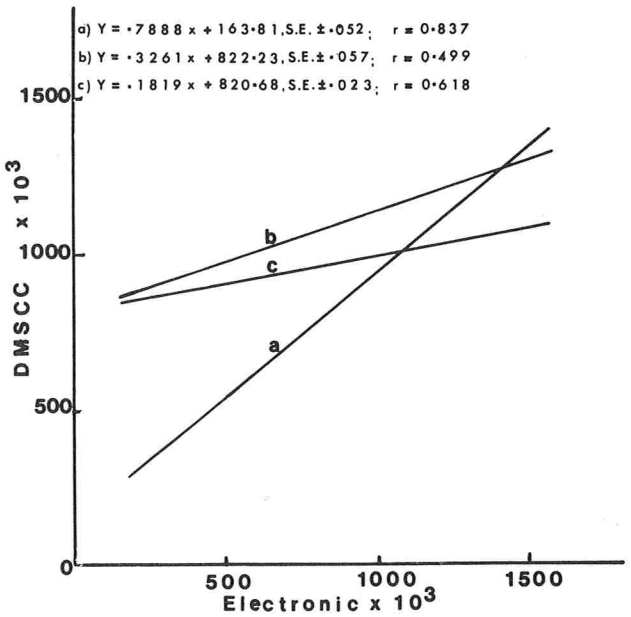

Figure 1. Regression of DMSCC on Electronic Counts in 78 samples of bulk tank milk. .02% unbuffered formalin added on day samples were taken; counts made after 24 h (*a*), 48 h (*b*), or 72 h (*c*) at room temperature.

equations of microscopic counts (Y) on electronic counts (X) for the three periods are shown in Fig. 2. The mean counts of the 78 samples for DMSCC at 0 h, and electronic counts after storage at room temperature for 24, 48, and 72 h were 12.12 ± 3.9 × 10^5, 13.29 ± 4.2 × 10^5, 11.96 ± 6.0 × 10^5 and 21.52 ± 13.4 × 10^5, respectively. It can be seen that

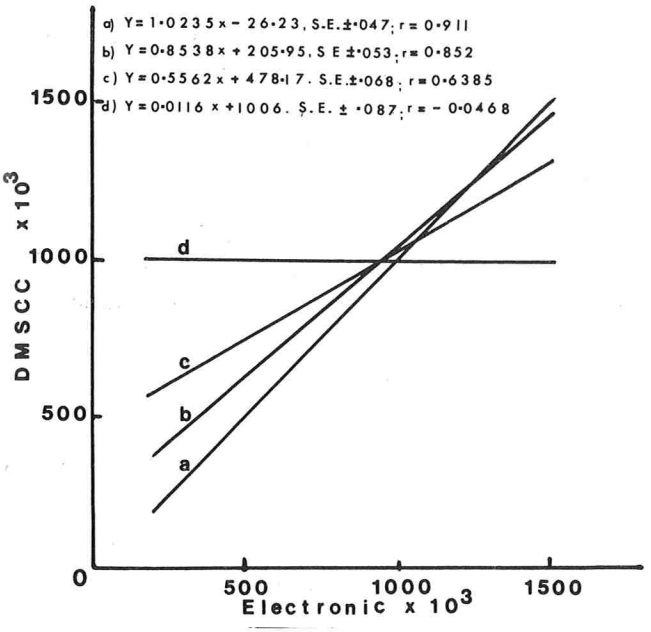

Figure 2. Regression of DMSCC on Electronic Counts in 100 samples on bulk tank milk. .02% unbuffered formalin added on day samples were taken *(a)*, or after 24 h *(b)*, 48 h *(c)*, 72 h *(d)*. Counts made after 24 h at room temperature after addition of formalin.

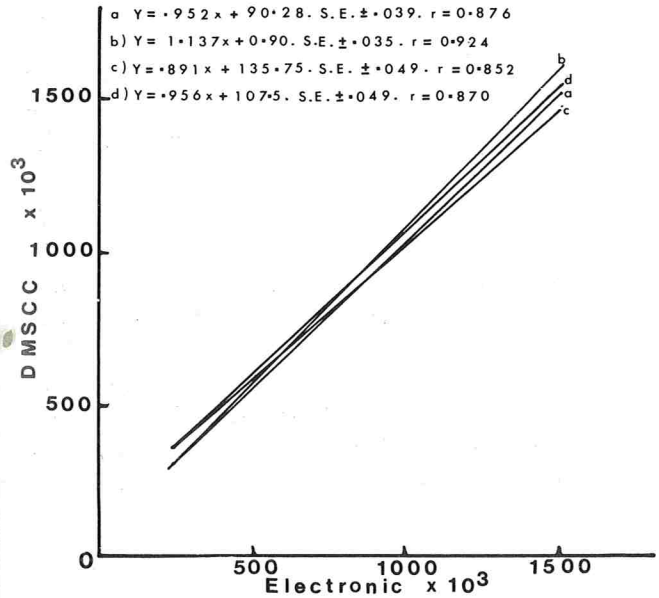

Figure 3. Regression of DMSCC on Electronic Counts in bulk tank milk. .02% buffered formalin added to 186 samples 24 h after samples were taken *(a)* or 48 h after *(b)* Buffered formalin added to 125 samples 24 h after samples taken, and counts made after 24 h at 32 C (*c*) or 24 h at 32 C plus 24 h at room temperature (*d*).

changes in the relationships between original microscopic counts and electronic counts following storage at room temperature after fixing were more extreme than those found following storage in the refrigerator before fixing.

These data did not support the expectations which we had previously for the technique, and thus, as explained earlier ,modifications were made in the methods used, modifications that appeared to overcome some of the erratic results encountered.

Reproductibility of modified methods

Results of several trials to determine reproductibility of the procedure are shown in Table 1. Since the variability shown includes that deriving from the original sampling together with that from the fixing, fat dispersion, and cell counting itself the modified procedure appeared to be quite satisfactory.

Comparison of storage times of samples both before and after fixing, using modified methods .

The correlation coefficients and regression equations of microscopic counts (Y) on electronic counts (X) for two storage times before addition of fixative, and two storage times after addition of fixative are shown in Fig. 3. The mean counts ± S.D. of 186 samples for DMSCC at 0 h, and electronic counts when fixed at 24 h and 48 h were 6.746 ± 3.54 × 10^5, 6.128 ± 3.27 × 10^5, and 5.929 ± 2.88 × 10^5 respectively.

The regression coefficients for the two storage times before fixation, *(a)* and *(b)* in Fig. 3, are significantly different (t=3.53, p=> 0.01), although the mean difference between the 24-h and 48-h electronic counts (119 × 10^3/ml) is not significant at the 5% level.

The mean counts ± S.D. of 125 samples for DMSCC at 0 h and electronic counts at 24 and 48 h after addition of fixative were 7.142 ± 3.73 × 10^5, 6.481 ± 3.57 × 10^5 and 6.345 ± 3.40 × 10^5 respectively. The regression coefficients for the two storage times after fixation are not significantly different (t=0.932), nor is the mean difference between electronic counts at 24 and 48 h after fixation (13.6 × 10^3/ml) at the 5% level.

Comparison of methods of adding formalin to milk samples

The effects of adding formalin to milk and milk to formalin were examined after samples were stored at 4 C for 24 h and 48 h. Results are shown in Table 2. While at both storage times the mean counts were slightly lower in these samples to which fixative was added to milk, the differences were very small and not statistically significant at the 5% level, nor are the regression coefficients.

DISCUSSION AND CONCLUSIONS

In the course of the work reported here we have investigated some variables, both in the handling of bulk tank samples and in treatment in the laboratory, in employing the chemical dispersion of fat in

electronic counting of somatic cells in farm bulk tank milk.

Throughout this work we have used the long (24 h) period for fixation, as originally recommended by Tolle et al. *(12)*. Although, rapid (30 min) fixation has been used *(7)*, this was suggested as an alternative where testing is urgent *(6)*. In a large scale program where several hundred samples must be counted in a day, unless some mechanical handling could be developed, the short fixation time is much less practical than the long one. It is more convenient to add formalin fixative to all samples that have reached the laboratory at a given time each day and store them in an incubator overnight, rather than having to use extra equipment and help for short time high temperature fixation during the same time that fat dispersion and counting is being done. In using the method for counting cells in fresh individual cow quarter or bucket milks we had experienced no difficulty with unbuffered formalin, nor with overnight fixation at ambient room temperature. However data from the present work indicate that farm bulk tank milks, as received in our area, required buffer in the formalin, and preferably a constant incubator temperature. Thus, our results confirm the observations of Philpot et al. *(7)* and Tolle et al. *(12)* that duration and temperature of fixing are critical. It would appear that this is possibly the most critical step in the counting procedure.

By use of the fixation technique as outlined we have been able to obtain a high degree of reproductibility of count in a variety of milks. Examination of the data suggest that a large proportion of the variation obtained probably resulted from sampling differences, since sampling with a dipper produced a significantly lower count and a higher coefficient of variation than did sampling with straws.

Of considerable benefit to those laboratories that receive samples from some distance, or that encounter other delays in shipments, is the observation that there was little or no difference in count in samples kept 24 and 48 h at refrigeration temperatures before fixing, for while the mean count of those samples fixed at 48 h was slightly lower the difference was not significant. Also, when 24-h old samples were fixed they could be treated for fat dispersion and counts made up to 48 h later without significant change in count. Some other data, not reported here, indicate that this period may be extended to 72 h with a slightly lower count, which is, however, not significantly different from the one at 24 h. Our experience agrees with that of Philpot and Pankey *(7)* that storage after initial fixing is better done at room temperature, as even short periods in the refrigerator cause erratic, unpredictable results.

We were unable to show any significant difference in count resulting from addition of formalin fixative to milk rather than vice versa. Presumably if formalin were merely dropped onto the top of a sample of milk some coagulation and precipation of protein could result, but where the fixative was added to the milk in a manner designed to give quick dispersion, there was no apparent problem, even when the milk was held for 48 h before addition of the fixative.

The objective for any counting procedure used in a quality control program must be for the counts obtained to reflect closely the actual somatic cell count in the milk at the time the samples are taken. Since the recognized standard against which other methods should be compared is the DMSCC *(4)* we have compared somatic cell counts determined by this method on the day milk samples were taken with the counts determined by the electronic procedure after storage of samples both before and after fixation. Our data indicate a very high correlation between counts determined by the two procedures, and indicate that by suitable regression equations it is possible to estimate the original DMSCC with a good degree of accuracy, since, using the regression equation $y = 90.28 + 0.952x$, the distribution of the differences between estimated and actual DMSCC had a S.D. of $\pm$ 119 $\times$ 10^3/ml, in a group of 76 samples with actual initial DMSCC ranging from 230 $\times$ 10^3/ml to 150 $\times$ 10^4/ml.

The procedure described by Pearson et al. *(6)*, modified by the use of buffered formalin, is accurate and reproducible. It shows promise of providing rapid, accurate counts on a large number of samples, since they may be stored for up to 48 h before fixation, and when fixed following 24 h storage can then be held for another 48 and probably 72 h before the dispersion of fat and actual counting of cells. These factors make for ease of fitting a counting program into the normal 5-day week of a service laboratory.

ACKNOWLEDGMENTS

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BOOK REVIEW

APPLIED FOODSERVICE SANITATION. *Published by the National Institute for the Foodservice Industry, 120 S. Riverside Plaza, Chicago, Ill., 1974, 224 p., $12.00.*

Anyone responsible for preparing and serving food must be acutely aware of the potential for an outbreak of foodborne illness. Food, if handled carelessly, can be easily contaminated with toxic materials or bacteria. Bacteria can grow in the food causing spoilage and illness. A single lapse in the application of good hygienic practices can result in a disastrous outbreak of food poisoning. Management has the responsibility for training its personnel in food sanitation. To aid supervisors in the training of foodservice workers, the National Institute for the Food Service Industry (NIFI) has prepared a text book entitled *Applied Foodservice Sanitation.* The book is divided into five parts. Part one devotes three chapters to sanitation and health and introduces the reader, in very simple terms, to the world of microorganisms and to the factors that favor or discourage their growth. The point is made here and elsewhere in the book that one of the most serious hazards lies in holding foods at moderate temperatures which enable bacteria to grow. Also, the point that clean is not necessarily sanitary is stressed. This section also contains a tabulated summary of some of the more commonly ocurring foodborne illnesses. In part two on sanitary food and food handling, the health and personal habits of the foodhandler are discussed as they relate to the safety of food. Part three is restricted to problems associated with maintaining a safe food environment. The designing of sanitary facilities and equipment, good sanitizing procedures, and pest control are explained. The point is made that a clean and sanitary establishment is the result of a planned program, properly supervised, and followed on schedule. The fourth section is short but pertinent; in it is a discussion of the response of the customer to an eating establishment and the suggestion is made that the manager view the facility through the eyes of the customer. The last section deals with such management responsibilities as employee training, regulations, and standards. In an appendix, guidelines for a self-inspection program are given.

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INHIBITION OF BACTERIA BY SOME VOLATILE AND NON-VOLATILE COMPOUNDS ASSOCIATED WITH MILK

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ABSTRACT

Nutrient broth inoculated with *Escherichia coli* was dispensed into epoxy-lined aerosol cans. Twenty-five milk-associated volatile or non-volatile compounds were then added individually to cans to yield final concentrations of 1, 10, 100, and 1000 ppm of the compound. Compounds used included fatty acids (formic, acetic, butyric, hexanoic, octanoic, and decanoic acid), aldehydes (formaldehyde, acetaldehyde, propionaldehyde, and glyoxal), ketones (acetone, 2-butanone, and diacetyl), amines (propyl- and hexylamine) alcohols (furfurol and methanol), sulfur compounds (methylsulfide, methylsulfone, methanethiol, and ethanethiol), acetronitrile, chloroform, ether, and ethylenedichloride. Bacteria were enumerated at intervals during incubation at 37 C.

Fatty acids at a concentration of 10 ppm and sometimes at 1 ppm were inhibitory to *E. coli;* formic acid was most inhibitory. Formaldehyde was more detrimental to *E. coli* than glyoxal, acetaldehyde, or propionaldehyde. The lower concentrations of aldehydes (10 and 1 ppm) were only marginally inhibitory. Diacetyl was most inhibitory of the ketones tested. Acetonitrile (10 ppm), chloroform (10 ppm), ether (100 ppm), ethylenedichloride (10 ppm), and methylsulfone (10 ppm) caused statistically significant inhibition of *E. coli.* Ethanethiol was more detrimental than methylsulfide or methanethiol. Generally, amines (1 ppm) were more inhibitory than alcohols.

Raw and heated milks contain numerous volatile and other compounds. We listed some of them in an earlier report *(8)*. The effect of such compounds on growth of bacteria has received only limited attention. Hedgecock and Jones *(6)* noted that diacetyl inhibited some gram-negative bacteria. Singh et al. *(12)* found that *Escherichia coli* was inhibited by the diacetyl contained in an aroma-producing starter distillate. Fatty acids also can inhibit *E. coli (3, 9, 11)*. Cutinelli *(2)* reported partial inhibition of this bacterium by DL-glyceraldehyde. Formaldehyde and ketones *(1, 4)* and acetaldahyde *(4)* also are detrimental to growth of *E. coli.* Mukherjee and Roy *(10)* found that bound formaldehyde had no bacteriostatic or bactericidal effect against *E. coli.* Vapors of some volatile anaesthetics, including chloroform and diethylether, in the presence of controlled concentrations of humidity and 0_2, reduced the survival time of *E. coli (7)*. Kulshrestha and Marth *(8)* tested 27 milk-associated volatile and non-volatile compounds against *E. coli* by the disc assay method. They reported that aldehydes, diacetyl, amines, and fatty acids inhibited growth of this organism.

The present report deals with results obtained when 25 volatile and non-volatile compounds were tested to determine their effect on growth and/or survival of *E. coli* when the bacterium and the compounds were confined in an air-tight vessel.

MATERIALS AND METHODS

Chemicals

The 25 chemicals used in this study included some of the compounds found to be present in raw or mildly heated milk. Others were representatives of the general classes of compounds reported to be present in such milks. Chemicals with a relatively low boiling point were selected to represent such groups of compounds. Chemicals used and their sources are: acetic acid, E. I. du Pont de Nemours and Company, Inc., Wilmington, Del.; acetaldehyde, chloroform, ether, formaldehyde, and methanol, Mallinckrodt Chemical Works, St. Louis; acetone, University of Wisconsin Stores; acetonitrile, ethylenedichloride, and formic acid, J. T. Baker Chemical Company, Phillipsburg, N. J.; 2-butanone, butyric acid, decanoic acid, furfuryl alcohol, hexanoic acid, hexylamine, methanethiol, methylsulfide, methylsulfone, octanoic acid, propionaldehyde, and propylamine, Eastman Organic Chemicals, Rochester, N. Y.; and diacetyl, ethanethiol, and glyoxal, Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. All chemicals were of the highest purity available commercially. Chemicals were not tested further for purity.

Culture

E. coli ML 30 obtained from Dr. J. Garver, Department of Biochemistry, University of Wisconsin, Madison was used as the test organism for these studies.

