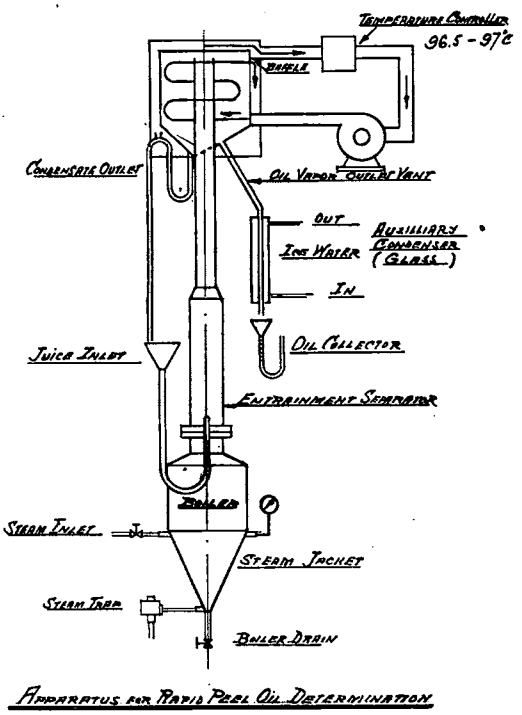
through a condenser at 97° C. and the condensate returned to the boiler. Peel oil, noncondensible at this temperature in the presence of steam, is vented into an auxiliary condenser, collected and measured.

A full scale machine with condenser capacity to handle an evaporation rate of 200 ml. per minute at a condensing temperature of 97° C. was built and used during the 1948-49 season



Apparatus for Rapid Peel Oil Determination

(fig. 1)

to test, prior to canning, each batch of single strength orange juice produced at Dunedin.

Referring to Fig. 1, operating procedure is as follows:

With the main condenser temperature controlled at 96.5° to 97° C. and ice water flowing through the auxiliary condenser, about 500 ml. of water is introduced into the apparatus, and the steam turned on. The water is allowed to boil to clear air from the system, and the boiling rate is adjusted to allow the escape of a small amount of steam from the main condenser vent. Then a 1 liter juice sample is poured in slowly (600 to 700 ml. per minute) so as not stop the evaporation. A 5-minute interval is allowed for the oil to be vaporized and to work through to the second condenser. The oil trap is then removed and the peel oil volume noted. With the machine in standby condition, a complete oil determination can be made in 7 minutes.

Some typical peel oil results on single strength orange juice follow. These are from our 1948-49 laboratory records.

Rapid Method	Clevenger
.028	.0235
.010	.010
.020	.016
.024	.0225
.032	.023
.017	.018*

*—Condenser water held at 40° F. to allow high boiling rate.

Conclusions

The apparatus furnishes a rapid and accurate oil determination method, but unless immediate results are needed for process control, the equipment will be found cumbersome and expensive as compared to the standard Clevenger method.

THE ISOLATION, CULTIVATION AND IDENTIFICATION OF ORGANISMS WHICH HAVE CAUSED SPOILAGE IN FROZEN CONCENTRATED ORANGE JUICE.

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During the 1949-50 season, this laboratory was requested to investigate spoilage in frozen concentrated orange juice. This spoilage was characterized by a "buttermilk" off-odor which was detectable in the blending tanks before the product was filled into the cans.

Samples of finished product had been cultured hourly in Lindegren's Agar by the control laboratory, yet the total count had not increased over that observed in the normal product before this spoilage had been encountered. A microscopic examination of the product revealed the presence of numerous rod-shaped bacteria which were absent from the smears of normal product. Organisms morphologically similar to these rod-shaped bacteria were observed to increase in numbers during continued

operation of the evaporators in those stages of the multi-stage, low temperature, high vacuum exaporators which produce the lower degree Brix concentrate, i.e. up to between 20 and 35° Brix. These microscopic observations indicated that the spoilage was microbial in origin and that the control medium, Lindegren's Agar, lacked nutrients essential to the growth of these organisms.

