The presence of such substances might be particularly significant in the case of media containing inhibitors in concentrations slightly below a level at which coliforms themselves are affected. These factors, in combination with an incubation temperature above the optimum for coliforms, could explain the inability of selective media to detect these organisms in some instances.

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STORAGE CHANGES IN CITRUS MOLASSES

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Introduction

Citrus molasses, which has become a familiar livestock feed in Florida has been produced commercially for less than ten years. Its wide acceptance and increasing popularity warrant more complete understanding of its physical and chemical properties. Buyers of this carbohydrate concentrate are interested in obtaining further information regarding the product, storage changes, and the ramifications of microorganisms associated with it.

Citrus molasses is produced from the rinds and pulp of citrus after the juice has been expressed. This waste residue is chopped, limed, and pressed to yield a press liquor of 10°-14° Brix (percent soluble solids content by weight) which when concentrated to 72°-75° Brix is the final molasses. Since citrus molasses can be produced only during the processing season, the processor is required to store millions of gallons to serve the year round needs of cattlemen. Certain changes take place during storage and they are the subject of this report to industry.

Before discussing storage changes in citrus molasses it might be well to examine Table 1 wherein the comparative analysis between this product and the common molasses obtained from sugar refining is presented. The average analysis for clarified citrus molasses represents samples made from several varieties of both grapefruit and orange. Clarified molasses refers to a product made from a clear press liquor. It is immediately noticeable that blackstrap is generally concentrated to a higher degree Brix, but has the disadvantage of having more than a proportionately higher ash content. Citrus molasses producers tend now to use 72° Brix as a minimum value with the average being maintained at a higher level.

Sugar Losses and Instability During Storage

In storage, citrus molasses has been found to slowly undergo both a physical and chemical transformation. Of paramount importance are the changes in sugar content which occur on storage. When ten samples of citrus molasses collected from ten commercial processors in January of 1948 were reanalyzed by the Lane-Eynon Volumetric procedure they were found to have lost

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TABLE 1.
COMPARATIVE DATA ON CITRUS AND BLACKSTRAP MOLASSES.

Analysis	Commercial[1] Citrus Molasses	Clarified[2] Citrus Molasses	Louisiana[3] Blackstrap	Cuban[3] Blackstrap
Brix°	72.0	73.1	90.7	87.2
Sucrose %	19.6	26.1	30.1	37.3
Reducing Sugars %	22.9	24.9	26.4	16.6
Total Sugars %	43.5	52.4	58.0	55.8
Carbonate Ash %	4.7	3.0	10.8	10.9
Nitrogen % X 6.25	4.1	3.6	1.6	2.1
pH	5.0	5.9	5.7	5.5

[1] Average of 36 samples.
2 Average of 16 samples (laboratory prepared).
[3] Fort (3). (See literature cited).

an average of 1.7 percent total sugars per year of storage. Similarly, 13 samples collected in April of 1948 from 11 producers were found to have lost an average of 3.2 percent total sugars per year of storage. In contrast, however, are 13 samples of clarified citrus molasses made in the laboratory from different varieties of both grapefruit and orange that showed an average increase of 0.4 percent total sugars per year of storage. These clarified citrus molasses samples precipitated considerable insoluble matter during storage and since only the supernatant liquid was analyzed it is understandable that the percent total sugars could increase even in the face of a slow deterioration of sugars during storage. Table 2 summarizes these data showing maximum and minimum values as well as a comparison of sugar losses noticed in blackstrap during storage. Owen (6) investigating the deterioration of blackstrap found that those samples having the highest total sugar values were most

TABLE 2.
COMPARISON OF SUGAR LOSSES DURING STORAGE OF CITRUS MOLASSES AS COMPARED WITH BLACKSTRAP.