Procedure

Nutrient broth was inoculated with sufficient of an 8 to 12-h old culture to yield a final concentration of 10^2 - 10^3 organisms/ml. Fifty milliliters of inoculated broth was aseptically dispensed into sterile epoxy-lined metal aerosol cans (99-ml capacity) supplied by Continental Can Co., Chicago, Ill. The required amount of the proper dilution of the chemical was added to the inoculated broth in the can to yield final concentrations of 1, 10, 100, and 1000 ppm. Cans were immediately sealed using stainless steel caps without dip tubes. Inoculated broth was plated using Plate Count Agar (Difco) to determine the initial number of organisms. Five cans containing each concentration of each chemical were incubated at

[1]Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

TABLE 1. DIFFERENCE IN POPULATIONS OF *Escherichia coli* IN NUTRIENT BROTH CAUSED BY ADDED FORMIC, ACETIC, AND BUTYRIC ACID

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Formic acid[1]	1	(−) 4.21*	(−) 0.19	(−) 2.13*	(−)0.74*	(−)0.37
	10	(−) 5.26*	(−) 0.57	(−) 2.84*	(−)2.59*	(−)1.56*
	100	(−)15.00*	(−)19.89*	(−)24.86*	(−)9.86*	(−)6.23*
	1000	NG[2]*	NG*	NG*	NG*	NG*
Acetic acid[1]	1	(+) 0.26	(+) 0.19	−	(−)0.25	(−)0.37
	10	−[3]	−	(−) 2.98*	(−)1.23*	(−)0.61*
	100	(−) 7.89*	(−)13.83*	(−)10.94*	(−)8.51*	(−)4.16*
	1000	NG*	NG*	NG*	NG*	NG*
Butyric acid[1]	1	(−) 0.53	(+) 0.19	(−) 0.99*	(−)0.49*	(−)0.24
	10	(−) 1.05	(−) 0.19	(−) 2.98*	(−)2.71*	(−)0.86*
	100	(−) 7.37*	(−) 7.58*	(−) 9.80*	(−)6.41*	(−)1.83*
	1000	(−)50.53*	NG*	NG*	NG*	NG*

[1]Control: Log of no. /ml: 3.80, 5.28, 7.04, 8.11, and 8.18 at 2, 5, 8, 11, and 14 h, respectively.
[2]NG: Less than 10 organisms/ml of test liquid.
[3]−: No difference.
*: Population significantly different from control at 5% level.

TABLE 2. DIFFERENCE IN POPULATIONS OF *Escherichia coli* IN NUTRIENT BROTH CAUSED BY ADDED HEXANOIC, OCTANOIC, AND DECANOIC ACID

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Hexanoic acid[1]	1	(−) 4.46*	(−) 2.47*	(−)0.46*	−[2]	(+)0.12
	10	(−) 7.80*	(−) 6.27*	(−)1.83*	(−)0.26	(−)0.50
	100	(−)10.03*	(−)10.08*	(−)6.27*	(−)6.70*	(−)3.49*
	1000	(−)25.35*	(−)69.58*	NG[3]*	NG*	NG*
Octanoic acid[1]	1	(−) 3.62*	(−) 0.19	(−)1.83*	(−)0.39	(−)0.12
	10	(−) 5.01*	(−) 4.56*	(−)3.21*	(−)2.37*	(−)0.62
	100	(−)10.31*	(−) 5.51*	(−)5.35*	(−)5.39*	(−)1.62*
	1000	NG*	NG*	NG*	NG*	NG*
Decanoic acid[1]	1	(−) 1.95*	(−) 3.42*	(−)1.07*	(−)0.13	(−)0.37
	10	(−) 4.46*	(−) 4.37*	(−)1.99*	(−)1.58*	(−)0.62
	100	(−) 8.36*	(−)10.08*	(−)5.50*	(−)4.99*	(−)1.34*
	1000	NG*	NG*	NG*	NG*	NG*

[1]Control: Log of no./ml: 3.59, 5.26, 6.54, 7.61, and 8.03 at 2, 5, 8, 11, and 14 h, respectively.
[2]−: No difference.
[3]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at 5% level.

37 C. Broth in cans attained the incubation temperature in about 3 h. One set of cans with each concentration of chemical represented was used to determine the number of organisms /ml after 2, 5, 8, 11, and 14 h of incubation. The plate count done according to standard methods *(5)* and with Plate Count Agar (Difco) was used to enumerate *E. coli*. All plates were incubated at 37 C for 24 to 36 h. The percentage difference in log of population/ml between the sample and the control was computed to determine the degree of inhibition or stimulation of the test organisms. Duncan's New Multiple Range test *(14)* was used to determine if the numbers of organisms in the samples treated with chemicals were significantly different from that of the control.

RESULTS AND DISCUSSION

Data recorded in Tables 1 and 2 were obtained when various concentrations of six different fatty acids were tested. Of all the fatty acids used only hexanoic acid at 1000 ppm failed to cause 50% or more reduction in numbers of *E. coli* in less than 2 h. At the same concentration formic, acetic, octanoic, and decanoic acid essentially inactivated the test organism in less than 2 h. A similar effect was noted after 5 h with butyric acid and after 8 h with hexanoic acid. A significant reduction in growth was always noted when each of the fatty acids was used at a concentration of 100 ppm. At 10 ppm, formic, hexanoic, octanoic, and decanoic acid almost always caused significant inhibition of *E. coli*. The inhibitory effect caused by formic, acetic, and butyric acid generally increased up to about 8 h and then declined, whereas when hexanoic, octanoic, and decanoic acid were used the effect was greatest in the beginning and then generally declined during the incubation. Only small

but occasionally significant differences in number as compared to the control were noted when 1 ppm of any of the fatty acids was used. Insignificant increases in growth also appeared after 5 h of incubation when 1 ppm of acetic or butyric acid was used. In general, of the fatty acids tested formic acid was most inhibitory to *E. coli*, followed by acetic acid. The remaining four acids had a similar effect on *E. coli* as is evident from data obtained when 100 ppm of the acids were used.

As is evident from data in Table 3, formaldehyde was more active against *E. coli* than were acetaldehyde and propionaldehyde. It caused almost complete inactivation of the organisms in less than 2 h when present at a concentration of 1000 ppm. A similar effect also was caused during later stages of incubation by acetaldehyde and propionaldehyde at the same concentration. With both acetaldehyde and propionaldehyde at 1000 ppm, inhibition increased with time. Formaldehyde even at 100 ppm was quite bactericidal and reduced the numbers of organisms to less than 10/ml in less than 11 h; the degree of inactivation increased with time. Relatively minor but always significant inhibition was caused by 100 ppm of acetaldehyde or propionaldehyde. Only marginal inhibition was noted when 10 ppm of the aldehydes were used, but such inhibition was statistically significant in the later stages of incubation especially when formaldehyde and acetaldehyde were used. None of the aldehydes were significantly inhibitory when used at 1 ppm.

The effect exerted by the three ketones on growth

TABLE 3. DIFFERENCE IN POPULATIONS OF *Escherichia coli* IN NUTRIENT BROTH CAUSED BY ADDED FORMALDEHYDE, ACETALDEHYDE, AND PROPIONALDEHYDE

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Formaldehyde[1]	1	–[2]	(–) 0.20	(+) 0.60	(–) 0.50	(+)0.12
	10	(–) 0.28	(–) 0.60	(+) 0.30	(–) 1.39*	(–)1.61*
	100	(–)34.84*	(–)57.00*	(–)70.45*	NG*	NG*
	1000	NG[3]*	NG*	NG*	NG*	NG*
Acetaldehyde[1]	1	(+) 1.13	–	–	(–) 0.25	(+)0.12
	10	–	(–) 1.00	(–) 0.91*	(–) 1.26*	(–)1.12*
	100	(–) 2.55*	(–) 6.80*	(–) 2.88*	(–) 6.68*	(–)3.35*
	1000	(–)21.53*	(–)46.40*	(–)62.42*	(–)79.07*	NG*
Propionaldehyde[1]	1	(+) 0.28	(–) 0.80	(+) 0.15	(–) 0.13	(+)0.12
	10	(–) 0.28	(–) 1.80	(–) 0.15	(–) 0.88*	(–)0.62
	100	(–) 1.98*	(–) 4.80*	(–) 1.67*	(–) 4.16*	(–)0.87*
	1000	(–)21.81*	(–)47.80*	(–)48.03*	NG*	NG*

[1]Control: Log of no./ml: 3.53, 5.00, 6.60, 7.93, and 8.07, at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
[3]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at 5% level.

TABLE 4. DIFFERENCE IN POPULATIONS OF *Escherichia coli* IN NUTRIENT BROTH CAUSED BY ADDED ACETONE, 2-BUTANONE, AND DIACETYL

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Acetone[1]	1	(–)0.27	(–) 0.38	(–) 0.45	–[2]	(–)0.12
	10	(–)0.81	(–) 2.47*	(–) 1.06	(–) 0.75*	(–)1.10*
	100	(–)2.70	(–) 4.93*	(–) 2.27*	(–) 2.87*	(–)1.96*
	1000	(–)4.85*	(–) 8.92*	(–) 5.75*	(–) 9.11*	(–)3.92*
2-Butanone[1]	1	(+)0.27	(+) 0.19	(–) 0.30	(–) 0.25	(–)0.24
	10	(–)1.35	(–) 2.09*	(–) 1.36	(–) 2.50*	(–)1.96*
	100	(–)3.50	(–) 4.17*	(–) 3.18*	(–) 7.74*	(–)4.65*
	1000	(–)5.12*	(–) 6.45*	(–) 7.56*	(–)11.49*	(–)7.10*
Diacetyl[1]	1	(–)1.89*	(–) 0.95*	(–) 0.45	(–) 0.37	–
	10	(–)5.12*	(–) 2.85*	(–) 1.36	(–) 3.50*	(–)1.84*
	100	(–)9.43*	(–)12.52*	(–)14.83*	(–)16.35*	(–)9.42*
	1000	NG[3]*	NG*	NG*	NG*	NG*

[1]Control: Log of no./ml: 3.71, 5.27, 6.61, 8.01, and 8.17 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
[3]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at 5% level.

TABLE 5. DIFFERENCE IN POPULATIONS OF *Escherichia coli* IN NUTRIENT BROTH CAUSED BY ADDED ACETONITRILE, CHLOROFORM, AND ETHER

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Acetonitrile[1]	1	(−) 3.06*	(−) 2.57*	(−) 3.71*	(−) 0.62	(−)0.24
	10	(−) 8.16*	(−) 4.22*	(−) 5.00*	(−) 2.11*	(−)1.59*
	100	(−)10.46*	(−) 5.32*	(−) 6.57*	(−) 4.59*	(−)3.55*
	1000	(−)11.48*	(−) 6.42*	(−) 7.43*	(−) 6.82*	(−)4.16*
Chloroform[1]	1	(−) 1.79	–[2]	(−) 1.00*	(−) 0.37	(−)0.12
	10	(−) 5.10*	(−) 4.40*	(−) 1.57*	(−) 1.86*	(−)1.59*
	100	(−) 6.12*	(−) 9.36*	(−)10.14*	(−) 6.82*	(−)2.33*
	1000	(−)33.16*	(−)22.20*	(−)20.14*	(−)23.08*	(−)4.28*
Ether[1]	1	–	(−) 0.37*	(+) 0.14	–	(−)0.24
	10	(−) 2.81*	(−) 1.28*	(−) 0.14	(−) 0.50	(−)0.37*
	100	(−) 4.08*	(−) 2.08*	(−) 3.71*	(−) 2.48*	(−)1.35*
	1000	(−) 6.12*	(−) 3.85*	(−) 5.29*	(−) 3.72*	(−)2.33*

[1]Control: Log of no./ml: 3.92, 5.45, 7.00, 8.06, and 8.17 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
*: Population significantly different from control at 5% level.

TABLE 6. DIFFERENCE IN POPULATIONS OF *Escherichia coli* IN NUTRIENT BROTH CAUSED BY ADDED GLYOXAL, ETHYLENEDICHLORIDE, AND METHYLSULFONE

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Glyoxal[1]	1	(−) 0.81	(−) 1.15*	(−) 0.46	(−) 1.13*	(−) 0.74*
	10	(−) 1.35*	(−) 3.64*	(−) 3.53*	(−) 9.02*	(−) 3.70*
	100	(−) 4.04*	(−) 3.83*	(−) 5.68*	(−)13.53*	(−)10.73*
	1000	(−)39.89*	NG[2]*	NG*	NG*	NG*
Ethylenedichloride[1]	1	(+) 1.35	(−) 0.38	–[3]	(−) 0.38	(−) 0.25
	10	(−) 0.27	(−) 3.45*	(−) 0.61*	(−) 5.26*	(−) 0.74*
	100	(−) 1.35*	(−) 8.24*	(−) 3.99*	(−) 8.52*	(−) 1.73*
	1000	(−) 9.43*	(−)13.22*	(−)13.82*	(−)16.67*	(−) 9.25*
Methylsulfone[1]	1	(+) 0.27	(−) 0.77*	(+) 0.31	(−) 0.13	(−) 0.62*
	10	(−) 1.08	(−) 2.49*	(−) 0.77*	(−) 0.88*	(−) 1.23*
	100	(−) 3.23*	(−) 4.02*	(−) 6.61*	(−) 9.40*	(−) 2.47*
	1000	(−) 4.31*	(−) 4.98*	(−) 9.37*	(−)14.54*	(−) 7.15*

[1]Control: Log of no./ml: 3.71, 5.22, 6.51, 7.98, and 8.11 at 2, 5, 8, 11, and 14 h, respectively.
[2]NG: Less than 10 organisms/ml of test liquid.
[3]–: No difference.
*: Population significantly different from control at 5% level.

of *E. coli* is summarized in Table 4. Diacetyl was most inhibitory and also inactivated the organisms in less than 2 h when used at a concentration of 1000 ppm. Marked and always significant inhibition was noted with 100 ppm of diacetyl in the medium. *E. coli* was always inhibited by 10 or 1 ppm of diacetyl; such inhibition was almost always significant with 10 ppm, but only significant early during the incubation when 1 ppm of diacetyl was used. Both acetone and 2-butanone at 1000 ppm caused up to about 10% reduction in growth of *E. coli*. Again the inhibition was significant. With 100 ppm of the chemical less inhibition was noted but it was always significant after 5 h of incubation. Only marginal and inconsistently significant inhibition was caused by 10 ppm of acetone or 2-butanone, whereas at the 1 ppm concentration, there was no significant effect.

Table 5 lists results obtained when acetonitrile, chloroform, and ether were used. Data for acetonitrile indicate that 1000 and 100 ppm of this chemical were virtually equally inhibitory to *E. coli*. When 10 ppm of acetonitrile were used, inhibition was somewhat less but continued to be significant throughout the incubation period. At the 1 ppm concentration limited inhibition was noted, which was significant only during early stages of incubation. Generally, the inhibitory effect of acetonitrile declined with the elapse of time. Chloroform was more inhibitory to *E. coli* than was acetonitrile and the inhibition increased when the concentration of chemical became greater. Chloroform always caused significant inhibition of *E. coli* even when as little as 10 ppm were

present. Only marginal and insignificant inhibition was caused by 1 ppm. Ether was the least effective of the three compounds listed in this table. Inhibition was always significant with 1000 or 100 ppm of this chemical and only occasionally significant when 10 ppm of ether were added to the medium. Only marginal inhibition that generally was insignificant was noted with 1 ppm of ether.

Glyoxal (Table 6) at 1000 ppm was so detrimental to *E. coli* that the organism was inactivated in 5 h or less. Inhibition with 10 or 100 ppm of glyoxal was substantially less but always significant. Ethylenedichloride was more inhibitory than methylsulfone at 100 ppm. Inhibition was minimal but almost always significant when either 10 or 100 ppm of either of these two compounds were used. With 1 ppm of either compound, insignificant inhibition or stimulation was noted.

Table 7 summarizes data obtained when methylsulfide, methanethiol, and ethanethiol were tested. Of the three compounds, ethanethiol was most inhibitory to *E. coli*. When 1000 ppm of ethanethiol were used, inhibition increased with time and terminated in complete inactivation of the organisms in 11 h or less. At 100 ppm this compound was most detrimental at the beginning of the incubation period, but the in-

TABLE 7. DIFFERENCE IN POPULATIONS OF *Escherichia coli* IN NUTRIENT BROTH CAUSED BY ADDED METHYLSULFIDE, METHANETHIOL, AND ETHANETHIOL

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Methylsulfide[1]	1	(+) 1.00	–[2]	(+) 0.33	(+) 0.27*	(+) 0.50
	10	(–) 1.33	(–) 3.75	(–) 0.16	(–) 0.27	(–) 0.12
	100	(–) 3.00*	(–) 6.75*	(–)15.03*	(–) 4.79*	(–) 1.36*
	1000	(–) 5.33*	(–)14.25*	(–)21.08*	(–) 9.03*	(–) 1.86*
Methanethiol[1]	1	–	–	–	–	(+) 0.37
	10	(–) 1.00	(–) 0.25	(–) 0.65*	(–) 0.27	(–) 3.34*
	100	(–) 3.67*	(–) 2.75*	(–)15.36*	(–)11.76*	(–) 7.18*
	1000	(–)13.00*	(–)29.50*	(–)50.98*	(–)45.14*	(–)32.80*
Ethanethiol[1]	1	–	(+) 0.75	(+) 0.49	(+) 0.14*	(+) 0.37
	10	(–) 0.67	(+) 0.25	(+) 0.49*	(+) 0.14	(–) 0.50
	100	(–) 9.67*	(–) 6.25*	(–) 5.07*	(–) 2.87*	(–) 4.83*
	1000	(–)40.67*	(–)60.00*	(–)83.66*	NG[3]*	NG*

[1]Control: Log of no./ml: 3.00, 4.00, 6.12, 7.31, and 8.08 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
[3]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at 5% level.

