DIACETYL TEST AS A QUALITY CONTROL TOOL IN PROCESSING FROZEN CONCENTRATED ORANGE JUICE

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Lactic acid bacteria are organisms of sanitary significance in processing frozen concentrated orange juice. They impart flavors and odors which have been characterized as similar to buttermilk (3) (7). The bacteria responsible are principally those organisms belonging to the genera *Lactobacillus* and *Leuconostoc.* They are present on and in the fruit when it arrives at the plant and grow well in single strength and partially concentrated orange juice. To prevent a buildup of these organisms during the processing operation, an efficient sanitation program must be maintained.

It soon became apparent among the early investigators that a rapid method was needed to detect the presence of lactic acid bacteria. The direct miscroscopic method was first used. It consisted of counting the number of cells present on a stained slide preparation. The difficulty in distinguishing between the bacteria and the debris present on the smear left much to be desired. Then in 1954, Hill et al. (4) and Byer (2) introduced to the citrus industry a rapid method for the detection of diacetyl and acetylmethylcarbinol (AMC), the metabolic

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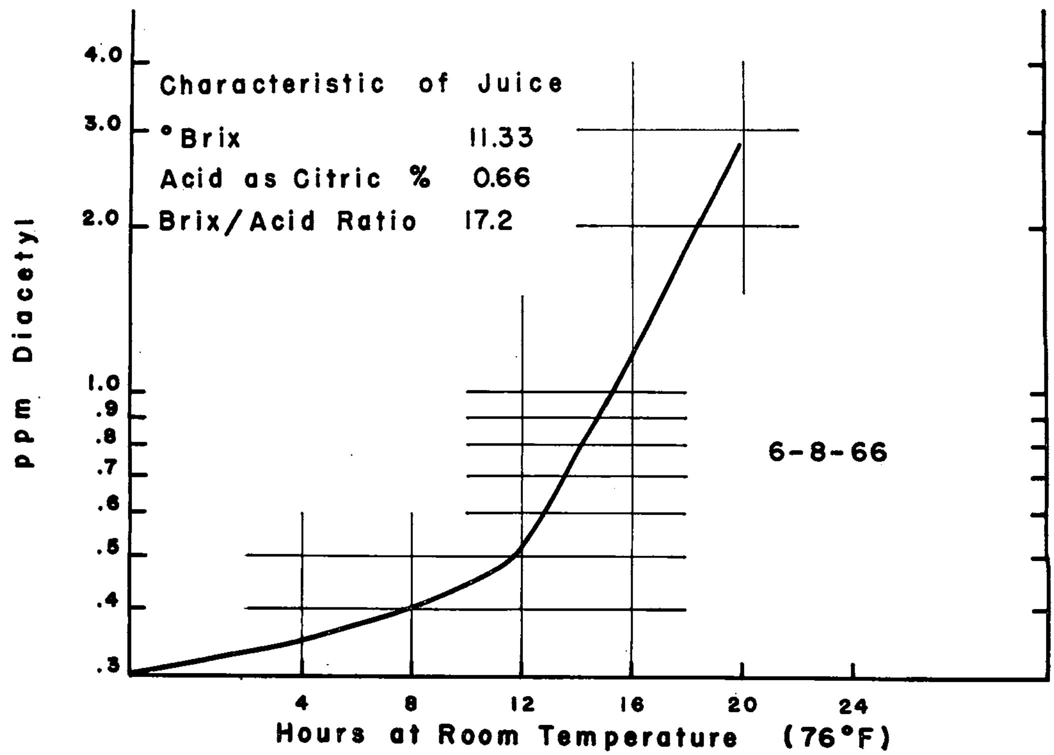
Fig. 1 DIACETYL VALUES OF ORANGE JUICE HELD AT ROOM TEMPERATURE Sample Obtained From Finishers



products of the growth of lactic acid bacteria in orange juice. The test, a modification of the Voges-Proskauer (VP) reaction, is extremely sensitive to diacetyl and will detect concentrations of less than 1 ppm. Most processors of frozen concentrated orange juice, as indicated by a survey made by Murdock and Dennis in 1964 (8), use some form or modification of the VP test for the detection of diacetyl and AMC in orange juice. Variations in preparation and handling of VP reactants were reported by Murdock in 1965 (9).