Description of Sample	No. of Samples	Type of Value	Change in Total Sugars per Year of Storage (Calculated %)
Commercial Citrus Molasses January 1948	10	Average	−1.7
		Maximum	−3.4
		Minimum	−0.6
Commercial Citrus Molasses April 1948	13	Average	−3.2
		Maximum	−7.7
		Minimum	−1.3
Clarified Citrus Molasses	13	Average	+0.4[1]
		Maximum	−1.3
		Minimum	+1.4[1]
Blackstrap[2], Factory No. 1	1	....	−6.3
Blackstrap[2], Factory No. 2	1	....	−11.9
Blackstrap,[2] Factory No. 3	1	....	−11.3

[1] Actually increased in percent total sugars (See text)
[2] Owen (6)

susceptible to actual deterioration in storage. Upon examining each of the 3 groups of citrus molasses samples previously mentioned, it was noted that within each group this same correlation generally held true for citrus molasses. The clarified samples of citrus molasses, however, did not deteriorate with the rapidity expected for their high total sugar content and is probably accounted for by the removal during clarification of some colloidal unstable organic substances contributing to this deterioration.

Although there has been a loss of total sugars in each of the commercial citrus molasses samples during storage, the corresponding change in degree Brix is so small as to be insignificant, being but a fraction of the percent of total sugars lost.

The froth fermentation or spontaneous foaming of molasses has been the subject of much inquiry, for even though it happens infrequently, it can be a serious economic loss. This phenomena occurs when molasses spontaneously heats to such high temperature as to "boil" and foam out of its storage tank leaving but a charred mass. Usually the molasses foams to some multiple volume that is greater than the storage space available. All attempts to correlate this instability with some other chemical or physical analysis have been futile to date. Owen (6) corroborates this and further states regarding blackstrap "that actual deterioration involving loss of sugars is accompanied by gas evolution, but it is also true that the latter cannot be taken as an indication of the destruction of sugars." Manometric measurement of gas evolution from various citrus molasses samples was similarly found not to correlate with loss of sugars. This gas formation in citrus molasses can also be found in concentrated orange juices and was investigated by Curl (2) who studied synthetic mixtures and found that mixtures of amino acids and sugars produced darkening and gas which was further accelerated by certain metallic ions. Hucker and Brooks (5) also demonstrated that gas is produced by mixtures of nitrogen compounds and glucose, a reaction which is more commonly known as the Maillard reaction. When various chemicals were added in small quantities to citrus molasses under manometric observation it was noticed that formaldehyde, though impractical to use, mitigated gas formation, and that pH changes on the acid side had little effect.

While studying this spontaneous foaming it was noticed that certain commercial citrus molasses samples had shown sub-surface gas formation during the first months of storage. When these samples were disturbed they tended to foam more readily much like a carbonated beverage. Analysis of a citrus molasses sample from a tank foaming excessively showed no significant difference from other samples on hand. This particular storage tank was finally controlled by aeration which may have helped only by its agitation action on the surface foam, and it follows also that the reaction may already have spent itself. It appears significant that of twenty samples of clarified citrus molasses made in the laboratory only two have shown any sign of sub-surface gas formation and both of these samples had an excessive precipitation of insolubles during storage. None of these samples showed any sign of surface foaming and they have the further advantage of having a less stable foam system. Among the conditions contributing to stable foams, are high viscosity and finely divided solids, both of which have been reduced by clarification. Hucker and Brooks (5) have demonstrated that high viscosities tend to increase the chances of spontaneous foaming and that high storage temperatures further aggravate this condition with 40°-45°C. being a critical temperature range.

Many explanations of froth fermentation have been advanced over the years, but most agreement has been found in two theories; one, the glucic acid theory which is favored by Browne (1) and relates that the action of lime on invert sugar produces unstable organic substances that further reacts with invert sugar. The second is the Maillard theory whereupon it is believed that the source of gas formation is the reaction between amino acids and invert sugar. Hucker and Brooks (5) seemed to have definitely established that microorganisms can be considered only a minor cause.