TABLE 8. DIFFERENCE IN POPULATIONS OF *Escherichia coli* IN NUTRIENT BROTH CAUSED BY ADDED FURFURYL AND METHYL ALCOHOL, PROPYLAMINE, AND HEXYLAMINE

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Furfuryl alcohol[1]	1	(–) 0.77	(–) 2.01*	(–) 1.41*	(–) 1.59*	–
	10	(–) 3.06*	(–) 2.38*	(–) 3.39*	(–) 4.04*	(–)0.85*
	100	(–) 4.34*	(–) 3.84*	(–) 7.77*	(–) 6.00*	(–)1.95*
	1000	(–) 6.12*	(–) 6.95*	(–)12.01*	(–) 8.69*	(–)3.29*
Methyl alcohol[1]	1	–[2]	(–) 1.83*	(–) 3.67*	(–) 2.69*	(–)0.37*
	10	(–) 2.30*	(–) 2.56*	(–) 5.51*	(–) 3.92*	(–)1.58*
	100	(–) 4.08*	(–) 4.39*	(–) 9.75*	(–) 5.63*	(–)2.92*
	1000	(–) 6.38*	(–) 8.96*	(–)13.84*	(–) 9.91*	(–)5.12*
Propylamine[1]	1	(–) 1.28	(–) 0.73*	(–) 2.54*	(–) 2.69*	(–)0.49*
	10	(–) 2.04*	(–) 2.38*	(–) 6.50*	(–) 5.88*	(–)6.70*
	100	(–) 7.91*	(–) 4.02*	(–) 9.89*	(–)11.87*	(–)8.16*
	1000	(–)75.00*	NG[3]*	NG*	NG*	NG*
Hexylamine[1]	1	(–) 1.53*	(–) 2.74*	(–) 3.25*	(–) 4.53*	(–)0.12*
	10	(–) 5.61*	(–) 5.30*	(–) 4.80*	(–) 5.26*	(–)1.83*
	100	(–) 8.67*	(–) 8.59*	(–) 7.63*	(–) 8.08*	(–)3.05*
	1000	NG*	NG*	NG*	NG*	NG*

[1]Control: Log of no./ml: 3.92, 5.47, 7.08, 8.17, and 8.21 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
[3]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at 5% level.

hibition continued to remain significant during the entire period of observation. Generally only marginal and insignificant inhibition or stimulation appeared when lesser amounts (10 or 1 ppm) of ethanethiol were present. However, in one instance slight but significant stimulation of growth occurred at 8 h when 10 ppm of this compound were used. Methanethiol, at 1000 ppm, was rather inhibitory to *E. coli*. This is evident from the substantial (up to about 50%) reduction in population present in treated as compared to control samples. At 100 ppm inhibition was less marked but still significant. At lower concentrations only slight and generally insignificant inhibition was observed. At 1000 ppm methylsulfide was least inhibitory of the three compounds listed in this table and caused up to about 21% reduction in the population of *E. coli* when compared to the control. At lower concentrations (100, 10, or 1 ppm) methylsulfide was about as inhibitory as methanethiol.

Results obtained when furfuryl and methyl alcohol and proply- and hexlylamine were tested are given in Table 8. The data indicate that amines were more inhibitory to *E. coli* than were the alcohols. Hexylamine at 1000 ppm caused complete inactivation of *E. coli* in less than 2 h, whereas propylamine did the same in 5 h. At 100 ppm both amines caused up to about 10% reduction in the population of *E. coli* as compared to the control. The inhibitory effect was less pronounced but almost always significant even when 1 and 10 ppm of either amine were present. Alcohols used always were significantly inhibitory when either was present at a concentration of 1000, 100, or 10 ppm. At 1 ppm both alcohols had marginal and inconsistently significant inhibitory activity.

It is evident from the data in this report that the chemicals tested generally inhibited and often even inactivated to *E. coli*. Although some compounds (fatty acids and amines) caused significant inhibition even at concentrations equal to or lower than the amounts that could be expected in milk, this was not true for the other test compounds. The amounts of these compounds needed to inhibit *E. coli* were far greater than the amounts likely to be in milk.

It was suggested by Podesta and Bertoldini *(11)* that fatty acids do not alter the growth conditions for *E. coli* and some other organisms in milk cultures. Hence it is difficult to postulate what effect these compounds might have in complex systems like milk or milk products.

Many workers have suggested different ways by which some of these compounds might exert an inhibitory effect on organisms. Spector *(13)* hypothesized that the effect exerted by fatty acids of corn oil or milk fat was dependent on the amount of growth-limiting amino acids and vitamins in the medium; generally, inhibition of growth was observed at high concentrations of vitamins, whereas with less vitamins present growth was either stimulated or was not affected. Daly et al. *(3)* reported that acetic and formic acid inhibited *E. coli* and that the inhibition was pH-dependent. Aldehydes have been reported to inhibit *E. coli* perhaps because of a bond between aldehyde and cysteine, which makes cysteine, an essential amino acid, unavailable for protein synthesis *(2)*. It also was suggested by Cutinelli *(2)* that inhibition of glycolysis might play a role in retarding growth of the bacterium. We found butyric acid to be less inhibitory than hexanoic, octanoic, and decanoic acid. This was also observed by Podesta and Bertoldini *(11)*. In the absence of any conclusive evidence as to the modes of action of these compounds, more detailed studies are needed to establish why they inhibited or inactivated *E. coli*.

We observed in these trials that chemicals with no apparent inhibitory action against *E. coli* when tested by the disc assay procedure *(8)* caused significant reduction in the growth of this bacterium. Hence it is believed that procedures used in this study give a more accurate assessment of the effect of these compounds on bacteria than does the disc assay method.

ADDENDUM

The following additional information is provided so the reader can better interpret the data reported in this paper. The boiling point *(C)* and solubility in water (s, soluble; ss, slightly soluble; i, insoluble) for chemicals used in the study are as follows: formic acid (100.7, s), acetic acid (117.9, s), butyric acid 163.5, s), hexanoic acid (205, i), octanoic acid (239.3, ss), decanoic acid (270, ss), formaldehyde (−21, s), acetaldehyde (20.8, s), propionaldehyde (48.8, s) glyoxal (50.4, s) acetone (56.2, s), 2-butanone (79.6, s), diacetyl (88, s), furfuryl alcohol (171, s), methyl alcohol (65, s), propylamine (47.8, s), hexylamine (130, ss), methylsulfide (37.3, i), methanethiol (6.2, ss), ethanethiol (35, ss), methylsulfone (238, s), acetonitrile (81.6, s), chloroform (61.7, ss), diethyl ether (34.5, ss), and ethylenedichloride (83.5, ss).

When 1 or 10 ppm of all compounds were added to nutrient broth, initial pH values were in the range of 6.45-6.70. Addition of 100 ppm of the compounds resulted in pH values of 6.2-6.6 except for formic, acetic, and butyric acid when the pH values were 5.6, 5.8, and 6.05, respectively. Use of 1000 ppm of the compounds was accompanied by pH values of 6.35-6.6 except for formic, acetic, butyric, hexanoic, octanoic, and decanoic acid, and propylamine and hexylamine which produced pH values of 3.6, 4.25, 4.45, 4.75, 4.85, 5.55, 9.3, and 8.9. Undoubtedly these low and high pH values contributed substantially to the antibacterial properties of these compounds when they were tested at the higher concentrations.

When the reduction in population of *E. coli* was 21.5% or greater, a given chemical at the appropriate concentration was bactericidal. A reduction in population of 0.6 to 21.1% represents bacteriostatic action by the chemical.

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SENSITIVITY OR RESISTANCE DAIRY STARTER AND ASSOCIATED MICROORGANISMS TO SELECTED ANTIBIOTICS

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Abstract

Forty-two strains of mesophilic lactic streptococci, enterococci, lactobacilli, leuconostoc, staphylococci, and other dairy- and food-related microorganisms were tested for their sensitivity to 30 antibiotic and antimicrobial agents. Elliker's lactic agar served as the growth medium; incubation temperature and times were varied according to individual culture growth requirements. Commercially available multitipped sensitivity discs were used. Almost all the microorganisms investigated, except for *Streptococcus thermophilus*, exhibited definite resistance to eight different sulfonamides. Most of the strains of starter streptococci and *Lactobacillus bulgaricus* were sensitive to all 16 antibiotic or antimicrobial agents (included in this survey) used for mastitis control. Strains of *Leuconostoc dextranicum*, *Streptococcus faecalis*, *Streptococcus durans*, and *Brevibacterium linens* were less affected.

Many antibiotics are used routinely in mastitis therapy, although antibiotic residues are excreted into milk, thus creating problems *(5)*. Also, most residual antibiotics are quite heat resistant *(5)*. If antibiotics are present in milk for cheesemaking, for example, it is more than likely that they will inhibit starter growth, thus affecting product quality *(4)*. Difficuties created by the presence of antibiotics in milk and milk products have been thoroughly discussed *(6)*. Most investigations have used a limited number of antibiotics or microbial strains. Our survey, however, determines the effect of a large number of antibiotics on a considerable number of strains of starter and other microorganisms associated with the dairy and food industry. It is possible that this information also could be of help in classification, differentiation, or speciation of these microorganisms.

Materials and Methods

Bacterial cultures

Five strains were used of each of *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus diacetilactis*, *Streptococcus thermophilus*, *Streptococus durans*, and *Lactobacillus bulgaricus*. Only four strains of *Streptococcus faecalis* were employed. Also included were three strains of *Brevibacterium linens*, two of *Leuconostoc dextranicum*, and one each of *Staphylococcus aureus*, *Staphylococus epidermidis*, and *Micrococcus varians*. Except for the *Brevibacterium linens* (Tryptic soy broth), all cultures were propagated in sterile, reconstituted nonfat dry milk.

Antibiotic sensitivity assays were conducted by using Elliker's lactic agar *(3)* at appropriate incubation temperatures.

Antibiotic agents

Commercially available Multidisks *(2)*, multitipped sensitivity discs manufactured by Colab Laboratories, Inc., Glenwood, Ill., were used.

Sensitivity test procedure. *(a)* Ten to 12 ml of sterile Elliker's lactic agar (pH 7.0) were poured into a level petri dish, solidified, and dried overnight at 37 C. *(b)* Two-tenths milliliter of actively growing bacterial culture was mixed with 7 ml of sterile, 45 C Elliker's agar and was poured onto each dried agar plate. *(c)* Antibiotic-impregnated Multidisks were then placed on each lawn of cells. *(d)* Inoculated agar plates, each with selected antibiotic discs, were incubated for specified periods at the appropriate growth temperature for each test organism: lactic streptococci, 32 C for 16 h; *Leuconostoc*, 32 C for 16 to 24 h; enterococci, lactobacilli, *S. thermophilus, and staphylococci*, 37 C for 16 h; *M. varians*, 28 C for 24 h; and *B. linens*, 21 C for 48 to 72 h. *(e)* Growth inhibition zones were measured from the tips of the antibiotic-impregnated discs to the edge of visible bacterial growth.

Results and Discussion

Interpretation of test results

Growth reactions to antibiotics when applied in two different concentrations were reported as: *sensitive*—a zone around the disc tip with the lower concentrations of antibiotic; *moderately sensitive*—a zone around the disc tip with higher concentration of antibiotic only; *resistant*—no zone around either disc tip.

Growth reactions to antibiotics applied in only a single concentration were reported as: *sensitive*—a zone around the disc tip; *resistant*—no zone around the disc tip.

Discussion of test results

Overall results are summarized in Tables 1 and 2. In both tables, the abbreviation "mcg" is used according to the manufacturer's designation instead of the more conventional symbol "μg." For full appreciation of the data with regard to mastitis therapy, note that 16 of the 30 agents listed are currently in use and are so designated in each table.

In addition to these antibiotics, sulfa drugs also

[1]Journal Paper No. J-7813 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa 50010. Project No. 1838.

TABLE 1. ANTIBIOTIC AND ANTIMICROBIAL AGENT SENSITIVITY PATTERNS[a, b] OF STRAINS OF LACTIC STREPTOCOCCI, *Streptococcus thermophilus, Lactobacillus bulgaricus,* AND *Leuconostoc dextranicum.*

ANTIBIOTIC OR ANTIMICROBIAL AGENT	CONCENTRATION PER DISC	BACTERIAL STRAINS																								
		STREPTOCOCCUS LACTIS					STREPTOCOCCUS CREMORIS					STREPTOCOCCUS DIACETILACTIS					STREPTOCOCCUS THERMOPHILUS					LACTOBACILLUS BULGARICUS					LEUCONOSTOC DEXTRANICUM	LEUCONOSTOC DEXTRANICUM B1165
		7963	7962	C_2F	10	SLE	HP	SC_1	DR_7	ML_4	SC_3	18-16	26-2	31-2	11007	DRC_3	ST_1	ST_2	ST_3	ST_4	ST_5	LB_1	LB_2	LB_3	LB_4	LB_5		
PENICILLIN G[c]	2 units	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	0	0
PENICILLIN G[c]	10 units	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2
POLYMYXIN B[c]	50 units	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	2
POLYMYXIN B[c]	300 units	0	0	0	0	1	1	2	2	1	2	1	1	1	1	1	1	1	1	1	1	2	2	0	0	0	3	3
PHENETHICILLIN	3 units	3	3	2	3	3	3	3	3	3	3	2	2	2	2	2	3	3	3	3	3	3	3	3	3	2	0	0
TETRACYCLINE[c]	5 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0	2	1	2	3	3
TETRACYCLINE[c]	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
VANCOMYCIN	5 mcg	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	2	3	3	3	2	2	2	0	0
VANCOMYCIN	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0
CHLORTETRACYCLINE[c]	5 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	1	2	3	3	3	3
CHLORTETRACYCLINE[c]	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
AMPICILLIN[c]	2 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	1	2	3	1	0	0
AMPICILLIN[c]	10 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	0	1
BACITRACIN[c]	2 units	2	2	3	2	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0
BACITRACIN[c]	10 units	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0
CHLORAMPHENICOL	5 mcg	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2	3	3	3	2	2	2	2	1	2	3	2	2
CHLORAMPHENICOL	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3
CEPHALOTHIN	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
ERYTHROMYCIN[c]	2 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	1	2	3	3	2	0	0
ERYTHROMYCIN[c]	15 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2
KANAMYCIN	5 mcg	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	0	0	0	0	0	0	0	2	1	2	2	2
KANAMYCIN	30 mcg	2	2	2	2	3	2	3	3	3	3	3	3	2	2	2	1	1	1	1	1	2	1	3	2	3	3	3
LINCOMYCIN[c]	2 mcg	3	3	1	3	3	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0
METHACYCLINE	5 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3
METHACYCLINE	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
METHICILLIN	5 mcg	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	0	0	3	3	0	0	0
NAFCILLIN	1 mcg	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	0	0	1	3	1	0	0
COLISTIN	2 mcg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
COLISTIN	10 mcg	0	0	0	0	0	0	1	1	1	2	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	3	3
CLOXACILLIN[c]	1 mcg	2	2	1	1	2	2	2	2	2	2	2	1	1	1	2	2	2	1	2	2	0	0	2	3	0	0	0
DEMETHYLCHLORTETRACYCLINE	5 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	1	2	2	3	3	3	3
DEMETHYLCHLORTETRACYCLINE	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
DOXYCYCLINE	5 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	2	2	3	3	3	3	3
DOXYCYCLINE	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
DIHYDROSTREPTOMYCIN[c]	2 mcg	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	0	0	1	1	2	2	1
DIHYDROSTREPTOMYCIN[c]	10 mcg	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	3	2	3	3	3	3	3	3	3
NOVOBIOCIN[c]	5 mcg	2	3	2	2	2	2	3	3	3	3	2	2	2	2	2	3	2	2	3	3	1	1	3	3	3	0	2
NOVOBIOCIN[c]	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3
NEOMYCIN[c]	5 mcg	1	0	1	1	1	1	2	2	2	2	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1
NEOMYCIN[c]	30 mcg	2	2	2	2	2	2	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2
OLEANDOMYCIN[c]	2 mcg	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2	3	3	3	3	3	1	0	2	3	3	0	0
OLEANDOMYCIN[c]	15 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0
OXACILLIN[c]	1 mcg	1	1	1	1	1	1	1	2	2	1	1	1	1	1	2	3	3	3	3	3	0	0	2	3	0	0	0
OXYTETRACYCLINE[c]	5 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	2	0	2	3	3	3	3
OXYTETRACYCLINE[c]	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3
FURADANTIN[c]	50 mcg	0	2	2	0	1	1	1	3	1	1	1	1	1	1	1	3	3	3	3	3	3	3	0	0	3	2	2
FURADANTIN[c]	100 mcg	1	3	3	1	2	1	2	3	2	2	1	1	1	1	2	3	3	3	3	3	3	3	1	1	3	3	3
MANDELAMINE	1.0 mg	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0
MANDELAMINE	2.5 mg	1	1	0	1	1	1	1	2	1	1	1	1	1	1	1	2	1	2	2	2	0	0	0	2	1	1	1
NALIDIXIC ACID	30 mcg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3
TRIBURON	250 mcg	3	3	3	3	3	2	3	3	3	3	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	1	1
TRIBURON	1 mg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2

[a] NO INHIBITION 0

[b] INHIBITION { 1 INHIBITORY ZONE ≤ 2 mm. ; 2 INHIBITORY ZONE > 2 mm. BUT < 5 mm. ; 3 INHIBITORY ZONE ≥ 5 mm.

[c] CURRENTLY USED IN MASTITIS THERAPY.