The purpose of this paper is to show how the diacetyl test, which requires less than one-half hour to perform, can be used as a quality control tool to determine the microbial activity in processing frozen concentrate orange juice.

Procedure

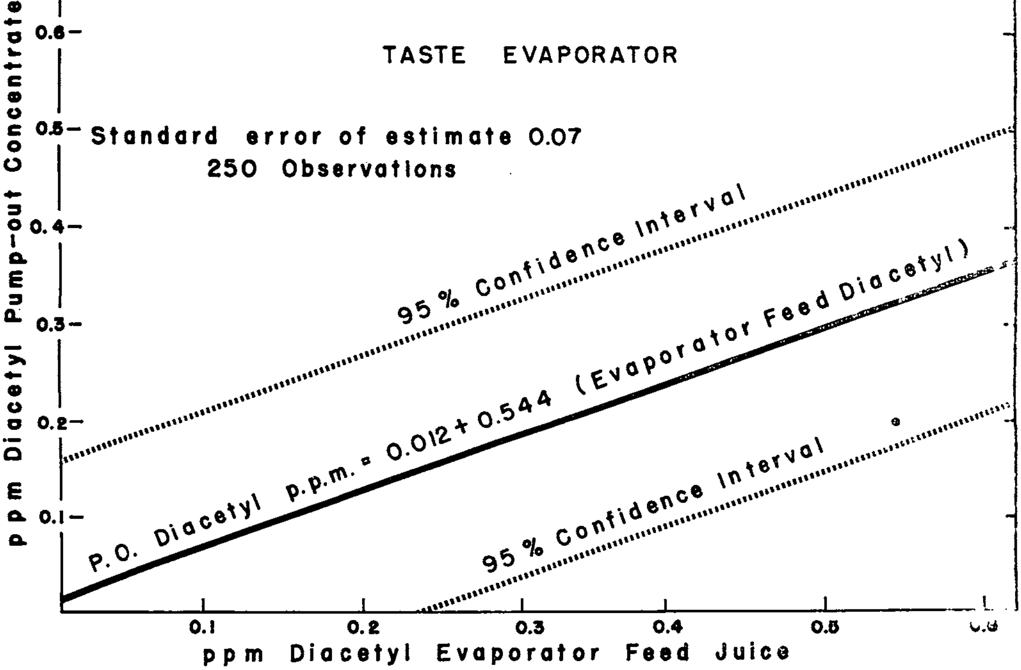
The diacetyl test used in this investigation was described by Murdock (Fla. State Hort. Soc. Proc., Nov. 1965). Briefly, it consisted of adding 300 ml of single strength or reconstituted orange juice (12° Brix) to a large boiling flask connected to a water condenser, collecting 25 ml of distillate, adding the VP reagents, and reading the color reaction on a Lumetron colorimeter 60 seconds after addition of the reagents. The results are expressed as ppm of diacetyl in the distillate.

Results and Discussion

Relationship of fruit quality to diacetyl values.—A study was made to determine the relationship of fruit quality to diacetyl values. It consisted of collecting at the concentrate plant ½ box (approx. 45 lbs.) of sound acceptable fruit (oranges) and a similar amount of reject fruit, the latter consisting of splits, partially decomposed, soft spots, etc. The fruit was brought to the laboratory where the juice was extracted from each lot by means of a hand extractor. The following diacetyl values were obtained:

Fig. 2

RELATIONSHIP OF DIACETYL VALUES IN EVAPORATOR FEED JUICE TO PUMP-OUT ORANGE CONCENTRATE FOR 1966 VALENCIA SEASON



Type of Fruit	*° Brix*	*Brix/acid Ratio*	*ppm Diacetyl*
Sound (Good)	12.16	14.47	0.15
Reject (Bad)	12.98	14.42	0.80

Beisel et al. (1) also found that juice from defective fruit was higher in diacetyl than it was from sound fruit. Murdock et al (10) found that there was a direct correlation between fruit quality and the microbial contamination of freshly extracted juice. Therefore, it is quite evident that if unsound fruit entering the plant is not removed during the grading operation it will not only "seed" the plant with microorganisms but will also result in an immediate surge in diacetyl during the processing operation.