Influence of pH During Storage

Although the initial pH of a citrus

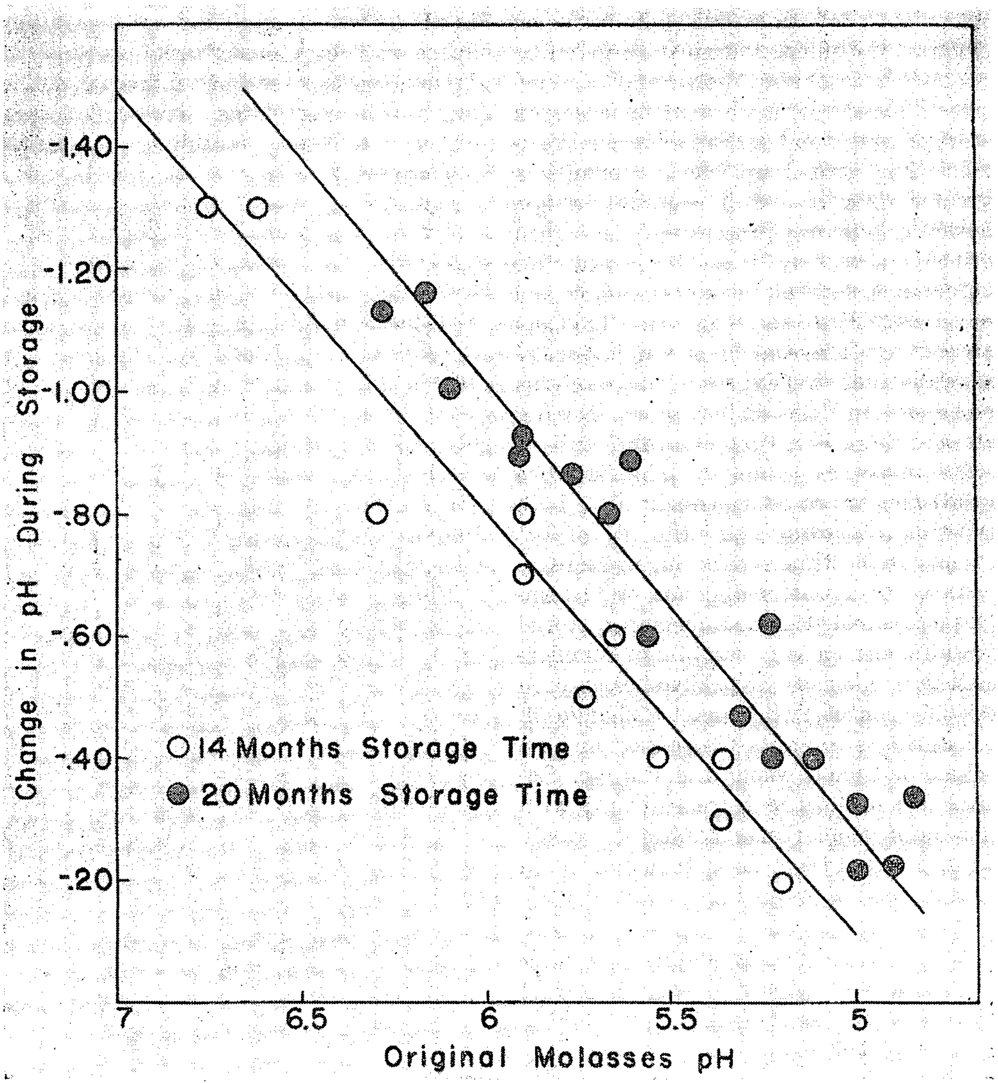

Figure 1. A scatter diagram comparing the change in pH of clarified citrus molasses after 14 months storage versus the change for commercial citrus molasses after 20 months.

