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R. G. Arnold
University of Nebraska-Lincoln

R.B. Maxcy
University of Nebraska-Lincoln

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Evaluating Circulation Cleaning by Analysis of Soil Depletion from Surfaces [1]

R. G. ARNOLD and R. B. MAXCY
Department of Food Science and Technology
University of Nebraska, Lincoln 68503

Abstract

The rate of soil removal and its nature were studied with a small commercial high-temperature short-time pasteurizer. The commercial recirculation system was altered to provide a single pass of cleaning solution through the equipment to observe progress of cleaning. A composite of the first 38 liters was taken after 1.5 minutes. Subsequent samples were taken after 3, 6, 12 and 21 to 22 minutes. Cleaning solutions were analyzed by centrifugal fractionation, solvent extraction, gas liquid chromatography, thin layer chromatography and infrared spectroscopy to determine the composition of residue and rate of its depletion.

Results showed both the nature of the tenacious material and the rate of removal. Triglycerides were the predominant material in the tenacious residue, which resisted removal by circulation cleaning. Triglycerides were in solutions taken after several minutes of cleaning. Triglycerides per se were removed during cleaning, thus contradicting the concept that saponification is an integral part of the cleaning process. The sensitivity achieved indicated the potential application of instrumental methods for evaluating cleanliness of food processing equipment.

Introduction

Traditional criteria for cleanliness of food processing equipment have been based on visual inspection. Modern methods of processing liquid products, however, generally are closed systems cleaned by circulation. Though considerable progress has been made in circulation cleaning, some soil remains even in processes universally considered to be acceptable (7, 9). The extent of soil remaining is influenced by imperfections resulting from corrosion of equipment.

The residuals from food processing and imperfect cleaning are generally referred to as milkstone in the dairy industry (12) with similar nomenclature related to hard residues in other food industries (10). The residue is particularly troublesome in operations of rapid heating to high temperatures. Work on the nature of this residue has been limited to observations on the gross composition, such as mineral salts, protein and fat (1); these results are applicable when the residue is abundant. When residues are below the threshold of visibility, less is known of their composition. Analyses for protein by the micro-Kjeldahl method and the method of Lowry et al. (4) proved to be little more accurate than visual examination (7). Analysis for calcium residue and removal has been used to evaluate cleaning processes (2). Residual soil below the limit detectable by these methods is still adequate to support the growth of microorganisms (5, 6).

The long range objective of this study is development of more sensitive methods for determining effectiveness of circulation cleaning.

Experimental Procedures

Equipment, soiling, and cleaning process. To obtain samples of residue to reflect the normal challenges of cleaning, a small commercial high-temperature short-time pasteurizer was used. The unit consisted of 61 plates with a regenerative efficiency of more than 80%. Pasteurization was 75C for 16 seconds. Residue accumulated on this equipment during processing of approximately 5,000kg of Grade "A" milk.

After normal processing, the unit was rinsed with tap water until it was clear at the outlet. For cleaning, 1,136 liters of 1% w/v phosphated caustic[2] at 77C was used. The solution was pumped through the pasteurizer at 52 liters per min, discharging to the drain. Cleaning solution, flow rate and temperature were essentially the same as had been proven through

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[2] Wyandotte Chemicals Corp., Wyandotte, Michigan.

routine use to clean the equipment beyond visual detection of soil. The single pass cleaning operation allowed sampling of the cleaning solution at progressive stages of cleaning. A composite of the first 38 liters was taken after 1.5 minutes. Subsequent samples were taken after 3, 6, 12 and 21 to 22 minutes.

Gross composition. Nitrogen was determined on cleaning solutions using a micro-Kjeldahl method to estimate protein. The Biuret test was also used for determination of proteinaceous material. Total solids were determined by a vacuum oven procedure. Ash was determined on aliquots of the dry matter.

Preliminary explorations of the nature of components removed by cleaning solutions were fractionation by centrifugation and extraction by various organic solvents. Nature of the organic materials in the various fractions was determined by infrared spectroscopy and gas liquid chromatography (GLC). Dried fractions were reacted with trimethylsilyl reagent for further characterization.

Sample preparation of organic solvent soluble material. Samples of cleaning solutions and a control of the cleaner were extracted by a modified Roese-Gottlieb procedure (8). Ammonium hydroxide was excluded. The extracts were concentrated in a rotary evaporator and a vacuum desiccator.

Samples analyses. Extracts were subjected to GLC analyses with a flame ionization gas chromatograph.[3] Various columns and temperatures determined the appropriate parameters for separation of the extracted materials. A 61cm by 3.2mm od stainless steel column packed with 2.5% SE-30 on 60 to 80 mesh VarAport 30[4] performed satisfactorily when programmed from 150 to 310C at 2 degrees per min. Columns were inserted into the injection port to provide on-column injection. Other instrument parameters were: injection port, 250C; detector, 320C; carrier gas, 25ml per minute N_2; range, 10^{-11}; attenuation, $\times$ 1 to $\times$ 256.