Diacetyl as an index of plant sanitation.—In citrus processing plants, as in other food plants, juice may occasionally remain stagnant in an unused pipeline; or a dead pocket may occur as a result of a change in the piping arrangement. Also, pulp adhering to finisher chutes may not always be removed during a flush operation. It was found that pulp under optimum conditions adhering to equipment, such as finisher chutes, would develop a sour odor in approximately 6 hrs. if not removed (11). When this occurred, the pulp usually had a total viable count of 1,000,000 organisms per gram and a diacetyl concentration of one or more ppm (6).

Fig. 1 shows that diacetyl values of orange juice held at room temperature (76° F) increased from 0.3 to 2.9 ppm in 20 hrs. The greatest increase in diacetyl occurred after 12 hrs. From these data, it is apparent that if orange juice is held in a dormant condition, such as in an unused pipeline and the equipment is not kept in a sanitary condition, an increase in diacetyl will occur.

Diacetyl values during evaporator operation.—Hill and Wenzel in 1957 (5) reported on the diacetyl values obtained in processing orange concentrate in a low temperature pilot and commercial evaporator. They noted an increase in diacetyl occurred during the concentrate process, usually after the evaporator had been operated 24 hrs. or more. The citrus industry within the last year or two has replaced nearly all of their low temperature evaporators with high temperature short holding time units, generally referred to as a TASTE evaporator (Thermal Accelerated Short Time Evaporator).

A study was made under commercial conditions comparing a TASTE unit with a 6-stage low temperature Buflovak evaporator in processing wash pulp concentrate. Samples for diacetyl analyses were drawn every 4 hrs. while the evaporators were in operation. Juice was collected before it entered the evaporator (evaporator feed) and when it left the units (pump-out concentrate). The Buflovak units were cleaned on a 48-72 hr. cycle and the TASTE evaporator every 10-12 hrs. A table of frequencies of diacetyl values (evaporator feed juice and pump-out concentrate) for the period the TASTE and Buflovak evaporators were in operation is presented in Table 1. It can be seen from the data that for the period April 6-13, 18 of the TASTE evaporator feed juice diacetyl values were in the range of 0.20 to 0.25 ppm. The distribution of the values leaving the evaporator were 14 in the range of 0.10 to 0.15 ppm, two in the range of 0.20 to 0.25, and two in the range of 0.30 to 0.35. The average diacetyl values for the test period and the percent change between evaporator feed juice and pump-out concentrate is shown in Table 2. Note that the average loss of diacetyl between these two points for the TASTE evaporator was 36%, which compares with a 15% increase in diaetyl for the Buflovak evaporator.

In another study, which was made at Plant A from April 29 through June 22, the orange juice entering the TASTE evaporator (evaporator feed) and pump-out concentrate 60° Brix were checked for diacetyl every 4 hrs. while the evaporator was in operation. A total of 250 analyses were made. The average loss of diacetyl for the season between the evaporator feed juice and the pump-out concentrate was 32% (Table 2). A table of frequencies of the diacetyl data for the test period is shown in Table 3. Also included are the diacetyl values obtained at Plant B while processing orange concentrate.

In analyzing the diacetyl data obtained from Plant A, the following regression equation was derived:

Pump-Out Diacetyl ppm = 0.012 + 0.544 x Evaporator Feed Diacetyl

This equation shows the functional relationship between the level of diacetyl in the evaporator feed juice entering the TASTE evaporator and the level of diacetyl in the 60° Brix concentrate pump-out. From the equation, it can be estimated that if the evaporator feed juice contains 0.4 ppm diacetyl there will be on the average 0.23 ppm diacetyl in the pump-out concentrate. The relationship for other diacetyl values

Table 1. Distribution of diacetyl data in processing wash pulp concentrate during 1966 Valencia season

