at pH 4.5 are possible and could take place during processing of orange juice resulting in the production of volatile carbonyls.

ACKNOWLEDGMENT

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RELATIONSHIP OF BACTERIAL CONTAMINATION IN ORANGE OIL RECOVERY SYSTEM TO QUALITY OF FINISHED PRODUCT

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Recent introduction by the beverage industry of drinks using citrus oils as one of their flavor components has created a large demand for some of the oils, particularly oil of grapefruit. Increased usage of these oils has attracted more processors not only to recover their oil, but also to seek refinements in their operation to increase yields.

To the best of the authors' knowledge, approximately 65% of the oils currently produced in Florida employ the FMC (Food Machinery Corp.) In-line extractor procedure. The remainder use the screw press method. A typical FMC In-line installation is shown in Fig. 1. This method consists briefly of a special oil cup assembly on the extractor which is used to recover the peel oil expressed during juice extraction. The oil is released by a shredding action. The shredded peel, generally referred to as peel grit slurry, along with a small amount of water is collected on the baffles on one side of the extractors. It is then conveyed, usually by means of a small screw conveyor, to one or more finishers where the solids are removed. The aqueous phase or oil finisher emulsion is then pumped to a desludger where most of the water is removed by centrifugal action. The oil emulsion (oil-rich fraction) from the desludger may now contain from 30 to 70% oil. It is polished and then winterized to remove the waxy materials. Orange peel oil is referred to as cold-pressed (c.p.) when

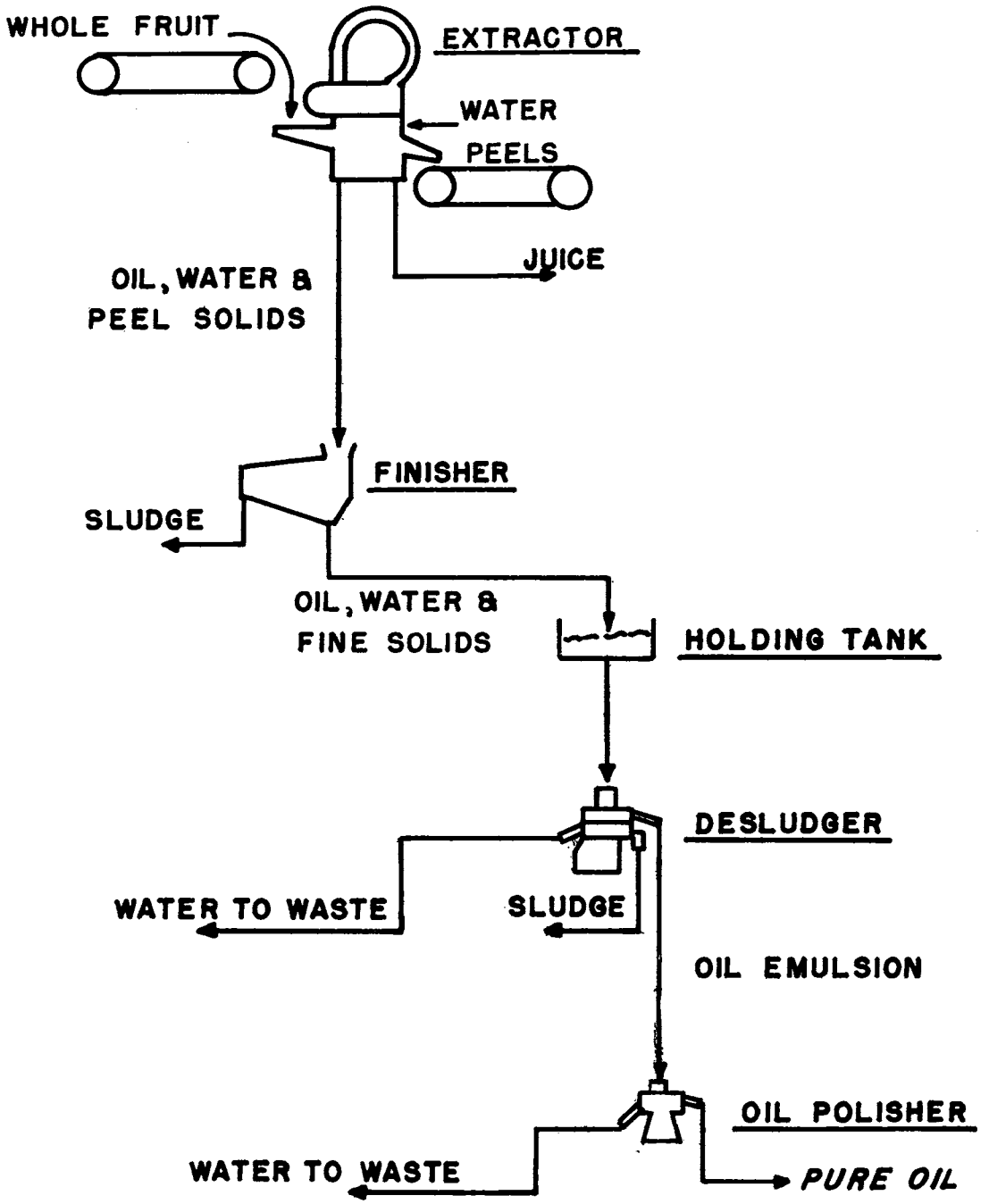

Fig. 1.—Typical FMC In-line extractor peel oil recovery system.

undistilled, and folded when concentrated by vacuum distillation.

It is known that many essential oils possess antimicrobial properties. Murdock and Allen (2) noted the fungicidal effect of orange peel oil and d-limonene against yeast in water and in single strength orange juice. Subba, et al. (4) found orange oil to exhibit a more inhibitory effect than lemon oil against the spores of *Bacillus subtilis*. These authors also noted yeasts, in general, were more sensitive than bacteria to orange oil.