Isolation and Cultivation of the Spoilage Organisms

The increase in numbers of these rod-shaped bacteria observed in the evaporator effluent during continued operation, circumstantially incriminated them as the cause of the spoilage. To prove definitely their role as the causative agent, it would be necessary to isolate the organisms in pure culture and show by inoccucation tests that they would reproduce the off-odor spoilage in low Brix concentrate. Therefore, a medium which would supply nutrients necessary for the growth of these organisms was essential to the solution of this problem.

It has been known for some time (1) that the juices or serums of many fruits contain factors which stimulate the growth of acid producing organisms, especially the lactobacilli. This laboratory has used tomato serum agar as a routine medium for examination of spoilage in acid products; consequently, the writer used this medium in the original investigation of the spoilage.

A colony count ten to a hundredfold greater was observed in the tomato serum cultures after 48 hours incubation at 30° C. of samples of the "off-odor" concentrate or of the evaporator effluent as compared to the duplicate Lindegren Agar cultures. The majority of the colonies developing in the serum agar were bacteria, while yeast colonies were the predominant type observed on the Lindegren Agar cultures. The bacteria growing in the serum agar were morphologically similar to those observed in the spoilage. These organisms were then isolated in pure culture from the serum agar plates and when inoculated into bottles of pasteurized evaporator effluent were found to reproduce the typical characteristics of the spoilage in concentrate up to strengths of 35° Brix.

The fruit being used was, no doubt, the original source of the spoilage organisms. These organisms gained access to the evaporators with the incoming juice and multiplied in the citrus solids which were not being continually washed free from the surfaces of the evaporators.

As the spoilage organisms grew readily in orange juice, the writer suggested the substitution of orange serum for tomato serum in the agar media. The organisms grew even better in this medium than in the tomato serum agar, so the orange serum agar was proposed for use in routine control checks for the presence of the spoilage bacteria.

Orange serum agar having the following general formula has subsequently been widely accepted as a control medium by the industry because of its ability to support the growth of the "off-odor" spoilage organisms, as well as yeast and other organisms commonly found in citrus products.

ORANGE SERUM AGAR

Tryptone	10 grams
Yeast Extract	3 grams
Dextrose	4 grams
Agar	17 grams
Orange serum	200 ml.
Distilled Water	800 ml.
Dipotassium Phosphate	2-3 grams*

*—The amount of phosphate to be used will depend upon the buffering capacity of the juice from which the serum is prepared. For satisfactory results the pH of the medium should be below 6.0, with an optimum pH of about 5.5.

Media for the Enumeration of Spoilage Organisms

A medium on which the spoilage organisms could easily be identified and enumerated would be helpful to the quality control laboratory personnel. The incidence of spoilage types could be used as a basis for scheduling equipment clean-up or for more rigid inspection of fruit going to the extractors.

Vaughn and Emard (2) have suggested the addition of sorbic acid to liver infusion broth or agar for the isolation or enumeration of catalase negative organisms in citrus products. Preliminary results indicate that orange serum agar to which 0.13 per cent sorbic acid has been added and having a final pH of 5.5 will permit the growth of lactobacilli and leuconostoc while inhibiting the growth of yeast.

Media and Methods Employed in the Identification of the Spoilage Organisms

The following basic medium (3) was used to establish the fermentation pattern and the products of fermentation of these spoilage organisms.

After sterilization, sterile carbohydrate solutions were added to give a final sugar concentration of 1.5 per cent.

Tryptone	5 grams
Yeast Extract	2½ grams
Tap Water	1000 ml.
Salt Solution A	5 ml.
Salt Solution B	5 ml.

Salt A. A solution containing 10% each of K_2HPO_4 and KH_2PO_4.

Salt B. A solution containing 4% $MgSO_4$ $7H_2O$, 0.2% each of $FeSO_4$ $7H_2O$, $MnSO_4$ $4H_2O$ and NaCl.