TASTE EVAPORATOR

April 6-13

Evaporator Feed Juice Diacetyl Range ppm	Total Freq.	Pump-Out Concentrate (60° Brix) Diacetyl Range ppm 0.00 0.05	0.10 0.15	0.20 0.25	0.30 0.35	0.40 0.45	0.50 0.55
		Frequencies					
0.40 - 0.45	1	1	0	0	0	0	0
0.30 - 0.35	14	1	2	9	2	0	0
0.20 - 0.25	18	0	14	2	2	0	0
0.10 - 0.15	2	1	1	0	0	0	0
	35						

BUFLOVAK EVAPORATOR

April 22- May 4

Evaporator Feed Juice Diacetyl Range ppm	Total Freq.	0.00 0.05	0.10 0.15	0.20 0.25	0.30 0.35	0.40 0.45	0.50 0.55
0.40 - 0.45	5	0	0	1	3	1	0
0.30 - 0.35	19	0	0	7	7	4	1
0.20 - 0.25	16	0	2	4	4	5	1
0.10 - 0.15	4	1	1	2	0	0	0
	44						

in evaporator feed juice to pump-out concentrate is shown in Fig. 2. Using the standard error of the estimate of 0.07 ppm, a 95% confidence interval was calculated to show the variability associated with the estimate. For an evaporator feed juice diacetyl of 0.4 ppm, we would expect 95% of the estimates of pump-out diacetyl ppm to fall within a confidence interval of 0.09 to 0.37 ppm.

Although the strength of the regression equation for the other TASTE evaporators was weak due to the limited number of samples collected, it appears that the same functional relationship applies.

Biological Control Program.—We have seen that diacetyl can be used as presumptive evidence of the quality of the incoming fruit and as an index of sanitation in a citrus concentrate plant. Since the diacetyl test will detect the end products of microbial growth in orange juice during processing operations, it can be used effectively as a quality control tool. In developing a biological control program, the following should be considered:

1. Sample stations
   Select definite check points to adequately cover the processing operation.

Table 2. Average Diacetyl Values for Test Period

Product	Evaporator	No. Tests	Avg. ppm of Diacetyl Evaporator Feed Juice	Avg. ppm of Diacetyl Pump-Out Concentrate	Diacetyl % Change
Wash pulp (60° Brix)	TASTE	35	0.265	0.171	36% (-)
Wash pulp (60° Brix)	Buflovak	44	0.282	0.323	15% (+)
Orange Conc. (65° Brix)	TASTE (1)	250	0.374	0.254	32% (-)
Orange Conc. (65° Brix)	TASTE (2)	36	0.260	0.160	38% (-)

(1) 40,000 lb. evaporator Plant A

(2) 50,000 lb. evaporator Plant B

2. Frequency of tests
   Check product from each station every 4 hrs. or more frequently if necessary.
3. Recording of data
   Log all diacetyl values. An example of a log in which the data can be plotted graphically as it is accumulated is shown in Fig. 3.
4. Interpretation of data
   Be able to correctly interpret the data as it is obtained. In order to assist Minute Maid personnel in this regard, a Guide for Interpretation of Diacetyl Analyses has been developed (Fig. 4).

Diacetyl values during normal and abnormal periods.—Fig. 3 is a copy of a log showing diacetyl values obtained during a normal plant run which started on May 10, 1966. Note the values remain more or less constant within a very narrow range.

Fig. 5 is a copy of a log showing a diacetyl buildup. Observe the steady increase in diacetyl in the wash pulp discharge liquor starting at 0100 on March 31 and the large increase in diacetyl between 0900 on April 2 and 1300 on that same day. During this time interval the diacetyl increased from 0.4 ppm to 0.8 ppm in 4 hours. This would indicate the contaminating organisms were in their optimum growth phase. If preventive measures are not immediately taken, spoilage will occur in a short time. In this instance, the juice room was shut down and the equipment thoroughly cleaned with a detergent solution. The source of contamination, when conditions exist as illustrated in Fig. 5, usually will be found in the juice room. It may occur from sour pulp in finisher chutes, stagnant juice in troughs or header lines, etc. If found, a flush down with chlorinated water is indicated and/or the equipment should be thoroughly cleaned with detergent solution.