TABLE 2. ANTIBIOTIC AND ANTIMICROBIAL AGENT SENSITIVITY PATTERNS[a, b] OF STRAINS OF ENTEROCOCCI, *Brevibacterium linens*, STAPHYLOCOCCI, AND MICROCOCCI.

ANTIBIOTIC OR ANTIMICROBIAL AGENT	CONCENTRATION PER DISC	BACTERIAL STRAINS													
		STREPTOCOCCUS DURANS					STREPTOCOCCUS FAECALIS				BREVIBACTERIUM LINENS			STAPHYLOCOCCUS AUREUS	STAPHYLOCOCCUS EPIDERMIDIS	MICROCOCCUS VARIANS
		15-20	9-20	36-11	52-13	38-2	51-2	22-23	24-23	47-13	1	2	3			
PENICILLIN G[c]	2 units	3	3	3	3	3	2	2	2	2	0	2	0	3	3	1
PENICILLIN G[c]	10 units	3	3	3	3	3	3	3	3	3	0	3	0	3	3	2
POLYMYXIN B[c]	50 units	0	0	0	0	0	0	0	0	0	1	2	1	0	0	0
POLYMYXIN B[c]	300 units	0	0	0	0	0	0	0	0	0	2	3	2	0	1	0
PHENETHICILLIN	3 units	2	2	3	3	2	2	2	2	2	0	0	0	3	3	3
TETRACYCLINE[c]	5 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
TETRACYCLINE[c]	30 mgc	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
VANCOMYCIN	5 mcg	2	2	2	2	2	2	2	2	2	3	3	1	2	2	2
VANCOMYCIN	30 mcg	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3
CHLORTETRACYCLINE[c]	5 mcg	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3
CHLORTETRACYCLINE[c]	30 mcg	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3
AMPICILLIN[c]	2 mcg	3	3	3	3	3	3	3	3	3	0	0	0	3	3	3
AMPICILLIN[c]	10 mcg	3	3	3	3	3	3	3	3	3	0	1	0	3	3	3
BACITRACIN[c]	2 units	3	2	2	2	3	1	2	1	1	3	3	2	1	2	1
BACITRACIN[c]	10 units	3	3	3	3	3	2	3	2	2	3	3	3	3	3	3
CHLORAMPHENICOL	5 mcg	2	2	2	2	2	2	2	2	2	3	3	2	2	2	3
CHLORAMPHENICOL	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
CEPHALOTHIN	30 mcg	2	2	3	3	3	2	2	2	2	2	3	1	3	3	3
ERYTHROMYCIN[c]	2 mcg	3	3	2	2	2	2	2	2	2	3	2	3	3	3	3
ERYTHROMYCIN[c]	15 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
KANAMYCIN	5 mcg	1	1	0	0	0	0	0	0	0	1	1	1	2	2	3
KANAMYCIN	30 mcg	2	2	1	1	1	1	1	1	1	3	3	2	3	3	3
LINCOMYCIN[c]	2 mcg	3	3	1	1	1	1	1	1	1	2	1	2	3	1	3
METHACYCLINE	5 mcg	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3
METHACYCLINE	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
METHICILLIN	5 mcg	1	0	3	3	0	1	1	1	2	0	0	0	3	3	3
NAFCILLIN	1 mcg	1	0	3	3	0	1	1	1	2	0	0	0	3	3	3
COLISTIN	2 mcg	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
COLISTIN	10 mcg	0	0	0	0	0	0	0	0	0	1	2	1	0	0	0
CLOXACILLIN[c]	1 mcg	0	0	2	2	1	0	0	0	0	0	0	0	3	3	3
DEMETHYLCHLORTETRACYCLINE	5 mcg	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3
DEMETHYLCHLORTETRACYCLINE	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
DOXYCYCLINE	5 mcg	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3
DOXYCYCLINE	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
DIHYDROSTREPTOMYCIN[c]	2 mcg	0	0	0	0	0	0	0	0	0	2	1	1	0	0	0
DIHYDROSTREPTOMYCIN[c]	10 mcg	0	0	0	1	1	0	0	0	0	3	3	2	2	2	1
NOVOBIOCIN[c]	5 mcg	3	3	3	3	3	2	1	1	2	3	3	2	3	1	3
NOVOBIOCIN[c]	30 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3
NEOMYCIN[c]	5 mcg	1	1	1	1	1	0	0	0	0	2	2	2	2	3	3
NEOMYCIN[c]	30 mcg	2	2	2	2	2	0	0	0	0	3	3	2	3	3	3
OLEANDOMYCIN[c]	2 mcg	2	2	2	2	1	2	2	2	2	3	3	3	2	2	3
OLEANDOMYCIN[c]	15 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
OXACILLIN[c]	1 mcg	0	0	3	3	1	0	0	0	0	0	0	0	2	2	3
OXYTETRACYCLINE[c]	5 mcg	3	3	3	3	3	1	3	3	3	3	3	2	3	3	3
OXYTETRACYCLINE[c]	30 mcg	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3
FURADANTIN[c]	50 mcg	3	2	1	2	1	3	3	3	3	0	0	0	3	3	3
FURADANTIN[c]	100 mcg	3	3	2	3	2	3	3	3	3	0	0	0	3	3	3
MANDELAMINE	1.0 mg	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0
MANDELAMINE	2.5 mg	0	0	0	0	0	0	0	0	0	1	2	1	2	2	1
NALIDIXIC ACID	30 mcg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
TRIBURON	250 mcg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
TRIBURON	1 mg	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

[a] NO INHIBITION 0

[b] INHIBITION
- 1 INHIBITORY ZONE ≤ 2mm.
- 2 INHIBITORY ZONE > 2mm. BUT < 5mm.
- 3 INHIBITORY ZONE ≥ 5mm.

[c] CURRENTLY USED IN MASTITIS THERAPY

are added to therapeutic preparations. We attempted to include some of the commonly used sulfa drugs in this survey, but found that Elliker's lactic agar could not be used as a test medium. Mueller-Hinton agar does not contain the protective factor(s) found in Elliker's lactic agar. Because many of the starter microorganisms used in our study are nutritionally fastidious, Mueller-Hinton agar, however, would be inadequate. Almost all the microorganisms investigated, except for S. *thermophilus*, exhibited definite resistance to eight different sulfonamides when grown on lactic agar. Strains of S. *thermophilus* were inhibited by several sulfa drugs. This might indicate severe sensitivity when tested by using more appropriate procedures.

Most of the strains of starter streptococci (S. *lactis*, S. *cremoris*, S. *diacetilactis*, and S. *thermophilus*) and *L. bulgaricus* were sensitive to all 16 antibiotic or antimicrobial agents (included in this survey) used for mastitis control. Strains of *L. dextranicum*, S. *faecalis*, *B. linens*, and S. *durans* exhibited resistance, in the same order, to 5, 5, 3, and 1 of the designated antimastitis agents.

The results presented in Tables 1 and 2 show that *L. dextranicum* may be more resistant to antibiotics than most other starter bacteria. On the basis of their resistance to the number of antibiotics included in this survey, starter and associated bacteria could be listed in this order of decreasing resistance: *L. dextranicum*, *B. linens*, S. *faecalis*, S. *durans*, *L. bulgaricus*, S. *lactis*, S. *diacetilactis*, S. *thermophilus*, and S. *cremoris*. Four of the 5 strains of S. *lactis* showed complete resistance to polymyxin B; the remaining strain was only moderately sensitive to this antibiotic. All 5 strains of S. *diacetilactis* were moderately sensitive to polymyxin B. Three of the 5 S. *cremoris* strains were completely sensitive to polymyxin B; the other strains were moderately sensitive.

Table 3 presents a list of antibiotics without effect (at use levels) on specific test organisms. For example, Furadantin, at 50 μg, does not inhibit *B. linens*. This inhibitory agent, therefore, might add selectivity to Cheese agar *(1)* used to isolate *B. linens*. From other data in Table 3, it seems that a combination of mandelamine plus polymyxin B, at some concentration, could be used to develop a selective medium for enterococci. Neomycin at 30 μg was inhibitory to the 5 test strains of S. *durans* investigated. Strains of S. *faecalis*, on the other hand, exhibited resistance to this use level. This observation might be exploited to develop a differential test for these enterococci.

From data presented, it should be possible to compare the pharmacological action of different antibiotics on the basis of their degree of inhibitory effect

TABLE 3. ANTIBIOTIC RESISTANCE OF TEST MICROORGANISMS[a]

Leuconostoc dextranicum	*Brevibacterium linens*	*Streptococcus faecalis*	*Streptococcus durans*	*Lactobacillus bulgaricus*	*Streptococcus lactis*	*Streptococcus diacetilactis*	*Streptococcus thermophilus*	*Streptococcus cremoris*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Micrococcus varians*
Phenethicillin	Methicillin	Polymyxin B	Polymyxin B	Nalidixic acid	Colistin	Colistin	Colistin	Nalidixic acid	Polymyxin B	Nafcillin	Polymyxin B
Vancomycin	Nafcillin	Colistin	Colistin Mandelamine Nalidixic acid		Nalidixic acid	Nalidixic acid	Nalidixic acid		Nafcillin Colistin Nalidixic acid	Colistin Nalidixic acid	Nafcillin Colistin Nalidixic acid
Bacitracin	Cloxacillin	Cloxacillin									
Lincomycin	Oxacillin	Dihydrostreptomycin									
Methicillin	Furadantin										
Nafcillin	Nalidixic acid	Oxacillin									
Cloxacillin		Mandelamine Nalidixic acid Neomycin									
Oleandomycin											
Oxacillin											

[a]The highest concentration of these antibiotics used is given in Tables 1 and 2.

on the several strains and species of bacteria used in this investigation.

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

II. OPTIMIZATION OF CONDITIONS

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Abstract

Attempts were made to maximize biomass production in the oxidation of fish oil by *Candida lipolytica* Y1094 and *Geotrichum candidum* Y552 in shake flasks. Maximum biomass was produced on 5% w/v oil in water containing 1.5% $(NH_4)_2SO_4$, 2.2% KH_2PO_4, 0.12% $MgSO_4$, 0.03% fish stickwater solids, 10 ppm $FeSO_4 \cdot 7H_2O$, 1.5 ppm $MnSO_4 \cdot H_2O$, and adjusted to pH 7.5. For *G. candidum* 40 ppb biotin were added to the medium. When inoculum size was varied from 1 to 8% v/v, the most appropriate inoculum size was about 2%. Aeration rates were increased by decreasing the medium volume per 500-ml shake flask. Maximum yields were obtained with 25 ml of medium. Cell recoveries were complicated by formation of oil-media emulsions. Several methods of biomass recovery were studied. Warming the acidified cell suspension in the presence of Triton X-100 allowed removal of oil from the cell mass. Extraction of the dried cell mass with ethyl ether yielded highest recovery of cell material compared to other methods. Dry cells contained 40.1 to 49.3% crude protein. These studies suggest the feasibility of obtaining 800 g of single cell biomass with a crude protein yield of 320 g from 1 kg of fish oil using batch or continuous culture systems.

Hottinger et al. *(1)* describe the utilization of fish oil in terms of the basic requirements of the system. A survey of the literature indicates these studies probably comprise the first comprehensive research on microbial utilization of triglycerides, especially fish oil.

The studies reported in the present paper are an extension of the previous report, and aim to optimize the system for maximal production of biomass at the shake-flask level.

Materials and Methods

The materials and methods are essentially those used in the previous communication *(1)*. The bulk of the studies reported here relate to maximum biomass produced at an oil concentration of 5%. Attempts were made to optimize the oxidation of fish oil by addition of the following components to the various media: $(NH_4)_2SO_4$, KH_2PO_4 and $MgSO_4$; trace metals such as $FeSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, and $ZnSO_4 \cdot 7H_2O$; thiamine, biotin; and Triton X-100 as a surfactant.

Further studies were adduced to determine the effects of inoculum size and rate of aeration on the oxidation. Aeration rates were increased by using a reduced volume in larger flasks. Finally, the depletion of fish oil as the propagation progressed was determined along with the yield of biomass under the optimized conditions

Results and Discussion

Maximum biomass production at 5% oil concentration

As reported earlier, the dry cell yield could be increased by increasing the oil concentration up to 5.0% w/v. Further gains in cell yield were not possible due to the inhibitory effect of low pH of the oxidized media. The amount of $(NH_4)_2SO_4$, KH_2PO_4 and $MgSO_4$ present corresponded to the needs for maximum biomass production in the presence of 1% fish oil. Subsequent experiments were initiated to improve the biomass yields at the 5.0% oil level by altering the various nutrient concentrations and supplementing them with additional growth-promoting ingredients. On the basis of the carbon content of 5.0% w/v fish oil the need for $(NH_4)_2SO_4$ would increase from 0.6% to about 2.8% (calculated on basis: yeast = 48.0% carbon; 7.5% nitrogen. Tristearin = 76.8% carbon and $(NH_4)_2SO_4$ = 21.2% N). Table 1 shows the

Table 1. Effect of ammonium sulfate concentration on the growth of *Candida Lipolytica* in 5% fish oil media

$(NH_4)_2SO_4$ (%)	KH_2PO_4 (%)	g/100 ml dry cell yield after 48 h	Doubling time (h)	Specific growth rate (h^{-1})
0.6	0.6	1.290	3.13	0.2213
1.5	0.6	1.195	3.25	0.2131
3.0	0.6	1.165	3.23	0.2143
4.2	0.6	1.185	3.24	0.2133
0.6	1.5	1.345	3.06	0.2267
1.5	1.5	1.682	3.11	0.2226
2.1	1.5	1.744	3.14	0.2203

effect of increased concentrations of $(NH_4)_2SO_4$ on growth of *Candida lipolytica*. These data indicate that changes in $(NH_4)_2SO_4$ concentration alone did not produce a change in growth unless the KH_2PO_4 concentration was increased from 0.6 to 1.5%. Thus, for subsequent experiments the concentration of $(NH_4)_2SO_4$ was increased to 1.5%. Two percent NH_4NO_3 or 1.1% $(NH_4)_2SO_4$ have been used in hydrocarbon fermentations *(8, 9, 10)*. Also, the highest concentration of KH_2PO_4 used in hydrocarbon fermentation was 0.7% *(5)*.

Table 2 shows that a rise from the 0.6% KH_2PO_4

TABLE 2. EFFECT OF POTASSIUM DIHYDROGEN PHOSPHATE CONCENTRATION ON THE GROWTH OF *Candida Lipolytica* IN 5% FISH OIL MEDIA

KH_2PO_4 (%)	$(NH_4)_2SO_4$ (%)	Dry cell yield after 48 h (g/100 ml)	Dry cell yield after 48 h based on initial oil content	Doubling time (h)	Specific growth rate (h-1)	g Nitrogen taken up per g dry cell after 48 h	N conent of dry cells (%)
0.6	1.5	1.195	0.239	3.25	0.2131	0.078	—
1.5	1.5	1.631	0.326	3.16	0.2193	0.044	—
2.1	1.5	1.804	0.361	3.16	0.2193	0.041	—
2.7	1.5	1.761	0.352	3.17	0.2183	0.037	
After complete supplementation of media[1]							
0.6	1.5	1.680	0.336	3.00	0.2311	0.071	6.20
1.5	1.5	2.025	0.405	2.90	0.2392	0.061	4.80
2.1	1.5	2.190	0.438	3.07	0.2253	0.058	4.70
2.7	1.5	2.310	0.462	3.13	0.2214	0.062	4.04

[1] $FeSO_4 \cdot 7H_2O$ 10 mg/l, $MnSO_4 \cdot H_20$ 1.5 mg/l, $MgSO_4$ 0.12%, fish stickwater 0.03%.

TABLE 3. EFFECT OF VARIOUS METAL IONS ON GROWTH OF *Candida Lipolytica* IN 5% FISH OIL MEDIA

Added metals[1]				Dry cell yield after 48 h (g/100 ml)	Dry cell yield after 48 h based on initial oil content	Doubling time (h)	Specific growth rate (h-1)	g/Nitrogen uptake per g dry cell after 48 h[2]
$MgSO_4$ (%)	$FeSO \cdot 7H_2O$ (mg/liter)	$MnSO_4 \cdot H_2O$ (mg/liter)	$ZnSO_4 \cdot 7H_2O$ (mg/liter)					
0.05	—	—	—	1.580	0.316	3.21	0.2157	0.0734
0.10	—	—	—	1.694	0.339	3.18	0.2177	0.0870
0.15	—	—	—	1.682	0.336	3.14	0.2208	0.0455
0.20	—	—	—	1.659	0.332	3.13	0.2211	0.0444
0.10	—	—	—	1.682	0.336	3.11	0.2226	0.0438
0.10	10	—	—	1.751	0.350	3.14	0.2205	0.0437
0.10	10	1.5	—	1.825	0.365	3.01	0.2239	0.0448
0.10	10	1.5	5	1.664	0.333	3.17	0.2186	0.0493

[1] 1.5% KH_2PO_4, 1.5% $(NH_4)_2SO_4$

[2] Nitrogen uptake measured after 33 h.

in the basic formulation to 1.5% KH_2PO_4 brought about a significant increase of the 48-h dry cell yield. Further increments in KH_2PO_4 increased cell yield but did not increase the specific growth rate. Similar observations were made with respect to KH_2PO_4 variations relative to a fully supplemented media (Fe^{++}, Mn^{++} added, fish stickwater doubled). As shown in Table 2, the yield coefficient per 5.0% oil increased from 0.336 to 0.462 by raising the KH_2PO_4 content of the medium to 2.7%. Since the increases in specific growth rate were limited to the KH_2PO_4 concentration change 0.6 to 1.5%, the latter concentration was used. The greater buffering capacity at higher KH_2PO_4 concentrations resulted in a pH shift of 0.5 unit or less. Also of interest is the decrease in nitrogen uptake while increasing the KH_2PO_4 concentration from 0.6 to 1.5%. The analysis of the corresponding dry cell matter also yielded a low nitrogen content, thus verifying this observation.