Sanitation has never played an important role in the peel oil recovery system in Florida since it has generally been the consensus that slurry, with its high oil content, would never support microbial growth. Consequently, some of the equipment is never cleaned throughout the processing season. The sanitary aspect of the orange peel oil process might be summed up by a statement obtained from one operator, "We just operate the system and no one worries about sanitation." The essential oil industry has never investigated the relationship of sanitation to the quality of the finished oil.

This paper concerns an investigation of an orange peel oil operation employing the FMC method of oil extraction, with particular emphasis on the microbiological aspects of the operation and the end products produced by microbial growth.

Procedure

Bacteriological

Samples of orange oil slurry and emulsion were obtained from various stages in the manufacture of c.p. orange oil during the 1963 and 1967 Valencia seasons. The product was pour plated in duplicate on orange serum agar, and the plates counted after 3 - 5 days of incubation at 30°C (86°F). Some samples were incubated for an extended period at this temperature. Orange oil emulsion from the desludger, besides being plated, was analyzed by gas chromatography (GC) at periodic intervals.

Suspensions were prepared from 4 different colony isolates using a special medium consisting of orange serum agar (BBL), plus 3% orange oil emulsion. Each isolate was grown on modified orange serum agar slants prepared in 8-oz screw-cap bottles and incubated 3 - 5 days at 30°C (86°F). Each slant was washed with a small amount of sterile peptone water, and the resulting suspension placed in a sterile bottle containing glass beads. The number of organisms present was determined by making plate counts on orange serum agar after 3 to 5 days incubation at 30°C (86°F). The suspensions were stored in a refrigerator when not in use.

Analytical

Oil was separated from the emulsion by centrifuging and then analyzed by gas chromatography (GC). The oil constituents, represented by the peaks in the chromatogram, were identified by infrared and mass spectroscopy. A Perkin-Elmer 226 Flame Ionization Gas Chromatograph was used to monitor the oil.

The alpha-terpineol fold in the oil was calculated from its chromatographic peak height. Any deviations, because of sample size or delivery loss of the sample into the gas chromatograph, were corrected using the natural n-decanal concentration in the oil as an internal standard.

The identification of the oil constituents was accomplished using a F & M Flame Ionization Gas Chromatograph Model 1609. The instrumnt was fitted with an effluent splitter and a ¼" x 18' packed column. The constituents of the oil represented by each peak were collected and examined by both infrared and mass spectroscopy. Identification of alpha-terpineol as well as the other oil constituents was accomplished by comparing the unknown spectra with that of authentic spectra. The Perkin-Elmer and F & M Gas Chromotographs were operated as follows:

Perkin-Elmer

Column:	300' x 0.01" Capillary Column coated with Carbowax 20M
Helium Flow:	Programmed from 30 - 120 psi at 1 psi min.
Temp.:	Programmed from 75° - 175°C at 1°/min.

F & M

¼" x 18'
10% Carbowax, 20M on Chromosorb W
Flow Rate: 50 ml/min.
Programmed 125° - 200°C at 2°/min.

The orange oil emulsion used for this phase of the study had the following analysis:

% Oil w/w Scott Method (3)	67.2%
° Brix (water phase)	3.4
pH	5.0
Gas Chromotography	Normal

Results and Discussion

The assumption that microbial growth is not a problem in processing c.p. orange oil, because of the high oil content, seems unfounded. Our investigation has shown that this process is an excellent medium for microbial growth. For example, a sample of orange oil emulsion from California increased in 24 hours at 30°C (86°F) from 175 to 12,000,000 org. per ml. Samples of product from a Florida oil operation also showed a very rapid rate of microbial growth when incubated 5 days at 30°C (86°F) (Table 1). These samples contained Voges-Proskauer (VP) reactants, as the data in Table 1 indicate. The oil emulsion from the desludger was VP positive after 24 hours, and all samples positive after 5 days of incubation at 30°C (86°F). This would indicate the presence of diacetyl and/or acetylmethylcarbinol. The pH and ° Brix changes are shown in Table 2.

In another study a sample of oil emulsion from the desludger was allowed to stand several days in the laboratory. As much of the oil as possible was then removed by decanting. The resulting slurry was diluted 1:1 with distilled water and then checked for VP reactants by the distillation procedure developed by Byer (1). The product was found to contain over 1 ppm diacetyl. The effect of diacetyl and/or acetylmethylcarbinol in the aqueous fraction during the manufacturing process on the quality of orange oil is not known.

Microbial Growth in Orange Oil Emulsion

Three different samples of orange oil emulsion were obtained from the desludger, one during the Valencia season in 1963, and two during that same portion of the pack in 1967. The product was stored at 30°C (86°F). It was plated on orange serum agar at periodic intervals, and at the same time a GC analysis of the oil fraction was made. Since all results were comparable, only one set of data will be presented. Typical curves are shown in Fig. 2. Data show oil emulsion is an excellent medium for microbial growth and that these organisms apparently produce alpha-terpineol, which increased in concentration even after the organisms in the emulsion began to die. Fig. 3 is an exploded view of a portion of the data showing an arbitrary line separating normal from abnormal orange oil. It was assumed a 2-fold or greater concentration of alpha-terpineol in the oil was abnormal. On this basis, oil in this experiment would be considered abnormal in 4 - 6 days. A sterilized control sample of orange oil emulsion

Table 1. Microbial growth and VP reaction during storage of components in processing c. p. orange oil.

Days 30°C	Peel grit slurry from extractor		Oil emulsion from finisher		Oil emulsion from desludger	
	org./ml	VP	org./ml	VP	org./ml	VP
0	130,000	-	220,000	-	500,000	-
1	24,000,000	-	14,000,000	-	TNTC	+
2	50,000,000	+	33,000,000	-	71,000,000	+
3	13,000,000	+	15,000,000	-	882,000,000	+
5	17,000,000	+	42,000,000	+	473,000,000	+

Table 2. Changes in pH and ° Brix during storage of components in processing c. p. orange oil.