Another abnormal condition is a sudden surge in diacetyl. This usually occurs when:

(1) A "slug" of sour juice is diverted into the system, ie. from an unused pipeline not cleaned prior to use,

(2) product remaining in an evaporator feed tank that has become stagnant, or

(3) a load of poor fruit being processed.

Fortunately, a sudden increase in diacetyl is usually followed by a corresponding decrease as the slug of contaminated product passes through the system. If diacetyl does not return to normal, a flush down or a clean up is indicated.

We have seen how the diacetyl test can be used as an effective quality control tool in the processing of frozen concentrated orange juice. It can also be used to a lesser degree as an indication of the quality of bulk concentrate which, at times, is purchased on the open market. If the product was produced in a TASTE evaporator, it must be remembered that approximately ⅓ of the diacetyl present in the evaporator feed juice will be lost during the

Table 3. Distribution of diacetyl data in processing orange concentrate in TASTE evaporator during 1966 Valencia orange season.

Evaporator Feed Juice Diacetyl Range ppm	Total Freq.	Pump-Out Concentrate (60° Brix) Diacetyl Range ppm 0.00–0.05	0.10–0.15	0.20–0.25	0.30–0.35	0.40–0.45	0.50–0.55
PLANT A April 29- June 22		Frequencies					
0.60 - 0.65	7	0	0	1	3	2	1
0.50 - 0.55	33	0	1	15	10	5	2
0.40 - 0.45	85	1	15	44	22	3	0
0.30 - 0.35	79	2	30	41	6	0	0
0.20 - 0.25	42	4	31	6	1	0	0
0.10 - 0.15	4	1	3	0	0	0	0
	250						
PLANT B April 11 - May 10							
0.40 - 0.45	3	0	3	0	0	0	0
0.30 - 0.35	12	1	10	0	1	0	0
0.20 - 0.25	13	4	9	0	0	0	0
0.10 - 0.15	8	1	6	1	0	0	0
	36						

concentrate operation. Therefore, if high diacetyl is found in bulk drummed product, the assumption can be made that the product was produced under insanitary conditions prior to evaporation.

SUMMARY

Data are presented to show that the diacetyl test can be used as an index of juice quality and poor sanitation in processing frozen concentrated orange juice. Approximately ⅓ of the diacetyl present in the evaporator feed juice was lost in a TASTE evaporator during the concentrate operation. An increase in diacetyl usually occurred when a low temperature evaporator was used.