Retention data and quantitative comparisons were related to tricaproin, incorporated as an internal standard.

Sample identification. Extracts from the cleaning solutions were subjected to thin layer chromatographic separation on pre-coated silica-gel plates[5] with ethylene dichloride as the solvent.

For infrared spectroscopy, samples were obtained by trapping appropriate fractions from gas chromatographic separations or from thin layer plates. A Beckman GC-cell[6] was used with carbon tetrachloride as the solvent.

Extracts were saponified, and the fatty acids were separated and esterified with methanol containing 1% sulfuric acid (3). The resulting methyl esters were chromatographed on a 366 cm by 3.2 mm od aluminum column packed with 7.5% diethyleneglycol succinate on 80–100 mesh Chromosorb W, programmed from 120 to 170C at 2 degrees per minute.

Results

Observations on gross composition of soil removed by the cleaning solution. Determination of total solids, nitrogen and ash indicated considerable amounts of ash, measurable organic matter and minor amounts of protein. Estimates of protein by the Biuret test confirmed the relatively low content of proteinaceous material, even in early phases of the cleaning process. Similar indications were obtained by infrared spectroscopy. Silation of fractions of cleaning solutions before GLC analysis indicated minor amounts of components with amino, hydroxyl, carboxyl or similar functional groups indicating the absence of carbohydrates. The combination of these observations suggested that the organic solvent soluble material was an important part of the material removed by the cleaning solution. Further work was directed toward an understanding of the properties of the organic solvent soluble fraction in the cleaning process.

Components from the cleaning process. Preliminary observations indicated high GLC temperatures were required to reveal any components which persisted in the cleaning process. As cleaning progressed there was a reduction in the number and quantity of components in the cleaning solution extracts, as shown by GLC analysis. Figure 1 shows a comparison of chromatograms of samples taken after 1.5, 3, 12, and 21 to 22 minutes of cleaning.

The chromatograms in Figure 1 are representative of a normal, satisfactory cleaning process. On other occasions less effective cleaning resulted in the persistence of components well into the cleaning process. For example, malfunction of the temperature control on one occasion resulted in considerably more residue on the pasteurizer plates than normal. In this instance the chromatogram of the sample from the last of the cleaning solution revealed size-

[3] Model 1740, Varian Aerograph, Walnut Creek, California.

[4] Varian Aerograph, Walnut Creek, California.

[5] Brinkman Instruments, Westbury, New York.

[6] Beckman Instruments, Fullerton, California.

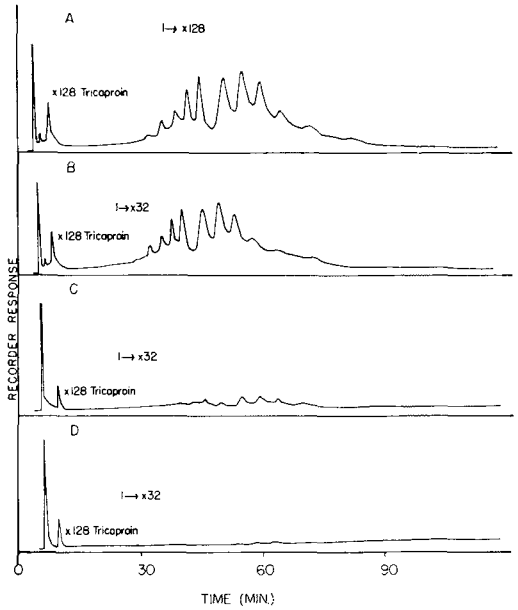

FIG. 1. Chromatograms of cleaning solution extracts: A, composite of first 38 liters; B, after 3 minutes; C, after 12 minutes; D, at the end of the cleaning process.

able peaks, indicating the removal of significant amounts of residue throughout the entire cleaning process.

There was a parallel in the persistence of compounds in the cleaning process and their slow movement on GLC. This relationship implied that the tenacious residue consisted of high molecular weight materials.

Nature of components of tenacious residue from equipment. Commercial literature and some authors (10, 11, 12) make frequent reference to the role of saponification in cleaning processes. Since our procedure involved extraction from samples of alkaline solutions, fatty acids presumably would not have been included. Therefore an alternate procedure was used to verify fatty acids. Before extraction, samples of cleaning solution were adjusted to pH 4.0. Extracts of samples were analyzed by GLC and the results were compared to those from alkaline extractions. This alternate procedure did not produce a detectable difference in the chromatograms. To confirm the efficacy of the alternate procedure to reveal free fatty acids, known quantities were added to cleaning solutions before acidification and extraction. Fatty acids, when present, were revealed by GLC analysis. Thus saponification had a minor role in the removal of tenacious residue from equipment.