Magnesium sulfate is generally added to hydrocarbon media for yeast fermentation. Reported concentrations vary from 0.02 to 0.2% *(5, 6)*. Trace metals commonly added to hydrocarbon fermentations are iron, manganese, and zinc. Good growth by *Candida hydrocarbofumarica (11)* was obtained in a medium containing 10 ppm $FeSO_4 \cdot 7H_2O$, 2 ppm $MnSO_4 \cdot H_2O$ and 5 ppm $ZnSO_4 \cdot 7H_2O$. Table 3 indicates that 0.10-0.15% $MgSO_4$ was superior to 0.05% $MgSO_4$. Likewise supplementation with $FeSO_4 \cdot 7H_2O$ and $MnSO_4 \cdot H_2O$ stimulated a further increase in growth, whereas $ZnSO_4 \cdot 7H_2O$ showed no growth promoting activity.

Propagation experiments done with stearic acid and *Geotrichum candidum* (unpublished data) indicated growth was stimulated by biotin. Since thiamine promotes yeast growth *(8)*, *C. lipolytica* and *G. candidum* were tested for their ability to utilize these two vitamins in fish oil media adjusted to pH 7.5 and pH 5.0. Results of these experiments, compiled in Tables 4 and 5, demonstrate that at pH 5.0, 100 μg/l of thiamine HCl in the absence of fish stickwater produced good growth of *C. lipolytica*. At pH 7.5 200 μg/liter thiamine HCl were needed to compensate for the growth effect of 0.015% fish stickwater. With *G. candidum* 200 μg thiamine HCl produced growth greater than that for 0.015% fish stickwater. Biotin did not promote growth in the absence of fish stickwater at pH 5 with *C. lipolytica*, whereas *G. candidum* under the same conditions showed an increase in growth. Also, at pH 7.5 biotin supported a slight increase in growth of *C. lipolytica* in the presence of

fish stickwater.

Concerning the nitrogen uptake in these experiments, Tables 4 and 5 indicate that in the absence of fish stickwater, but in the presence of biotin at pH of 5.0 *C. lipolytica* took up more nitrogen than in the presence of fish stickwater alone. *G. candidum* had a higher nitrogen uptake also in the presence of thiamine HCl at pH 5.0.

Doubling the addition of fish stickwater from 0.015% to 0.03% brought about a 10% increase in the 48-h dry cell yield from 1.694 g to 1.865 g. The yield coefficient per 5% oil rose from 0.339 to 0.373, but doubling time and specific growth rate remained essentially the same.

Table 6 illustrates the additive effect of the various growth-promoting supplements. For *G. candidum* the combined addition of iron, manganese, biotin, and doubling the amount of fish stickwater increased the yield coefficient from 0.296 to 0.368. With *C. lipolytica* the combination of iron, manganese, and doubling the amount of fish stickwater had further growth-promoting effect by increasing the yield coefficient from 0.376 to 0.465. With the addition of biotin, growth of *C. lipolytica* declined.

Thus, manipulation of the medium composition containing 5% fish oil resulted in increases in yield coefficient from 0.229 to 0.465 and specific growth rate (h^{-1}) from 0.2095 to 0.2346 and a shorter doubling time, from 3.31 to 2.95 h for *C. lipolytica*. For *G. candidum* the changes in formulation resulted in increases in yield coefficient from 0.196 to 0.368 and specific growth rate (h^{-1}) from 0.2101 to 0.2180 and a decrease in the doubling time from 3.30 to 3.18 h.

Effect of surfactant added to the media

Fish oil added to media formed an oil-in-media emulsion, which, during the course of microbial growth, became quite homogeneous and stable. Possibly, if this tendency of the fish oil to emulsify could be enhanced, further increase in growth would be obtainable due to enlarged ratio of oil droplet surface area to microbial surface area. Consequently, the growth of *C. lipolytica* in media at pH 5 and pH 7.5 to which were added increasing concentrations of Triton X-100 was studied. The data indicated that surfactant had no effect on growth at pH 7.5, and at pH 5.0 its effect was rather inhibitory.

Microscopic examination of the medium at pH 5 resulted in the following observations: (*a*) In surfactant-free medium: cells and hyphae were covered with small oil droplets; cells and hyphae formed agglomerates in which large oil droplets were enmeshed. (*b*) In medium with 0.01% surfactant: cells and hyphae were only slightly covered with small oil droplets. Medium and large size oil droplets were enmeshed in hyphae agglomerations. (*c*) In medium with 0.1% surfactant: Practically no oil droplets attached to the surface of the cells and hyphae. Cells, hyphae and oil droplets were evenly distributed in medium. No agglomeration was observable. Oil droplets were large.

These observations indicate that addition of Triton X-100 reduces the attraction between the microbial cell and the oil droplets. These observations also indicate that the degree of oil utilization is dependent on the number of small oil droplets present and the extent of their adsorption to the microbial cell surface.

Effect of inoculum size an growth of Candida lipolytica

As shown in Table 7 and Fig. 1, doubling the

TABLE 4. GROWTH OF *Candida Lipolytica* AS AFFECTED BY ADDITIONS OF BIOTIN AND THIAMINE

Additions			Growth of *Candida lipolytica*				
Biotin (μg/l)	Thiamine HCl (μg/l)	Starting pH	48 h dry cell yield (g/100 ml)	48 h yield coefficient based on initial oil content	Doubling time (h)	Specific growth rate (h-1)	g/Nitrogen taken up per g dry cell after 48 h
–	–	7.5	1.682	0.336	3.11	0.2226	0.044
2	–	7.5	1.755	0.351	3.16	0.2190	0.047
4	–	7.5	1.940	0.388	3.09	0.2242	0.040
–	–[1]	5.0	0.583	0.117	3.87	0.1792	0.066
4	–[1]	5.0	0.426	0.085	3.77	0.1838	0.129
40	–[1]	5.0	0.416	0.083	3.79	0.1827	0.145
–	–	7.5	1.682	0.336	3.11	0.2226	0.044
–	100	7.5	1.685	0.371	3.05	0.2269	0.045
–	100[1]	7.5	1.506	0.301	3.13	0.2211	0.040
–	200[1]	7.5	1.659	0.332	3.13	0.2212	0.039
–	–	5.0	1.060	0.212	2.99	0.2317	0.078
–	100	5.0	1.290	0.258	2.92	0.2373	0.072
–	–[1]	5.0	0.583	0.117	3.86	0.1793	0.066
–	100[1]	5.0	1.295	0.259	2.90	0.2394	0.081
–	200[1]	5.0	1.120	0.224	2.98	0.2329	0.063

[1]Medium contained no fish stickwater.

TABLE 5. GROWTH OF *Geotrichum Candidum* AS AFFECTED BY THE ADDITIONS OF BIOTIN AND THIAMINE

Additions			Growth of *Geotrichum candidum*				
Biotin (μg/l	Thiamine HCl (μg/l	Starting pH	48 h dry cell yield (g/100 ml)	48 h yield coefficient based on initial oil content	Doubling time (h)	Specific growth rate (h-1)	g/Nitrogen taken up per g dry cell after 48 h
–	–	7.5	–	– not tested	–	–	–
2	–	7.5	–	– not tested	–	–	–
4	–	7.5	–	– not tested	–	–	–
–	–[1]	5.0	0.600	0.120	3.82	0.1815	0.108
4	–[1]	5.0	0.571	0.114	3.40	0.2040	0.134
40	–[1]	5.0	0.762	0.152	3.43	0.2022	0.090
–	–	7.5	–	– not tested	–	–	–
–	100	7.5	–	– not tested	–	–	–
–	100[1]	7.5	–	– not tested		–	–
–	200[1]	7.5	–	– not tested	–	–	–
–	–	5.0	0.794	0.159	3.31	0.2092	0.090
–	100	5.0	0.795	0.159	3.31	0.2096	0.080
–	–[1]	5.0	0.601	0.120	3.82	0.1816	0.108
–	100[1]	5.0	0.812	0.162	3.41	0.2030	0.092
–	200[1]	5.0	1.200	0.240	3.32	0.2086	0.134

[1]Medium contained no fish stickwater.

TABLE 6. ADDITIVE EFFECT OF GROWTH-PROMOTING SUPPLEMENTS

Composition of media[1]				Growth values				
$FeSO_4 \cdot 7H_2O$ (mg/l)	$MnSO_4 \cdot H_2O$ (mg/l)	FSW[2] (%)	Biotin (μg/l)	48 h dry cell yield (g/100 ml)	48 h yield coefficient based on initial oil content	Doubling time (h)	Specific growth rate (h-1)	g Nitrogen taken up per g dry cell after 48 h
				Candida lipolytica				
–	–	0.015	–	1.880	0.376	3.03	0.2289	0.057
20	3	0.015	–	2.002	0.400	3.02	0.2291	0.051
20	3	0.030	–	2.327	0.465	2.95	0.2346	0.048
20	3	0.030	40	1.750	0.350	3.03	0.2287	0.060
				Geotrichum candidum				
–	–	0.015	–	1.480	0.296	3.37	0.2057	0.056
20	3	0.015	–	1.530	0.306	3.26	0.2127	0.056
20	3	0.030	–	1.590	0.308	3.24	0.2140	0.059
20	3	0.030	40	1.840	0.368	3.18	0.2180	0.054

[1]Fish oil 5.0%, $(NH_4)_2SO_4$ 1.5%, KH_2PO_4 1.5%, $MgSO_4$ 0.12%.

[2]Fish stickwater

inoculum from 1% to 2% v/v produced the most significant increase in cell growth. Four percent inoculum produced a further but smaller increase in growth. At 8% inoculum the growth rate was favored but the 48-h dry cell yield declined. In a test of inocula effect on growth rate of *Candida utilis*, Mian et al. *(4)* showed that the growth rate increased with a decrease in inocula size. They considered inocula of 0.21 to 3.10%. In the present work an inoculum range from 1.0 to 8.0% was used. This difference in inoculum may account for the observed difference in results. Our data indicate the most appropriate inoculum size to be about 2.0%.

Effect of aeration on growth of Candida lipolytica

As Fig. 2 indicates, with each decrement of media per shake flask, the specific growth rate for *C. lipolytica* and the yield coefficient increased. In the experiment on pH optimum, a maximum cell yield of 0.78 g per gram of oil was obtained. From this yield and the molecular weight for tristearin an oxygen requirement of 2.43 g per gram of dry cells was calculated *(3)*. Thus, 50 g of oil per liter of media would require 94.8 g of oxygen to produce 39.0 g cells. This would correspond to an oxygen-absorption rate of 1.03 mmoles/l/min for the duration of 48 h. The 500-ml flask, containing 50 ml media, nearly satisfied this minimum requirement. However, the yield coefficient was 0.415 instead of the desired 0.780 indicating that the aeration rate should have been about 2.20-2.30 mmoles of oxygen/l/min to attain the estimated yield. In this instance, Erlenmeyer shaker flasks with baffles or indentations would be needed *(7)*.

Aspects of cell recovery

Dry cell yields as influenced by the method of cell harvest. Two methods of cell recovery were studied:

TABLE 7. EFFECT OF INOCULUM SIZE ON GROWTH OF *Candida Lipolytica*

Inoculum size (% v/v)	48 h dry cell yield (g/100 ml)	Doubling time (h)	g Nitrogen taken up per g dry cell after 48 h
1.0	1.574	3.17	0.050
2.0	1.697	3.05	0.050
4.0	1.734	3.07	0.046
8.0	1.660	3.03	0.049

(a) oil was separated from outer cell surfaces by extraction with Skellysolve B, and *(b)* oil removal was accomplished by warming the acidified cell suspension in the presence of Triton X-100. Method *(b)* gave higher yields on both the 1% and the 5% oil media, as documented in Table 8. As a consequence, the dried residue derived from method *(b)* was washed with anhydrous ethyl ether until the supernatant ethyl ether remained clear, and the solids were redried and reweighed. The ether washes did not change the weights of the residues from the 1% oil media materially, except for the 11-h sample. On the other hand, considerable loss was encountered with ether washing of the residue from the 5% fish oil media. However, the yield of cell mass remaining was still higher than the yield obtained by the Skellysolve extraction method. This also held true for the experiments involving the 1% fish oil media. To verify this observation more tests should be done and also the residual cell mass should be analyzed for fat content.

Resistance of the cell mass-oil emulsion to separation by centrifugation. As indicated before, the growing cell mass formed a stable emulsion with the oil. This emulsion greatly hindered direct cell harvest by centrifugation. This problem was reduced if the harvest was delayed until practically all of the oil was consumed. To illustrate the magnitude of the problem, distribution of oil and cells between the cell suspension, which creams up, and the cells which sediment during centrifugation was measured. These measurements were made on 22 and 33-h samples. The results of these tests are tabulated in Table 9. *G. candidum* has a greater tendency to cream and also to form a more persistent cream than *C. lipolytica*. This difference might be due to the greater mycelium formation of *G. candidum* and to a possible lower specific weight. The extent of mycelium formation seems to correlate with the degree of enmeshing of oil droplets within the hyphal network, which in turn might help to retain cells in the cream layer that otherwise would settle. Since harvesting of cells, as done in our two laboratory procedures, might not work at a scaled up level because of economics, or, in the case of solvent extraction, undesirable nutritional effects, two other possibilities of harvesting the cells were studied. With the first method, the cream layer was transferred to phosphate buffer in an Erlenmeyer flask such that the total volume was equal to the original volume of the sample. To this was added 0.075% Triton X-100 followed by shaking on a reciprocal shaker for 60 min. The samples were then centrifuged to yield a clean separation of oil and cell mass with *C. lipolytica* but no separation at all with *G. candidum*. With the second method, the cream layer was transferred to a 75/25 mixture of phosphate buffer and energy-free medium without added surfactant for a second fermentation. After shaking for 5 h all the *C. lipolytica* cell mass sedimented on centrifugation, whereas the *G. candidum* would not sediment, and the organism remained in the cream layer. The amount of oil remaining associated with the sedimented cells was not determined, but these last two experiments indicate that the *C. lipolytica* cell mass could be harvested in keeping with high nutritional standards, the requirement being that in the surfactant method an edible one be used.

Utilization of Fish Oil and Production of Single Cell Biomass on Fully Supplemented Media at a Volume of 25 ml Media Per 500 ml Shake Flask:

Table 10 illustrates how fish oil was utilized during fermentation and single cell biomass was produced. The formula for the medium was optimized on the basis of results of the preceding experiments. Also, maximal aeration was applied by reducing the fill per

TABLE 8. DRY CELL YIELDS OF *Candida Lipolytica* AS INFLUENCED BY THE METHOD OF CELL HARVEST

Propagation time (h)	Dry cell yields as % of original oil content of media					
	Skellysolve B extracted	Triton X-100 treated, Ether wash		Skellysolve B extracted	Triton X-100 treated, Ether wash	
		Before	After		Before	After
	(1% oil)			(5% oil)		
5	–	–	–	1.2	2.1	1.2
11	28.6	45.1	35.5	5.1	6.3	5.3
18	66.7	83.9	83.9	–	–	–
22	–	–	–	21.9	34.9	29.5
24	69.8	80.3	80.3	–	–	–
33	75.1	72.8	72.7	42.3	64.4	46.8
48	63.4	69.4	69.3	56.4	74.9	60.6

TABLE 9. DISTRIBUTION OF CELL MASS AND FISH OIL AFTER CENTRIFUGATION OF CULTURE BROTH (BASED ON GROWTH IN 3% W/V OIL MEDIA)

Distribution of oil and cell mass[1]	*Candida lipolytica* 22 h	*Candida lipolytica* 33 h	*Geotrichum candidum* 22 h	*Geotrichum candidum* 33 h
Cream phase:				
% of total residual oil	83.9	86.6	90.7	89.1
% of total cell mass	33.6	23.3	44.2	52.9
Oil % w/w	80.5	66.4	75.3	53.2
Cell mass % w/w	19.5	33.6	24.7	46.8
Sediment:				
% of total residual oil	16.1	13.4	9.3	10.9
% of total cell mass	66.4	76.7	55.8	47.1
Oil % w/w	29.8	8.7	20.0	12.0
Cell mass % w/w	70.2	91.3	80.0	88.0
Total residual oil as % of initial oil content	54.0	27.0	53.3	30.2
Yield coefficient	.3284	.4873	.3561	.4530

[1]Reported as % dry cell mass.

500 ml shake flask to 25 ml. As a consequence, the specific growth rate of *C. lipolytica* grown on the 5% fish oil medium increased slightly from 0.2346 (Table 6) to 0.2398, and the 48-h dry cell yield increased considerably from 2.327 g (Table 6) to 2.822 g resulting in a yield constant of 0.665. Except for the growth of *C. lipolytica* on the 5% oil medium, it appeared that as fermentation progressed the consumption of fish oil per gram of dry cell increased from about 1.1 g after 11 h to about 1.5 g after 48 h of fermentation. In the same medium *G. candidum* specific growth increased from 0.2180 (Table 6) to 0.2450 and dry cell yield rose from 1.840 g (Table 6) to 3.104 g, producing a yield constant of 0.710. Maximum yields of biomass for the 1% fish oil media were obtained after 33 h of fermentation: 0.751 g dry cells with a yield constant of 0.805 for *C. lipolytica*, and 0.678 g dry cells with a yield constant of 0.735 for *G. candidum*. These yield values were obtained from hexane washed cells and are within the range of yield values reported for hydrocarbon utilizing yeasts *(2, 5)*. Nitrogen values obtained on dry cells indicate a crude protein content of 40.1 to 44.2% for *G. candidum*, and 46.8 to 49.3% for *C. lipolytica*.