The volatile and non-volatile acids produced by the fermentation of the above medium were separated by steam distillation (4) and the non-volatile acids recovered from the residue by continuous ether extraction. The optical activity of the lactic acid was determined on its lithium salt.

Lactic acid determinations were also made by the colormetric method of Baker and Summerson. (5)

Identification of Spoilage Organisms

Both leuconostoc and gram negative rods, probably Erwinia, have been isolated from orange juice supplied to the evaporators, yet none of these organisms have been observed by the writer to grow in 25° to 35° Brix concentrate. Thus, this identification study has been limited to those organisms which will grow readily in those stages of evaporation producing up to 35° Brix concentrate. The identification of such organisms has been of extreme interest to the industry.

The organisms isolated either from spoilage or from orange juice being supplied to the evaporators and capable of growing in concentrated orange juice of 35° Brix have fallen into two species. Although many isolates have been obtained, the first of these species was represented by two cultures in this study.

1. These organisms are non-motile, Gram positive rods occurring singly and in pairs and measuring .5 to .8 by 1.5 to 4 microns, although an occasional cell may be 5 to 7 microns in length. Many cells of older cultures stain Gram negative.
2. Catalase is not produced by these organisms.
3. Luxuriant growth is noted in orange serum broth containing 5% NaCl, 2.5% citric acid or 40% sucrose.
4. These organisms ferment dextrose, fructose, sucrose, galactose, maltose, and arabinose with the production of acid. Lactose and mannose are not fermented.
5. The optimum temperature of these microaerophilic organisms appears to be between 30° and 37° C. Growth has been observed between 15° and 40° C.
6. Inactive lactic acid, acetic acid, and carbon dioxide are products of the fermentation of dextrose.

The above cultural characteristics and the products resulting from the fermentation of dextrose identifies these cultures as being *Lactobacillus brevis.* (6) and (7)

The other species was also represented by two cultures in this study.

1. These Gram positive rods measuring .6 to .8 by 2.0 to 4.0 microns, occurring singly and in pairs, are actively motile up to 48 to 72 hours. These cells possess peritrichous flagella. Like the L. brevis cultures, many of these cells stain Gram negative in older cultures.
2. Catalase is not produced by these organisms.
3. Luxuriant growth is noted in orange serum broth containing 5% NaCl, 2.0 to 2.5% citric acid, or 40% sucrose.
4. These organisms ferment dextrose, fructose, sucrose, galactose, maltose, arabinose, and mannose with the production of acid. Lactose is not fermented.
5. The optimum temperature of these microaerophilic organisms is between 30° and 37° C. Slight growth has been observed at 4.4° C. and good growth from 15° to 40° C.
6. Inactive lactic acid and a trace of acetic acid but no carbon dioxide are the products of the fermentation of dextrose.

While these cultural characteristics differ somewhat from the characteristics listed in Bergey's Manual (6) particularly with respect to motility, these organisms are considered as motile strains of *Lactobacillus plantarum.* Such a variety is recognized by Harrison and Hansen (8) who have given the name *Lactobacillus plantarum var mobilis* to such organisms.

Lactic acid, acetic acid, diacetyl, and carbon dioxide have been identified as products of the fermentation of single strength orange juice by both the *Lactobacillus brevis* and *Lactobacillus plantarum var mobilis* cultures.

Summary

1. Microorganisms were observed to develop in those stages of multi stage evaporator which produce concentrate up to 35° Brix.
2. Microorganisms were isolated from the final product as well as from evaporator effluent which were capable of producing the typical spoilage when inoculated into normal concentrate of not over 35° Brix.
3. Tomato serum and Orange serum agar were found to be suitable media for the isolation of these spoilage organisms. Addition of 0.13 per cent sorbic acid to orange serum agar adjusted to pH 5.5 appears to be a good medium for determining the concentration of these organisms for control purposes.
4. Typical spoilage organisms have been identified as **Lactobacillus brevis** and **Lactobacillus plantarum var mobilis,** which utilize citric acid in the production of diacetyl. This compound is probably responsible for the "butter-milk odor" characteristic of this spoilage.

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