Fig. 3 DIACETYL VALUES OBTAINED DURING NORMAL OPERATION

DIACETYL LOG

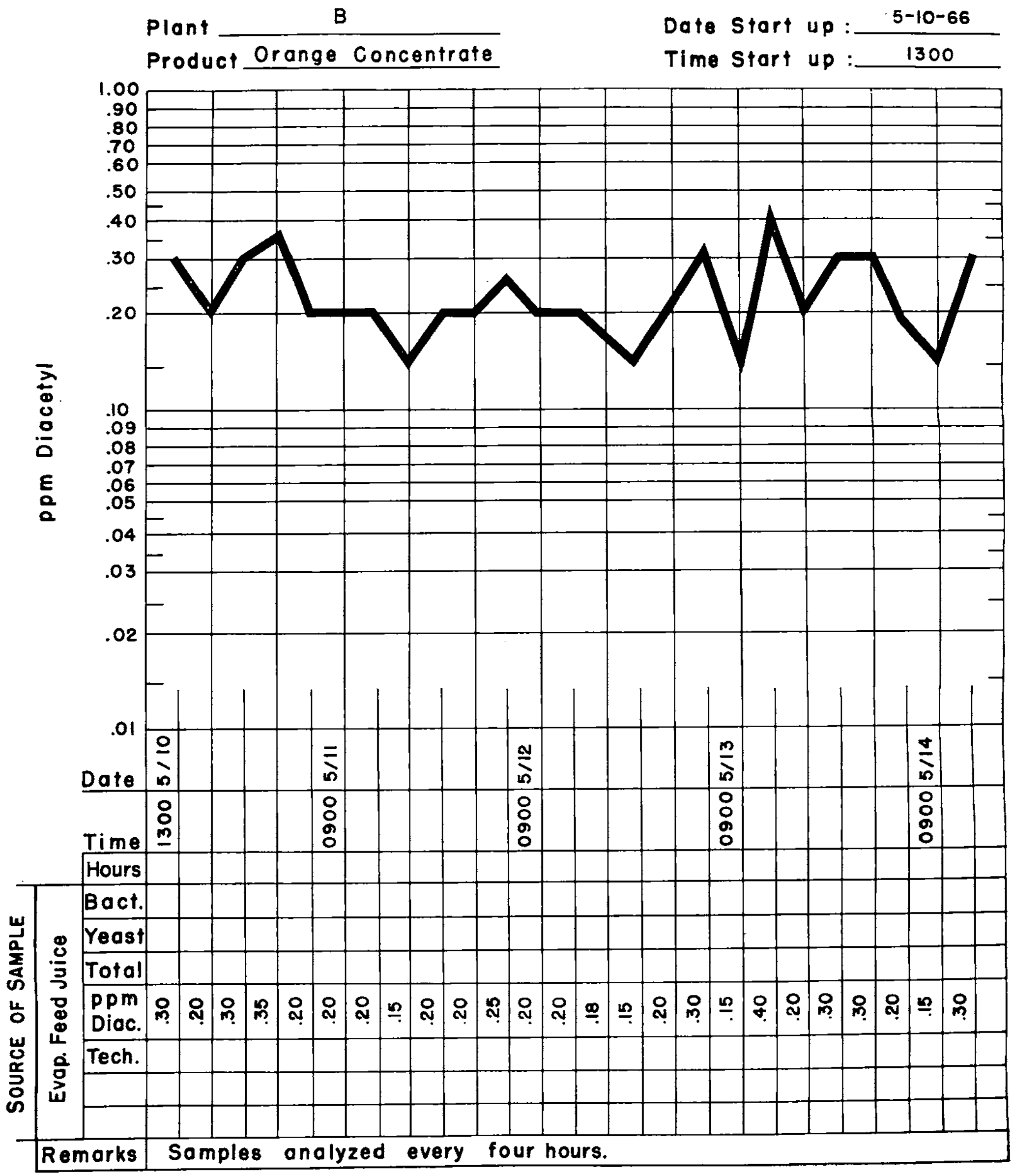
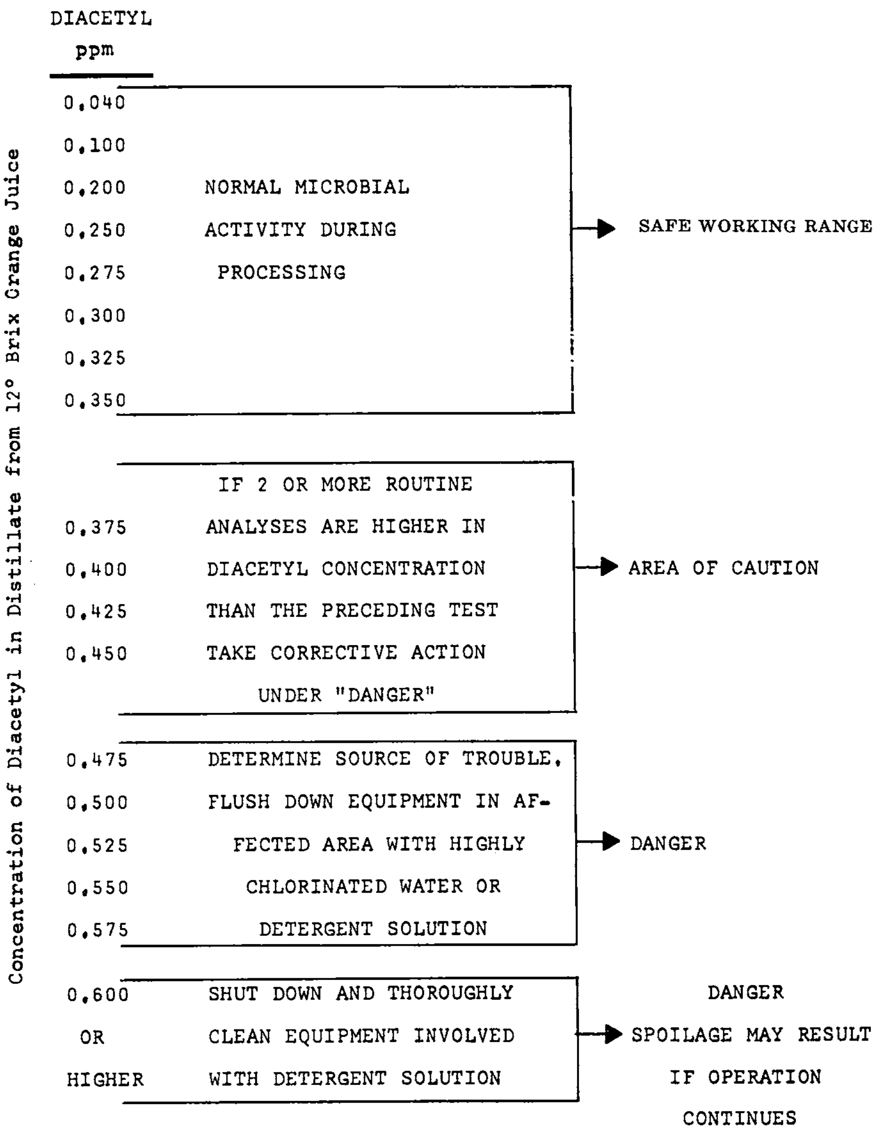