A sample of butteroil was chromatographed with conditions identical to those described for extracts from the cleaning solutions. Several components in the butteroil had identical retention times as components from the cleaning solutions (Fig. 2). The inference is that triglycerides are an important part of the tenacious residue.

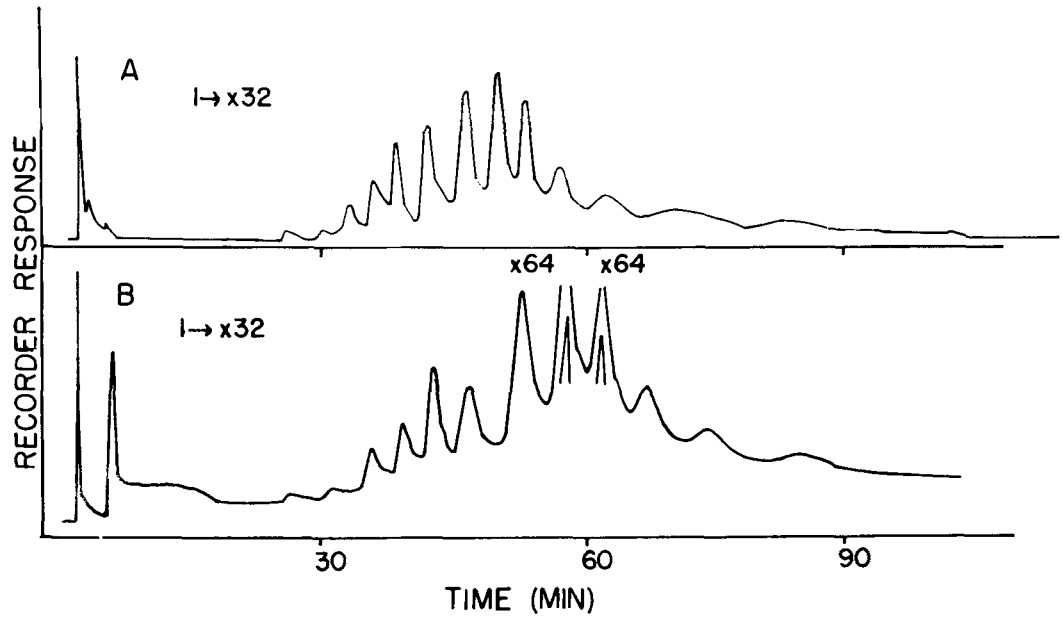

FIG. 2. Chromatograms of A, extract of cleaning solution after 3 minutes of cleaning; B, butteroil.

Retention data for pure triglycerides were obtained, and compared to components of the extracts of the cleaning solution. Excellent agreement was again demonstrated.

To obtain additional information as to the nature of the high molecular weight material, extracts from the cleaning solutions by alkaline extraction as well as the alternate acid extraction were subjected to thin layer chromatography. Extracted components moved at the same rate as known triglycerides. An example of the results is given in Figure 3. Free fatty acids appeared in the thin layer chromatograms only in the samples from the first 38 liters of cleaning solution, suggesting removal of free acids absorbed on surfaces of equipment. The triglyceride spot appeared even after a major part of the cleaning process.

Chromatograms of the methyl esters resulting from saponification and methylation of cleaning solution extracts and milk fat were similar (Fig. 4). Retention times of standard methyl esters were similar to components derived from the extracts and from milk fat.

Infrared spectra of fractions of cleaning extracts revealed an intense absorption band at 5.74μ, which is the characteristic carbonyl absorption band of an ester.

Discussion

The single pass system of cleaning enabled sampling at different times in the cleaning process. As cleaning progressed, the extent of removal of the residue decreased. The concentration and nature of the materials at the terminal stages are most important, as they are the most difficult compounds to remove.