press liquor is almost entirely controlled by the quantity of hydrated lime added to the chopped citrus peel, there are certain other factors to be considered in arriving at the final pH of a citrus molasses sample in storage. Attempt should be made to control the initial pH of citrus molasses between the limits of 6.0 to 6.5 with due thought given to the destructiveness and other inherent disadvantages of excessive alkalinity on sugars. Consideration must be given by processors to the corrosive and destructive influence of a too acid molasses on storage equipment. It is generally realized that grapefruit peel demands a greater quantity of lime than orange, however, during processing and upon prolonged heating it is to be further noted that both citrus press liquor and molasses will decrease in pH. This decrease in pH averaged one unit for 14 samples that were processed to citrus molasses in the laboratory. During storage there is a further drop in pH of citrus molasses samples. The decrease in pH appears to be dependent upon both the time of storage and the pH of the sample at the time it was put in storage. Figure 1 is a scatter diagram of 11 samples of clarified citrus molasses stored for 14 months and 17 samples of commercial citrus molasses that have been stored for 20 months, wherein the change in pH during storage is plotted against its pH at the time it was put in storage. Below a pH of 4.5 the decrease in pH with time is of smaller magnitude than would be anticipated from this figure and would appear to be approaching a point of little change.

Prior to storage the quantity of total sugars found as reducing sugars in citrus molasses is definitely related to the pH of the processed molasses. As was similarly found in the analysis of Valencia orange juice by Roy (7), the lower the pH the greater the ratio of reducing to nonreducing sugars. In storage it was noticeable that almost without exception the percent of total sugars as invert sugar had increased with there being a tendency for the samples having the lower pH to show the greater percent change.

In studying the clarification of citrus molasses it was found that in storage clarified molasses precipitated considerable insoluble matter and that variations in pH between 4 and 8 did not perceptibly decrease the quantity precipitating. It is also to be noted that pH could not in any way be correlated with sugar losses, or spontaneous frothing of citrus molasses.

Physical Changes During Storage

It has undoubtedly been previously recognized that citrus molasses upon storage tends to increase in viscosity, sometimes appearing to gel, but hitherto an explanation has been lacking. This increase is strikingly seen in Figure 2 in which is plotted the viscosities of 18 samples of commercial citrus molasses after over one year of storage against their Brix, versus 11 samples of commercial citrus molasses that had been in storage only one month. The regression lines show a considerable increase in viscosity with time. Four other molasses samples whose Brix were between 68° and 73° had solidified and are not shown in this diagram. The wide variation of viscosities for samples of similar Brix is largely due to the quantity of suspended insolubles present. When the viscosities of many clarified molasses samples were plotted in similar fashion against those of nonclarified citrus molasses by Hendrickson (4), the regression line for clarified molasses showed its viscosity to be seven times smaller and showed less viscosity variation between samples. The influence of temperature and Brix upon the viscosity of an excellent sample of commercial citrus molasses prior to storage can be seen in Figure 3.