Some additional microbial oxidations using a 1% fish oil medium with *C. lipolytica* (shake flask filled to 120/80 ml) produced the yield values shown in Table 11.

The analysis of the dry cell mass from the above oxidations indicated an average crude protein content of 42.7% with a range from 39.3-51.2%. These values, obtained under shake flask conditions, suggest that it would be feasible, using the observations made in this study, to obtain in batch–or continuous culture systems from 1.0 kg fish oil 800 g single cell biomass with a crude protein yield of 320 g.

ACKNOWLEDGMENTS

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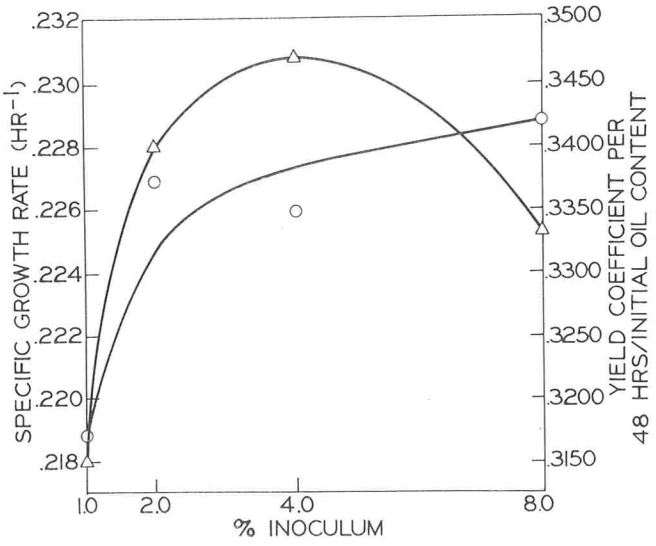

Figure 1. Effect of inocula size on specific growth rate (h^{-1}), open circles, and yield coefficient per 48 h, open triangles, for *Candida lipolytica*.

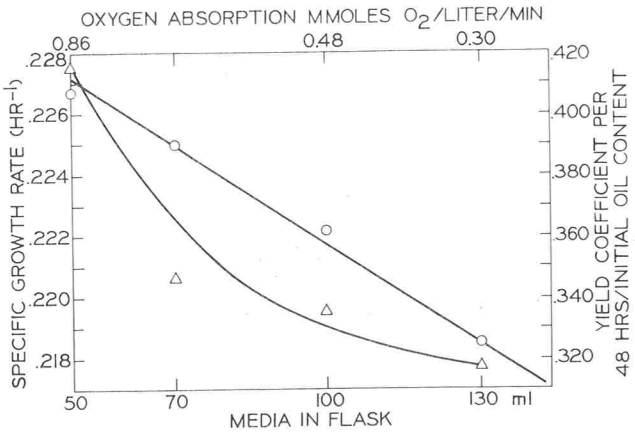

Figure 2. Effect of aeration on specific growth rate (h^{-1}), open circles, and yield coefficient per 48 h for *Candida lipolytica*, open triangles.

TABLE 10. UTILIZATION OF FISH OIL AND GROWTH OF BIOMASS ON FULLY SUPPLEMENTED MEDIA AT A VOLUME OF 25 ML MEDIA PER 500 ML SHAKE FLASK[1]

Microorganism	Initial oil content of medium (% w/v)	Test criteria	Propagation (h) 11	18	22	24	33	48	Specific growth-rate (h-1)	Doubling time (h)
Candida lipolytica	1.0	Oil consumed % w/v	0.340	0.855	—	0.919	0.933	0.948	0.2172	3.19
		g oil consumed/g dry cell	1.19	1.28	—	1.31	1.24	1.50		
		Dry cell yield g/100 ml	0.286	0.666	—	0.698	0.751	0.634		
		Yield constant	0.842	0.780	—	0.761	0.805	0.668		
		N uptake g/g dry cell	0.072	—	—	0.065	—	0.071		
		Dry cell analysis:								
		Nitrogen %	7.48	—	—	7.86	—	7.88		
		Protein %	46.77	—	—	49.12	—	49.27		
Candida lipolytica	5.0	Oil consumed % w/v	0.443	—	1.712	—	3.217	4.244	0.2398	2.89
		g oil consumed/g dry cell	1.75	—	1.56	—	1.52	1.50		
		Dry cell yield g/100 ml	0.253	—	1.094	—	2.112	2.822		
		Yield constant	0.571	—	0.642	—	0.657	0.665		
		N uptake g/g dry cell	—	—	—	—	—	—		
		Dry cell analysis:	—	—	—	—	—	—		
Geotrichum candidum	1.0	Oil consumed % w/v	0.322	—	0.816	—	0.924	0.938	0.2239	3.10
		g oil consumed/g dry cell	0.88	—	1.29	—	1.36	1.72		
		Dry cell yield g/100 ml	0.353	—	0.634	—	0.678	0.546		
		Yield constant	1.130	—	0.776	—	0.735	0.582		
		N uptake g/g dry cell	0.066	—	0.063	—	—	0.078		
		Dry cell analysis:								
		Nitrogen %	6.42	—	7.07	—	—	6.52		
		Protein %	40.15	—	44.18	—	—	40.73		
Geotrichum candidum	5.0	Oil consumed % w/v	0.538	—	1.431	—	3.136	4.371	0.2450	2.77
		g oil consumed/g dry cell	1.15	—	1.27	—	1.32	1.40		
		Dry cell yield g/100 ml	0.480	—	1.125	—	2.366	3.104		
		Yield constant	0.892	—	0.786	—	0.754	0.710		
		N uptake g/g dry cell	—	—	—	—	—	—		
		Dry cell analysis:	—	—	—	—	—	—		

[1]Media composition: 5.0% w/v Fish oil medium: $(NH_4)_2SO_4$ 1.5%, KH_2PO_4 2.2%, $MgSO_4$ 0.12%, $FeSO_4 \cdot 7H_20$ 10 mg/liter, $MnSO_4 \cdot H_2O$ 1.5 mg/liter.
Fish stickwater solids 300 mg/liter, (For *G. candidum*: Biotin 40 μg/liter).
1.0% w/v Fish oil medium: $(NH_4)_2SO_4$ 0.6%, KH_2PO_4 0.8%, $MgSO_4$ 0.06%, $FeSO_4 \cdot 7H_2O$ 5 mg/liter, $MnSO_4 \cdot H_2O$ 1 mg/liter.
Fish stickwater solids 300 mg/liter, (For *G. candidum*: Biotin 10 μg/liter).

TABLE 11. UTILIZATION OF FISH OIL BY *Candida Lipolytica*

Initial pH of media	Yield coefficient based on initial oil content of 1%	Specific growth rate (h-1)	Doubling time (h)
5.8[1]	0.358 - 0.756	0.1827 - 0.2271	3.05-3.79
7.5[2]	0.760 - 0.870	0.2145 - 0.2450	2.83-3.23

[1]Ranges for 8 experiments.

[2]Ranges for 7 experiments.

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ABSTRACTS OF PAPERS PRESENTED AT THE SIXTY-FIRST ANNUAL MEETING OF IAMFES

ST. PETERSBURG, FLORIDA

AUGUST 12-14, 1974

The 61st Annual Meeting of IAMFES was marked by the presentation of 14 contributed research papers. The abstracts of those papers appear below. In addition, 15 invited papers were presented and the abstracts of most of them also appear below. The complete text of many of these papers will appear in future issues of the *Journal of Milk and Food Technology*.

Contributed Research Papers

ENTEROTOXIN RECOVERY IN FOODS UNDER CONDITIONS OF POLYELECTROLYTE EXCLUSION, R. W. Bennett, D. L. Archer, and W. T. Amos, *Food and Drug Administration, Food Microbiology Branch, HFF-124 200 C Street, S.W., Washington, D. C. 20204.*

To develop a more refined method for separation of staphylococcal enterotoxins from chromatographically similar proteins in foods, enterotoxin recovery studies were done to confirm the effectiveness of low ionic strength buffer on separation of enterotoxin with exclusion of interfering food proteins. These studies were done by ion exchange column chromatography utilizing carboxymethyl cellulose (CMC). To 100-g amounts of a variety of food extracts were added 1-μg amounts of staphylococcal enterotoxin types A and B (SEA and SEB). These extracts were diluted, and percolated through 1.0-g amounts of CMC-22 equilibrated with 0.005 M phosphate buffer, pH 5.7. Columns were desorbed utilizing high ionic strength buffer (150 ml of 0.2 M PO_4-NaCl buffer, pH 7.4) and replicate columns under conditions of lower ionic strength (200 to 250 ml of 0.05 M PO_4-NaCl buffer, pH 6.5). Enterotoxin recovery was determined by the micro-slide gel diffusion test. All columns desorbed with 0.05 M PO_4-NaCl buffer, pH 6.5 showed a recovery of SEA equal to or greater than (0 to +44%) that obtained with 0.2 M PO_4-NaCl buffer, pH 7.4. Generally, the same trend was observed for the recovery of SEB.

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THE IRON CONTENT OF SEPARATOR AND CLARIFIER SEDIMENT, B. J. Demott, *Department of Food Technology and Science, University of Tennessee, Knoxville 37901.*

Raw milk received at the University of Tennessee Dairy Plant was separated at 40 F and on alternate days the raw milk was clarified at 135 F. Ten samples each of clarifier and separator sediment from the bowl were analyzed for water, protein, and iron concentration. The clarifier sediment was lower in moisture and higher in ash than the separator sediment. On a dry matter basis, there was no difference between the two sediments as related to iron concentration (76 ppm), protein concentration (77%), or the ratio of the iron to protein. The quantity of sediment was related to the quantity of milk processed; roughly the amount of dry sediment was 0.01% of the milk processed. Assuming an iron concentration in milk of 0.5 ppm, about 1.5% of the natural iron in milk is removed by clarification or separation; a negligible amount.

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SOME ENVIRONMENTAL, CULTURAL, AND NUTRITIONAL FACTORS THAT AFFECT RUBRATOXIN FORMATION, C. O. Emeh and E. H. Marth, *Department of Food Science, University of Wisconsin, Madison, Wisconsin.*

Penicillium rubrum P-3290 was grown in a glucose-salts medium with different additives and cultures were incubated under various conditions. Maximum mold growth was obtained with 10% glucose, whereas 20% favored maximum production of rubratoxin. The highest yield of rubratoxin A appeared in 6 days and of rubratoxin B in 12 days. Rubratoxin A disappeared from cultures after 22 days of incubation and rubratoxin B was gone after 26 days. All amino acids tested supported synthesis of rubratoxin B; highest yields were obtained when the medium contained glutamic acid or asparagine. Serine, methionine, and glycine supported good yields of rubratoxins A and B, whereas aspartic acid, lysine, tyrosine, and tryptophan allowed production of only rubratoxin B. Yields of toxin were enhanced by 0.2-0.3% ammonium sulfate and growth was maximal when 5% was in the medium. Inorganic phosphate generally enhanced toxin production; it was optimal at 0.6% and inhibitory at 9.6%. Copper, ascorbic acid, cobalt, thiamine, and barium did not enhance toxin production. Zinc and iron were required for toxin production but manganese and magnesium were not. A temperature of 25-28 C was optimal; reducing it to 18 C caused a 65% decline in toxin production. Increasing the temperature to 37 or 45 C caused a 60-85% decline in, or complete inhibition of, toxin synthesis. A pH value of 4-5 enhanced toxin synthesis although limited toxin production occurred at pH 10 but not pH 1. A relative humidity of 67% allowed rubratoxin production by 7-day cultures, of 77% supported highest yields of toxin, and of 85% allowed accumulation of moderate amounts of rubratoxin.

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CONTROL OF AFLATOXIN PRODUCTION IN WILD RICE, J. F. Frank, G. S. Torrey, E. H. Marth, D. A. Stuiber, R. C. Lindsay, and D. B. Lund, *Department of Food Science, University of Wisconsin-Madison, Madison, Wisconsin 53706.*

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

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ASSESSING SAFE EXPOSURE LEVELS OF METHYL MERCURY, A. G. Hugunin, R. L. Bradley, Jr., and W. E. Ribelin, *Department of Food Science, 1605 Linden Drive, Babcock Hall, University of Wisconsin-Madison, Madison, Wisconsin 53706.*

To determine the amount of mercury tolerated in mammals, guinea pigs were selected because of their known sensitivities to methyl mercury. Feeding trials were done using 10 guinea pigs at 5 dietary levels: control (0.0 ppm), 0.02, 0.2, 2.0, 20.0 ppm of methyl mercury hydroxide. Sacrifices were at 30, 90, and 120 days except that all animals on a dietary level of 20 ppm were sacrificed at 30 days. This latter group showed typical signs of mercury poisoning such as lack of activity, tremors, and ataxia. In the fourth week, three animals died in this group because of bronchopneumonia. No deaths or typical symptoms of mercury poisoning or irregular growth rates were recorded in any of the other groups. No changes were observed when serum samples from guinea pigs were analyzed for these enzymes: alkaline, phosphatase, lactic dehydrogenase, and serum transaminases (SGOT). Mercury levels in guinea pig hair, determined by neutron activation, showed logarithmic increases paralleling dietary levels. Stabilization occurred at 90 days of feeding.

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PATULIN PRODUCTION IN CHERRIES BY *penicillium* AND *aspergillus* SPECIES, Joseph Lovett, Brenda Boutin, and Rubin G. Thompson, *Food and Drug Administration, Cincinnati, Ohio 45226.*

The purpose of this experiment was to determine the potential for mycotoxin contamination in mold damaged cherries. Ten patulin producing strains of *Aspergillus* and *Penicillium* were grown on canned tart cherries packed in water (no added carbohydrate). Cultures were assayed for patulin on days 6 and 8. Ethyl acetate extracts were analyzed by TLC. Plates were developed in benzene:methanol:acetic acid (90:5:5) and air dried. After being sprayed with 0.5% 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate, plates were heated for 15 min at 135 C for color development. Patulin was produced in concentrations of $>$ 100 μg/ml by *A. clavatus* (NRRL 1980), *P. claviforme* (NRRL 2149 and NRRL 1002), *P. expansum* (NRRL 973), and *P. patulum* (ATCC 24550, M1172 and M1165). The following produced little or no patulin: *A. clavatus* (ATCC 9598 and ATCC 9599) and *P. griseofulvum* (NRRL 2300). Cherries could provide an excellent substrate for mycotoxin production.

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A RECOMMENDED PROGRAM FOR MILK DISTRIBUTION AND RETAIL HANDLING, C. K. Luchterhand and Ward K. Peterson, *Wisconsin Division of Health, Madison, Wisconsin 53701 and Milwaukee, Wisconsin 53222.*

The Grade A Pasteurized Milk Ordinance has been recognized as an excellent document that has caused the general public to be provided with a safe, wholesome milk supply. The next revision should include an optional ordinance designed to control milk through retail outlets. We have moved from a situation of local dairies delivering fresh milk daily to their customers, to large processing plants that market their products across state lines. In so doing the dairy processor has lost control over his products. A survey of milk distribution practices was conducted in 1968 on several local health jurisdictions in Wisconsin. It was apparent that changes taking place in the marketing of dairy products resulted in Grade A Milk Products deteriorating in flavor because of longer storage and at storage temperatures above 45 F. Local health departments were encouraged to adopt an ordinance to control milk handling at the retail level. Also a "community rating system" was devised.

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ENTEROPATHOGENIC *Escherichia coli* IN FOODS, Ira J. Mehlman, A. C. Sanders, N. T. Simon and J. C. Olson, Jr., *Food and Drug Administration, Food Microbiology Branch, Washington, D. C. 20204.*

Recent studies on behavior of enteropathogenic *Escherichia coli* (EEC) have enlarged the concept of the species concerning its role in foodborne illness. To accommodate variant behavior among strains necessitates modification of the usual procedure for its recovery. Serotyping is an important element in this regard. Although pathogenic behavior has been recognized for 50 years, *E. coli* and other coliforms continue to serve as indicators of the sanitary conditions of processing and storage of foods in contrast to *Salmonella* and *Shigella* which are regarded as overt pathogens. Routine application of tests for capacity to produce infection or intoxication is precluded at present both for technical and practical reasons. Improvement in enrichment methods and development of simple and valid tests for determination of pathogenicity must precede implementation of routine analytical examination of foods for the presence of EEC. In the interim serotyping of isolates and the application of appropriate tests for evaluation of pathogenic capacity are recommended for the identification of biotypes generally associated with human illness.