Our results indicate factors other than saponification are involved in initial removal of lipids from equipment surfaces, since the lipids occur in cleaning solutions as neutral fats rather than as fatty acids. These findings do not agree with the concept that saponification is a major factor in alkaline cleaning. The difference in conclusions may be attributed to our single pass cleaning system. A common recirculation system involves prolonged exposure to alkaline solution, high temperature and agita-

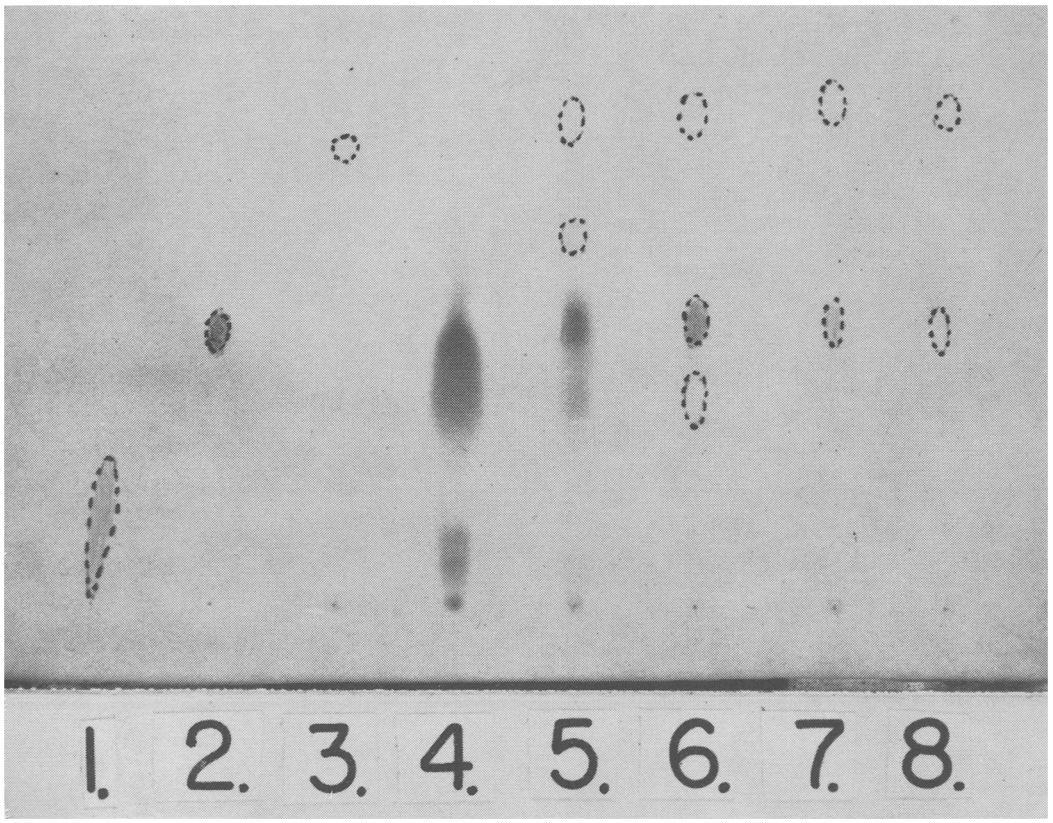

FIG. 3. Thin layer chromatogram of: 1, known free fatty acid mixture; 2, known triglyceride mixture; 3, extract of control cleaning solution; 4 to 8, extracts of cleaning solutions from first 38 liters, then after 3, 6 and 12 minutes, and at the end of the cleaning process, respectively.

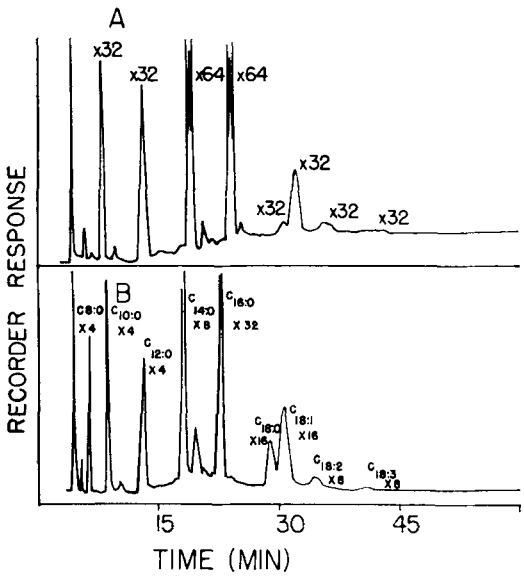

FIG. 4. Chromatograms of methyl ester mixture resulting from saponification and methylation of: A, 3 minute cleaning solution extract; B, butteroil.

tion. These combined factors may saponify some fat after removal from surfaces.

With the single pass system of removing soil from equipment, rate of removal can be determined. Concentration of soil in the aliquots is directly related to the amount remaining on surfaces. Thus removal rate can be determined. Extrapolation of removal rates should enable prediction of the end point of soil removal.

Specific procedures in our work could be used to study effectiveness of various cleaning processes. This approach could be extended to the evaluation of cleaning materials, equipment design, other soiling conditions and other foods as related to cleaning. This work will be continued to include observations on other components and the interaction of microorganisms with the residue.

Acknowledgments

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