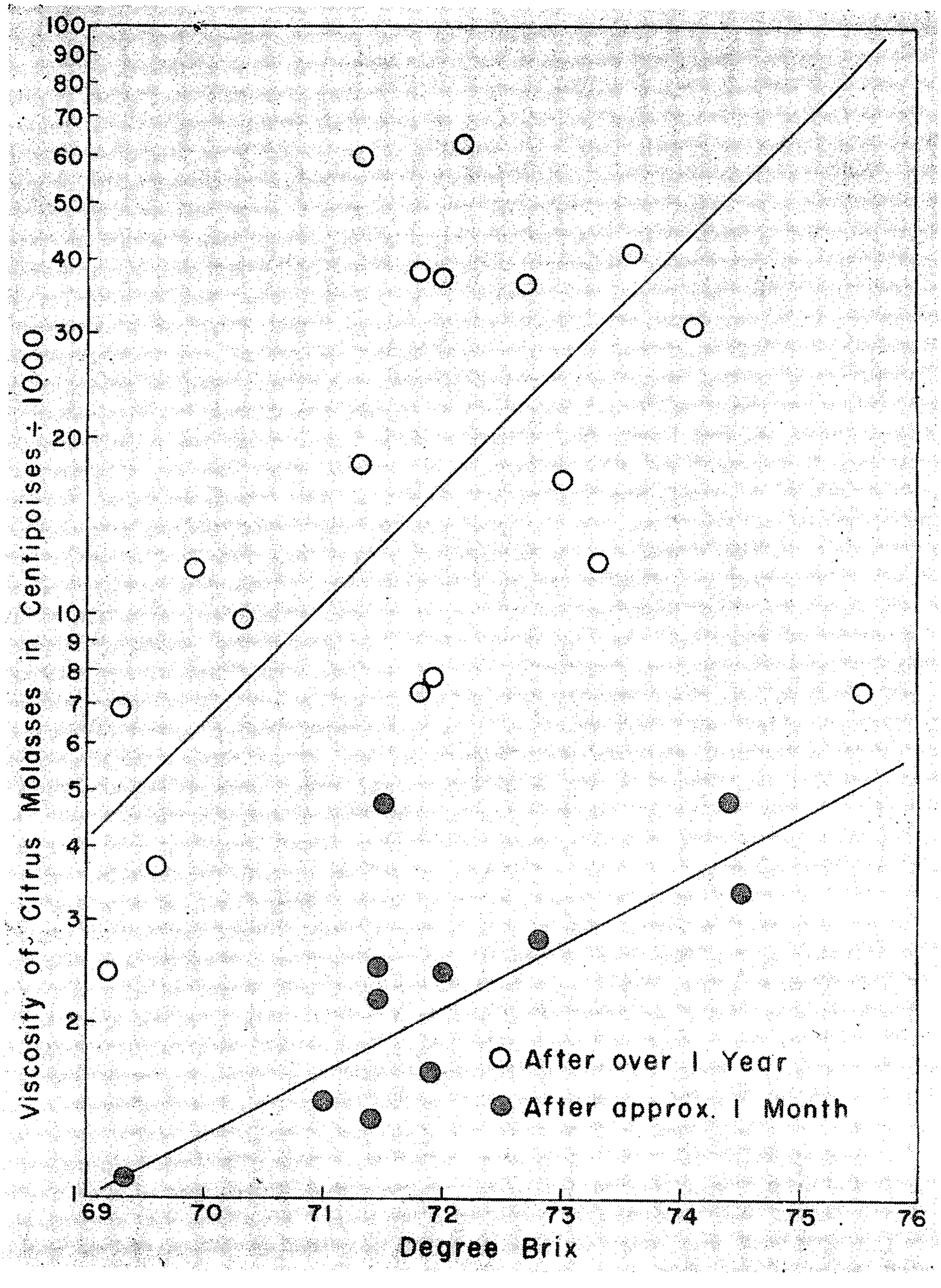

Figure 2. A scatter diagram comparing the viscosity at 30°C. of citrus molasses samples stored for over one year versus samples that had been in storage approximately one month.