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INCIDENCE AND PROLIFERATION OF *geotrichum candidum* IN REFRIGERATED CAKE YEAST AND FACTORS AFFECTING ITS GROWTH AND DETECTION, P. B. Mislivec, S. M. Cichowicz, and V. R. Bruce, *Division of Microbiology, Food and Drug Administration, Washington, D. C. 20204.*

Geotrichum candidum, the etiological agent of the human disease, Geotrichosis, was found as a contaminant in fresh active pressed cake yeast which had been held under refrigeration. To determine the growth kinetics of *G. candidum* in cake yeast at refrigerated temperatures, four separate sets of 12 cakes were held for 25 days (up to 6 days beyond expiration dates) at 4 C and 8 C, 6 cakes per set at each temperature. Counts were determined periodically in wort and potato dextrose agar pour plates incubated at 26 C. The organism did not grow in potato dextrose agar. However, in wort agar, counts increased steadily at both temperatures, the resultant population actually being higher at 4 C than at 8 C. Culture medium greatly influenced growth of *G. candidum*. Utilizing 11 agar media at 26 C, with growth measurement based on colony extension with time, rapid growth occurred on wort and Sabouraud maltose agars. Growth, in smaller amounts, occurred on seven of the nine remaining media. There was no growth on either malt-salt or potato dextrose agars, the latter medium used routinely in determining yeast and mold counts in foodstuffs. Effects of temperature on *G. candidum* on three agar media showed a growth range from 4 C through 37 C, optimum at 26 C.

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SHELF-LIFE OF PASTEURIZED MILK STORED AT 4.5 AND 7.0 C, L. E. Mull, S. V. Pilkhane, R. L. Richter, and K. L. Smith, *Department of Dairy Science, University of Florida, Gainesville.*

Sixty-one samples of freshly pasteurized milk were obtained from milk processors in Northeast Florida during the period April, 1973 to January, 1974. Split samples were stored at 4.5 and 7.0 C. Organoleptic analysis of each sample at both temperatures was made until the sample was judged unacceptable. It was found on the average that samples stored at 4.5 C had a shelf-life of 5 days longer than those stored at 7.0 C. This difference was not uniform for all samples, and tended to be less than 5 days for short shelf-life samples and greater than 5 days for long shelf-life samples. It was calculated that the shelf-life at 4.5 C was 28.8% longer than that at 7.0 C. The correlation between the log of the Moseley Count and the shelf-life was $-.64$ at 4.5 C storage temperature and $-.62$ at 7.0 C storage. If the Moseley count is 31,000/ml or less, 90% of the samples should have a shelf-life of 10 days or longer at

4.5 C and if the count is 450/ml or less, 95% should keep 14 days or longer.

BETALAINES AS COLORANTS IN DAIRY PRODUCTS, J. H. Pasch, J. H. von Elbe, and R. J. Sell, *Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706; and Chr. Hansen's Laboratory, Milwaukee, Wisconsin.*

Color in foods is one of the most important attributes and is subject to great changes not easily controlled. Therefore, to control appearance, addition of artificial dyes is often required. In recent years safety of artificial dyes has been questioned and, as a result, their use in the future will be limited if not eliminated. It is for this reason that interest has developed in obtaining pigments from natural sources. The red beet is a rich source of pigments, and their use as colorants in dairy products warrants investigation. In addition, beet powder is permitted as a colorant under the 1960 Color Additive Amendment. The term betalaine refers to the class of beet pigments which contains both betacyanines (red) and betaxanthines (yellow). The betanine content (major red pigment) in beets is in excess of 100 mg per 100 g fresh weight and juice concentrates and powders available range in dye content between 0.2 and 1%. Evaluation of betalaines as colorant and color stability of betalaines in yogurt, ice cream, and sherbert have been conducted. Subjective and objective color values were measured and, as an example, yogurt colored with 45 ppm pigment, calculated as betanine, was judged a raspberry shade.

RECOVERY OF AFLATOXIGENIC MOLDS FROM FOODS IN THE HOME, G. S. Torrey and E. H. Marth, *Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706.*

A silica-gel medium containing glucose and mineral salts was developed to detect aflatoxigenic fungi. Under ultraviolet light, outer green and inner blue fluorescent rings were visible around 4- and 5-day old mold colonies that produced aflatoxins B_1 and G_1 on the medium. Samples of refrigerated and nonrefrigerated foods, and soil from refrigerator surfaces were collected from households participating in the Wisconsin Expanded Food and Nutrition Education Program, and in other volunteer organizations. The silica-gel medium was used to detect the presence of aflatoxigenic fungi in samples. All molds isolated on the medium were retained for identification. A total of 190 molds were isolated from 240 samples. Nine of the isolates produced aflatoxin. Of the samples containing aflatoxigenic molds, three were refrigerated foods (yeast, cheese, cottage cheese) and six were nonrefrigerated (breads, cake, spices). These data indicate that molds able to produce aflatoxins can be present in food stored in the home. Molds isolated from the samples were species of the genera *Penicillium* (44%), *Aspergillus* (15%), *Mucor* (3%), and a variety of others (38%).

DEVELOPMENT OF AN IMPROVED METHOD FOR THE ISOLATION OF *Salmonella* FROM ACTIVE YEAST, C. R. Wilson, P. L. Poelma, and W. H. Andrews, *Food and Drug Administration, Division of Microbiology, Washington, D. C. 20204.*

Examination of dried active yeast for *Salmonella* is currently made by pre-enrichment in 1% Tryptone broth using a sample/broth ratio of 1 to 5 with subsequent transfers to selenite-cystine (SC) broth and tetrathionate (TT) broth before streaking onto selective agars. An improved method has been developed which employs Trypticase Soy (TS) broth as the pre-enrichment medium using a sample/broth ratio of 1 to 10 with subsequent transfers to Lauryl Sulfate Tryptose (LST) broth and TT broth before streaking onto selective agars. The improved method was evaluated, by MPN technique, for effectiveness with pressed active yeast as well as the dried active yeast. With the dried active yeast the improved procedure developed up to 700 times more *Salmonella* in the pre-enrichment broth than did the previous method. The improved procedure was more productive with pressed yeast than the previous method, but not to the extent noted with dried yeast. Both forms of yeast have unique characteristics which seem to preclude a universal examination procedure.

PRELIMINARY STUDY TO DETERMINE THE FEASIBILITY OF USING A 0.20-INCH DIAMETER DISK TO MEASURE SEDIMENT IN FLUID MILK, Earl O. Wright, Donald K. Hotchkiss, and Warren S. Clark, Jr., *Departments of Food Technology and Statistics, Iowa State University, Ames, Iowa 50010; and American Dry Milk Institute, 130 North Franklin Street, Chicago, Illinois 60606.*

A preliminary collaborative study was done to determine whether laboratory personnel could grade raw milk for sediment content using 0.20-inch diameter as well as 0.40-inch diameter sediment disks. The 0.40-inch disk presently is used for grading sediment in mixed bulk milk samples. Technicians in 10 separate laboratories made 800 determinations (80 per laboratory) of known sediment amounts on 0.20-inch and 0.40-inch diameter standard sediment disks; 225 (63.7%) of 400 determinations made on the 0.20-inch diameter disk were graded accurately, whereas 187 (46.8%) of the 400 determinations made on the 0.40-inch diameter sediment disks were graded correctly. Based on these results, it is recommended that photoprint standards be developed for 0.20-inch diameter sediment disks and that a further collaborative study be undertaken.

INVITED PAPERS

TREATMENT OF RAW MILK WASTES BY A MULTI-STAGED BIOLOGICAL SYSTEM, William L. Arledge, *Dairymen, Inc.; Louisville, Kentucky 40202, and* John K. Sullins, *Sullins Ecology Engineering, Inc., Kingsport, Tennessee.*

The Bristol, Virginia Division of Dairymen, Inc. is a storage and transfer operation for raw milk. As production increased and effluent quality regulations became more stringent, waste treatment progressed from land irrigation, to a two stage biological system including: *(a)* full mixed anaerobic; *(b)* facultative - high rate trickling filter and non solids controlled aeration; *(c)* quiescent anaerobic; and *(d)* facultative - high rate trickling filter and activated sludge.
Treatment efficiencies are:

	Raw	Treated	% Efficiency
BOD_5	3920	73	98
COD	3300	40	99
TOC	1750	20	99
Total Nitrogen	168	22	87
Total Suspended Solids	570	56	90
Oil and Grease	1200	0	100

MILK QUALITY IN THE PUBLIC SCHOOL SYSTEM, David K. Bandler, *Department of Food Science, Cornell University, Ithaca, New York 14850.*

It may be that children are learning more in school than we would prefer. A current study at Cornell indicates that many are learning to dislike milk. Surveys at 693 schools in New York State show a high incidence of off-flavor in milk and excessively high storage and serving temperatures in the schools. Complaints by school lunch managers and children should become cause for concern by milk producers, processors, and distributors. Research has also uncovered some basic problems in school milk sales—lack of information on the part of cafeteria workers; poor quality control on the part of the dairies; and

questionable design of refrigeration equipment in the schools. A practical program to solve these problems is proposed. In New York State, when school is in session, over 10% of the Class I milk sales are made to schools. (*Note*: This research, and subsequent remedial program is supported in part by a $48,900 grant from the New York State Dairy Promotion Order and the New York State Department of Agriculture and Markets.)

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PROCEDURES FOR SURVEILLANCE OF BULK MILK SAMPLING, Raymond A. Belknap, *Laboratory Development Section, Division of Microbiology, Bureau of Foods, Food and Drug Administration, Cincinnati, Ohio 45226.*

Surveillance of methods for collection of raw milk samples and their transmittal to laboratories is essential to interpretation of laboratory results for official grading purposes. At least 15,000 farm bulk tank truck drivers collect milk samples each day. Accuracy of thermometers and sanitization and/or sterilization of sample transfer instruments, agitators and containers is important. Agitation of milk, collection of temperature controls and samples, and storage in insulated sample cases, containing a suitable refrigerant during transmittal to the laboratory, are necessary critical operations in the collection of representative samples. Accurate records indicating time, date, and temperature at collection must accompany each series of samples. Samples must arrive at the laboratory between 32-40 F and examinations should begin within 36 h after collection. Surveillance of sample collectors is conducted at least biennially by FDA approved State milk sampling evaluation officers who have met FDA criteria for certification.

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TEMPERATURE PROFILES OF PERISHABLE DAIRY PRODUCTS IN RETAIL FOOD STORES, F. W. Bodyfelt and W. D. Davidson, *Department of Food Science and Technology and Extension Service, Oregon State University, Corvallis, 97331.*

A direct reading, quick response electronic thermometer was employed to conduct thorough, systematic temperature profiles of perishable dairy products in 25 Oregon retail food stores. Sensitive thermistor surface probes permitted measurement of product temperatures from the exterior surfaces of two adjacent containers to within 0.28 C (0.5 F). A switch box facilitated the simultaneous use of up to six surface probes for monitoring product, air blower, and air "curtain" temperatures at various cabinet shelf locations. Fluid milk temperatures for the outside rows of shelves averaged 1.9 C higher than the inside rows of product. The average temperatures (C) for products in display cabinets were: fluid milk and cream, 6.4; cottage cheese, 7.1; hard cheese, 9.4; and frozen dairy desserts, −17.0. At least 35% of the products profiled exceeded the maximum legal temperature of 7.28 C (45 F) and 71% of the product temperatures exceeded the generally accepted optimum of 4.5 C (40 F). The data indicate a definite need for improvement in food store temperature control for extended shelf life of perishable dairy products. A retail food store temperature profile form was designed to simplify and efficiently record temperature data.

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STATE SHELLFISH PROGRAMS AND THE NATIONAL SHELLFISH SANITATION PROGRAM, J. David Clem, *Shellfish Sanitation Branch, Division of Food Technology, Bureau of Foods, Food and Drug Administration, Washington, D. C. 20204.*

The sanitary control of fresh and fresh frozen oysters, clams, and mussels has long been achieved through an interlocking cooperative program between coastal State control agencies, Food and Drug Administration, and the shellfish industry. This program, known as the National Shellfish Sanitation Program (NSSP), is now being questioned for its effectiveness in assuring that only safe and wholesome shellfish are offered the consumer. The 1925 public health principles upon which the NSSP was founded are still valid today; however, the administrative framework, procedural mechanisms, and recognition of present day legal requirements necessitates modernization and formalization of the existing NSSP. In consideration of the need to have broader participation and understanding of shellfish safety control measures by local, State, and Federal officials, the Food and Drug Administration is presently developing new Federal shellfish regulations. The objective of these new regulations is to make the NSSP a more effective consumer protection program and ensure that appropriate State and Federal shellfish control measures are fairly and adequately applied. The reasons why FDA is prompted to develop such regulations are presented.

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TEMPERATURE RECORDING—ITS EFFECTS ON QUALITY AND MARKETABILITY OF FLUID MILK, Joseph Hansberry, *The Partlow Corporation, 2 Campion Road, New Hartford, New York 13413.*

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

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MICROBIOLOGICAL QUALITY OF GROUND BEEF AND CHICKEN PARTS FROM RETAIL STORE MEAT CASES, Robert J. Hasiak and William S. LaGrange, *Department of Animal Science, Iowa State University, Ames, Iowa 50010.*

Ground beef and chicken breast samples were purchased from eight different retail food stores on three different days of each of two separate weeks. Duplicate microbial analyses were done on each of the samples to determine the standard plate count, psychrotrophic count, *Clostridium perfringens, Staphylococus aureus*, and *Salmonella.* This study was done to determine the microbiological quality of fresh meat within a given store, and between stores. Standard plate count results indicated significant differences existed between stores as well as within stores in a given week. Similar differences were observed in the psychrotrophic counts. This study indicated that prepackaged meat generally had lower bacterial counts than did that which was wrapped after purchase. All samples tested were negative for *Salmonella,* and only rarely were *C. perfringens* or *S. aureus* isolated from chicken samples, while the incidence of these organisms was significantly greater in the ground beef samples. Results of this study indicate a need to intensify sanitary practices at the retail food store.

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PREVENTING PROBLEMS IN THE FOOD INDUSTRY, E. H. Marth, *Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706.*

The food industry is continually faced with such problems as food additives, food-borne diseases, consumer pressures, economic concerns, and difficulties associated with a given operation. Unsolved problems lead to crises and no organization can effectively operate on a crisis basis for any length of time. Problems must be anticipated and prevented before they occur. Prevention of problems involves: *(a)* adequate knowledge, *(b)* effective use of manpower, and *(c)* proper com-

munications. Adequate knowledge entails familiarity with the processing operation, raw materials, the finished product, the environment, the laboratory and its results, and regulations. To use manpower effectively people and jobs must be matched properly, what is expected of employees must be adequately explained, criticism or correction must be done properly, workers must be rewarded adequately for their services, and a sense of loyalty must be developed among employees. Clear, meaningful, and sufficiently detailed communications are needed to minimize many of the people-related problems in the food industry.

BOTULISM IN CANNED FOODS, R. B. Read, Jr. and D. A. Kautter, *Food and Drug Administration, Division of Microbiology, 200 C Street, S.W., Washington, D. C. 20204.*

Occasionally, a problem thought to be well under control returns to plague us. This is true of botulism in commercially canned foods. These foods have had a remarkably good record over the last 45 years with about 3/4 of a trillion cans of commercially canned foods being consumed with only three known deaths from 1925 through 1969. Since then, however, botulinal toxin and/or *C. botulinum* has been found in commercially canned vichyssoise, chicken vegetable soup, peppers, tuna fish, and in 41 cans of mushrooms from 20 lots of seven domestic and two foreign producers. The typical cause of botulism in canned foods is underprocessing which may result from inadequate equipment, improper operating procedures, and scheduled processes which are not appropriate for the actual operating conditions being used.

ENERGY CONSERVATION IN THE FOOD INDUSTRY, A. L. Rippen, *Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824.*

Reduced supplies and rising costs of energy emphasize the importance of conservation and increase the feasibility to invest in fuel recovery systems which more fully use utilities. The methods of processing may shift to those requiring less net energy. Some products require considerably more energy than others. One ton of canned green beans ready for distribution require approximately 3 million K. Cal., whereas, for one ton of canned peaches the requirement is about 1.4 million. Canning requires slightly more energy than freezing up to the point of warehouse storage. Indications show that with some adjustments in methods and following prudent conservation practices most food processors can reduce energy consumption probably 15% as a minimum.

THE PERFORMANCE OF PRESENT-DAY CIP SYSTEMS RELATED TO WATER AND CHEMICAL UTILIZATION, Dale A. Seiberling, *Equipment-Engineering Division, Economics Laboratory, Inc., Beloit, Wisconsin 53511.*

CIP systems are available in two different forms. Multi-tank recirculating units utilize the same wash solution for a large number of cleaning operations during the production day, adding to this solution as required to maintain strength and cleaning ability. Single-use systems operate on the basis of making up smaller volumes of solution automatically to the required concentration, using it once at the lowest possible strength, and discharging it to the sewer at the end of each cycle. Several dairy organizations have evaluated the two systems under controlled conditions, on limited applications such as tanker washing operations, but the data have not been made available to the industry. Published "comparisons" of the two systems have been based on theory, rather than field evaluation of operating systems achieving comparable levels of performance. Further, an effective comparison can be based only on total system design and application including *(a)* design of the CIP supply-return system, *(b)* engineering design and arrangement of circuits and vessels to be cleaned, and *(c)* location of CIP recirculating units, rather than the type of unit alone. This report is based on evaluation of seven re-use systems installed in three different plants and eight single-use systems installed in four other plants; one of each being located in one of the plants under study. Three of these plants include multiple units in multi-product plants processing fluid milk products, cottage cheese, and ice cream, and the design capacity and the equipment installed in each of these plants was comparable. Systems were programmed to achieve comparable levels of performance based on physical evaluation of the equipment being cleaned and the quality of the finished product handled through this equipment. Cost data were obtained for each individual circuit, vessel, or HTST pasteurizing system, after which the daily cleaning cost for each plant at a normal operating load was established. These data were further used to estimate the cost of cleaning a theoretical plant by applying individual cycle costs to a standard "typical" cleaning load. Estimates are provided regarding the operation of a single-use solution recovery system which combines the advantages of the two basically different approaches.