Days 30°C	Peel grit slurry from extractor		Oil emulsion from finisher		Oil emulsion from desludger	
	pH	° Brix	pH	° Brix	pH	° Brix
0	-	-	-	-	-	-
1	4.9	4.0	4.6	6.0	4.9	-
2	4.5	3.6	4.0	5.0	4.3	-
3	-	-	-	-	-	-
5	4.0	3.4	4.0	4.6	4.2	-

showed no increase in alpha-terpineol during the test period.

Fig. 4 and 5 are gas chromotograms of normal and abnormal oils, respectively. Fig. 5 shows an increase in the alpha-terpineol concentration as the result of microbial growth.

Growth of Test Organisms in Orange Oil Emulsion

An examination of orange serum agar pour plates showed a predominance of extremely small pinpoint colonies, a type which heretofore had never been observed in any of our agar plates, perhaps because they usually were not discernible after 48 hours of incubation at 30°C (86°F).

Four different strains were isolated, based on colony characteristics, and were numbered 2, 4, 6 and 7. All organisms except Strain No. 6 were medium-sized rods of varying morphology. Strain No. 6 was a long thick rod and produced a colony characteristic of a lactic acid organism. It was noted the growth rate of the isolates could be enhanced by the addition of orange oil emulsion to orange serum agar. Good growth was obtained by the addition of 3% orange oil emulsion to this medium. Suspensions were prepared from each strain, as previously discussed.

Orange oil emulsion from the same lot used in the previous experiment was sterilized 15 min at 250°F. Four different samples were prepared. Each lot was inoculated with one of the test organisms so that the emulsion, after inoculation, contained approximately 1000 org. per ml. A heated uninoculated sample served as a control. All samples were incubated at 30°C (86°F). The emulsion was plated at periodic intervals and the oil phase analyzed by GC, as indicated in a previous experiment. The results obtained are shown in Fig. 6, 7 and 8. All strains produced alpha-terpineol except Strain No. 6, the results which are not shown. Strain No. 4 produced a higher concentration of alpha-terpineol than any of the other organisms investigated. The heated control showed no change insofar as alpha-terpineol was concerned.

In examining the bacterial and alpha-terpineol curves, it is apparent that in each case alpha-terpineol continued to increase even after the organisms began to die. One possible explanation is that as microbial growth occurred alpha-terpineol was being produced. The concentration steadily increased which continued even after the organisms reached the peak of their population and then started to level off. During the death phase of the growth curve—i.e., when the organisms were dying faster than they were reproducing—alpha-terpineol was still being produced. Since alpha-terpineol did not break

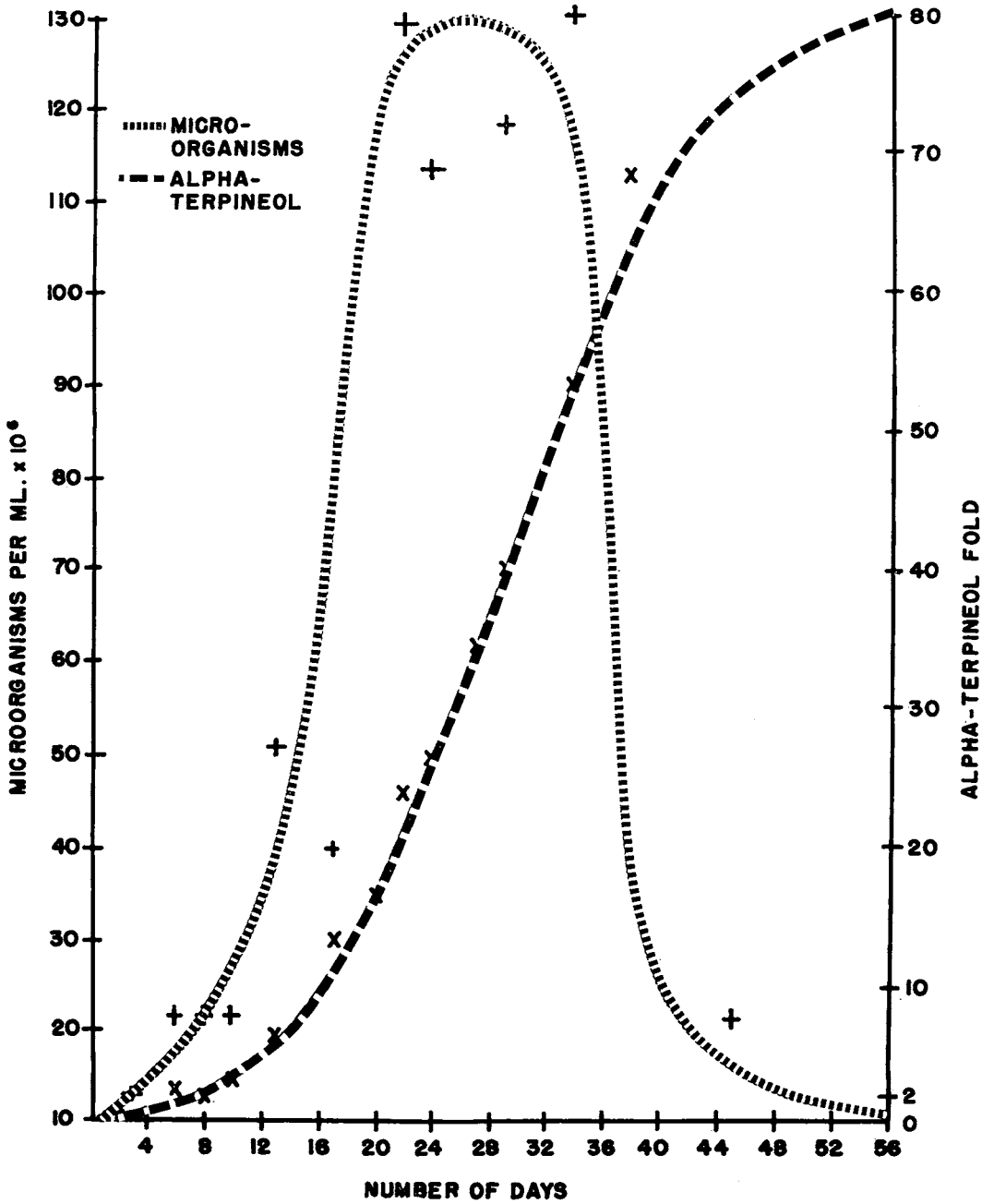

Fig. 2.—Growth of microflora in orange oil emulsion and increase of alpha-terpineol in orange oil.