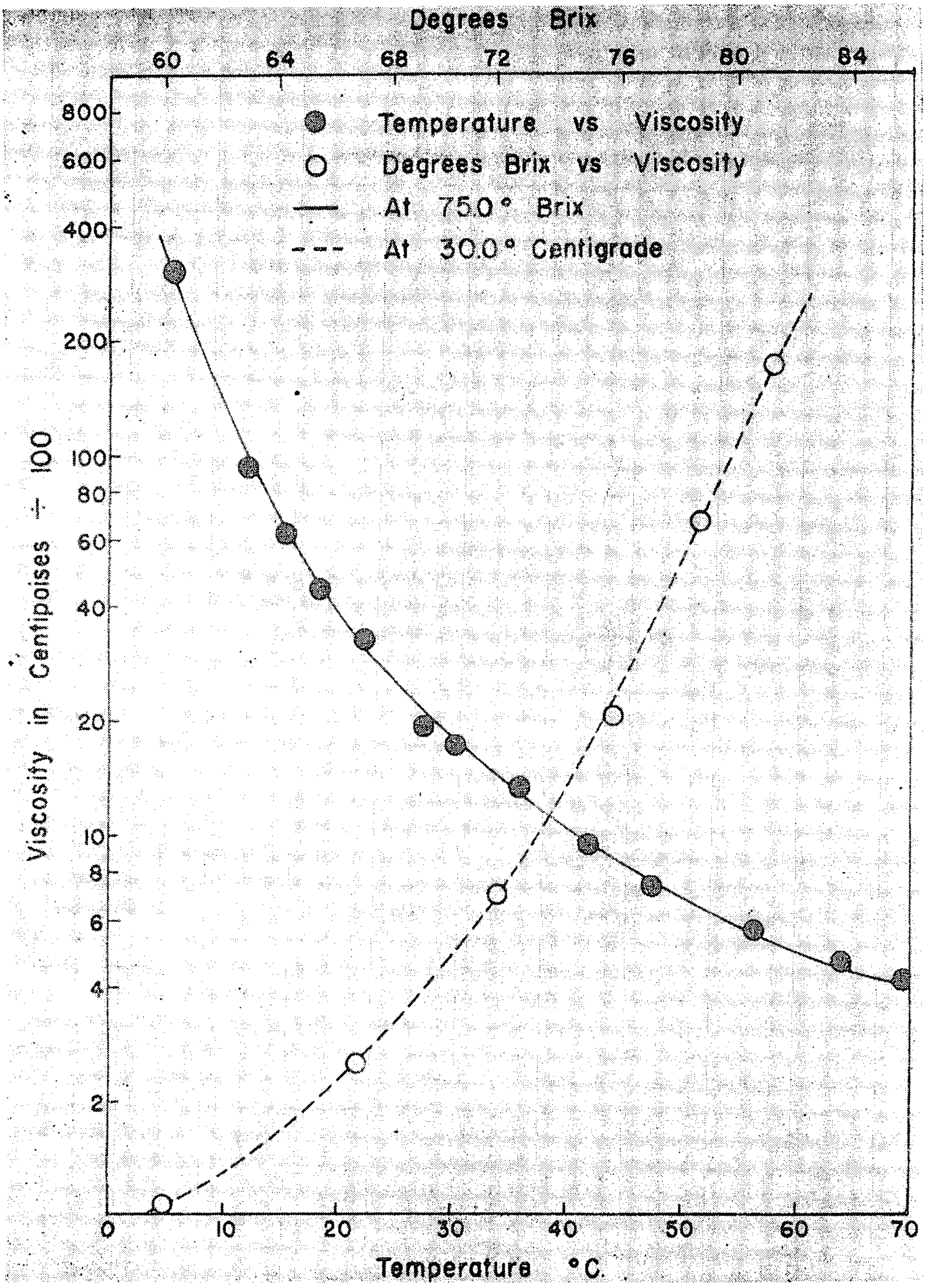

Figure 3. Influence of concentration and temperature upon the viscosity of citrus molasses.

Since the insoluble matter is mostly responsible for the wide variations in viscosity, it is well to examine the source of insolubles. Prior to concentrating citrus press liquor to citrus molasses it has been noted as having anywhere from 0.2-0.5 percent by weight insoluble matter depending upon how it has been screened and the degree of liming. During the process of evaporation another one to three percent, on a citrus molasses basis, of calcium organic salts are estimated to precipitate. In storage a considerable quantity of insoluble matter has been noted to precipitate which is especially noticeable in clarified citrus molasses samples. A greater quantity of insolubles was found to precipitate from clarified grapefruit molasses than from the clarified orange samples, with one sample of grapefruit molasses precipitating 5.6 percent insolubles by weight, two-thirds of which was soluble in alcohol. It is not strange then for the amount of insolubles to build up to a rather high percentage. For example, one commercial molasses sample that had solidified was observed to have 9.6 percent insolubles.