AN APPROACH FOR ATTACKING WORLD FOOD PROBLEMS, W. F. Wedin, *World Food Institute, Iowa State University, Ames, Iowa 50010.*

Approaches to solving food problems have often been too specific, both here at home and abroad. In developing countries, chronic food problems have often been attacked with a technology, the adoption-diffusion of which, if non-appropriate to mores and customs of the people, has in the long-run been counter-productive. Through the World Food Institute at Iowa State University, we propose to identify problems, analyze them, bring competencies to bear on solving them, provide a continuing feed-in of educated, competent people geared to a problm-solving, interdisciplinary attack, and study the interrelationships to Iowa and the United States. We propose a continuing thrust from our University utilizing pertinent components of the land-grant mission which permitted problems to be solved in Iowa. Through this outward thrust in the broader, international scale, we hope to improve the nutrition and hope for hunger avoidance of humans elsewhere, and simultaneously thereby to increase our own understanding. We look to the interchange of food-related knowledge which, in the ultimate, knows neither borders nor political leanings. In 1976, we will host a World Food Conference where the role of the professional in feeding mankind will be highlighted.

PRESIDENTIAL ADDRESS, Earl O. Wright, *413 Kellogg Avenue, P. O. Box 701, Ames, Iowa 50010.*

The year 1974 will go down in our history as a year of big changes in our organization. There was a change in Executive Secretary and the headquarters were moved from Indiana to Iowa. This year considerable effort has been directed toward strengthening the affiliate organizations and reviving some affiliates that were not active in recent years. It has been very gratifying to receive approximately 350 new members this year. Several states or areas do not have organized affiliates even though large memberships made up of direct members are on file. It should be a challenge to us to organize these units. The study with NEHA on a possible future merger of the two organizations was continued. Committee work is the backbone of our organization, and every effort has been put forth to strengthen the committees. The IAMFES is an active and a strong organization only because of the input of its individual members.

ASSOCIATION AFFAIRS

A. K. "KELLY" SAUNDERS

A. K. "Kelly" Saunders passed away on March 11, 1974.

Born in Okotoka, Alberta, Canada on September 28, 1908, he and his family moved to Bowen, Illinois where his father was actively engaged as a Minister. After graduating high school in Bowen, Illinois, his family moved to Sycamore, Illinois where Kelly attended DeKalb Teachers College and then taught school for eight years.

It was at this point that "Kelly" became associated with the Dairy Industry . . . and for the rest of his life.

Kelly's accomplishments and activities are too numerous to mention; however, this partial listing capsules his active role in associating himself with the Dairy Industry.

After 18 years with Diversey Corporation, he became head fieldman for the Litchfield Creamery in Litchfield, Illinois for 15 years. Kelly then held position with Monarch Chemical Co. & Lazarus. "Kelly" then moved to DeLaval Separator Co. where he developed their Sanitation and Detergent Program. Kelly retired from DeLaval in the fall of 1973; however, he continued as a consultant.

Kelly was an active member of the International Milk, Food & Environmental Sanitarians Association for over 20 years and served as an outstanding chairman of the Farm Methods Committee from 1962 through 1973. In addition, he was a member of the National Mastitis Council where again he worked effectively on committee assignments. He received the IAMFES Citation Award in 1968.

Kelly is survived by his wife, Alice and two children; Richard of Hawaii and Diane Karneboge of Georgia. In addition, Kelly and Alice raised eight foster children including Ernest; Charlotte and Heddy. Kelly also has eight grandchildren and two great grandchildren.

"Kelly", as he was known to all segments of the Dairy Industry was responsible, directly and indirectly, for many of the high standards this dairy industry maintains today.

NOTICE

IAMFES AWARDS 1975

Eeach year IAMFES recognizes outstanding contributions 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 equally 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 following Awards, which will be considered for presentation at our 1975 IAMFES Annual Meeting.

1. *The Sanitarian's Award* of $1000 to a state or Federal 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 University or Industry employee who has made outstanding contributions to food safety and sanitation.
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 Walter F. Wilson, Chairman of the IAMFES Recognition and Awards Committee, County Los Angeles Health Dept., 313 N. Figueroa St., Los Angeles, Ca. 90012.

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, 1975, 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, Klenzade 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 62nd annual meeting of the Association which is to be held at Toronto, Canada.

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 62nd annual meeting at Toronto, Canada.

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 recepient.

   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 within 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 renomination for this Award.

Nominations

Nominations of condidates 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 subsmsiion of nominations may be obtained upon request from Earl O. Wright, Executive Secretary, International Association of Milk, Food and Environmental Sanitarians, Inc., P. O. Box 701, Ames, Ia. 50010.

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, 1975. Nominations should be submitted to Walter F. Wilson, 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.

Walter F. Wilson
Committee on Recognition and Awards,
Los Angeles Health Dept.
313 Figuerao St.,
Los Angeles, Ca. 90012

LETTER TO THE EDITOR

Joe S. Taylor memorial scholarship announced

The Joe S. Taylor Memorial Scholarship has been established to provide scholarships for undergraduate students enrolled in the College of Agriculture at The Pennsylvania State University.

Taylor, who died July 19 at 59 had been in charge of dairy extension programs at Penn State since 1954 and was an outstanding leader in the state's dairy industry. He served as an advisor to many organizations and had been president of the Pennsylvania Junior Dairy Show since it was started in 1955.

The monies for scholarships will be made available from interest accrued through investment of the principal fund. Scholarships will be awarded to one or more full time undergraduate students. Preference will be given to students who have an interest in the dairy industry and who are enrolled in one of the animal majors such as Animal Industry, and Animal Science, or their equivalent. If there are no eligible applicants in these categories, the scholarship may be granted to any other student enrolled in the College of Agriculture. Students will be selected on the basis of academic record and financial need.

The number of scholarships, and the amount of each will vary from year to year depending on the income realized from the principal fund. Awards will be made in an amount at least equivalent to full tuition for one academic term. Scholarships may be renewed at the discretion of the selection committee if the recipient continues as a full time student in the College of Agriculture and meets other conditions of eligibility.

The recipients of these scholarships shall be selected by the Extension and Resident faculty in the field of dairy science, in consultation with the Committee on Scholarships of the College of Agriculture. The latter Committee will recommend the awardees to the Office of Student Aid in accordance with University policy.

Individuals or organizations wishing to contribute to the scholarship fund should send checks to the Office of Gifts and Endowments, 116 Old Main, University Park, Pa. 16802. Checks should be made payable to the Pennsylvania State University and they should be designated for the *Joe S. Taylor Memorial Fund.*

HERBERT C. GILMORE
Department of Dairy Science
The Pennsylvania State University
University Park, Pennsylvania 16802

LETTER TO THE EDITOR

Scholarship fund to commemorate P. H. Tracy

DEAR SIR:

We were all saddened by the news of the passing of Dr. Paul H. Tracy who for 39 years actively served on our staff. He will be remembered for his research contributions that are still benefiting the dairy industry; for his extension activities that brought technical knowledge to all levels of dairy plant personnel in a form that was to serve as a model for the rest of the country; and perhaps most importantly for the imprint he made on all of his students, friends, and associates. His continued concern for the well-being and pride for the high level of performance of his former students, at both the University of Illinois and extension level, was testimony to his dedication to his life's work as a teacher.

In gratitude for all that he has done for us and to insure that his influence is long remembered, we are establishing the Paul H. Tracy Memorial Scholarship Grant for undergraduate students studying Food Science at the University of Illinois. Any individual, company, or society is invited to share in this tribute by contributing to the scholarship fund. Checks made out to the University of Illinois Foundation and directed to this specific purpose may be sent to the University of Illinois Foundation, 224 Illini Union, Urbana, Illinois 61801.

We hope to hear from many of you.

JOSEPH TOBAIS, R. MCL. WHITNEY, V. L. PORTER
Paul H. Tracy Memorial Committee, Department of Food Science, University of Illinois, Urbana, Illinois 61801

NOTICE TO MEMBERSHIP

In accordance with IAMFES Constitution and By-laws, which requires our Second Vice-President and Secretary-Treasurer to be elected by mail ballot, you are hereby notified that President P. J. Skulborstad, at the annual meeting in St. Petersburg, Fla., August 1974, appointed the following members to the nominating committee for 1975: Al N. Myhr, Harold Wainess, Karl Jones, Elmer Marth, Ray Belknap, Mel Jefferson and Bill Kempa.

Nominations for the office of Second Vice-President and Secretary-Treasurer are now open and any member wishing to make a nomination should send a picture and biographical sketch of his nominee to the Nominating Committee not later than November 15, 1974. To maintain proper balance on Executive Board nominees should be selected from industry, preferably food processing area.

Al Myhr, Chairman
Nominating Committee
Univ. of Guelph, Dairy Dept.,
Guelph, Ontario, Canada

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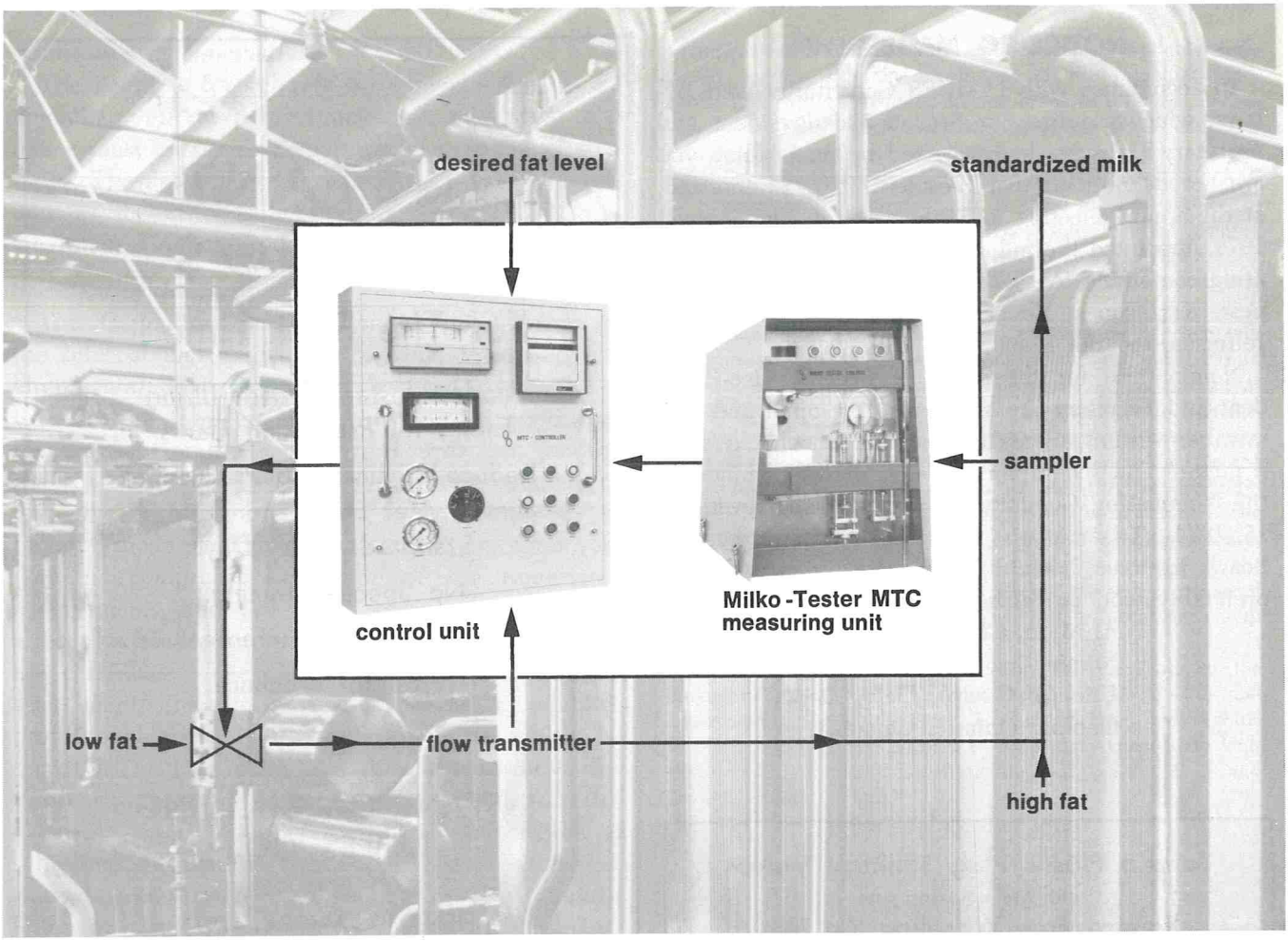

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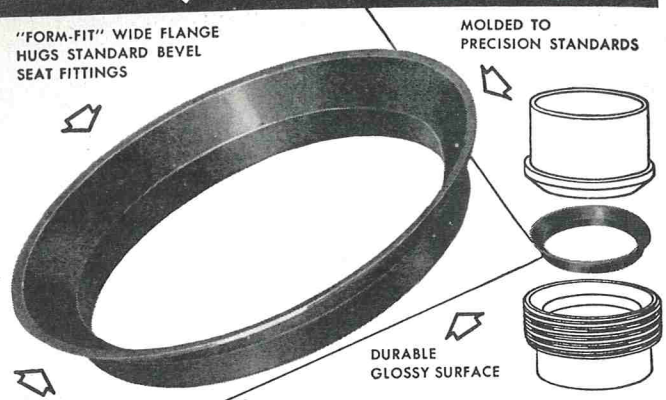

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Dairy authorities speak out on better cow milking

William L. Arledge
Director of Quality Control
Dairymen, Inc.

Quality milk makes consumers happy and helps dairymen prosper.

Dairy farmers are unique. In most cases their primary source of income is their milk check. A major influence on that milk check is quality, and it has been shown that, as the quality of milk in a given market improves, so does consumer demand, and so does income to the dairy farmer.

High quality milk starts with the cow but it must be continued each step of the way until the milk is purchased and consumed.

TASTE MAKES FRIENDS

The nutritional value of milk to the human diet is never disputed. The relative dollar value of these nutrients, particularly protein in relation to other food costs, is also not disputed. However, consumers could care less about protein and nutritional value if the milk does not taste good due to poor quality control practices somewhere between the cow and the consumer. People simply will not buy poor quality milk.

Over 90% of consumer complaints are the result of post-pasteurization contamination and improper temperature control of the bottled product after pasteurization. This is dramatized by the fact that seven Grade A dairy plants recently increased the shelf life of their total packaged products from an average of 6 days to more than 21 days by improving in-plant controls over post-pasteurization contamination. Little or no change was made in the quality of the raw milk coming into the plant. (This shows that significant improvements can be made in dairy plants as well as on farms.)

REVIEW YOUR EFFORTS

Your role of producing quality milk daily can be as simple as you desire to make it.

It has been proven many times that it is cheaper to spend whatever is necessary to thoroughly clean your bulk tank, pipeline, or bucket milkers (all milk contact surfaces) than it is to do an improper job.

Follow these recommendations:

1. Read the label of your chlorinated cleaner detergent and *measure* the correct amount of water to be added to the correct amount of cleaner.
2. Be sure when circulating cleaning systems to start your wash cycle with 160° F. water and *stop* circulating when the temperature drops to 110° F. (If you don't, you will redeposit soil and fat.)
3. In colder areas, the use of a heating element in the wash tank is recommended to help maintain wash temperatures above 110° F., preferably 130-140° F. (Six to ten minutes is usually enough*).
4. Many dairy barns do not have hot water heaters that will deliver water at 160-180° F. and even if they do—the recovery of temperature in the heater is too slow and you end up trying to wash in lukewarm to cool water.
5. To prevent loss of *hot* water for cleanup, many dairymen install a second hot water heater and set the regulator at around 110-120° F. for use in hand washing of cows' teats and udders prior to milking as well as other manual cleaning chores. This leaves your other hot water heater available for the important job of cleaning equipment.
6. If you have an electric hot water heater, install fast recovery heating elements to prevent cool down of your hot water. For safety make sure all heaters are equipped with an approved *pressure* and *temperature* relief valve.
7. After thorough washing of equipment, rinse in an acid rinse and then immediately prior to milking always *sanitize* all milk contact surfaces with an approved sanitizer.

CARELESSNESS IS A LUXURY

As surprising as it may seem, we still find some people rinsing equipment with plain water after sanitizing. This causes great problems affecting milk quality since all water supplies contain from a few to large numbers of the "cold loving" psychrothrophic bacteria that can and do cause some very undesirable off-flavors in milk. Granted these organisms are killed by pasteurization but, by carelessness, dairymen can alter the flavor of milk prior to its leaving the farm by 1) lack of sanitizing, or 2) rinsing equipment rather than washing, or by 3) only rinsing a bulk tank.

Too many times we see dairymen doing a good job in their milking management practices, but disregarding their water supply, temperature of cleaning solutions and sanitization, thus nullifying all other good practices.

Quality of your *only* product affects *your only* income; your milk check. You can do more concerning the quality of your milk! Follow the routine and procedures you know to be correct and with these few quality tips, you may prevent the shipment of a tank of less-than-superior-quality milk.

We must all relate to the consumer. Do a quality job in your personal operation and expect the same throughout the chain of events to the consumer. You will reap the benefits through personal pride "all the way to the bank".

*Refer to local Health Department regulations

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