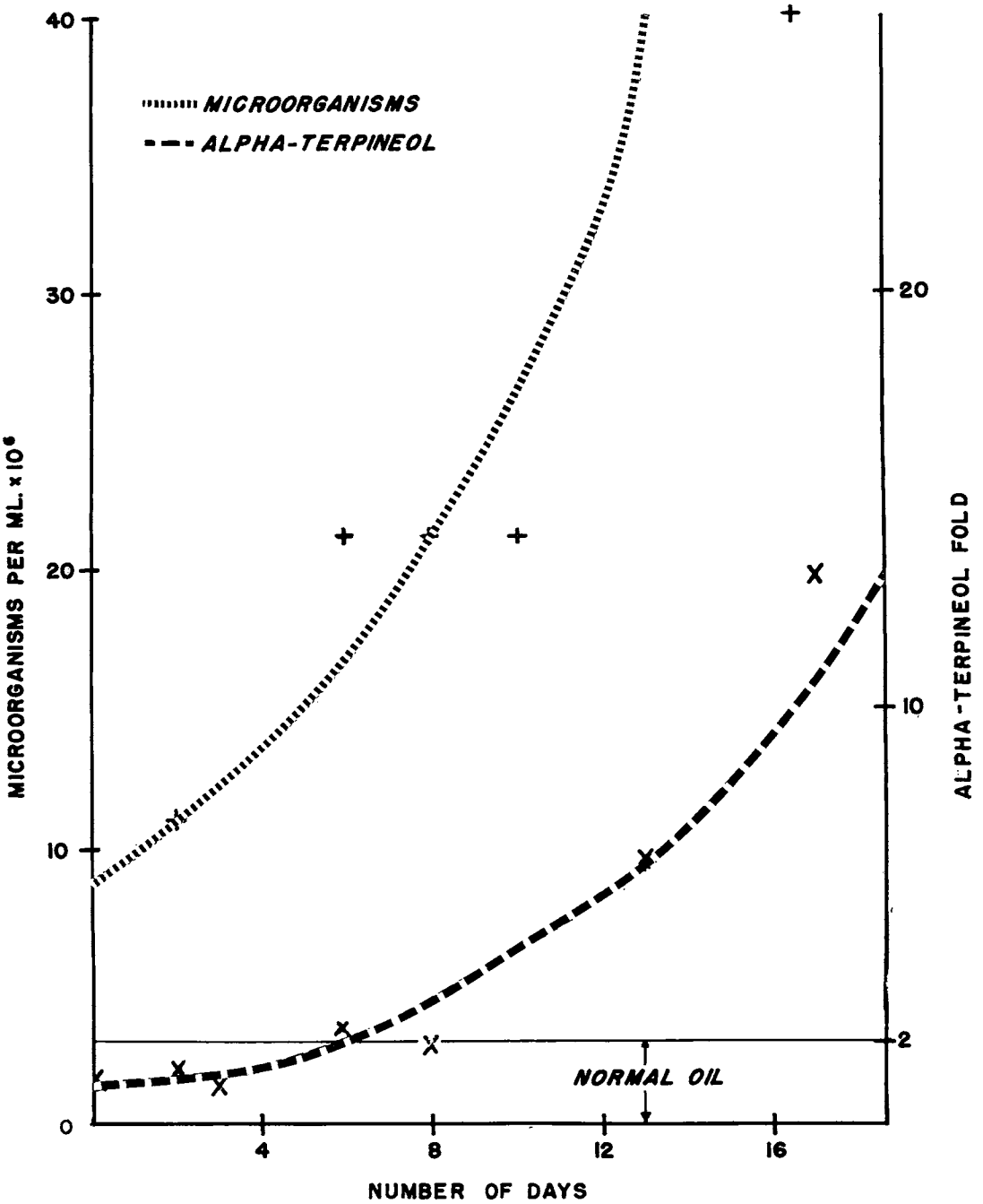

Fig. 3.—Exploded view of data in Fig. 2 showing arbitrary line separating normal from abnormal orange oil based on alpha-terpineol fold.