Examining more closely the voluminous quantity of insolubles precipitating from grapefruit molasses samples it was noted that the majority of insolubles were crystalline and appeared as clusters of needles growing from common centers. In Figure 4 is shown a photomicrograph of these crystals which were subsequently identified as naringin. The very bulkiness of these crystalline needle formations, as well as the quantity of it, and the percent of calcium organic salts precipitating in storage points to the cause of increasing viscosities in storage. By heating the citrus molasses the naringin crystals will, by virtue of their increased solubility, go back into solution and remain as a supersaturated solution for some time. Hesperidin has not been isolated from orange molasses to date.

Conclusions

In retrospect, citrus molasses was found to lose an average of 2-3 percent total sugars per year of storage while clarified citrus molasses showed little if any loss. There was little change in degree Brix of these samples, being but a fraction of the sugar loss. Those molasses samples having the highest total sugars appeared most susceptible to loss of sugars in storage although other unknown factors would appear to be equally important. The spontaneous foaming of citrus molasses was investigated, but could not be correlated with any chemical or physical analyses. Clarified citrus molasses, however, showed a perceptible improvement in stability.

The pH of citrus molasses was noted to decrease with time and was greatest for those samples having the higher pH. Below a pH of 4.5 the samples appeared to approach a point of little change. The ratio of reducing to nonreducing sugars was found dependent in part upon pH and there was a tendency for samples having the lower pH to show the greater increase in percent inversion.

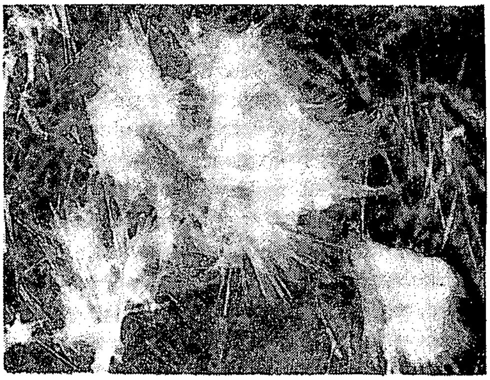

Figure 4. A microphotograph of naringin in citrus molasses. (Magnified 50 X)

During storage, there was an increase in viscosity which was felt to be caused by the quantity of insolubles precipitating. Upon closer examination some of the insolubles were found to be naringin which crystallized in needle fashion while another portion was found to have considerable calcium content. Upon heating, the naringin is redissolved and remains in solution for a considerable length of time. The viscosity of the molasses sample meanwhile will have been greatly reduced, allowing more ease in handling the product.

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AN INDEX OF PASTEURIZATION OF CITRUS JUICES BY A RAPID METHOD OF TESTING FOR RESIDUAL ENZYME ACTIVITY[1]

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In the course of some investigations on the effect of different temperatures and rates of heating used in the pasteurization of citrus juices, it became evident that there was need for a simple test for determining the presence of the pectinesterase enzyme. An investigation was started and a test was developed that is more rapid than those now available. It has been found useful in the experimental work and should be of value in determining the adequacy of pasteurization in commercial packs. The test is simple, rapid, gives positive results, and is suitable for routine use in control laboratories of canning plants.

The test is based on the activity of the pectinesterase enzyme which hydrolyzes methyl ester groups to give acid groups which increase the acidity. This enzyme also destroys cloud stability in the canned product and if it is not inactivated during pasteurization, the juice in the upper portion of the can will be clarified and a sludge will settle to the bottom or a curd may form. Of the enzyme systems tested, the pectinesterase enzyme requires the highest temperature for inactivation. Canned juice should be as stable as possible and to this end it is considered desirable that all enzyme systems be inactivated. Excessive heating is also to be avoided to decrease the danger of scorching and the development of undesirable flavor changes. In general, the organisms which will grow actively in citrus juices are destroyed at temperatures below those required for enzyme inactivation.

One of the procedures investigated was that suggested by Jansen (1). This method was to centrifuge the cloud from the juice as much as possible; extract this cloud material with quarter molar sodium chloride, maintaining the pH at 7.5 and after filtration, assaying this solution for pectinesterase by the procedure described by Lineweaver and Ballou (2) and MacDonnell, Jansen and

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[1] Report of a study made under the Research and Marketing Act of 1946.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.