Fig. 4 GUIDE FOR INTERPRETATION OF DIACETYL ANALYSIS IN PROCESSING FROZEN CONCENTRATED ORANGE JUICE



Development of biological control program using the diacetyl test as a quality control tool is discussed. Interpretation of the diacetyl data and preventive measures used in a concentrate plant to prevent spoilage during periods of high microbial activity are mentioned.

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Fig. 5

LOG SHOWING DIACETYL BUILDUP

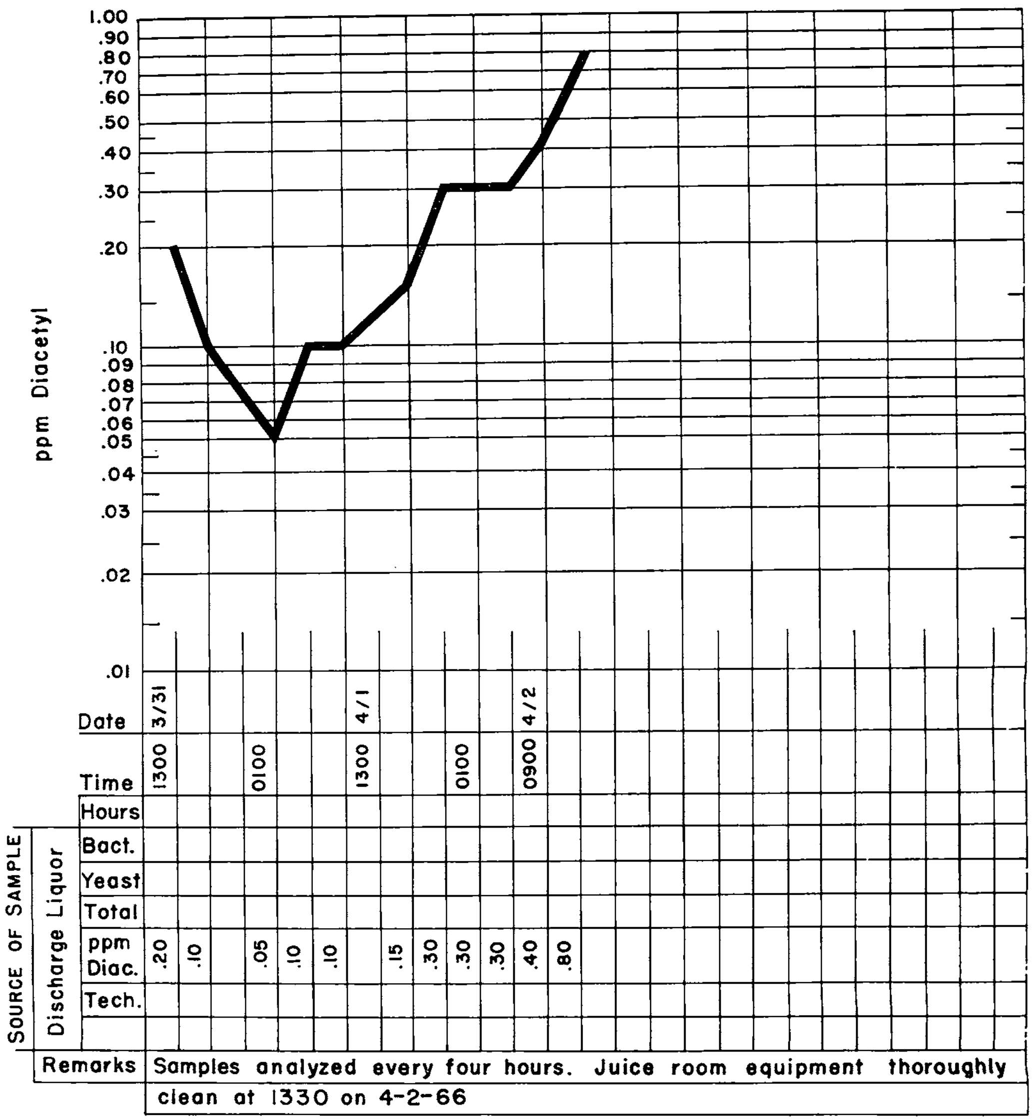
DIACETYL LOG

Plant A

Product Wash Pulp (W.P.)

Date Start up: 3-31-66

Time Start up: 1300



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COMPARISON OF SUBJECTIVE AND OBJECTIVE METHODS FOR DETERMINING THE COLOR OF RECONSTITUTED FROZEN CONCENTRATED ORANGE JUICE[1]

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ABSTRACT

One subjective and two objective methods were used to determine the color of reconstituted frozen concentrated orange juice. The USDA color scores for 21 samples ranged from 32 to 37 points when this subjective method was used. Two objective methods were used: (a) the Hunter Color and Color Difference Meter to obtain tristimulus color values and (b) the Bausch and Lomb Spectronic 505 recording spectrophotometer to obtain spectral curves. Hunter *Rd* values ranged from 20.3 to 27.1; the *a* values values from —7.5 to —2.8; and the *b* values from 28.1 to 29.8. Dominant wavelengths computed from the spectral curves for the reconstituted juices, ranged from 576 to 581 mμ; purity from 59 to 90%; and brightness from 20.3 to 33.8%. As the visual color score increased, the Hunter *a* value increased and the *Rd* value decreased; also, brightness decreased but no trend was evident between either the dominant wavelength or purity and the color score.

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INTRODUCTION

Color to different disciplines of science means various things. To the chemist it is dye and pigments. To the physicist color is a phenomena in the field of optics and electromagnetc radiation. To the physiologist and psychologist color denotes a sensation to the human observer.

There are many procedures in use today that compare the color of one object with that of another or give a value for the color difference between them. Such methods are either subjective or objective, the former depending upon a visual evaluation while the latter uses different instruments.

Subjective methods.—The Maerz and Paul Dictionary of Color (5) contains examples of many colors. Plate 10 on page 43 of this book shows various colors that could apply to orange juice.

The Macbeth-Munsell Disk Colorimeter (3, 4) can be used so that overlapping color wedges are spun and the resulting color compared to that in samples of orange juice. By varying the amount of white, gray, yellow, and orange, the color of orange juice may be matched.

Another example of a subjective method is the use of USDA color comparator tubes to obtain a color score for orange juice. A trial set at such tubes consisted of colored viscous plastic in capped glass tubes. These were avail-