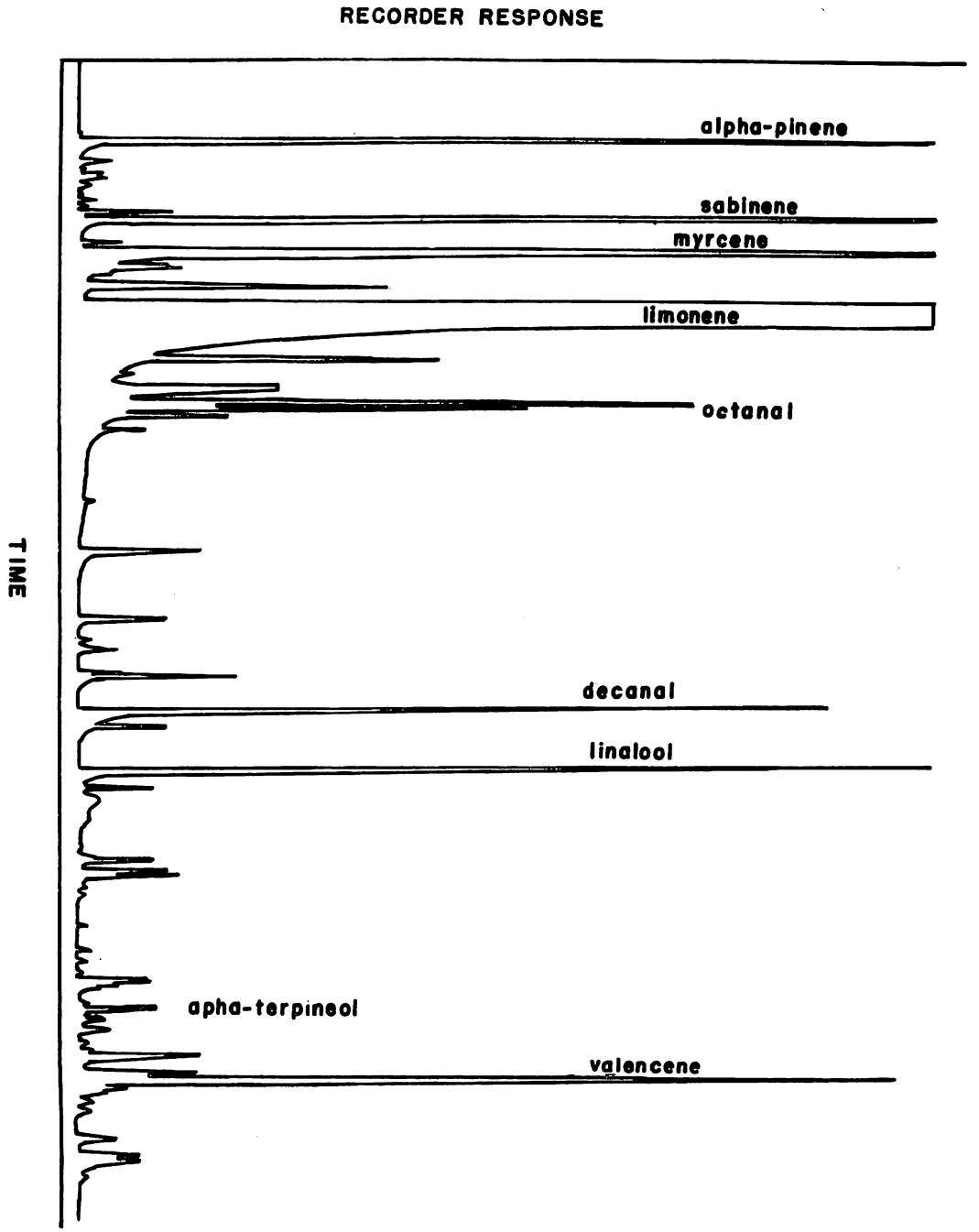

Fig. 4.—Chromatogram of normal c.p. orange oil.

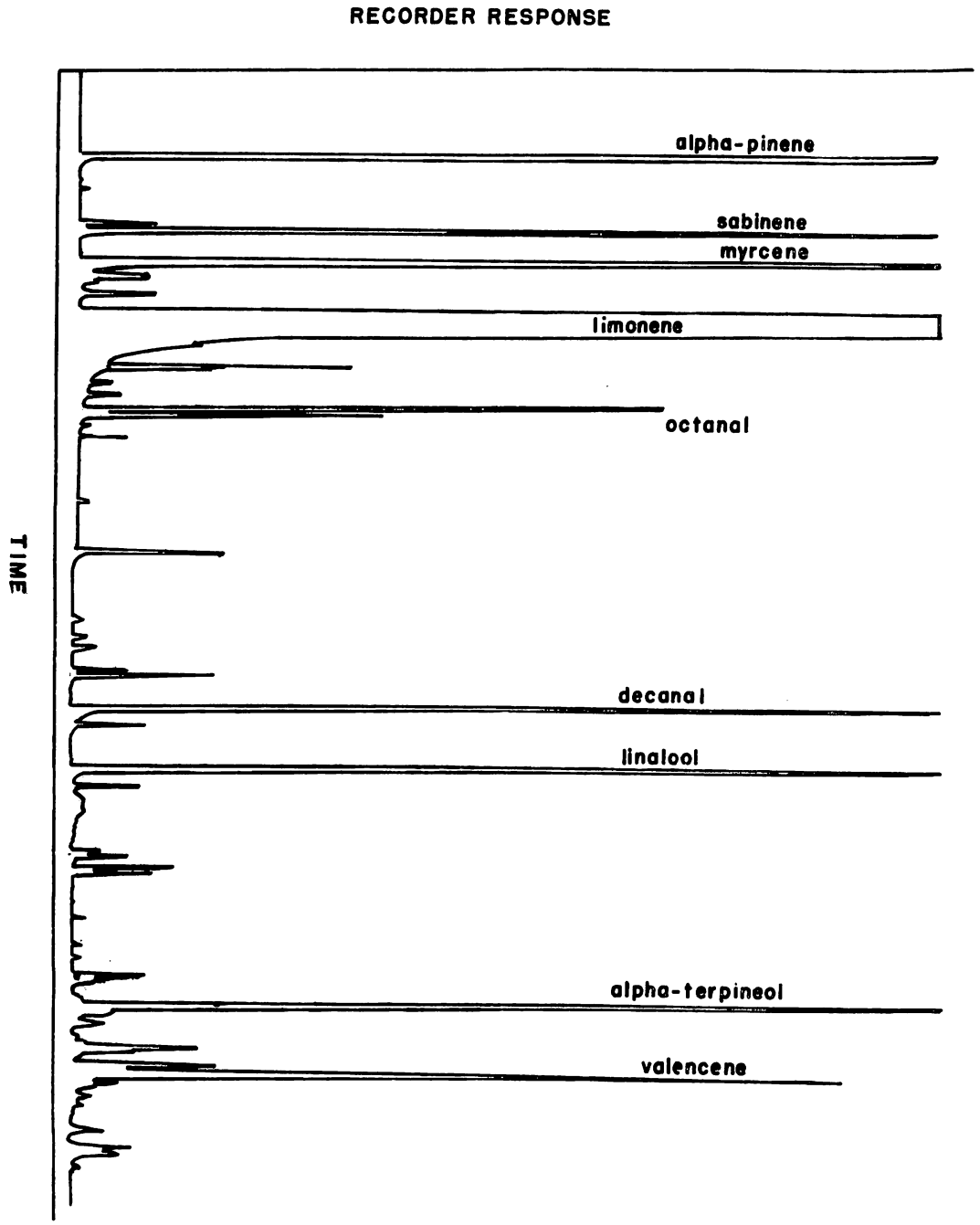

Fig. 5.—Chromatogram of abnormal c.p. orange oil showing a high alpha-terpineol peak.

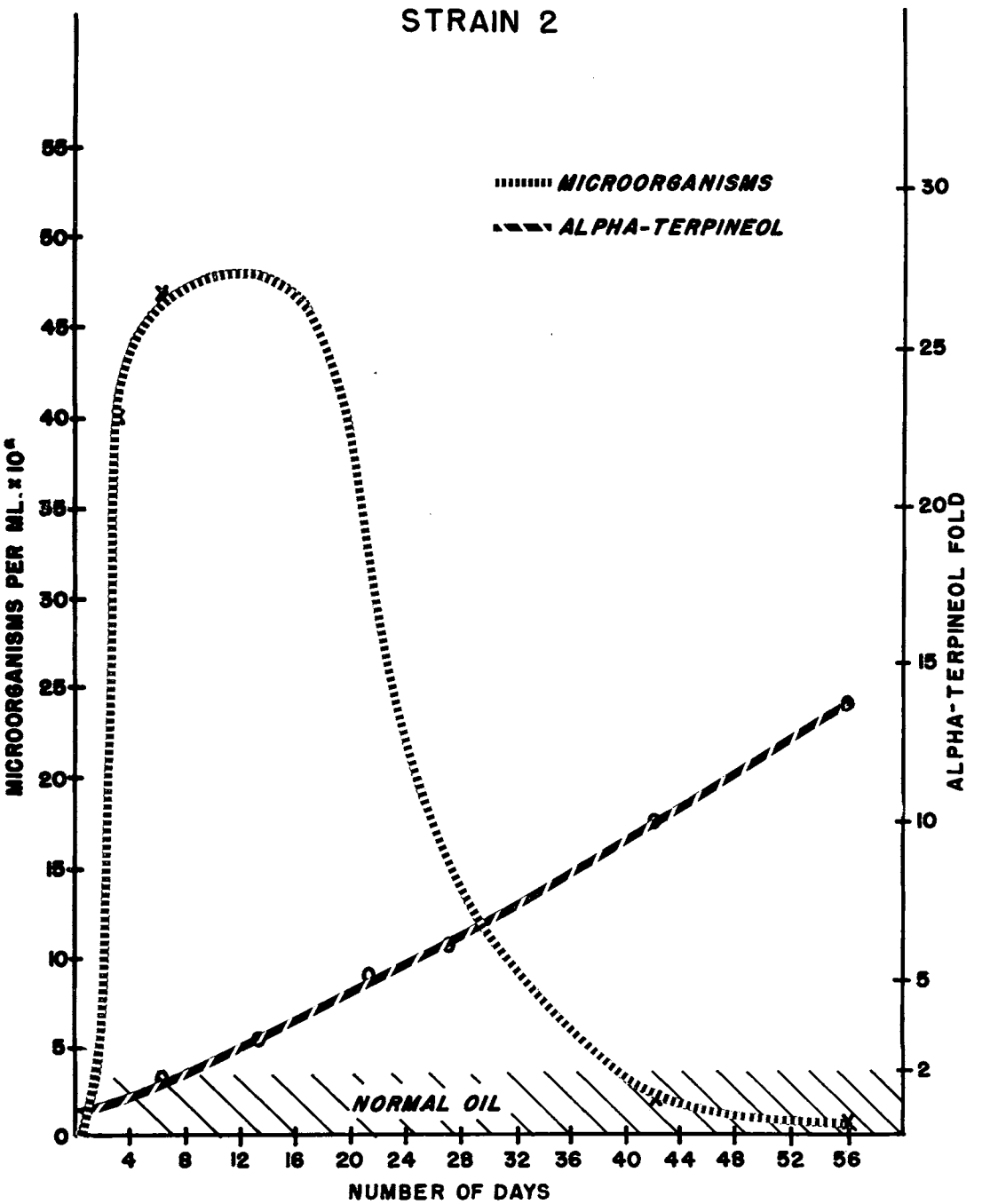

Fig. 6.—Orange oil emulsion inoculated with test organism showing microbial growth and production of alpha-terpineol.

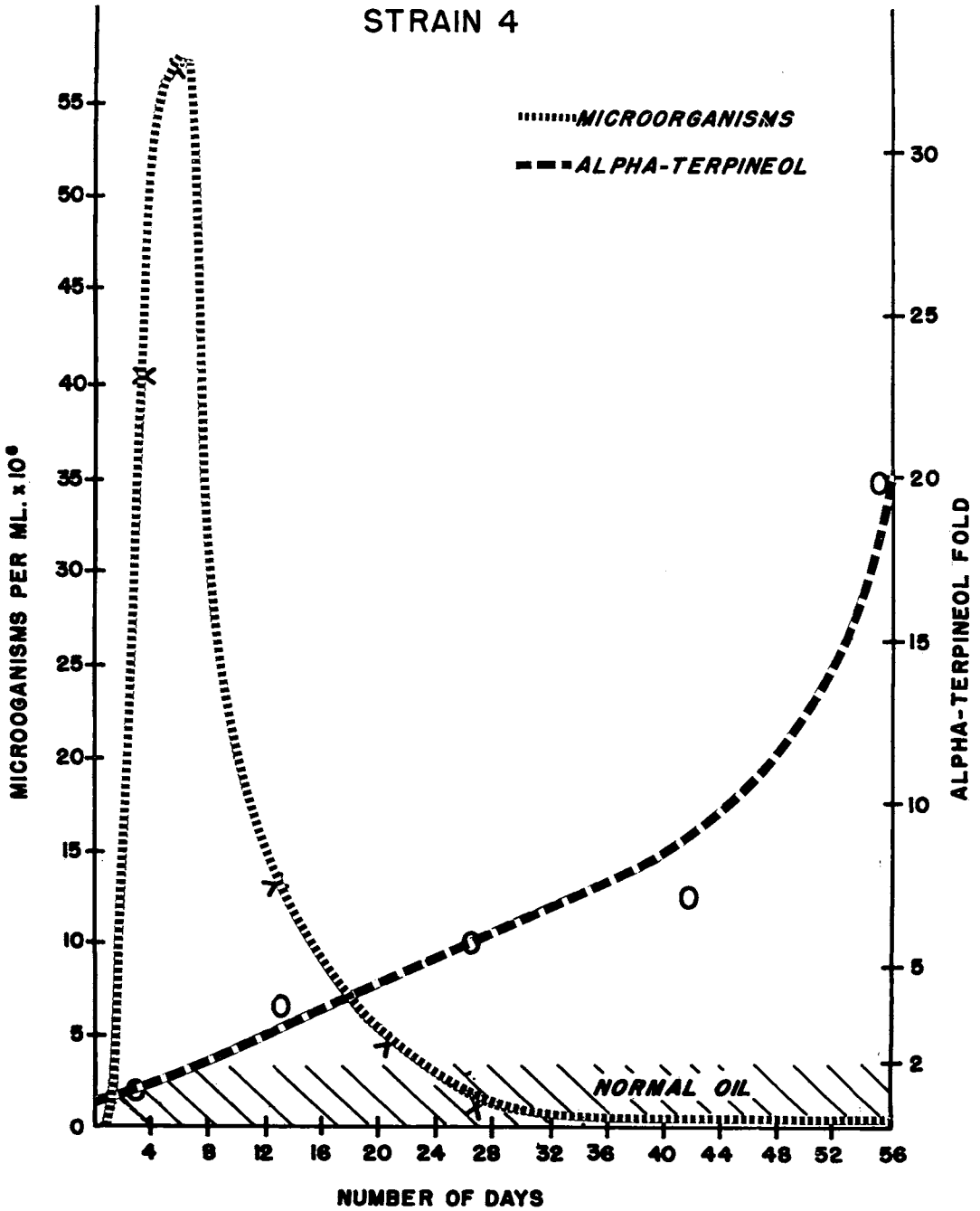

Fig. 7.—Orange oil emulsion inoculated with test organism showing microbial growth and production of alpha-terpineol.

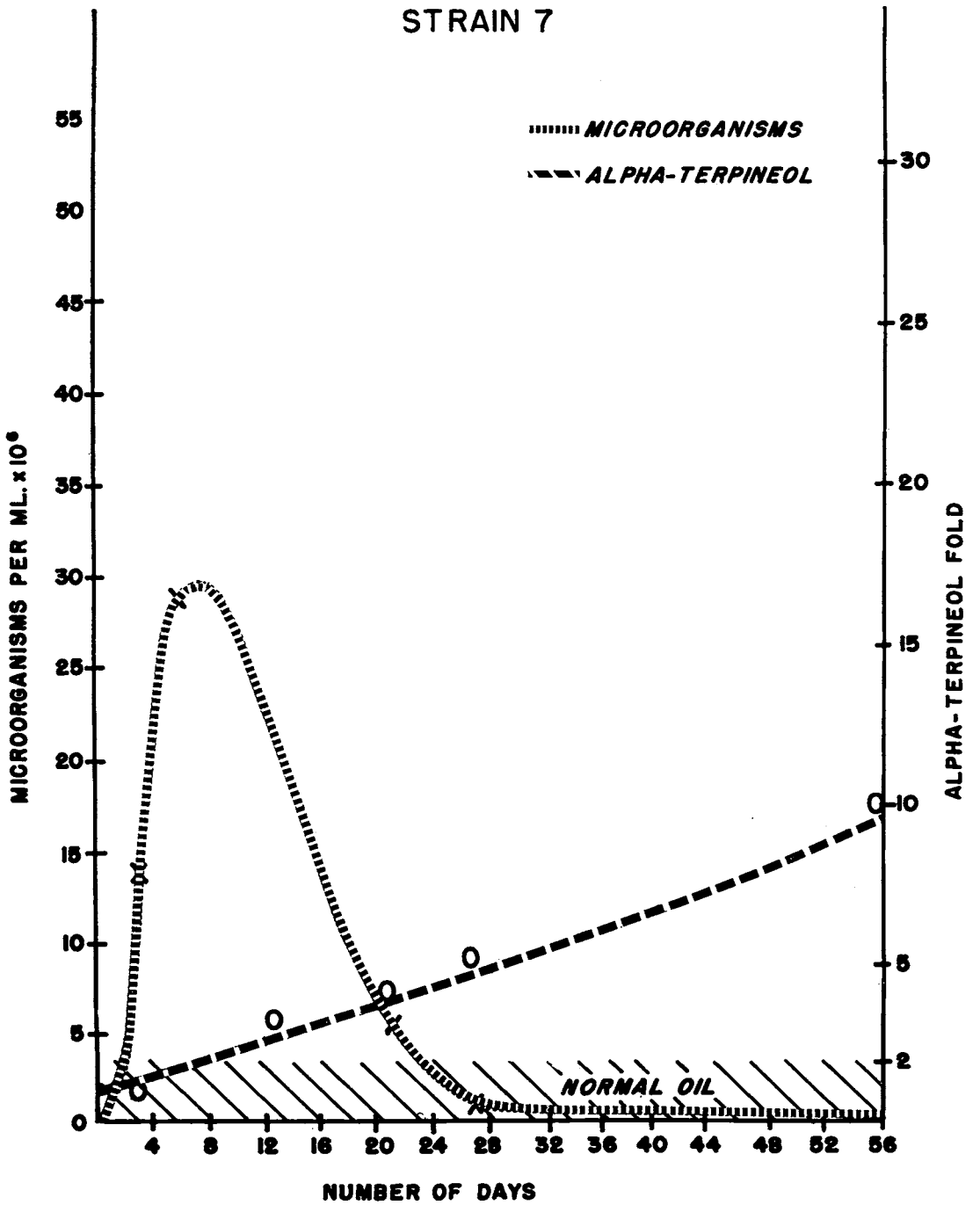

Fig. 8.—Orange oil emulsion inoculated with test organism showing microbial growth and production of alpha-terpineol.

Table 3. Microorganisms found in orange oil emulsion from desludger when produced under insanitary and sanitary conditions.

Date 1963	Insanitary Org. per g	Date 1967	Sanitary Org. per g
05-7	1,400,000	06-7	9,000
05-8	1,500,000	06-8	4,000
05-15	1,100,000	06-16	170,000
		06-18	100,000
		06-21	10,000

down, the concentration steadily increased, as data indicate.

From the data presented herein it appears alpha-terpineol in orange oil is an end product of bacterial growth since, without microbial growth, the alpha-terpineol content in the oil remains more or less constant. Growth apparently occurs in the aqueous or oil-free phase of the slurry and/or emulsion. However, it is possible that certain strains may have developed a tolerance for high concentrations of oil. On the other hand, plate counts made of the finished oil have shown, for all practical purposes, that it is free from micro-organisms.

Sanitary Aspects of C. P. Oil Process

It is evident orange oil slurry, prior to polishing, is an excellent medium for microbial growth. Consequently, the peel oil system can become heavily contaminated in a very short time. Therefore, in order to control contamination it is necessary good sanitary practices be maintained, which means the whole system should be thoroughly cleaned at periodic intervals. It is also important that equipment employed be of sanitary design. Justification for producing oil under sanitary conditions might be borne out by a statement obtained from an operator at one plant visited this past season. He stated orange oil emulsion becomes sour during warm weather if held 8 or more hours prior to polishing, and sour slurry produces bad oil.

Oil emulsion produced under insanitary conditions was found to be heavily "seeded" with microorganisms, as the data in Table 3 indicate. In this operation the equipment was not cleaned on a routine basis, and some of it never cleaned during the season. Oil emulsion held 3 days at 30°C (86°F) produced oil which had an abnormal concentration of alpha-terpineol. On the other hand, oil produced in a plant where an efficient sanitation program was maintained, the microbial population of the emulsion was held to a minimum (Table 3). In this case, the system was flushed from the extractors to the desludger every 4 hours with highly chlorinated water, finisher screens were changed every 8 hours or more frequently if required, and the entire peel oil recovery system thoroughly cleaned every 24 hours.

Additional studies are contemplated to investigate lemon and grapefruit oil recovery processes. It is hoped, however, from the data presented that the citrus industry will become more cognizant of the importance of maintaining a sanitary peel oil operation.

Summary

Orange oil slurry is an excellent medium for microbial growth, and when incubated at 30°C

(86°F) contained Voges-Proskauer (VP) reactants. Over 1 ppm of diacetyl was found in one sample analyzed by the distillation procedure. Oil analyzed by gas chromatography showed alpha-terpineol was produced which increased throughout the test period.

Four test organisms were isolated from orange serum agar pour plates. All grew rapidly when inoculated into sterile orange oil emulsion. One did not produce alpha-terpineol.

Production of alpha-terpineol in orange oil is believed to be a result of bacterial growth, and not a chemical and/or oxidation process.

Good sanitary practices must be maintained in order to control contamination during the orange peel oil recovery operation.

ACKNOWLEDGMENT

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RELATION OF PRESSURE AND MOISTURE FOR DENSIFYING FOAM-MAT DRIED ORANGE AND GRAPEFRUIT JUICE CRYSTALS[1]

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ABSTRACT

Previous studies have indicated that foam-mat dried citrus juices require densification for improved reconstitution properties. This may be achieved by application of pressure. A study was made of the relation between pressure applied, moisture content of the citrus crystals, and the degree of densification achieved in orange and grapefruit foam-mat dried crystals. Samples were prepared which varied in moisture content from 0.9 to 1.6% and these were subjected to pressures of 3,800 to 19,100 psi (pounds per square in.) by a ram driven hydraulic press. This treatment formed small, firm, citrus "discs" which were then tested in a shear press to determine the amount of shear required to break them. This shear strength was then taken as an index of the degree of firmness, or densification achieved. Some differences were found in the behavior of orange and grapefruit samples. Orange crystals generally increased in firmness of the discs with pressure at all moisture levels. With grapefruit samples, however, there was a definite peak in degree of densification at about 13,400 psi. At pressures of 15,300 and 19,100 psi, the firmness of the formed discs dropped sharply. With orange crystals the degree of densication at a given pressure generally increased with increased moisture, but with grapefruit optimum moisture content was 1.3%.

INTRODUCTION

Increasing interest is being shown in methods for dehydration of citrus juices. Most methods presently under consideration for this purpose produce a very low density dried material with a coarse structure. This is particularly true of the foam-mat drying process which has been under study at the U. S. Fruit and Vegetable Products Laboratory, Winter Haven. This low bulk density has been found impractical for commercial purposes and methods have been investi-

[1]Cooperative research of the Florida Citrus Commission, the Western and Southern Utilization Research and Development Divisions, Agricultural Research Service, U. S. Department of Agriculture.
References to specific commercial products do not constitute endorsement.